

New understanding of an old phenomenon: uncontrolled factors and misconceptions that cast a shadow over studies of the 'male effect' on reproduction in small ruminants

Trina JORRE DE ST JORRE, Penny Alison Rhian HAWKEN, Graeme Bruce MARTIN*

School of Animal Biology and UWA Institute of Agriculture, University of Western Australia, Crawley, Australia

Received: 17.04.2014 • Accepted: 05.05.2014 • Published Online: 24.10.2014 • Printed: 21.11.2014

Abstract: In female sheep and goats, exposure to males can be used to induce ovulation and thus control and synchronise fertility. This 'male effect' offers simple, cost-effective, and hormone-free management of reproduction in flocks and herds but, despite decades of research, significant gaps in our knowledge impede commercial application. Many of these gaps can be traced back to misconceptions in the early documentation of the male effect, and others are the result of uncontrolled factors in experimental design. Consequently, it is often difficult to draw conclusions from past research, to further our understanding of the phenomenon, and to make recommendations for industry application. Here, we describe 2 aspects of the male stimulus used to elicit the male effect that are often uncontrolled – the novelty of the stimulus males and factors that contribute to variability in the potency of the male stimulus. We discuss the physiology that underpins novelty, including the individual specificity of the sociosexual signals emitted by the male, and the concomitant ability of females to distinguish among individual males and remember them. Importantly, an understanding of the issue of novelty can make it easier to use the male effect in commercial practice. We also discuss difficulties in interpretation caused by variation in endpoints of the male effect (from neuroendocrine reaction to birth). We conclude that more rigour is needed in the control and reporting of male novelty, in preexperimentation separation of the sexes, and in extrapolation from detection of early responses to the male stimulus to reproductive outcomes.

Key words: Male effect, sheep, goat, behaviour, industry, pheromone

1. Introduction

In sheep and goats of both sexes, exposure to a prospective mate can elicit a dramatic, almost instantaneous change in the secretion of reproductive hormones. For example, in anovulatory females, exposure to males increases the tonic (pulsatile) secretion of luteinising hormone (LH) and this response can lead to a preovulatory surge of LH and ovulation. This phenomenon, known as the 'male effect', has generated considerable interest since it was initially documented for sheep in the 1940s (1) and for goats in the 1960s (2). The interest might have fluctuated over the decades (3) but research on the male effect continued primarily because of its potential value for controlling reproduction, a simple and cost-effective way to produce out-of-season offspring, and to synchronise mating in a flock or herd so that parturition, weaning, and marketing can be better managed (4). Recently, new levels of interest have been generated by increasing consumer demand for hormone-free methods of animal production, with the male effect now seen as an alternative to exogenous hormones and thus a cornerstone of 'clean, green, and

ethical' livestock management (5–7). However, despite decades of research, significant gaps in our knowledge still impede the commercial application. Some of the most important of these gaps originate from misconceptions reported in the early documentation of the male effect that have led to uncontrolled factors in experimental design, as reviewed by Delgado et al. (8). As a consequence, it is often difficult to draw conclusions from past research, further our understanding of the male effect, and make recommendations for using it as a management tool.

Perhaps the most obvious example is the importance of the novelty of males as a critical determinant of their ability to elicit the male effect (9–14), despite which the novelty of stimulus males used in experiments was rarely reported. In hindsight, it seems obvious that novelty has been largely overlooked because, in the initial documentation of the phenomenon, a key observation was misinterpreted: in 3 independent studies, researchers found that ewes in continual contact with rams did not cycle indefinitely but entered seasonal anoestrus. This outcome was interpreted as evidence of habituation (or

* Correspondence: martin@uwa.edu.au

refractoriness) to the male stimulus (1,15,16). The authors did not consider the possibility that ewes were only habituated to the specific males with which they were in continual contact, and might still respond to unfamiliar, or novel rams. Consequently, for both sheep and goats, the dogma evolved that females needed to be preconditioned by a period of complete separation from all males if the male effect was to induce ovulation. Some 3 decades later, Oldham and colleagues correctly suggested that females might only become habituated and unresponsive to specific males (17,18). However, the dogma persisted and, since then, very few studies have described how male novelty was controlled.

This issue is particularly important when the experimental design has involved repeated exposure of females to males. For example, in their study on the effects of body condition and short-term nutritional supplementation on the response of ewes to the male effect, Scaramuzzi et al. (19) rotated rams regularly to ensure an even stimulus and avoid the variability associated with individual rams. This was a logical protocol, but the management of ram novelty was not considered. The male effect clearly worked, but it is impossible to say whether the outcome, with respect to responses to treatment, would have been affected.

Failure to control for male novelty has also led several authors to make a second misleading recommendation – that a month of separation or less is sufficient to renew the ovulatory response of females to familiar males (20–22). Again, it is not clear whether the males used in these studies were novel or ‘familiar’. Recent studies in our laboratory have challenged this recommendation from 2 perspectives: first, separation of the sexes did not enhance the response of females to new, novel males; second, a month of separation was not sufficient to ensure a full LH response (i.e. increased LH pulse frequency followed by an LH surge), even in the highly sensitive Merino genotype (13).

It is thus difficult to interpret the outcomes of many studies because the authors rarely state the length of time that females were separated from males, or whether the same or different males were reintroduced after the period of separation. This situation is further complicated by ambiguity over the physical distance or barriers necessary to prevent transmission of sociosexual stimuli between treatments (few authors report the method of separation or the degree of separation).

