Oestrus pheromones in farm mammals, with special reference to cow

SUMANT VYAS[1], C BRIANT[2], P CHEMINEAU[3], C LE DANVIC[4] and P NAGNAN-LE MEILLOUR[5]

National Research Centre on Camel, P.B. 07, Bikaner, Rajasthan 334 001 India

ABSTRACT

Pheromones are defined as chemical signals that are released from one individual and induce specific endocrine or behavioural reactions in another individual of the same species. Odours play a significant role in signalling the stage of reproduction of the cow, and the bull can use a combination of factors displayed by the female to determine its receptivity. During oestrus, females release olfactory molecules, thus signalling the stage of their cycle and stimulating sexual behaviour and functions of males. Attempts were made to review the chemical nature of olfactory signals of oestrus in milk, urine, blood, saliva, skin gland secretion during the proestrous and oestrous cycle. Several behavioural studies indicated that bovine milk from different stages of the oestrous cycle had different odours. Bulls have also been found to detect pheromone odours and differentiate between oestrus and non-oestrus urine. Bioassay involving rats revealed the presence of maximal pheromone activity during proestrus. Vaginal fluid is also reported to act as a chemical signal. Saliva, serum, skin gland secretions and faeces have also been studied for their pheromonal properties. Systemic evaluation of either urine or vaginal secretions over the whole cycle has yet to be investigated. The use of artificial olfaction could enable more accurate detection of oestrus and potentially increase fertility in cows. The current status of chemical signals (pheromones) of oestrus and their identification in farm animals is reviewed in this article with emphasis on cows.

Key words: Cattle, Farm Animals, Oestrus, Pheromone

The term pheromone was introduced by Karlson and Lüscher (1959) as substances secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction, for instance a definite behaviour or developmental process. Afterwards this term was extended to any form of innate intraspecific chemical communication i.e. to define chemicals emitted by living organisms to send messages to individuals of the same species. Most pheromones consist of blends of two or more chemicals, which need to be emitted at exactly the right proportions to be biologically active and to prevent mismating between species. These pheromones are associated to binding proteins and very specific receptors responsible for olfactory perception in the target animal (Ha and Smith 2006). This perception induces stereotyped behaviour (releaser pheromone) and/or physiological changes (primer

Present address : [1]Senior Scientist (sumantv@scientist.com).

[2,3]UMR 6175 INRA/CNRS/Université François Rabelais de Tours/Haras Nationaux- Unité de Physiologie de la Reproduction et des Comportements F 37380 Nouzilly, France.

[4,5]UMR 8576 CNRS/Université Lille 1 Unité de Glycobiologie Structurale et Fonctionnelle Bâtiment C9- F-59655 Villeneuve d'Ascq CEDEX, France.

pheromone). The pheromones are different from the recently coined term "signature mixtures" which are defined as a variable chemical mixture (a subset of the molecules in an animal's chemical profile) learned by other conspecifics and used to recognize an animal as an individual (e.g. Lobsters, mammals) or as a member of a particular social group such as a family, clan or colony (e.g. ants, bees, mongoose). A key difference between pheromones and signature mixtures is that in all taxa so far investigated it seems that signature mixtures need to be learnt (Wyatt 2009 and 2010). Both pheromones and signature mixtures are detected by main olfactory system or accessory olfactory system or both depending on pheromone and species. The accessory olfactory system consists of a sensory organ, the vomeronasal organ, and its central projection areas– the accessory olfactory bulb, which is connected to the amygdale and hypothalamus, and also to the cortex (Mucignat-Caretta 2010). Most of our knowledge on pheromones relies on insects, due to the clear-cut and readily identifiable response that insects have to these molecules, which has allowed the identification of pheromones from over three thousand species (www-pherolist.slu.se/pherolist.php) and has led to new concepts of pest control. Contrary to the large number of known insect pheromones, very few were characterised

in mammals, despite the importance of these chemical cues in regulating social and reproductive behaviours. Chemical communication plays an important role in mammalian sexual behaviour and reproductive processes (Brennan and Zufall 2006). Animals communicate information concerning reproduction to conspecifics in order to co-ordinate reproductive activities (Rekwot *et al.* 2001).

In farm animals, the generalised use of artificial insemination (AI) has made reproduction more effective. Efficiency of AI partly depends on the precise detection of female receptivity, which is made by breeders on visual cues or with the help of detector males. Novel 'pheromonal cues' based methods are required, which could not only help in detection of oestrus in the farm animals but also satisfy the social requirements of sustainable environment and animal welfare. Thus, it is necessary to understand fundamental biology of the target livestock species, in particular the identification of chemical signals and their precise involvement in oestrus detection by the males. The present article attempts to review the literature available on the pheromones of oestrus and their identification in farm animals, especially cows.

Types of pheromones

If the original definition of pheromones relies on insects, it can easily be applied to mammals– it refers to chemical cues that are exchanged between congeners and either elicits a stereotyped and innate behaviour or induce a physiological change in the recipient endocrine or reproductive system (Doty 1976, Izard 1983). The major divergence with the original definition is the chemical nature of pheromone components, which were originally supposed to be air-borne volatiles, suggesting a long-range attraction. But increasing knowledge on the chemical nature of pheromones in both insects (cockroach, Korchi *et al.* 1999) and vertebrates (mice, Morè 2006) has demonstrated that proteins or peptides could act in the transfer of intraspecific information. Pheromones can be classified into following types:

Releaser pheromones: These pheromones elicit an immediate behavioural response. Rabbit milk contains a pheromone 2-methyl, but–2-enal which elicits stereotyped nipple-search behaviour in rabbit pups, and is vital for them to locate the nipples during brief daily period of suckling (Schaal *et al.* 2003). In the pig, the male stimulates the immobilization reflex of the sow by using sex pheromones contained in its saliva (Signoret and du Mesnil du Buisson 1961, Signoret and Mauleon 1962). These pheromones were identified as the steroids 5α-androst-16-en-3-one and 5α-androst-16-en-3α-ol (Patterson 1966, Patterson 1968), which are synthesized in the testes and released in boar saliva (Claus 1979).

Primer pheromones: These pheromones mediate slow developing and longer-lasting changes to the endocrine state or development. A testosterone dependent constituent of male mouse urine, a-farnesene, is a primer pheromone. It accelerates puberty of pre-pubertal female mice (Novotny *et al.* 1999, Novotny 2003). In domestic mammals, especially small ruminants, priming pheromones from the male seems to have an influence on the induction of puberty, the termination of seasonal anoestrus and shortening of postpartum anoestrus (Gelez and Fabre-Nys 2004).

Signaller pheromones: These pheromones convey the information about the sender, such as individual or group identity, which are important for parent-offspring recognition and mate choice (Potts *et al.* 1991, Yamazaki *et al.* 2000).

Modulator pheromones: These pheromones affect mood and thought processes in humans. This category has not gained wide acceptance (Wysocki and Preti 2004).

Major urinary proteins (MUPs) and Odorant binding protein (OBPs): Major urinary proteins (MUPs) and α2u proteins are lipocalins that were first described in mouse and rat, which are synthesized in the liver and excreted in the urine (Finlayson *et al.* 1965, Shaw *et al.* 1983). Their roles are 1) to transport the pheromone in biological fluids, 2) to extend the period of bioavailability of the pheromone by delaying its liberation, and 3) to modulate the pheromone activity (Hurst and Beynon 2004). Odorant binding protein (OBPs) discovered in the nasal tissues of several vertebrates have strong similarity with MUPs (Pelosi *et al.* 1982, Cavaggioni *et al.* 1987). They are believed to shuttle odorants from the environment to the underlying odorant receptors, for which they could potentially serve as odorant presenter (Pugalenthi *et al.* 2007). In some cases, the same genes have been found to be expressed in both the nose and in the liver (Utsumi *et al.* 1999). Presence of endogenous ligands only in MUPs, make them different from OBPs. These endogenous ligands have been recognised as specific pheromones (D'Innocenzo *et al.* 2006).

