J. Steroid Biochem. Molec. Biol. Vol. 39, No. 4B, pp. 639–645, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

SEXUAL PHEROMONES IN THE DOMESTIC SHEEP: IMPORTANCE AND LIMITS IN THE REGULATION OF REPRODUCTIVE PHYSIOLOGY

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Summary—In mammals, primer pheromones are considered as the most important signals involved in socio–sexual stimulation of the reproductive processes. In the domestic sheep, male–female interactions induce changes in the pulsatile rhythm of the LH secretion in both sexes which influences reproductive endocrinology. In the female, the odor of the ram's fleece induces LH secretion and ovulation. The acid sub fraction of the methylene chloride extract under C16 and diols appear to contain the active components. An interspecific action has been observed, as male goat's hair extract is similarly active. In the male, although the receptive female is the most effective to stimulate LH release, no pheromonal action has been demonstrated. In the female, lesions of the vomeronasal system do not eliminate the endocrine response to pheromonal stimulation. Furthermore, in both sexes, anosmia does not impair the response to direct interaction with the sexual partner. In domestic sheep, the existence of primer pheromones has been demonstrated, but other sensory cues could replace olfaction during socio–sexual interactions interfering with the control of reproductive endocrinology.

In most vertebrate species, the major aspects of reproduction—spermatogenesis, oestrous cycle and ovulation—can take place in the isolated individual, as a result of a series of neuro-endocrine events interacting irrespective of the environment. In seasonal breeders, climatic factors, especially the light/dark cycle and its variations around the year account for the determination of the period of reproduction. However, the observation of the spontaneous timing of reproduction under "normal" conditions of living shows that in a number of species, the socio–sexual interactions could markedly influence reproduction.

In mammals, most of such situations result from stimulations conveyed by the sense of smell to the point that pheromones acting as "primers" are becoming synonymous with the effect of socio–sexual stimulation of reproductive processes. The existence of discrete events such as the occurrence of ovulation and the oestrous cycle makes it easier to establish such phenomenon in females. In fact, a series of so-called "effects" involving pheromonal stimulation have been extensively studied in female rodents: modification of the oestrous cycle or "Whitten effect", acceleration of puberty or "Vandenbergh effect" and blockage of implantation or "Bruce effect". An experimental analysis of the sensory as well as physiological mechanisms has been performed together with attempts to determine the chemical composition of the pheromone. Such an approach has been extended to the male of these species.

However, under natural conditions, pheromones rarely operate alone in the absence of other sensory stimulations. For some macro-osmatic species, mostly nocturnal, and living in burrows such as rodents, pheromones are logically especially important. As rodents are the most classical laboratory animals, it is not surprising that they play a major role in the literature relevant to this topic. The existence of similar phenomenons in animals belonging to different zoological groups could provide information on the possibility of extending the results obtained in rodents.

The aim of the present report is to review the scientific evidence obtained in a non-conventional species, the domestic sheep, on the mechanisms by which socio–sexual interactions influence the reproductive physiology, the role of pheromones and the possibility of interference from other sensory stimulations.

Proceedings of the International Symposium on Recent Advances in Mammalian Pheromone Research, Paris, France, 6–9 October 1991. Sponsored by the EROX Corporation.

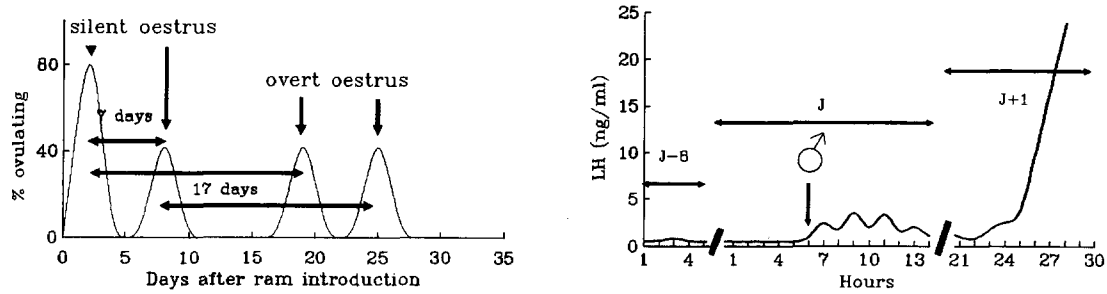

Fig. 1. Physiological consequence of the male's presence in anoestrous ewes. (a) Behavioural and ovarian responses (reproduced from Ref. [6] and (b) effect on LH secretion (samples at 20 min intervals). Reproduced from Ref. [4].