Another factor that is often not controlled or reported is the ‘potency’ of the stimulus males – the ability of males to elicit a neuroendocrine response in females is influenced by previous exposure to females and nutrition, age, sexual behaviour, and perhaps sexual experience (23–27).

Finally, interpretation is complicated by variation among authors in the definition of the endpoint of their

experiment (e.g., LH pulse frequency, LH concentration, ovulation, births). Extrapolation from one endpoint to the next is risky, especially where there is no clear statement about whether the same males were present from initial stimulation (e.g., LH secretion) through to the preovulatory LH surge and ovulation, or whether males were exchanged with ‘new’ (perhaps novel) males as the experiment progressed.

In this paper, we will identify and discuss the uncontrolled factors that have led to, or may lead to, misconceptions about the male effect. Specifically, we will focus on the problems associated with uncontrolled factors related to the male stimulus, and on the difficulties in interpreting the outcomes from studies with different endpoints.

2. Separation of the sexes – is it really necessary?

In the early documentation of the male effect in sheep, ewes in continual contact with rams did not cycle indefinitely but showed normal seasonal patterns of reproductive activity (1,15,16). For example, Riches and Watson (15) observed that ewes in continuous association with rams become anoestrus, and interpreted this outcome as evidence of habituation (or refractoriness) to the male stimulus. Schinckel (16) came to the same conclusion and suggested that the male effect relied upon the ‘sudden’ introduction of ewes to rams after a long period of separation, and that continuous association of ewes with rams depressed breeding activity and extended the length of anoestrus. Subsequent studies, based on simultaneous observation of ovulation as well as oestrus, confirmed the early findings (28) (Figure 1).

For decades, therefore, it was accepted that females needed to be preconditioned by a period of complete separation from all males to enable the male-induced ovulation (8). This understanding evolved into a general recommendation that females should be separated from males for a minimum of 1 month to optimise their ovulatory response to the male effect (29), although it is not clear how or when this recommendation came about. There certainly seems to be little scientific justification to support it and many researchers have chosen to err on the side of caution and separate the sexes for several months before an experiment (30–34). In other cases, it has been suggested that less than a month of separation is sufficient to renew the responsiveness of the ewes. For example, Oldham and Cognié (21) reported that the proportion of Ile-de-France ewes ovulating in response to rams did not differ between periods of separation of 21 days and 120+ days. Similarly, Oldham (20) found that ewes ovulated in response to rams after as little as 17 days of separation. Cohen-Tannoudji and Signoret (22) suggested that as little as 24 h of separation of the sexes was sufficient for rams

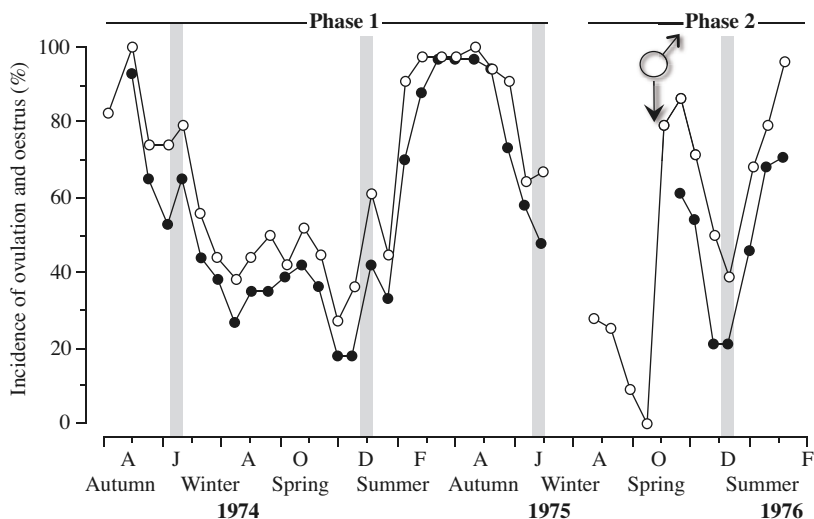


Figure 1. The annual patterns of oestrus (●-●) and ovulation (○-○) in a flock (n = 34) of 4-year-old Merino ewes in the southern hemisphere. The ewes were maintained at an approximately constant live weight of 40 kg. During Phase 1, the ewe flock was maintained continuously with vasectomised rams. During Phase 2, the flock was isolated from rams until 16 October 1975, when the vasectomised rams from Phase 1 were reintroduced (male symbol and arrow). The shaded columns represent the winter and summer solstices. From Oldham et al. (28).

to retain their ability to stimulate pulsatile LH secretion in ewes. However, none of these reports specified whether the males were familiar (i.e. the ewes were reexposed to the same rams) or novel to the ewes. We now know that this distinction is important, both for interpretation of the data and for practical application of the male effect. We directly addressed the issue by observing changes in the pulsatile secretion of LH in ewes exposed to the same rams after 15 min or 1 month of separation and comparing their responses with those observed in ewes exposed to novel rams (13,14). After a month of separation, reexposure of ewes to the once-familiar rams increased pulsatile LH secretion ($P < 0.05$) in 6 out of 8 ewes. However, the LH response was smaller than that seen in ewes exposed to completely novel rams and led to a preovulatory LH surge in only 2 of the 6 ewes. Furthermore, in a second experiment we showed that reexposure to familiar rams after 17 days of separation was not sufficient to alter even the pattern of pulsatile LH secretion in ewes (13).