The boar's salivary gland (submaxillary gland) also secretes lipocalins which contain two components of the boar sex pheromonal system as endogenous ligands, namely, 5α-androst-16-en-3-one and 5α-androst-16-en-3α-ol (Marchese *et al.* 1998). These proteins are also expressed in the nasal tissues of both sexes in pig but in this case these are devoid of ligands (Scaloni *et al.* 2001).

A general scheme of olfactory coding has hypothesised that pheromones are detected by sensory neurons of the vomeronasal organ (VNO), while general odours are detected by the main olfactory epithelium (MOE) sensory neurons (Dulac and Torello 2003, Brennan and Keverne 2004). But there is growing evidence that the coding of olfactory signals is more complex. Some pheromone-mediated behaviours are still effective after VNO lesions (Hudson and Distel 1986, Dorries *et al.* 1997, Lévy *et al.* 2004). Conversely, mouse VNO neurons can be stimulated by odorants not emitted by the same species, such as floral and woody smelling compounds (Sam *et al.* 2001).

Role and importance of olfactory compounds and pheromones in reproductive processes

The odoriferous compounds, potentially pheromones, may be released by males to influence female ovarian functions and oestrus behaviour as shown for sheep (Knight *et al.* 1983, Signoret 1991), goats (Chemineau *et al.* 1986, Claus *et al.* 1990), cattle (Izard and Vandenbergh 1982) and pigs (Signoret and Mauleon 1962, Claus and Schams 1990, Claus 1994). However in cattle, the role of pheromones, especially the "oestrus pheromones" in cattle reproduction is not as clearly defined as it is in other species such as sheep, goat and pig, possibly due to nutritional and other environmental stresses (Rekwot *et al.* 2001). Mating within a narrow (12 to 22 h) period on the day of oestrus is a prerequisite for optimal fertilization in cow (Schams *et al.* 1977). These time requirements are mainly regulated by female lordosis behaviour, which allows copulation. In unrestrained cows, mating is preceded by a complex sequence of bull-cow interactions that starts fewdays before oestrus (day 0). In pro-oestrus, bulls separate cows from the rest of the herd (Schloeth 1961, Reinhardt 1983). To determine female's receptivity, a bull uses a combination of factors displayed by the female, including visual, tactile, auditory and olfactory stimuli (Izard 1983, Zalesky *et al.* 1984). Visual and acoustic stimuli from cow were found important for optimal detection of oestrus by a bull (De Vuyst *et al.* 1964, Blaschke *et al.* 1984). In contrast, there are several reports that clearly show that there is an olfactory component, independent of vision, in the stimulation of male reproductive behaviour (Sambraus and Waring 1975, Paleologou 1977, Jacobs *et al.* 1980, Izard and Vandenbergh 1982, Klemm *et al.* 1987). Indeed, females seem to release olfactory molecules during oestrus, thus signalling the stage of their cycle, and stimulating sexual behaviour and endocrine functions of males. Such effects were shown for horse (Stahlbaum and Houpt 1989), sheep (Lindsay 1965, Schanbacher *et al.*1987, Gonzales *et al.* 1991, Walkden-Brown *et al.* 1993), goat (Ladewig and Hart 1980, Blissitt *et al.* 1994) and bovine species (Hradekcky *et al.* 1983, French *et al.* 1989). Presence of pheromones was indicated in equine urine which may act as markers for detection of oestrus (Ma and Klemm 1997). For cows it was demonstrated by use of a bioassay (involving rats and not bulls) that maximal pheromone activity is present in urine prior to the onset of behavioural oestrus (Dehnhard *et al.* 1991).

The behavioural pattern of bulls and a possible involvement of the olfactory cues or pheromonal substances, in a group of oestrus-synchronised cows have been established (Masaki and Ohta 1990). Bulls routinely investigate the anogenital region or urine of females (Hafez and Bouissou 1975). Sniffing, licking and nuzzling of the anogenital region usually elicits urination from females under investigation. The male then puts its nose and mouth directly in the stream of urine or sniffs and licks the urine-soaked substrate. The sniffing and licking behaviours follow with the male showing a typical posture of raised head with open mouth and curled upper lips, called "flehmen". This behaviour is seen in many ungulate species in both males and females in response to several odours (Estes 1972). Oestrus-signalling compounds from urine and vaginal secretions were suggestive to lead bulls to flehmen reaction and later to a more intensive mounting behaviour (Hradecky *et al.* 1983, Garcia *et al.* 1986). The vaginal mucus and urine of oestrus cows, when rubbed on the vaginal membranes of non-oestrus cows was reported to attract and stimulate bulls through olfaction and a volatile odour in the cervico-vaginal mucus of oestrus cows was believed to be a source of sexual attraction for bulls (Hart *et al.* 1946).

The presence of maximal pheromone activity during pro-oestrus suggests that it is more important to signal the imminence of oestrus to the bull, than for stimulating precopulatory male sexual behaviour test in male rats and not in bulls (Dehnhard *et al.* 1991). Similarly, investigation of olfactory stimuli in a dairy herd revealed that it reaches a maximum in pro-oestrus (French *et al.* 1989). In contrast, Hradecky *et al.* (1983) observed that the bull flehmen reaction is most frequent on theday of oestrus. Similarly, the odour intensity was maximal on day 0, as detected by trained dogs (Kiddy and Mitchell 1981). This discrepancy was explained by a field study (Blazquez *et al.* 1988a) on a grazing dairy herd, which showed that cows attracted bulls the day before oestrus and that this attraction is maintained throughout oestrus. Dehnhard *et al.* (1991) observed that pheromone activity still remained, though there was a decline in the activity on the day of oestrus. If all sensory stimuli contribute to the finding of a partner and to sexual behaviour, the olfactory stimuli, however, seem to be more important for attracting partners rather than for stimulating the mating.

Assays for the oestrus pheromones

Analytical assays: The identification of the oestrous pheromones is attempted by analytical techniques comprising first, extraction of compounds using organic solvents (Ramesh Kumar *et al.* 2000) or without organic solvents i.e. Solid Phase Micro Extraction (SPME) (Guiraudie-Capraz *et al.* 2005), followed by Gas Chromatography-Mass Spectrometry (GC-MS) (Ramesh Kumar *et al.* 2000, Guiraudie-Capraz *et al.* 2005).

Although several sequence based search methods were exploited for protein family prediction, less effort was devoted to the prediction of OBPs from sequence data and this area is more challenging due to poor sequence identity between these proteins. Recently Pugalenthi *et al.* (2007) have proposed a new algorithm that uses Regularized Least Square Classifier (RLSC) in conjunction with multiple physicochemical properties of amino acids to predict OBPs from sequence derived properties irrespective of sequence similarity. It was reported that this method predicts 92.8%

of 56 OBPs non-homologous to any protein in the Swissprot database and 97.1% of the 414 independent database proteins.

Bioassays: The availability of a highly practicable bioassay is essential for assessing the biological activity of the oestrus-related pheromones characterized by physicochemical techniques. Several attempts were undertaken to characterize the biological and chemical properties of an oestrus-specific odour in cow using bulls (Hradecky 1986), dogs (Kiddy *et al.* 1978) or rats (Ladewig and Hart 1981, Dehnhard and Claus 1988) for bioassays. The species of the detecting animal has tremendous importance for the validation of biological activity. Thus, the use of conspecific males is relevant to characterize the pheromonal nature of a chemical signal emitted by females of the same species. In this case the behavioural response is expected to be stereotyped (courtship sequence) and non-learned, even if it is well known that adult males mate more successfully than young naïve males (Reinhard 1983). Besides, the use of trained detector animals such as dogs or rats strongly suggests that one or several odours could be common to the oestrus specific secretions of these species. In that case, the perception of one or several odours being part of the pheromone of their female can evoke one or several reactions associated with the sexual behaviour (e.g. penile erection). As a matter of fact, none of these studies reported the observation of a full stereotyped courtship behaviour, which could be obtained in response to conspecific females in oestrus or their body fluids (Dehnhard and Claus 1988, Rampin *et al.* 2006).