THE CONSEQUENCES OF SOCIO-SEXUAL INTERACTIONS IN THE DOMESTIC SHEEP

The female sheep is a seasonal breeder, experiencing regular 16–18 day oestrous cycles in Summer–Autumn, and presenting a period of Winter–Spring anoestrus, the duration of which varies according to the breed. However during this period, the introduction of a male into a group of previously isolated females, can induce a resumption of sexual activity. Ovulation and oestrus are observed in the characteristic form of two peaks, seven days apart, 18 to 25 days after the introduction of the rams [1] [Fig. 1(a)].

In fact the long delay from the stimulation by the male to the observed oestrus involves a series of more rapid and complex physiological events. The ovarian activity is resumed rapidly, as ovulations are generally observed as early as 48 h after the introduction of the male. However, such ovulations are not associated with sexual receptivity, i.e. they are "silent". Furthermore, they appear in a proportion of the cases as abnormal: the corpus luteum, instead of being active in secreting progesterone for 12 to 14 days, regresses prematurely, after 5–7 days, and the female reovulates [2]. Such ovulations are generally not associated with oestrous behaviour. The necessity for progesterone priming before secretion of oestrogens to induce sexual behaviour in the female sheep probably accounts for these silent ovulations. The next ovulations, the second or the third, depending on the existence of a first abnormally short cycle, occur after a normal cycle. As progesterone has been secreted for more than 10 days, they are associated with normal sexual receptivity, this accounts for the two peaks of observed oestrus [Fig. 1(a)].

The rapid induction of ovarian activity is the consequence of a quite immediate change in the secretion of pituitary gonadotropins. A pulse of LH is observed within minutes of the presentation of the anoestrous female to the male [Fig. 1(b)]. Then, the rhythm of pulsatile secretion of LH remains high for several hours, and a preovulatory surge takes place within 36 h, with an important variability [3, 4]. The pituitary secretion of FSH does not undergo such rapid changes, but only increases at the time of the preovulatory surge of LH. The LH pulses probably stimulate the ovarian secretion of oestradiol that, via a positive feed-back at the hypothalamic level induces the preovulatory surge. The permanent presence of the male is required to obtain the ovulation: when the ram is removed after some hours, the secretion of LH rapidly returns to the low level characteristic of the seasonal anoestrus [5].

The ovulation-inducing capacity of the stimulation by the male differs according to the breed and the season. It is permanent throughout the seasonal anoestrus in the merino whereas in European breeds, ovulation can only be induced at the end of seasonal anoestrus (May–June). However, the short-term change in LH secretion is observed in all cases, even when ovulation cannot be obtained such as in European breeds in Winter [6].

The induction of an LH pulse in an anoestrous female gives an obvious and reliable measure of the effectiveness of the stimulation by the male and has been used for the experimental analysis of this phenomenon.

In the male, it has been reported that the presence of females could result in increased testis weight and circulating LH and testosterone [7]. The high spontaneous rhythm of LH secretion

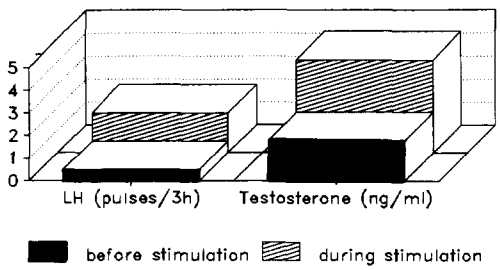

Fig. 2. Endocrine response of rams to the presence of an oestrous female. Reproduced from Ref. [8].

during the sexual season makes it difficult to demonstrate the existence of acute changes similar to that which has been observed in females. However, during the non-sexual season, when the spontaneous rhythm of LH secretion is reduced, the presentation of oestrous females increases LH pulse frequency, as well as mean circulating levels of LH and testosterone [8] (Fig. 2). A transitory rise of cortisol is also detected. When observing the hormonal profiles for some hours before and after contact with females, such changes are observed only in a proportion of the rams. In non-reacting males, the prestimulation level of LH is higher, which suggests that a spontaneous pulse of LH could be followed by a refractory period during which another release of this hormone is no longer possible.

Whatever the season, a diurnal rhythm of LH secretion has been observed, the lowest frequency occurring just after dawn, and the highest 4 h later. As the results obtained during the non-sexual season suggest the existence of a post secretion refractory period, it could be hypothesized that the higher the spontaneous frequency of LH pulses, the lower would be the probability of inducing a response by the presence of a female. In fact, an increase in LH pulsatility and testosterone can be demonstrated during the sexual season, but only at sunrise [9].