These observations are important for 3 reasons: first, they challenge the standard recommendation of a month of separation for application of the ram effect; second, they challenge the assumption that the male-induced increase in pulsatile LH secretion definitively leads to an LH surge (not to mention ovulation and lambing); third, there seem to be degrees of novelty – completely familiar rams have no effect on LH secretion, but familiar rams taken away for a month and then returned elicit only a partial response, and completely novel rams elicit the full LH response.

The interpretation of results is confused also by ambiguity over the definition of ‘separation’, particularly with respect to guaranteeing blockade of transmission of all of the male stimuli. Male odour alone can elicit an LH response in anovulatory females in both sheep and goats (35–38), but the response to the male effect is maximised by exposure to the full complement of male sociosexual stimuli (9,39). Despite wide acceptance of the potency of these stimuli, few studies report how they prevented their transmission between ‘separated’ males and females. Furthermore, no studies have specifically tested the distance over which females can receive olfactory, visual, or auditory stimuli from males. It is therefore often impossible to determine whether separation was actually achieved and thus whether the stimulus males were partially or completely novel to the females. Given the sensitivity of females to olfactory stimuli, it is also possible that animal handlers moving between treatments could have accidentally transferred stimulatory signals. In some cases, the authors themselves have recognised that the degree of separation used was not sufficient – for example, Walkden-Brown et al. (24) reported that 100 m was not sufficient to prevent the transmission of sociosexual stimuli between male and female goats in one of their experiments. We simply do not yet know the minimum distances needed, or the types of barriers that will be effective, in preventing transmission of sociosexual stimuli from males to females.

In summary, the recommendation that a month of separation or less is sufficient to guarantee a full response

to the male effect is potentially misleading and may even have led to flawed experimental designs in many studies. Furthermore, it is simply not possible to either accept or reject the conclusions of many studies because, in the reports describing them, there was no explicit statement about the duration and distance of separation prior to the experiment, and no explicit statement about how they managed the novelty of the stimulus males. These issues are obviously important for research on the male effect itself, but they are also important for any study of reproductive physiology in which the researchers might not be aware of interference from sociosexual stimuli.

3. Novelty of the male stimulus

Male novelty first emerged as a factor in the efficacy of the male effect when Pearce and Oldham (9) showed that anovulatory ewes maintained with rams ovulated if they were exposed to new, novel rams. Cushwa et al. (10) also showed that separation of ewes from all rams prior to mating with novel rams did not increase the synchrony of lambing compared to ewes that had been maintained with rams. The power of novelty is even evident in cyclic ewes in which replacement of rams every 17 days can shift the distribution of oestrus compared to ewes maintained with the same rams (12). These observations were mirrored in goats when Véliz et al. (11) showed that maintenance of does with bucks did not reduce the proportion of does that ovulated in response to novel bucks. In a recent study, we directly measured the neuroendocrine response of females

to novel males and showed that exposure to novel rams increased tonic secretion of LH and induced an LH surge in all ewes (13,14). Importantly, this series of neuroendocrine events did not occur in ewes reexposed to familiar rams after only 15 min of separation (Figure 2). Together, these observations indicate that females that are continuously with males do not completely lose their ability to respond to all male stimuli, as suggested in the original reports (1,15,16). Instead, the ewes appear to become habituated to specific males and only males that are sufficiently novel can induce a neuroendocrine response typical of the male effect.

4. The biology of ‘novelty’ in the male stimulus

The fact that female sheep and goats respond to novel, but not familiar males, allows us to infer 2 important aspects of the male effect: i) each individual male must emit specific signals that differentiate him from other individual males; and ii) females use these signals to differentiate between individual males and then form a memory of those individual males. These assumptions raise many questions regarding individual recognition, memory formation, and recall in sheep, all of which lie at the heart of determining what makes a novel male ‘novel’.

4.1. Is identity encoded by olfactory stimuli from the male?

It would be no surprise that, for an animal that normally lives in large groups, surrounded by the sights, sounds, and smells of their neighbours, prospective mates, and

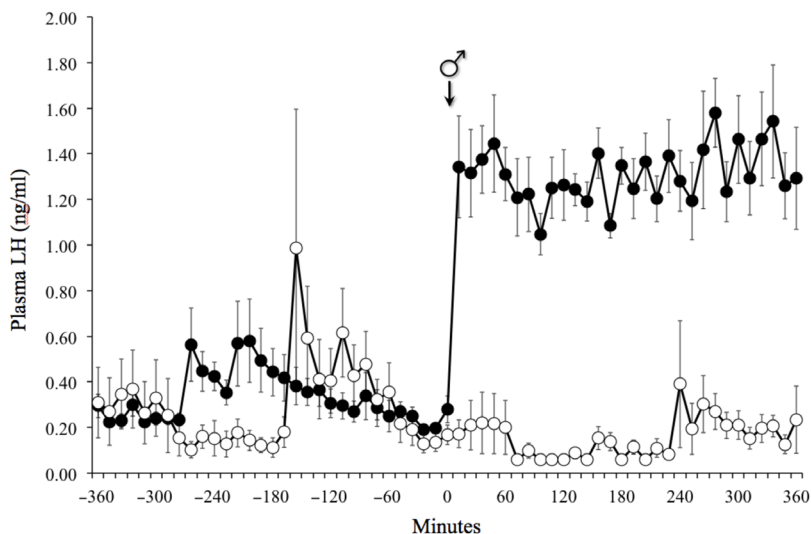


Figure 2. Mean \pm SEM concentrations of LH in female sheep in the presence of familiar males (-360 to 0 min), and then after the familiar males were removed for 15 min and returned (O-O), or after the familiar males were removed and replaced 15 min later with novel males (●-●). The male symbol and arrow at 0 min indicates the time of male exchange. Redrawn after Hawken et al. (14).