Nevertheless, the increase of Flehmen frequency is only one of the behavioural reactions that are expected in response to the perception of a pheromone. The oestrus specific odours can be ultimately qualified of "pheromone" at the condition they evoke stereotyped, non-learned behavioural sequence in conspecific males (Rivard and Klemm 1989).

In a bioassay, using rats trained to detect olfactory differences between estrous and diestrous cow urine. The oestradiol administration resulted into estrus symptoms observed by trained herdsman but urine from such cows were not identified by rats as estrous urine. This suggests that the presence of the ovary seems to be necessary for oestrus-specific odour detected by trained rats used in the study (Dehnhard *et al.* 1991). It is not clear, if the odour identified by rats in the oestrus urine from cyclic cows is caused by molecule other than the "pheromone specific to estrus" because the same was not identified by rats in urine from oestradiol treated ovariectomised cows expressing signs of estrus.

The identification of a pheromone specific of a physiological stage in one species therefore must satisfy two conditions: (i) the molecule can be identified in that stage and (ii) molecule induces a physiological effect in this species. Tests in other species do not indicate that the molecule is a pheromone.

Physiological sources of pheromones

The chemical nature of pheromones is not precisely known, but several body fluids have been reported as potential sources of oestrus signalling compounds. The volatile compounds termed or suggestive of pheromonal properties in different bodily fluids of animals are being summarised in Table 1.

Oestrus pheromones in milk: Milk volatiles can be transferred directly from forage via the rumen (Honkanen *et al.* 1964) and the respiratory tract (Shipe *et al.* 1962) or produced by metabolism (Virtatnen and Lampila 1967, Dumont and Adda 1978). The volatile constituents of bovine milk are of great interest to food scientists because those constituents contribute to flavour (Gordon and Morgan 1972, Dumont and Adda 1978, Badings 1991, Shiratsuchi *et al.* 1994). The volatile compound γ-12:2 lactone, identified in cow's milk was shown to be an active odorant (Bendaall 2001) and the lower rumen pH of cows fed on a concentrate diet might facilitate the production of such lactones (Urbach and Stark 1978). Since many flavour qualities are due to odour detection (Gordon and Morgan 1972), some volatile flavour compounds may possibly function as pheromones for olfactory communication. Indeed, the components of the

Table 1. Volatile compounds termed or suggestive of pheromonal properties in different bodily fluids of animals

Animal	Pheromonal compunds	Biological fluid	Reference
Boar	5α-androst-16-en-3-one 5α -androst-16-en-3 α -ol,	Saliva	Patterson (1966, 1968)
Rabbit	2-methylbut-2-enal.	milk	Schaal *et al.* (2003)
Male mouse	α-farnesene	urine	Novotny *et al.* (1999)
Cow	Acetaldehyde	Oestrus blood	Klemm *et al.* (1994)
	γ-12:2 lactone	milk	Bendall (2001)
	n-propylphthalate	Oestrus urine	Ramesh Kumar *et al.* (2000)
	1–iodoundecane	Oestrus urine	Ramesh Kumar *et al.* (2000)
		Oestrus faeces	Sankar and Archunan (2006)
	Acetic acid	Oestrus faeces	Sankar and Archunan (2006)
	Propionic acid	Oestrus faeces	Sankar and Archunan (2006)
Elephant	(Z)-7-dodecen-1-yl-acetate	Oestrus urine	Rasmussen *et al.* (1996)

pig appeasing mixture (Pageat 2001) were characterized in milk by Solid Phase Micro Extraction (SPME)-GC-MS (Guiraudie-Capraz *et al.* 2005). Using a combination of PAGE and mass spectrometry for protein identification evidence of a putative odorant binding protein was obtained in bovine colostrum. Its biological function is unclear, but pheromone transport could be considered (Fukuda *et al.* 2009).

The headspace sampling technique (GC-MS) and consequent reduction of sample pre-treatment allowed the identification of low-molecular weight volatile compounds in milk samples (Toso *et al.* 2002). Acetaldehyde, which had previously been found to be a marker of oestrus from headspace GC of bovine blood (Klemm *et al.* 1994) and vaginal secretions (Ma *et al.* 1995), was not detected in the milk samples because this compound has a shorter retention time than the solvent (diethyl ether). However, the results suggested that the property of three types of milk (pro-oestrus, oestrus and di-oestrus) samples were distinctly different from one to another (Weidong *et al.* 1997). Only one behavioural study was conducted and has failed to support a pheromone role for acetaldehyde in bovine (Prescicce *et al.* 1993).

Oestrus pheromones in urine: The role and importance of urine chemical signals in reproductive behaviour was clearly established in several species of mammals, especially rodents (Xia *et al.* 2006). In cattle, the bulls can detect pheromone odours and differentiate between oestrus and non-oestrus urine (Sambraus and Waring 1975). Urine is known to contain a large array of compounds that may confound the isolation of pheromones (Albone *et al.* 1986) and it is also believed to be an important source of oestrous signals (Crowell-Davis and Houpt 1985).

Several attempts were made to isolate and characterize the oestrus-signalling pheromones from cow's urine. Ramesh Kumar *et al.* (2000) analysed the GC-MS profiles of the volatile compounds of cow's urine extracted at three different stages: preovulatory (3–5days before oestrus), ovulatory and postovulatory (2–4days after oestrus). In a preliminary study, nine organic solvents were used to extract the compounds from pooled urine (n-hexane, acetone, methanol, ethanol, petroleum ether, diethyl ether, chloroform, dichloromethane and benzene). The maximum response was obtained when dichloromethane (DCM) was used for extraction, and the chemical profile of oestrus urine was distinguished by the presence of two specific compounds, di-n-propylphthalate and 1-iodo undecane, that were not found in other samples. As oestrous urine was found to elicit sexual behaviour in cattle, these two compounds may represent important chemical compounds that elicit signals that allow the bull to detect 'oestrous odours'. However the role of such compounds identified in the oestrus phase needs to be confirmed by their effects on the behaviour of bulls.

Oestrus pheromones in vaginal mucus: Though urine is considered to be primary source for chemical communication,

vaginal fluid is also reported to act as a chemical signal. Moreover, it is possible that urine and vaginal fluid may act together for completion of the pre-copulatory behaviour and successful mating (Klemm *et al.* 1987). Evidence for a pheromone was indicated by studies where dogs or rats were trained to detect oestrus by smelling cervico-vaginal secretions or urine (Kiddy *et al.* 1978, Ladewig and Hart 1981). Klemm *et al.* (1987) identified over 20 compounds: alcohols, diols, alkenes, ethers, diethers, ketones, primary amines, and aromatic alkanes. Nine compounds were found in samples that had been validated as being sexually stimulating; six of these compounds were validated as positive with another bull when randomly tested after 1 year of storage.

The dialyzable fraction of vaginal mucus and the neutral fraction prepared by ion-exchange chromatography of the dialyzable solution of vaginal mucus had a mounting inducing activity on the herd mates, as did the application of an animal's own vaginal mucus, suggesting that mounting–inducing pheromones are relatively low molecular weight, neutral substances (Nishimura *et al.* 1991). Another experiment suggested that vaginal mucus might act as an additional/ secondary source along with urine in eliciting copulatory behaviour and executing coitus in bulls during oestrus (Sankar and Archunan 2004).

Oestrus pheromones from faeces: The faeces is also suggested to act as pheromone cues in bovine bio-communication. The chemical profiles of oestrus faeces (bovine) were found to be distinguished significantly from other phases by the presence of three specific substances, viz. acetic acid, propionic acid and 1-iodo undecane. Bulls were observed to exhibit significantly higher (P<0.001) repeated flehmen and mounting behaviour when the mixture of these compounds was applied to genital region of non-estrus (dummy) cows (Sankar and Archunan 2008).

The levels of fatty acids, aldehydes, amines and alkenes in the faeces of oestrus mares were found to be significantly higher than their respective levels in the faeces of non-oestrus mares (Kimura 2001). Faeces of oestrus mares, foxes and rats were demonstrated to elicit more penile erection in rats than faeces of di-oestrus females of the same species did not (Rampin *et al.* 2006).