To conclude, the effect of socio–sexual interactions have been clearly established in both sexes in the domestic sheep, the first step being the rapid release of a pulse of LH secretion. The delay of a few minutes suggests direct control of the process by sensory information, which makes this a very convenient criteria for analyzing the sensory mechanisms involved.

EXISTENCE OF PHEROMONAL STIMULATION IN THE SOCIO–SEXUAL STIMULATION OF SHEEP REPRODUCTIVE PROCESSES

The ewes are anoestrous when stimulated by the male. Consequently, the activity of the male is limited to a few sexual displays at first contact; most of the interactions involve olfactory control of the newcomer. Furthermore, it has been shown that direct contact is not even necessary to induce ovulation [10]. Fleece and antorbital secretion from rams can induce ovulation in anoestrous ewes [11]. The presentation of the odours in a mask is extremely effective in inducing LH release, and has been used for further studies (Fig. 3). Fleece from any part of the male's body as well as the secretion from

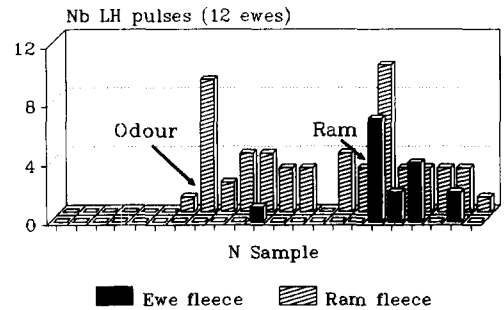

Fig. 3. LH response of ewes to the odour of fleece extract. Blood samples are taken at 20 min intervals for 3 h prestimulation. Then the extracts are presented in a mask, for an additional 3 h, and the reaction of the ewes is then tested when presented to males for 2 h. Reproduced from Ref. [12].

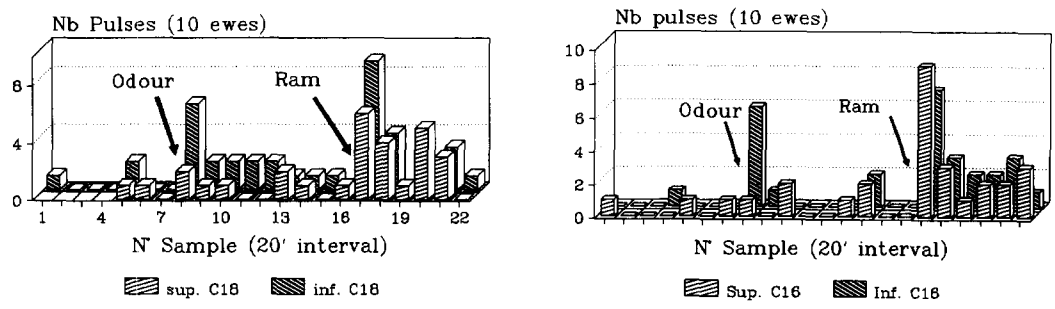

Fig. 4. Endocrine responses to purified fractions of ram's fleece, acid fraction inf. and sup. at C18 + diols 1–2 (a) and male goat's hair extract, acid fraction, inf. and sup. at C16. (b). Same protocol as in Fig. 3.

antorbital glands are equally effective as are ethanol or methylene chloride extracts of rams fleece, but not urine nor inguinal wax [12]. Surprisingly, interspecific pheromonal stimulation has been observed: hairs of male goats, or the methylene chloride extract are extremely effective in stimulating LH release in ewes, as they are in does [13].

The chemical study of the pheromone has been performed using the methylene chloride extract of rams fleece. Precipitation of fatty acids after treatment with sodium bicarbonate produced two fractions with a limited mutual contamination. Individually inefficient, they have to be reassociated to stimulate LH release. Adding hexadecane diol-1,2 and octadecane diol-1,2 to the acid fraction of the extract was sufficient to obtain an effective compound [12]. Further attempts to identify the acid components of the pheromones suggest that the subfraction containing branched fatty acids under C16 could be involved [Fig. 4(a)]. A parallel study on male goat's pheromone indicates that a similar fraction is present [Fig. 4(b)].

In the male, the stimulation of LH and testosterone secretion occurs when the stimulus female is in oestrus and when she is not. However, the response is greater in the presence of an oestrous female, although being independent of the level, and even the display of any type of sexual activity, as sexually inhibited rams presented a clear endocrine response [14]. The ocurrence of an ejaculation did not influence the LH and testosterone responses, but induced a significant rise in cortisol.