offspring, it is important to be able to recognise and remember other individuals. Indeed, in sheep, recognition of individuals has been demonstrated in several contexts – they can differentiate between their own lamb and alien offspring (40), show preferences for specific individuals (41), remember the faces of specific individuals for over 2 years (42), and prefer to graze near flock-mates with whom they have been raised from birth (43). The evolutionary background and complex social environment undoubtedly explain the profound effect of sociosexual stimuli on the reproductive physiology and behaviour of sheep and goats, but no studies to date have investigated how female ungulates discriminate between individual males.

To address this issue, it is logical to begin with olfactory stimuli because there is general agreement that male odour has an important impact on female reproductive physiology and behaviour (44) and, alone, can induce the entire male effect, from the initial neuroendocrine response through to ovulation (44–50). Olfactory stimuli are known to mediate individual recognition between males and females of other species (51,52) so a logical hypothesis is that male identity is encoded primarily by an olfactory chemical ‘fingerprint’ or ‘olfactory signature’. The ram ‘pheromone’ is purportedly a mix of long-chain fatty acids (37) and, given the vast variety in such molecular structures, it seems feasible that such a mix could be sufficiently complex to encode differences that would enable ewes to distinguish between individual males. This concept of an ‘olfactory signature’ is supported by the importance of olfactory memory and recall in maternal recognition. In the sheep, the ‘odour signature’ of the newborn lamb is carried in the amniotic fluid, and the mother learns this signature through brain processes that involve recognition and recall (‘olfactory memory’). As with the male effect, the chemistry of the molecules that are responsible for mother–young recognition is not known, but fatty acids and olfactory binding proteins appear to be involved (53).

Given the complexity of olfactory stimuli and the potential for these stimuli to ‘encode’ individual identity, it is difficult to see how a single molecule can be ‘the pheromone’ of male goats, as suggested in a recent study (54). One possible explanation is that a single major molecule carries the basic stimulatory signal for the male effect, and that other molecules in the mix modulate the response of the female to the major molecule. Interestingly, the pheromonal activity of this molecule was dependent on oxidation that was, in turn, associated with the formation of new compounds (55). Could these compounds encode the identity of that specific male? Further research, possibly using this unique model for studying the physiological effects of olfactory stimuli, hopefully holds the answer.

The response of ewes to rams is reportedly maximised by exposure to the full complement of sociosexual stimuli

from rams (9,39). Therefore, it is clear that nonolfactory stimuli also are important to the male effect and may be involved in the ability of females to differentiate between novel and familiar rams. Indeed, the remarkable ability of sheep to recognise and remember faces of other sheep has been documented in detail (56–59). In a recent study by our group, ewes were seen to respond to photographs of rams with a small increase in LH secretion – the response was muted compared to ewes exposed to the full complement of sociosexual stimuli but it can be inferred that visual stimuli do play a role in mediating the neuroendocrine response of female ungulates to the male effect. Few studies have evaluated the role of auditory stimuli in the male effect but, in the same study, auditory signals from a movie of ewes and rams mating had no effect on pulsatile secretion of LH (50). Nevertheless, auditory stimuli might still be involved in individual recognition: first, auditory signals assist the recognition of lambs by their mothers, particularly from a distance (60); second, some breeds are more reliant on vocal cues than others (57) and, in turn, this could affect the mode or method of individual recognition between males and females.

4.2. How do females determine who is ‘novel’ and who is ‘familiar’?

Clearly, if females respond only to signals from sufficiently novel males, they must be able to remember the individuals and, if odour is the major identification system, then they must form an ‘olfactory memory’ that they recall and, over time, ‘forget’. This leads into the area of memory formation and the potential role of neurogenesis in the brain. For the higher vertebrates, the long-held dogma was that the brain of the mature animal has a fixed number of cells, with no scope for repair or growth. During the 1990s, studies with song birds, in particular, suggested that this was incorrect, at least for brain regions involved with memory. This was borne out by studies in mice showing that male pheromones that help females to distinguish between subordinate and dominant males induce neurogenesis in the dentate gyrus of the hippocampus, a brain region fundamentally associated with memory (61). We also found that the acute LH response of ewes to novel rams is associated with a rapid (within 2 h) and robust increase in the rate of cell proliferation in the dentate gyrus of the hippocampus (14). Importantly, the odour of the newborn lamb induces cell division in the same region of the brain, indicating that neurogenesis is involved in both cases of the formation of olfactory memory (62).