Oestrus pheromone from skin glands: The increased proportion of bull olfactory behaviours during an experiment, elicited by an increased perineal skin gland discharge, adds support to hypothesis that the perineal skin glands are also source of an oestrous pheromone in the cow (Blazquez *et al.* 1988b).

Oestrus pheromone from serum and other body fluids: The serum may act as transport medium of pheromone in body from its origin to place of excretion. The serum taken at oestrus was observed to evoke sexual behaviours such as flehmen and penis protrusion in bulls (Rivard and Klemm 1989). The saliva was observed to be an important oral cue

used by females in the selection of socio-sexual partners in cows (Sankar and Archunan 2004). Similarly adult female Mongolian gerbils were found preferentially attracted to saliva from adult non-sibling males when paired with saliva from their male siblings (Smith and Block 1991).

Apparently, these sources, regardless to the site of the production are distributed throughout the cow's bodily fluids, probably by the circulatory system. It is reported that the hormones, such as, FSH, LH and steroids which used to be measured in blood have now been quantified in human urine (Shimizu *et al.* 2003) and saliva (Loewit *et al.* 1987). The steroid androstenol, was identified in both human urine and sweat and believed to act as pheromone (Gower and Ruparelia 1993). These reports indicate that compounds present in one source are likely to be present in other sources as well. Therefore, the source of the oestrus pheromones may originate in organs other than the genitals, such as skin or mouth. However it is not known whether the olfactory signals present in various bodily fluids, which help in oestrus detection, are of the same nature.

Physiological regulation of pheromone secretion

In cow, both the inhibitive effect of progesterone and a stimulating effect of oestrogen for the occurrence of oestrus are well known (Dozier and Pritt 1987, Thomas *et al.* 1988). A highly significant correlation exists between progesterone concentrations and rat responses, which demonstrates that the synthesis of pheromone is blocked by high progesterone concentration and increases with the onset of luteolysis (Dehnhard *et al.* 1991). The optimal response of rats in a bioassay could only be obtained when the original pH of the urine was not altered. A transient shift of either acid or basic pH values had irreversible effects on the pheromone activity and thus the reaction of the rats. The degree of loss of activity depended on the pH, which was maintained for 3 h. Thus, a transient change of pH below 7.0 or above 9.5 was sufficient to abolish any specific reaction of the rats. Therefore, all attempts to isolate the pheromones should be carried out at a pH in the order of native urine as suggested by Dehnhard and Claus (1996). An assumed function of a cow pheromone which is to inform bulls of imminent oestrus one day prior to mating (Dehnhard *et al.* 1991), explains the instability of the pheromones as shown by a transient shift of pH in this study. Thus, it appears that the intrinsic instability limits the information to a short stage of the cycle and thus avoids confusion. In contrast, male pheromones in various species have to provide long-lasting information on dominance and on territories (Johnston and Lee 1976) and are consequently more stable substances (Claus 1979). The mechanism of this phenomenon was explained by exhaustive studies conducted by Rasmussen and collaborators on the Asian elephant oestrus pheromones. The oestrus pheromone was identified as (Z)-7-dodecen-1-yl-acetate (Rasmussen *et al.* 1997) and is present at high concentrations in the pre-ovulatory urine. This compound is able to elicit in males the same range of behavioural responses the pre-ovulatory urine (Rasmussen *et al.* 1982 and 1996). This molecule is a part of hundred pheromones in Lepidoptera species, which indicates a strong evolutionary convergence between animal classes. Moreover, this work shed light on the implication of carrier proteins in pheromone liberation, which occurs via conformational modifications of the protein during the hormone-controlled pH decrease of urine (Rasmussen 2001, Lazar *et al.* 2002). Most of mammal pheromones are secreted in association with carrier proteins of the lipocalin family. The best-studied system is the association in mouse urine of small volatile pheromones and Major Urinary Proteins (Hurst and Beynon, 2004). Such information is not available in farm mammals, and it is not known if oestrus pheromones are bound and released by proteins in urine.

Molecular mechanism of olfactory perception

Buck and Axel (1991) cloned and characterized 18 different members of an extremely large multigene family that encodes seven transmembrane domain proteins whose expression is restricted to the olfactory epithelium. Members of this novel gene family were supposed to encode a diverse family of odorant receptors. A family of genes encoding candidate pheromone receptors was expressed in rat vomeronasal neurons. These receptors (V1R) consisted of seven transmembrane receptors. Individual V1R genes are composed of 1,000 nucleotides without introns and are expressed in vomeronasal sensory neurons whose cell bodies are located in the apical part of the VNO epithelium (Dulac and Axel 1995). Another type of pheromone receptor, V2R was reported simultaneously by three research groups (Herrada and Dulac 1997, Matsunami and Buck 1997, Ryba and Trinidelli 1997).

In rodents, two super families of seven transmembrane G protein-coupled receptors, V1Rs and V2Rs, serve as pheromone receptors (Dulac and Torello 2003). These cells also express a G protein subunit named Gαi2. In contrast, the multiexon V2R genes are characterized by a long, highly variable N-terminal domain and are coexpressed with Gαo in sensory neurons whose cell bodies are basally located. V2Rs are related to the Ca^{2+} sensing receptor and metabotropic glutamate receptors. V2Rs are expressed at high levels in small subpopulations of VNO neurons. V2Rs are primarily expressed in a different layer of VNO neurons from V1Rs, thus both gene families are likely to encode mammalian pheromone receptors (Ryba and Tirindelli 1997).

The V1R repertoires in cow and dog are substantially smaller than those in mouse and rat, which contain 187 and 102 putatively functional genes, respectively (Shi *et al.* 2005). Dog and cow have only 8 and 32 intact V1R genes respectively (Grus *et al.* 2005). Similarly Young *et al.* (2005) also found that dog, human and chimpanzee have very few intact V1Rs (8, 2, and 0 respectively). These findings were

unexpected because cows (Salazar *et al.* 2008) and dogs (Dennis *et al.* 2003) like mouse and rat, possess a functional VNO. Further, humans and chimpanzees appear to have suffered even more extreme deterioration of their vomeronasal organ and pheromone signalling components, perhaps as a result of dominant visual system. It was proposed that other gene families, such as V2Rs in the VNO or olfactory receptors in the nose, might be much more important than V1Rs in pheromone perception in dogs and other mammals (Young *et al.* 2005). It is unlikely that the small V1R repertoire could be compensated by a large V2R repertoire because all V2Rs identified from dogs and cows were pseudogenes (Grus *et al.* 2005). Furthermore, all V2R genes identified from the goat genome are pseudogenes and may not act as receptors (Wakabayashi *et al.* 2002). Since V1Rs are expressed in Gαi2 –positive neurons and V2Rs are expressed in Gαo –positive neurons (Mombaerts 2004) it is possible that functional V2Rs exist only in rodents and opossums among mammals. This suggests that mouse and rat may be atypical mammals in terms of their pheromone receptor genes and pheromone sensitivities.

Importance of detection of oestrus behaviour in cattle

The main problem in cattle reproductive management is the high frequency of silent and weak oestrous symptoms (Williamson *et al.* 1972, Bulman and Lamming 1978, Claus *et al.* 1983). Failure to detect oestrus or erroneous diagnosis of oestrus results in an enormous economic loss to the dairy industry worldwide. It is generally agreed that detection efficiency is <50% in most dairy herds (Bozworth *et al.* 1972, Barr 1975). Furthermore, research using milk and blood progesterone assays indicates that between 5 and 30% of all inseminations occur in cows that are not in oestrus (Appleyard and Cook 1976, Senger 1994). The single most important problem limiting the high reproductive efficiency in the national dairy herd (cows only) of America is reported to be poor detection of oestrus (Senger 1994). This is also true for Indian Dairy herd (cows and buffalo) because silent oestrus or poor expression of oestrus is more common in buffaloes (Awasthi *et al.* 2007, Perera 2011). As individual dairy herd increased in size, the problem of poor detection of oestrus has amplified due to a decrease in manpower input. There is an urgent need to develop and to apply new technologies that will provide highly effective automated methods for identifying cows and buffaloes in oestrus, thus eliminating the need for visual observation and marginally effective facilitators for detection (Senger 1994, Singh *et al.* 2000). The success of any new technology for oestrus detection depends on three factors: 1) the technology must solve the problem at a very high rate of effectiveness 2) the technology must be genuinely cost effective and contribute to increased profitability of the dairy enterprises and 3) the technology must make life easier for the management team. Technology that satisfies these three requirements will make a long-term positive impact worldwide.