In the absence of direct association of the display of sexual activity and endocrine responses, a pheromonal communication could be hypothesized, especially as olfactory control is the most frequent interaction between males and females. The effect of potential sources of pheromone from the oestrous female (urine, vaginal secretions and fleece) have been tested. In contrast to what has been observed in females, fitting the rams with masks containing such odorants did not change any endocrine parameters, although in controls, the actual presence of an oestrous female did increase LH pulse frequency and circulating testosterone [15] (Table 1). Such a negative result does not allow clear conclusions: the source of sexual pheromones could be different from those arbitrary chosen. The mode of presentation in a mask enforces a distance of 1–2 cm between the muzzle and the product tested. The lack of response could be due to insufficient stimulation, although the same methods have been effective and reliable in females.

Table 1. Effect of female odour on male endocrine secretions

	Prestimulation	Stimulation
	LH pulses/3 h	
Odour	0.66	0.83
Oestrous ewe	0.5	1.75*
	Testosterone (ng/ml)	
Odour	2.07	2.24
Oestrous ewe	1.8	4.09*

*Significant at $P < 0.05$. Reproduced from Ref. [15].

ROLE OF THE CHEMICAL SENSORY RECEPTORS IN THE STIMULATION OF PHYSIOLOGICAL PROCESSES BY SOCIO-SEXUAL INTERACTIONS

The "effect" male in sheep, because of its chemo-sensory basis and the rapidity of action appear similar to that which has been extensively studied in rodents. In these species, the vomeronasal or accessory olfactory system has been demonstrated as receiving and conveying the chemical signals acting on the neuroendocrine mechanisms [16]. Lesions of the vomeronasal organ eliminates the pheromonally-mediated suppression of oestrus [17] and acceleration of puberty [18] in female mice, as well as female-induced testosterone surges in males [19]. The vomeronasal organ is well developed in sheep [20] and is involved in the specific display of "flehmen", taking place after olfactory control of female genitalia and urine [21]. Its role has been investigated in the sexually experienced female sheep.

In this species, vomeronasal organs are large and visible structures, they consist of two epithelial tubes of *ca* 8 cm long, surrounded by a cartilage of *ca* 1 cm height, lying on either side of the nasal septum. Two surgical techniques have been designed to eliminate the accessory olfactory system. Electrocauterization, which allows complete destruction of both organs, and removal of a section of vomeronasal nerve which interrupts the efferent pathways. In both cases, apart from the macroscopic control of the completeness of the lesion, the histological examination of the glomerular layer of the accessory olfactory bulb showed complete disorganisation and atrophy. The effectiveness of the nerve section was controlled by the interruption of the transport of an anterograde tracer (Rhodamine acetate). In both cases, the presentation of the odour of rams fleece induced an LH secretion similar to that of controls [22]. The

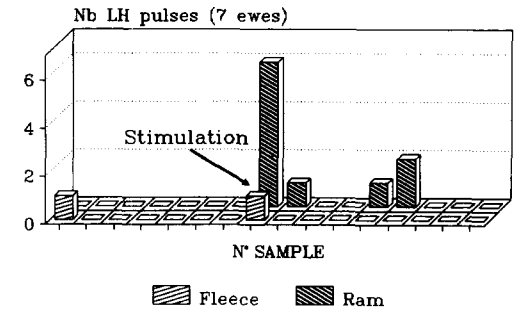

Fig. 5. Effect of anosmia in the response of ewes to pheromonal stimulation and to the contact with males (blood sampling at 20 min interval for 3 h pre and post-stimulation). Reproduced from Ref. [24].

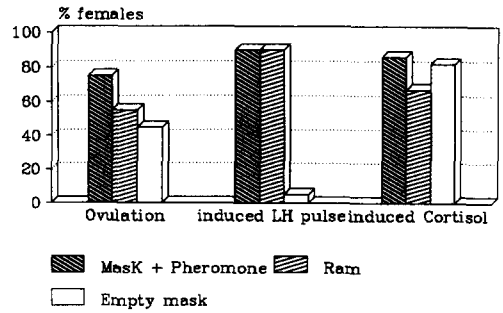

Fig. 6. Effect of pheromone presentation, in a mask, control (mask without pheromone) and rams on ovulation, LH and cortisol response of anoestrous ewes.

main olfactory system has been spared and remained functional in both cases, and, consequently appears able to mediate the pheromonal stimulation as before. As no selective lesion of the olfactory bulbs or mucosa has been performed, we can conclude that if the vomeronasal system is not necessary to the pheromonal stimulation of endocrine function, its involvement cannot be ruled out in sheep.