The complex neural processes associated with the neuroendocrine responses of females to males have been investigated (63–65), but none of these studies have specifically differentiated between novel and familiar males or focused on the role of memory. However, as with the involvement of neurogenesis in individual recognition

described above, there are many similarities between the regions of the ewe brain activated by rams (65) and by their new-born lamb (66), so it seems logical to begin with the premise that similar pathways are involved in the formation and recall of identity in both circumstances. Gelez and Fabre-Nys (65) found that the cortical nucleus of the amygdala was critical in mediating the LH response of ewes to rams. This structure projects to other parts of the amygdala involved in olfactory learning, memory, and discrimination of individuals (67,68), and also projects to several cortical regions, including the piriform and entorhinal cortices, both of which are involved in memory and learning (68–71). The response of ewes to ram olfactory stimuli seems to be at least partially dependent on learning the characteristics of rams (38), so these brain regions may be responsible for processing olfactory stimuli from rams and for combining them with known characteristics of the ram, thus allowing identification of novelty and the appropriate neuroendocrine response (64).

5. Potency of the male stimulus

The ability of males to induce an endocrine and/or ovulatory response in females depends on the presence of androgens, as clearly demonstrated by early studies showing that wethers (rams castrated before puberty) or ewes can induce ovulation if they are treated with testosterone (72–74). Likewise, in goats, androgenised wethers and does are as effective at inducing oestrus as intact bucks (75). Interestingly, high doses of exogenous oestrogen also confer wethers and females with the ability to induce the male effect (72–74) and to display male sexual behaviour (76), suggesting that the aromatase present in the male sheep brain (77,78) might mediate these processes. Importantly, the circulating concentrations of androgens can directly influence both factors that have a clear impact on the potency of the male stimulus: the expression of sexual behaviour and the production of olfactory stimuli.

In both sheep and goats, males that exhibit high levels of sexual behaviour induce ovulation in a higher proportion of females than do males with low levels of sexual behaviour (11,24,25,39,74). Differentiating between the effects of androgen concentrations on olfactory stimuli and sexual behaviour is difficult, but Vielma et al. (79) attempted to do so in goats by using sedation to control the behaviour of bucks. Exposure to sedated bucks initially led to an increase in LH pulsatility in does but the response declined within 4 h. In contrast, LH pulsatility remained elevated for up to 24 h in does exposed to nonsedated bucks (79). Thus, in goats at least, male behaviour appears to directly contribute to the potency of the male stimulus. Importantly, the difference between treatments in the persistence of the endocrine response in the females highlights again the danger of extrapolating from one endpoint to the next:

the interpretation of these results would be quite different if the experiment had been terminated at, for example, 2 h. Interpretation of the impact of these results on our understanding of the male effect is challenging but it does seem possible that the pheromone alone elicits the initial neuroendocrine response, after which the persistence of a high LH pulse frequency depends on other signals, including male behaviour. Either way, it seems that if the male effect is to be successful, anovulatory females need a sustained and intense exposure to olfactory, behavioural, visual and auditory stimuli from males (80). Furthermore, if the males produce stimuli of low quality and intensity, they could fail completely to induce ovulation or, at the very least, induce poor synchrony of oestrus cycles in the flock. However, even if we focus on only the aspects of male potency that depend on androgens, we need to consider the wide variety of factors that affect the hypothalamic–pituitary–testicular axis. This is the topic of the next section of this review.

5.1. Seasonality and genotype

In seasonal genotypes, the secretion of gonadotrophin (and thus testosterone) is suppressed in males and sexual behaviour declines during the nonbreeding season (27,81–84). A priori, we would expect low pheromone production, thus providing a combination of factors (chemical, behavioural) that limits the ability of males to elicit the male effect during the nonbreeding season (Figure 3). The degree to which the male reproductive axis is suppressed by seasonal changes in photoperiod varies with genotype (6,85), so it is logical to expect differences among genotypes in the ability of the male to induce the male effect. This hypothesis has been supported by several studies with sheep – Dorset rams are more effective than Suffolk, Romney, Romney × Finn, or Coopworth rams, with Merino rams being intermediate between Dorset and Romney rams (86–90).

In the more seasonal genotypes of sheep or goats, photoperiod is the dominant factor that determines annual changes in sexual activity in males (49). Consequently, at least in goats, treatment of males with artificial short days, characteristic of the breeding season, considerably improves the ability of males to stimulate females during the nonbreeding season (91). In rams, exogenous melatonin (a short-day signal) also improves plasma testosterone concentrations, libido, and their ability to induce ovulation in anovulatory ewes (92). In a more complex scenario, exposing goat bucks to artificial long days for 2.5 months, followed by a return to natural photoperiod, or by exogenous melatonin, greatly improves the proportion of does that exhibit oestrous behaviour (91,93). Together, these studies indicate a direct relationship between photoperiod and the capacity of males to stimulate ovulation in female ungulates.