One interesting way of oestrus marker detection is the possibility to use trained detector animals such as dogs or rats (Kiddy and Mitchell 1981, Ladewig and Hart 1981, Dehnhard and Claus 1988, Rampin *et al.* 2006) to detect cycle-dependent odour differences in vaginal secretions and in urine of cows. For example, acetaldehyde is one compound associated with oestrus that the dogs might be able to detect by smelling. Published studies on blood (Klemm *et al.* 1994), vaginal secretions (Ma *et al.* 1995), and milk (Weidong *et al.* 1997) indicated that concentration of acetaldehyde increased prior to oestrus. Contrary to these results, Dehnhard and Claus (1988) observed that several classes of chemical substances or even individual compounds from urine and cervico-vaginal mucus, which were suggested as candidates for the cow pheromone (Nishimura *et al.* 1984, Preti 1984, Hradekcky 1986, Klemm *et al.* 1987) did not alter the sexual behaviour of trained rats. These results demonstrate that choice of the molecule as oestrus marker in such bioassays is of crucial importance for the efficiency of oestrus detection.

The use of artificial olfaction could enable more accurate detection of oestrus and has the potential to increase fertility in cows. Changes in perineal odour as oestrus marker were monitored through the use of an electronic nose (Lane and Wathes 1998). Twelve conducting polymer sensors were used to quantify odours in terms of a change in sensor resistance. Oestrus was identified using ovarian ultrasound, behavioural observations, and plasma assay for progesterone and oestradiol. Samples were taken from the dorsal lateral perineal (perivulval) area using cotton bud swabs and presented to an electric nose. It was revealed that odour signals between the luteal phase and oestrus could be distinguished for a group of five cows. In another experiment, samples were obtained daily from eight cows during the midluteal phase and fromday 2 today 8 of the cycle (day 0 asday of oestrus, induced with cloprostenol). Of the 12 sensors, 7 showed a significant change in resistance that was dependent on the day of the oestrous cycle. Basal values were those taken in the luteal phase; values peaked on day 1, rose transiently on day 3, and returned to baseline on days 5 to 6. The pattern was strongly correlated with plasma oestradiol concentration.

Involvement of pheromonal cues in oestrus detection by the bull is well assessed by bioassays. Females release the olfactory molecules during oestrus, thus signalling the stage of the cycle, and stimulating sexual behaviour and endocrine function of males. Efforts were made to characterize the pheromone in milk, urine, vaginal mucus, serum and skin gland secretion during the different phases of oestrous cycle but the chemical nature of pheromone components is still unknown. Failure to detect oestrus or erroneous diagnosis of oestrus results in an enormous economic loss to the dairy industry worldwide. New technologies for the solutions of this problem must be more effective than visual observation

and aids currently used to detect oestrus. Therefore, it is conceivable to use artificial noses or other biotechnologies to detect specific markers of oestrus, the pheromone components and first to gain knowledge on the chemical nature of oestrus-indicating pheromones. Moreover, oestrus-related chemicals might also be useful as sexual stimulants for increasing the libido of unmotivated males. These techniques, based on a better and more natural utilisation of animal behaviour within their environment may contribute to lower and/or suppress the use of exogenous compounds and may then be more acceptable by the consumer. Moreover, as they will facilitate farmer's work and reduce manpower needs related to heat detection, these bio techniques should greatly contribute to the development of sustainable production systems integrating the new tools for multiple character genetic selection.

REFERENCES

Albone E S, Blazquez N B, French J, Long S E and Perry G C. 1986. Mammalian semiochemistry issues and futures with some examples from a study of chemical signalling in cattle. *Chemical signals in vertebrates* pp. 27–36. (Eds) Duvall D, Müller-Schwarze D and Silverstein R M, Plenum Press, New York.

Appleyard W T and Cook B. 1976. The detection of oestrus in dairy cattle. *Veterinary Record* 99: 253–56.

Awasthi M K, Kavani F S, Siddiquee G M, Sarvaiya N P and Derashri H J. 2007. Is slow follicular grwth the cause of silent estrus in water buffaloes. *Animal Reproduction Science* 99: 258–68.

Badings H T. 1991. Milk. *Volatile compounds in Foods and Beverages* p. 91 (Eds) Maarse H, Marcel Dekker, New York.

Barr H L. 1975. Influence of oestrus detection ondays open in dairy herd. *Journal of Dairy Science* 58: 246–47.

Bendaall J G. 2001. Aroma compounds of fresh milk from New Zealand cows fed different diets. *Journal of Agriculture and Food Chemistry* 49: 4825–32.

Blaschke C F, Thompson D L Jr, Humes P E and Godke R A. 1984. Olfaction, sight and auditory perception of mature bulls in detecting estrual responses in beef heifers. *Proceedings of 10th International Congress on Animal Reproduction and Artificial Insemination* 3: 284–86 University of Illinois, USA.

Blazquez N B, French J M, Long S E and Perry G C. 1988a. Bovine oestrous odours: behavioural and histological investigations. *11th International Congress on Animal Reproduction and Artificial Insemination* 4: 550–52 Dublin, Ireland.

Blazquez N B, French J M, Long S E and Perry G C. 1988b. A pheromonal function for the perineal skin glands in the cow. *Veterinary Record* 123: 49–50.

Blissitt M J, Bland K P and Cottrell D F. 1994. Detection of oestrous related odour in ewe urine by rams. *Journal of Reproduction and Fertility* 101: 189–91.

Bozworth R W, Ward G, Call E P and Bonewitz E R. 1972. Analysis of factors affecting calving intervals of dairy cows. *Journal of Dairy Science* 55: 334–44.

Brennan P A and Keverne E B. 2004. Something in the air? New insights into mammalian pheromones. *Current Biology* 14: 81–89.

Brennan P A and Zufall F. 2006. Pheromonal communication in vertebrates. *Nature* 444: 308–15.

Buck L B and Axel R. 1991. A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell* 65: 175–87.

Bulman D C and Lamming G E. 1978. Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. *Journal of Reproduction and Fertility* 54: 447–58.

Cavaggioni A, Sorbi R T, Keen J N, Pappin D J and Findlay J P. 1987. Homology between the pyrazine-binding protein from nasal mucosa and major urinary proteins. *FEBS Letters* 212: 225–28.

Chemineau P, Levy F and Thimonier J. 1986. Effects of anosmia on LH secretion, ovulation and oestrus behaviour induced by males in the anovular Creole goat. *Animal Reproduction Science* 10 : 125–32.

Claus R. 1979. Pheromone bei Säugetieren unter besonderer Berücksichtigung des Ebergeruchsstoffes und seiner Beziehungen zu anderen Hodensteroiden. Beiheft 10: Fortschritte in der Tierphysiologie und Tierernahrung. Hamburg, Berlin: Paul Parey.

Claus R. 1994. Pheromone. *Veterinarmedizinische Endokrinologie* Pp. 691–712. (Ed) Docke F. Gustav Fischer Verlag, Jena, Stuttgart.

Claus R and Schams D. 1990. Influence of mating and intrauterine oestradiolinfusion on peripheral oxytocin concentrations in sow. *Journal of Endocrinology* 126: 361–65.

Claus R, Over R and Dehnhard M. 1990. Effect of male odour on LH-secretion and the induction of ovulation in seasonally anoestrus goats. *Animal Reproduction Science* 22: 27–38.