Although the sense of smell has been demonstrated to mediate the stimulation of neuro-endocrine response to socio–sexual interactions, the possibility for other sensory cues that act synergistically has to be considered. In fact, full physical contact with rams induces ovulation in a higher proportion of anoestrous ewes than fence-line contact or isolated odourous ram's fleece [23]. In anosmic ewes, after complete surgical removal of main and accessory olfactory bulbs, the direct contact with males induces an LH release similar to that of controls [24] (Fig. 5). Similarly, in rams, anosmia does not modify the LH and testosterone response to the presence of an oestrous female [15] (Table 2).

To conclude, it appears that, in sheep, the stimulations conveyed by other sensory canals other than olfaction have a cumulative action, and could eventually replace the pheromonal stimulation to induce the process of ovulation. Such results suggest that the neural pathway controlling the release of gonadotropins is not under the control of some kind of direct "labelled line" conveying pheromonal stimulation, but of all sensory information from the environment.

Table 2. Effect of anosmia on the endocrine response of rams to the presence of an oestrous female

	Prestimulation	Stimulation
	LH pulses/3 h	
Control	0.16	0.83*
Anosmic	0.41	1.08*
	Testosterone ng/ml	
Control	1.10	3.16*
Anosmic	1.45	3.35

*Significant $P < 0.05$. Reproduced from Ref. [25].

An additional complexity results from the observations that non-specific stimulations are also able to facilitate ovulation: it has been observed that fitting anoestrous ewes with an empty mask could be sufficient to induce ovulation. A simultaneous release of LH and cortisol is observed in ewes stimulated by rams pheromones presented in a mask, but the control females, fitted with empty masks, ovulate without immediate LH release, but with a similar cortisol response (Fig. 6). The effect of stress on reproductive functions is generally considered as inhibitory However, occasional observations suggest that ovulation could also be induced by some kind of stress, as in the prepubertal gilt [25]. In the case of the ewe, the rapid release of LH that has been taken as criteria of the effectiveness of pheromonal stimulation does not appear as necessary. It is possible that the stimulation of the hypothalamic–pituitary–adrenal axis by some kind of stress interacts with the gonadotropin release. Furthermore, corticosteroid could interfere in the complex interplay of feed-back mechanisms that results in the release of a preovulatory surge of LH.

CONCLUSIONS

The effect of socio–sexual interactions on reproductive physiology has been demonstrated in male and female sheep: sensory information interferes with the nervous control of gonadotropin secretion. Most of the experimental evidence has been obtained in females. The results have:

1. allowed initial progress in the identification of ram pheromone;

2. shown that the vomeronasal system is not necessary;
3. shown that the chemical signal could be replaced by other sensory cues; and
4. shown some kind of stress or emotional reactions could also be efficient in inducing ovulation.

The chemical signal from the male is present in the fleece, and is probably a mixture of several compounds. The diols C16 and C18 appear to be important. The fatty acids with a long ramified chain could also be involved.

The fact that the odour of the male goat is effective in stimulating the ewe is surprising, as pheromones are generally highly characteristic of a given species, and very efficient interspecific barriers have been described. However, the active extracts from male goat's hair appear to contain similar components. The action could be a result of the presence of similar effective compounds of a slightly different chemical structure in each species. No direct evidence of a primer pheromone from the female has been obtained.

In contrast to rodents, the vomeronasal system is not necessary for the pheromonal stimulation of the female sheep. This system is implicated in physiological and stereotyped responses, whereas the nervous output of the main olfactory bulbs terminate widely over the brain, including some areas considered as necessary for cognitive processing. The fact that the main olfactory system is involved in the male effect on the ewe suggests that some treatment of the sensory information at a high level of the brain could take place before reaching the areas controlling gonadotropin release. The primer pheromonal action does not result in this species from a stimulation conveyed by a nervous "labelled line", as hypothesized from the anatomy of the vomeronasal system, but from stimulations integrated before acting on the nervous centres controlling LH release.

Such an hypothesis is supported by the fact that other sensory cues could replace the pheromone in stimulating the endocrine secretion. This suggests the existence of a common final pathway activated either by primer pheromones or by other sensory canals, and the possibility of the intervention of more elaborate treatments of information such as learning, associations, etc.

The intervention of some kind of stress makes the situation more complex again, as not only the specific sensory stimulations, but also the emotional state could act on endocrine balance, hence, there is a possibility that the general psycho–social environment could modulate the physiological processes of reproduction.

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