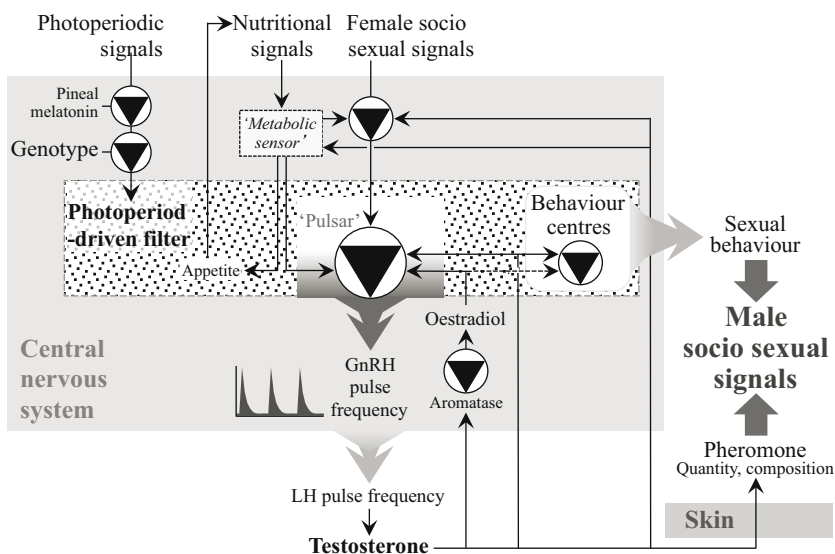


Figure 3. Hypothetical relationships among factors that affect the production of testosterone and thus the quality and intensity of the male sociosexual signals that induce the 'male effect' in sheep. Circles containing triangles represent important control points. Environmental inputs operate through a variety of pathways that ultimately affect the pulsatile secretion of gonadotropin-releasing hormone (GnRH) that, in turn, controls the frequency of pulses of luteinising hormone (LH) and the concentrations of testosterone. An interaction between genotype and photoperiod is thought to act as a 'filter' (the stippled area) that alters the neuroendocrine responses to environmental inputs, as well as the endocrine and behavioural responses to the sex steroids (96).

5.2. Exposure to females (the 'female effect')

In both sheep and goats, exposure of males to oestrous females, before or at the same time as their introduction to anoestrous females, can also improve the ovulatory response of anoestrous females to those males (23,24). This increase in the potency of the stimulatory males is probably a result of increased secretion of androgen, sexual behaviour, and olfactory stimuli induced in the males by the sociosexual stimuli from the oestrous females (Figure 3). Conversely, Rosa et al. (94) found that exposure to oestrous ewes increased the sexual behaviour and circulating plasma concentrations of testosterone in rams but had no effect on the proportion of ewes that ovulated. However, as the authors conceded, the ovulatory response was high with all treatments, leaving little scope for improvement.

5.3. Nutrition

Nutrition has been shown to increase plasma testosterone levels, sexual behaviour, and the odour of goat bucks, and to improve their ability to induce ovulation in anoestrous does (25). Hillbrick and Tucker (95) also showed that nutritional supplementation increased the lipid content and concentration of ethyl-branched fatty acid in buck fleece, suspected sources of the putative buck 'pheromone'. Rams also show an increase in gonadotrophin and testosterone secretion following nutritional supplementation (96), but

there is currently no evidence that nutrition can be used to improve the ability of rams to induce ovulation.

5.4. Age and sexual experience

Adult rams are more effective at inducing ovulation and oestrus in ewes than yearling rams (26), and oestrous ewes prefer to spend time near old, large, and sexually active rams (97). These differences could be attributed to differences in the physical appearance and behaviour of adult males. Alternatively, they may reflect variability in the quality of the ram pheromone because more anoestrous ewes ovulated after exposure to wool from adult rams than to wool from yearling rams (26). Previous sexual experience affects the expression of sexual behaviour by rams, and sexually experienced rams mate, mount, and lick ewes more frequently than sexually inexperienced rams (27). Therefore, it seems likely that differences between young and adult rams in their capacity to induce ovulation are due to the combined effects of maturity and sexual experience on pheromone production and on their expression of sexual behaviour.

5.5. Implications of variation in male potency

In summary, there is good evidence that a variety of factors affect the ability of males to induce the male effect in female ungulates (Figure 3). Most reports explain or control for female factors that affect the response, but

rarely acknowledge or report the relevant male factors. This omission makes precise interpretation of the data difficult. Moreover, variation in male factors that contributes to variation in the response of females could be a major limitation to the effective incorporation of the male effect into the management of commercial flocks (98). Clearly, factors that affect androgen production, pheromone production, and sexual behaviour in the males used to induce the male effect are important areas for investigation. Furthermore, researchers should be careful to control and clearly report factors that might affect the potency of the male stimulus in future studies.

6. Experimental protocols – variation in endpoint

The literature to date shows wide variation in the experimental endpoints of the response to the male effect. For example, some studies use ovulation detected by laparoscopy as an endpoint [e.g., Pearce and Oldham (9)], whereas others use the distribution of lambing [e.g., Cushwa et al. (10)]. Clearly, both of these endpoints imply stimulation of the hypothalamic–pituitary–gonadal axis, but both also involve a significant delay (days to months) between the neuroendocrine response and observed response. This distinction is important because many other factors could have exerted disruptive effects (e.g., nutrition, stress, novel males) on the outcome, thus making it a challenge to interpret the data. On the other hand, if the early response of the reproductive endocrine axis (e.g., changes in the pulsatile LH secretion) is used as an endpoint, it is risky if not ‘undisciplined’ to extrapolate to sustained changes in LH pulsatility, positive feedback, LH surge, and ovulation. For example, in our own study, all ewes reexposed to familiar rams after a month of separation exhibited an increase the secretion of LH, but few went on to have an LH surge (13). The risk of extrapolation from an initial increase in LH secretion to ovulation is compounded by our poor understanding of whether the continued presence of the stimulatory males is necessary for every stage of the process (47,80) and leads to a very basic question: which endpoint should we use to define the ‘male effect’? Such issues highlight the need to report whether males are present through to ovulation and, if so, whether the same males were used throughout. Clarity on this issue is also necessary for observations after the first ovulation because, if the stimulatory males are removed at that point, the ewes do not continue to cycle and become anovulatory again (74). Apparently, for goats, male presence needs to be continued but can be intermittent – for example, daily exposure to bucks for 4 h for 15 days led to ovulation in over 90% of does with no difference in the proportions of does that ovulated following exposure to bucks for 4, 8, 12, or 16 h per day (99).