Claus R, Karg H, Zwiauer D, von Butler I, Pirchner F and Rattenberger E. 1983. Analysis of factors influencing reproductive performance of the dairy cow by progesterone assay in milk fat. *British Veterinary Journal* 139: 29–37.

Crowell-Davis S and Houpt K A. 1985. The ontogeny of flehmen in horses. *Equine Veterinary Journal* 33: 739–45.

De Vuyst A, Thines G, Henriet L and Soffié M. 1964. Influence des stimulations auditives sur le comportement sexuel du taureau. *Experientia* 20: 648–50.

Dehnhard M and Claus R. 1988. Reliability criteria of a bioassay using rats trained to detect specific-specific odour in cow urine. *Theriogenology* 30: 1127–38.

Dehnhard M and Claus R. 1996. Attempts to purify and characterize the estrus-signalling pheromone from cow urine. *Theriogenology* 46: 13–22.

Dehnhard M, Claus R, Pfeiffer S and Schopper D. 1991. Variation in oestrus-related odors in the cow and its dependency on the ovary. *Theriogenology* 35: 645–52.

Dennis J C, Allgier J G, Desouza L S, Eward W C and Morrison E E. 2003. Immunohistochemistry of the canine vomeronasal organ. *Journal of Anatomy* 203: 329–38.

D'Innocenzo B, Salzano A M, D'Ambrosio, Gazzano, Niccolini A, Sorce C, Dani F R, Scaloni A and Pelosi P. 2006. Secretory proteins as potential semiochemical carriers in the horse. *Biochemistry* 45: 13418–28.

Dorries K M, Adkins R E and Halpern B P. 1997. Sensitivity and behavioural responses to the pheromone androstenone are not mediated by the vomeronasal organ in domestic pigs. *Brain, Behaviour and Evolution* 49: 53–62.

Doty R L. 1976. Mammalian Olfaction, Reproductive processes and behaviour. Academic Press, New York, USA.

Dozier L A and Pritt J H. 1987. Sexual behaviour of Holstein cows treated with progesterone and oestradiol benzoate. *Journal of Dairy Science* **70** (Suppl. 1): 205.

Dulac C and Axel R. 1995. A novel family of genes encoding putative pheromone receptors in mammals. *Cell* **83**: 195–206.

Dulac C and Torello A T. 2003. Molecular detection of pheromone signals in mammals: from genes to behavior. *Nature Reviews Neuroscience* **4**: 551–62.

Dumont J P and Adda J. 1978. Flavour formation in dairy products. *Progress in Flavour research* (Eds.) Land D G and Nursten H E. p 245. Applied Science Publication, London, England.

Estes R D. 1972. The role of the vomeronasal organ in mammalian reproduction. *Mammalia* **36**: 315–18.

Finlayson J S, Asofsky R, Potter M and Runner C C. 1965. Major Urinary Protein complex of normal mice: origin. *Science* **149**: 981–82.

French J M, Moore G F, Perry G C and Long S E. 1989. Behavioural predictors of oestrus in domestic cattle, *Bos taurus*. *Animal Behaviour* **38**: 913–19.

Fukuda K, Senda A, Ishii T, Urashima T, Morita M and Terabayashi T. 2009. Short Communication: Evidence for the presence of a putative odorant-binding protein in bovine colostrums. *Journal of Dairy Science* **92**: 4992–96.

Garcia M C, McDonnell S M and Kenney R M. 1986. Bull sexual behaviour tests: stimulus cow affects performance. *Applied Animal Behavior Science* **16**: 1–10.

Gelez H and Fabre-Nys C. 2004. The "male effect" in sheep and goats: a review of the respective roles of the two olfactory systems. *Hormonal Behaviour* **46**: 257–71.

Gonzales R, Levy F, Orgeur P, Poindron P and Signoret J P. 1991. Female effect in sheep. II. Role of volatile substances from the sexually receptive female, implication of the sense of smell. *Reproduction Nutrition and Development* **31**: 103–09.

Gordon D T and Morgan M E. 1972. Principal volatile compounds in feed flavoured milk. *Journal of Dairy Science* **67**: 392.

Grus W E, Shi P, Zhang Y and Zhang J. 2005. Dramatic variation of the vomeronasal pheromone receptor gene repertoire among five orders of placental gene repertoire among five orders of placental and marsupial mammals. *Proceedings of National Academy of Science USA* **102**: 5767–72.

Gower D B and Ruparelia B A. 1993. Olfaction in humans with special reference to odorous 16-androstenenes: their occurrence, perception and possible social, psychological and sexual impact. *Journal of Endocrinology* **137**: 167–87.

Guiraudie-Capraz G, Slomianny M C, Pageat P, Malosse C, Cain A H, Orgeur P and Nagnan-Le Meillour P. 2005. Biochemical and chemical supports for a transnatal olfactory continuity through sow maternal fluids. *Chemical Senses* **30**: 241–51.

Ha T S and Smith D P. 2006. A pheromone receptor mediates 11-cis-vaccenyl acetate-induced responses in *Drosophila*. *Journal of Neuroscience* **26**: 8727–33.

Hafez E S E and Bouissou M F. 1975. Mating behaviour in animals. In The behaviour of domestic animals (Ed) Hafez E S E. pp. 203–45. Bailliere, London.

Hart G H, Mead S W and Regan W M. 1946. Stimulating the sex drive of bovine males in artificial insemination. *Endocrinology* **39**: 221–23.

Herrada G and Dulac C. 1997. A novel family of putative pheromone receptors in mammals with a topologically organized and sexually dimorphic distribution. *Cell* **90**: 115–22.

Honkanen E, Karvonen P and Virtanen A I. 1964. Studies on the transfer of some flavor compounds to milk. ***Acta Chemica Scandinavica*** **18**: 612.

Hradecky P. 1986. Volatile fatty acids in urine and vaginal secretions of cows during reproductive cycle. *Journal of Chemical Ecology* **12**: 187–96.

Hradecky P, Sis R F and Klemm W R. 1983. Distribution of flehmen reactions of the bull throughout the bovine estrous cycle. *Theriogenology* **20**: 197–204.

Hudson R and Distel H. 1986. Pheromonal release of suckling in rabbits does not depend on the vomeronasal organ. *Physiology and Behavior* **37**: 123–29.

Hurst J and Beynon R. 2004. Scent wars: the chemobiology of competitive signalling in mice. *Bioassays* **26**: 1288–98.

Izard M K. 1983. Pheromones and reproduction in domestic animals. *Pheromones and reproduction in mammals* Pp. 253–85. (Ed.) Vandenbergh J G. Academic press, New York.

Izard M K and Vandenbergh J G. 1982. The effect of bull urine on puberty and calving date in crossbreed heifers. *Journal of Animal Science* **55** (11): 1160–68.

Jacobs V L, Sis R F, Chenoweth P J, Klemm W R, Sherry C J and Copcock E E. 1980. Tongue manipulation of the palate assists oestrus detection in the bovine. *Theriogenology* **13**: 197–204.

Johnston R E and Lee N A. 1976. Persistence of the odor deposited by two functionally distinct scent marking behaviours of golden hamster. *Behavioral Biology* **16**: 199–210.

Karlson P and Luscher M. 1959. "Pheromone": a new term for a class of biologically active substances. *Nature* **183**: 55–56.

Kiddy C A and Mitchell D S. 1981. Estrous related odors in cows: time of occurrence. *Journal of Dairy Science* **64**: 267–71.

Kiddy C A, Mitchell D S, Bolt D J and Hawk H W. 1978. Detection of estrus-related odors in cows by trained dogs. *Biology of Reproduction* **19**: 389–95.

Kimura K. 2001. Volatile substance in faeces, urine and urine-marked faeces of feral horses. *Canadian Journal of Animal Science* **81** (4): 411–20.

Klemm W R, Hawkins G N and De Los Santos E. 1987. Identification of compounds in bovine cervico-vaginal mucus extracts that evoke male sexual behaviour. *Chemical Senses* **12**: 77–87.

Klemm W R Y, Rivard G F and Clement B A. 1994. Blood acetaldehyde fluctuates markedly during bovine estrous cycle. *Animal Reproduction Science* **35**: 9–26.

Knight T W, Terwit H R and Lynch P R. 1983. Effects of boar pheromones, rams wool and presence of bucks on ovarian activity in anovular ewes early in the breeding season. *Animal Reproduction Science* **6**: 129–34.

Korchi A, Brossut R, Bouhin H and Delachambre J. 1999. cDNA cloning of an adult male putative lipocalin specific to tergal gland aphrodisiac secretion in an insect (*Leucophora maderae*). *FEBS letters* **449**: 125–28.

Ladewig J and Hart B L. 1980. Flehmen and vomeronasal organ function in male goats. *Physiology and Behavior* **24**: 1067–71.

Ladewig J and Hart B L. 1981. Demonstration of oestrus-related odors in cow urines by operant conditioning of rats. *Biology of Reproduction* **24**: 1165–69.

Lane A J and Wathes D C. 1998. An electronic nose to detect changes in perineal odors associated with oestrus in the cow.

Journal of Dairy Science **81**: 2145–50.

Lazar J, Greenwood D, Rasmussen L E L and Prestwich G D. 2002. Molecular and functional characterization of an odorant binding protein of the Asian Elephant, *Elephas maximus*: Implications for the role of lipocalins in mammalian olfaction. *Biochemistry* **41**: 11786–94.

Lévy F, Keller M and Poindron P. 2004. Olfactory regulation of maternal behavior in mammals. *Hormones and Behavior* **46**: 284–302.

Lindsay D R. 1965. The importance of olfactory stimuli in the mating behaviour of the ram. *Animal Behaviour* **13**: 75–8.

Loewit K, Huber J, Ortlieb A, Kraft H G, Widhalm R and Wolfram G. 1987. Salivary LH as an ovulation indicator comparison between salivary LH, serum LH and ultrasonic findings. *Geburtshilfe Frauenheilkd* **47**: 800–02.

Ma W and Klemm W R. 1997. Variations of equine urinary volatile compounds during the oestrus cycle. *Veterinary Research Communications* **21**: 437–46.

Ma W, Clement B A and Klemm W R. 1995. Cyclic changes of volatile constituents of bovine vaginal secretions. *Journal of Chemical Ecology* **21**: 1895–1906.

Marchese S, Pes D, Scaloni A and Pelosi P. 1998. Lipocalins of boar salivary glands binding odours and pheromones. *European Journal of Biochemistry* **252**: 563–68.

Masaki J and Ohta M. 1990. Mating behaviour of a bull for estrous synchronised cows and a possible involvement of pheromonal factors. *Pheromones and reproduction* (Eds) Sagara Y and Seto K. pp 63–75. The Parthenon Publishing Group Limited, Carnforth, UK.

Matsunami H and Buck L B. 1997. A multigene family encoding a diverse array of putative pheromone receptors in mammals. *Cell* **90**: 775–84.

Mombaerts P. 2004. Genes and ligands for odorant, vomeronasal and taste receptors. *Nature Reviews Neuroscience* **5**: 228–50.

Morè L. 2006. Mouse major urinary proteins trigger ovulation via the vomeronasal organ. *Chemical Senses* **31**: 631–34.

Mucignat-Caretta C. 2010. The rodent accessory olfactory system. *Journal of Comparative Physiology* **A 196**: 767–77.

Nishimura K, Okano T, Utsumi K and Yuhara M. 1984. Partial separation of mounting-inducing pheromones from vaginal mucus of Holstein heifer. *10th International Congress on Animal Reproduction and Artificial Insemination*, Pp. 291–93. University of Illinois, USA.

Nishimura K, Utsumi K, Okano T and Iritani A. 1991. Separation of mounting-inducing pheromones of vaginal mucus from estrual heifers. *Journal of Animal Science* **69** : 3343–47.

Novotny M V. 2003. Pheromones, binding proteins and receptor responses in rodents. *Biochemical Society Transactions* **31**: 117–22.

Novotny M V, Weidong M, Wiesler D and Zidek L. 1999. Positive identification of the puberty-accelerated pheromone of the house mouse: the volatile ligands associating with the major urinary protein. *Proceedings of the Royal Society of London B series, Biological Sciences* **266**: 2017–22.

Pageat P. 2001. Pig appeasing pheromones to decrease stress, anxiety and aggressiveness. *US Patent n°6* 169:113.

Paleologou A M. 1977. Detecting oestrus in cows by a method based on bovine sex pheromones. *Veterinary Record* **100**: 319–20.

Patterson R L S. 1966. 5a-androst-16-en–3-one: compound responsible for taint in boar fat. *Journal of the Science of Food and Agriculture* **19**: 31–37.

Patterson R L S. 1968. Identification of 3a-hydroxy-5a-androst-16-en-ol as the musk odour component of boar submaxillary salivary gland and its relationship to the sex odour taint in pork meat. *Journal of the Science of Food and Agriculture* **19**: 434–38.

Pelosi P, Baldaccini N E and Pisanelli A M. 1982. Identification of a specific olfactory receptor for 2-isobutyl-3-methoxypyrazine. *Biochemical Journal* **201**: 245–48.

Perera B M. 2011. Reproductive cycles of buffaloes. *Animal Reproduction Science* **124** : 94–99.

Potts W K, Manning C J and Wakeland E K. 1991. Mating patterns in seminatural populations of mice influenced by MHC genotype. *Nature* **352**: 619–21.

Presicce G.A, Brockett C C, Cheng T, Foote R H, Rivard G F and Klemm W R. 1993. Behavioural responses of bulls in artificial breeding to compounds presented for smelling, licking or with topical nasal application. *Applied Animal Behaviour Science* **37**: 273–84.

Preti G. 1984. Methods for detecting bovine oestrus by determining methyl heptanol concentrations in vaginal secretions. *US patent no4* 467: 814.

Pugalenthi G, Tang K, Suganthan P N, Archunan G and Sowdhamini R. 2007. A machine learning approach for the identification of odorant binding proteins from sequence-derived properties. *BMC Bioinformatics* **8**: 351.

Ramesh Kumar K, Archunan G, Jeyraman R and Narasimhan S. 2000. Chemical characterization of bovine urine with spezial reference to estrous cycle. *Veterinary Research Communication* **24**: 445–54.

Rampin O, Jerome N, Briant C, Boué F and Maurin Y. 2006. Are oestrous odors species specific? *Behavioural Brain Research* **172**: 169–72.

Rasmussen L E L. 2001. Source and cyclic release pattern of (*Z*)-7-dodecenyl acetate, the pre-ovulatory pheromone of the female Asian elephant. *Chemical Senses* **26**: 611–23.

Rasmussen L E L, Schmidt M J, Henneous R, Groves D and Daves G D. 1982. Asian bull elephants: flehmen-like responses to extractable components in female elephant estrous urine. *Science* **217**: 159–62.

Rasmussen L E L, Lee T D, Roelofs W L, Zhang A and Daves G D Jr. 1996. Insect pheromone in elephants. *Nature* **379**: 684.

Rasmussen L E L, Lee T D, Zhang A, Roelofs W L and Daves G D Jr. 1997. Purification, identification concentration and bioactivity of (2)-7-dodecen-1-yl-acetate: sex pheromone of the female asian elephant, *Elephas maximus*. *Chemical Senses* **22**: 417–38.

Reinhardt V. 1983. Flehmen, mounting and copulation among members of a semi-wild cattle herd. *Animal Behaviour* **31**: 641–50.

Rekwot P I, Ogwu D, Oyedipe E O and Sekoni V O. 2001. The role of pheromones and biostimulation in animal reproduction. *Animal Reproduction Science* **65**: 157–70.

Rivard G and Klemm W R. 1989. Two body fluids containing bovine estrous pheromone(s). *Chemical Senses* **14**: 273–79.