7. Future perspectives

Since its discovery over 70 years ago, the male effect has fascinated reproductive biologists because of its dynamic and robust impact on the hypothalamic–pituitary–gonadal axis of female sheep and goats. However, in the words of Albert Schweitzer, “as we acquire more knowledge, things do not become more comprehensible but more mysterious”. Through this review, we have highlighted misconceptions about the male effect that, with the benefit of hindsight, have led to uncontrolled factors in experimental design that, in turn, complicate interpretation of the data. They also inhibit the application of the male effect to commercial practice. The following questions need to be considered for future experiments, and for formulating recommendations for on-farm application of the male effect:

- 1) How long were females separated from the males that will be used to elicit the male effect?
- 2) What distance and barriers were used to separate treatments and keep the females separate from the males that will be used to elicit the male effect?
- 3) What factors may have affected the potency of the male stimulus?
 - a) Have they had previous contact with females?
 - b) What was their age and sexual experience?
 - c) Were they on a high or low plane of nutrition?
 - d) Had they been exposed to changing/stimulatory photoperiod?
- 4) What intensity and length of male exposure is required to maximise ovulation and conception?
- 5) Does intermittent exposure of females to males maintain ‘male novelty’?

We propose that careful consideration of these questions during experimental design, along with clear reporting of these factors, will increase the clarity and ease of interpretation of the literature describing the male effect.

8. Conclusion

When it comes to the male effect, it is clear that not all males are ‘created’ equal, nor do they ‘remain’ equal throughout their lives. Variability in the male stimulus is driven by factors that affect their capacity to produce androgens, and thus the potency of the male stimulus and how ‘novel’ they are to the females. Male novelty is clearly a critical determinant of the response of females to the male effect and the mechanism through which males ‘gain’ and ‘lose’ their capacity to stimulate females (i.e. novelty) is particularly intriguing. Future research into the brain regions involved in memory formation and recall of ‘novel’ males is likely to be guided by findings in the field of maternal recognition of offspring, because of uncanny parallels between the 2 phenomena. From a practical

perspective, it is vital to determine how long females actually need to be separated from specific males for those males to gain the ability to induce ovulation. We showed that 1 month of separation, often used as a standard [e.g., Ungerfeld et al. (29)], is not actually sufficient for those 'familiar' males to induce an LH surge in all ewes – so how long is actually needed? Furthermore, this 'optimal' period of separation is likely to be affected by factors that affect androgen production and sexual behaviour (e.g., nutrition, photoperiod), but this hypothesis is yet to be tested. Addressing these issues will significantly further

our understanding of the field and our ability to make robust and accurate recommendations for use of the male effect on farm.

Acknowledgements

T Jorre de St Jorre was supported by a PhD scholarship from the University of Western Australia and by funding from the UWA School of Animal Biology. PAR Hawken was supported by an Australian Research Council Discovery Grant (DP0558952) and by Meat & Livestock Australia (Project No. B.MGS.0027).