Ryba N J and Tirindelli R. 1997. A new multigene family of putative pheromone receptors. *Neuron* **19**: 371–79.

Salazar I, Sanchez-Quinteiro P, Aleman N and Prieto D. 2008. Anatomical, immunohistochemical and physiological

characteristics of the vomeronasal vessels in cows and their possible role in vomeronasal reception. *Journal of Anatomy* **212**: 686–96.

Sam M, Vora S, Malnic B, Ma W, Novotny M V and Buck L. 2001. Neuropharmacology. Odorants may arouse instinctive behaviours. *Nature* **412**: 142.

Sambraus H H and Waring Z. 1975. Effect of urine from estrous cows on libido in bulls. *Zoo. Saug.* **40**: 49–54.

Sankar R and Archunan G. 2004. Flehmen response in bull: role of vaginal mucus and other body fluids of bovine with special reference to oestrus. *Behavioural Processes* **67**: 81–86.

Sankar R and Archunan G. 2008. Identification of putative pheromones in bovine (*Bos Taurus*) faeces in relation to estrus detection. *Animal Reproduction Science* **103**:149–53.

Scaloni A, Paolini S, Brandazza A, Fantacci M, Bottiglieri C, Marchese S, Navarrini A, Fini C, Ferrara L and Pelosi P. 2001. Purification, cloning and characterization of odorant- and pheromone- binding proteins from pig nasal epithelium. *Cellular and Molecular Life Sciences* **58**: 823–34.

Schaal B, Coureaud G, Langlois D, Ginies C, Semon E and Perrier G. 2003. Chemical and behavioural characterization of the rabbit mammary pheromone. *Nature* **424**: 68–72.

Schams D, Schallenberger E, Hoffmann B and Karg H. 1977. The oestrous cycle of the cow: hormonal parameters and time relationships concerning oestrous, ovulation and electric resistance of the vaginal mucus. *Acta Endocrinologica* **86**: 180–92.

Schanbacher B D, Orgeur P, Pelletier J and Signoret JP. 1987. Behavioural and hormonal responses of sexually-experienced ile-de-France rams to estrous female. *Animal Reproduction Science* **14**: 293–300.

Schloeth R. 1961. Das Sozialleben des Camarque-Rindes. Qualitative und quantitative Untersuchungen uber die sozialen Beziehungen-insbesondere die soziale Rang ordnung- des halbwilden franzosischen Kampfrindes. *Zeits fur Tier* **18**: 574–627.

Senger P L. 1994. The oestrus detection problem: New concepts, technologies and possibilities. *Journal of Dairy Science* **77**: 2745–53.

Shaw P H, Held W A and Hastie M D. 1983. The gene family for major urinary proteins: Expression in several secretory tissues of the mouse. *Cell* **32**: 755–61.

Shi P, Bielawski J P, Yang H and Zhang Y P. 2005. Adaptive diversification of vomeronasal receptor 1 genes in rodents. *Journal of Molecular Evolution* **60**: 566–76.

Shimizu K, Douke C, Fujita S, Matsuzawa T, Tomonaga M, Tanaka M, Matsubayashi K and Hayashi M. 2003. Urinary steroids, FSH and CG measurement for monitoring the ovarian cycle and pregnancy in the chimpanzee. *Journal of Medical Primatology* **32**:15–22.

Shipe W F, Ledford R A, Peterson R D, Scalan R A, Geerken H F, Dougherty, R W and Morgan M E. 1962. Physiological mechanism involved in transmitting flavours and odors to milk II. Transmission of some flavor components of silage. *Journal of Dairy Science* **45**: 477–80.

Shiratsuchi H, Shimoda M, Imayoshi K, Noda K and Osajima Y. 1994. Volatile flavor compounds in spray-dried skim milk,powder. *Journal of Agriculture and Food Chemistry* **42**: 984–88.

Signoret J P. 1991. Sexual pheromones in the domestic sheep:

Importance and limits in the regulation of reproductive physiology. *Journal of Steroid Biochemistry and Molecular Biology* **39**: 639–45.

Signoret J P and du Mesnil du Buisson F. 1961. Etude du comportement de la truie en oestrus. Proceeding of 4th International Congress of Animal Reproduction. *The Hague* **2**: 171–75.

Signoret J P and Mauleon P. 1962. Action de l'ablation des bulbes olfactifs sur le mécanismes de la reproduction chez la truie. *Annales de Biologie Animale, Biochimie, Biophysique* **2**: 167–74.

Singh J, Nanda A S, Adams G P. 2000. The reproductive pattern and efficiency of female buffaloes. *Animal Reproduction Science* **60–61**: 593–604

Smith B A and Block M L. 1991. Male saliva cues and female social choice in Mongolian gerbils. *Physiology and Behavior* **50**: 379–84.

Stahlbaum C C, Houpt K A. 1989. The role of flehmen response in the behavioural repertoire of the stallion. *Physiology and Behavior* **45**: 1207–14.

Thomas S, Becker W C and Wiebold J L. 1988. Influence of various progesterone levels on behavioural oestrus in ovariectomized cows. *Proceedings of Society of Animal Science* **36**: 368–70.

Toso B, Procida G and Stefanon B. 2002. Determination of volatile compounds in cows milk using headspace GC-MS. *Journal of Dairy Research* **69**: 569–77.

Urbach G and Stark W. 1978. The effect of diet on the g- and ä-lactone and methyl ketone potentials of bovine butterfat. *Journal of Dairy Research* **45**: 223–29.

Utsumi M, Ohno K, Kawasaki Y, Tamura M, Kubo T and Tohyama M. 1999. Expression of major urinary protein genes in the nasal glands associated with general olfaction. *Journal of Neurobiology* **39**: 227–36.

Virtanen A I and Lampila M. 1967. The chemistry and physiology of flavours. AVI Publ. Co., Westport, CT.

Wakabayashi Y, Mori Y, Ichikawa M, Yazaki K and Hagino-Yamagishi K. 2002. A putative pheromone receptor gene is expressed in two distinct olfactory organs in goats. *Chemical Senses* **27**: 207–13.

Walkden-Brown S W, Restall B J and Henniawati. 1993. The "male effect" in Australian cashmere goats: 2. role of olfactory cues from the male. *Animal Reproduction Science* **31**: 55–67.

Weidong M A, Clement B A and Klemm W R. 1997. Volatile compounds of bovine milk as related to the stage of the estrus cycle. *Journal of Dairy Science* **80**: 3227–33.

Williamson N B, Morris R S, Blood D C and Cannon C M. 1972. A study of oestrous behaviour and oestrus detection methods in a large commercial dairy herd. *Veterinary Record* **91**: 50–57.

Wyatt T D. 2009. Fifty years of pheromones. *Nature* **457**: 262–63.

Wyatt T D. 2010. Pheromones and signature mixtures: defining species-wide signals and variable cues for identity in both invertebrates and vertebrates. *Journal of Comparative Physiology A* **196**: 685–700.

Wysocki C J and Preti G. 2004. Facts, fallacies, fears, and frustrations with human pheromones. *The Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology* **281**:1201–11.

Xia J, Sellers L A, Oxley D, Smith T, Emson P and Keverne E B. 2006. Urinary pheromones promote ERK/At phosphorylation,

regeneration and survival of vomeronasal (VR2) neurones. *European Journal of Neuroscience* **24**: 3333–42.

Yamazaki K, Beauchamp G K, Curran M and Boyse E A. 2000. Parent–progeny recognition as a fnction of MHC odortype identity. *Proceedings of the National Academy of Science,* USA **97**: 10500–02.

Young J M, Kambere M, Trask B J and Lane R P. 2005. Divergent V1R repertoires in five species: Amplification in rodents, decimation in primates, and a surprisingly small repertoire in dogs. *Genome Research* **15**: 231–40.

Zalesky D D,day M L, Garcia-Winder M, Imakhawa K, Kittok R J, D'Occhio M J and Kinder J E. 1984. Influence of exposure to bulls on resumption of estrous cycles following parturition in beef cows. *Journal of Animal Science* **59** (11): 1135–39.