References

- Underwood EJ, Shier FL, Davenport N. Studies in sheep husbandry in Western Australia. V. The breeding season in Merino crossbred and British Breed ewes in the agricultural districts. *J Agric (West Aust) II* 1944; Series 2: 135–143.
- Shelton M. The influence of the presence of the male on initiation of oestrus cycling and ovulation in Angora does. *J Anim Sci* 1960; 19: 368–375.
- Ungerfeld R. Sixty years of the ram effect (1944-2004): how we have learned what we know about it? *J Anim Vet Adv* 2005; 4: 716–718.
- Martin GB. An Australasian perspective on the role of reproductive technologies in world food production. *Adv Exp Med Biol* 2014; 752: 181–197.
- Martin GB, Milton JTB, Davidson RH, Banchemo Hunzicker GE, Lindsay DR, Blache D. Natural methods of increasing reproductive efficiency in sheep and goats. *Anim Reprod Sci* 2004; 82–83: 231–246.
- Martin GB, Tjondronegoro S, Boukhliq R, Blackberry MA, Briegel JR, Blache D, Fisher JS, Adams NR. Determinants of the annual pattern of reproduction in mature male Merino and Suffolk sheep: modification of endogenous rhythms by photoperiod. *Reprod Fertil Dev* 1999; 11: 355–366.
- Martin GB. The 'clean, green and ethical' concept in animal production. *Agrociencia* 2009; 13: 1–7.
- Delgado JA, Gelez H, Ungerfeld R, Hawken PAR, Martin GB. The 'male effect' in sheep and goats – revisiting the dogmas. *Behav Brain Res* 2009; 200: 304–314.
- Pearce GP, Oldham CM. Importance of non-olfactory ram stimuli in mediating ram-induced ovulation in the ewe. *J Reprod Fertil* 1988; 84: 333–339.
- Cushwa WT, Bradford GE, Stabenfeldt GH, Berger YM, Dally MR. Ram influence on ovarian and sexual activity in anestrus ewes: effects of isolation of ewes from rams before joining and date of ram introduction. *J Anim Sci* 1992; 70: 1195–1200.
- Véliz FG, Poindron P, Malpoux B, Delgado JA. Maintaining contact with bucks does not induce refractoriness to the male effect in seasonally anestrus female goats. *Anim Reprod Sci* 2006; 92: 300–309.
- Hawken PAR, Beard AP. Ram novelty and the duration of ram exposure affects the distribution of mating in ewes exposed to rams during the transition into the breeding season. *Anim Reprod Sci* 2009; 111: 249–260.
- Jorre de St Jorre T, Hawken PAR, Martin GB. Role of male novelty and familiarity in male-induced LH secretion in female sheep. *Reprod Fertil Dev* 2012; 24: 523–530.
- Hawken PAR, Jorre de St Jorre T, Rodger J, Esmaili T, Blache D, Martin GB. Rapid induction of cell proliferation in the adult female ungulate brain (*Ovis aries*) associated with activation of the reproductive axis by exposure to unfamiliar males. *Biol Reprod* 2009; 80: 1146–1151.
- Riches JH, Watson RH. The influence of the introduction of rams on the incidence of oestrus in Merino ewes *Aust J Agric Res* 1954; 5: 141–147.
- Schinckel PG. The effect of the ram on the incidence and occurrence of oestrus in ewes. *Aust Vet J* 1954; 30: 189–195.
- Pearce DT, Oldham CM. Ovulation in the Merino ewe in the breeding and anoestrous seasons. *Aust J Biol Sci* 1988; 41: 23–26.
- Pearce GP. The ram effect: is isolation required? *Wool Tech Sheep Breed* 1988; 36: 81–86.
- Scaramuzzi RJ, Oujagir L, Menassol JB, Freret S, Piezel A, Brown HM, Cognié J, Fabre Nys C. The pattern of LH secretion and the ovarian response to the 'ram effect' in the anoestrous ewe is influenced by body condition but not by short-term nutritional supplementation. *Reprod Fertil Dev* 2013; <http://dxdoiorg/101071/RD13139>.
- Oldham CM. A study of sexual and ovarian activity in Merino sheep. PhD, The University of Western Australia, Crawley, WA, Australia, 1980.
- Oldham CM, Cognié Y. Do ewes continue to cycle after teasing? *Proc Aust Soc Anim Prod* 1980; 13: 82–85.
- Cohen-Tannoudji J, Signoret JP. Effect of short exposure to the ram on later reactivity of anoestrous ewes to the male effect. *Anim Reprod Sci* 1987; 13: 263–268.
- Knight TW. Are rams necessary for the stimulation of anoestrous ewes with oestrous ewes? *Proc New Zeal Soc Anim Prod* 1985; 45: 49–50.

24. Walkden-Brown SW, Restall BJ, Henniawati. The male effect in the Australian cashmere goat. 3. Enhancement with buck nutrition and use of oestrous females. *Anim Reprod Sci* 1993; 32: 69–84.
25. Walkden-Brown SW, Restall BJ, Henniawati. The male effect in Australian cashmere goats. 1. Ovarian and behavioural response of seasonally anovulatory does following the introduction of bucks. *Anim Reprod Sci* 1993; 32: 41–53.
26. Ungerfeld R, Ramos MA, González-Pensado SP. Ram effect: adult rams induce a greater reproductive response in anoestrous ewes than yearling rams. *Anim Reprod Sci* 2008; 103: 271–277.
27. Borg KE, Esbenshade KL, Johnson BH, Lunstra DD, Ford JJ. Effects of sexual experience season and mating stimuli on endocrine concentrations in the adult ram. *Horm Behav* 1992; 26: 87–109.
28. Oldham CM, Lindsay DR, Martin GB. Effects of seasonal variation of live weight on the breeding activity of Merino ewes In: Oldham CM, Martin GB, Purvis IW, editors. *Reproductive Physiology of Merino Sheep: Concepts and Consequences*. Perth, Australia: School of Agriculture (Animal Science), The University of Western Australia; 1990. pp. 41–58.
29. Ungerfeld R, Forsberg M, Rubianes E. Overview of the response of anoestrous ewes to the ram effect. *Reprod Fertil Dev* 2004; 16: 479–490.
30. Martin GB, Oldham CM, Lindsay DR. Increased plasma LH levels in seasonally anovular Merino ewes following the introduction of rams. *Anim Reprod Sci* 1980; 3: 125–132.
31. Knight TW, Hall DRH, Wilson LD. Effects of teasing and nutrition on the duration of the breeding season in Romney ewes. *Proc New Zeal Soc Anim Prod* 1983; 43: 17–20.
32. Atkinson S, Williamson P. Ram-induced growth of ovarian follicles and gonadotrophin inhibition in anoestrous ewes. *J Reprod Fertil* 1985; 73: 185–189.
33. Rosa HJD, Silva CC, Bryant MJ. The effect of ram replacement and sex ratio on the sexual response of anoestrous ewes. *Small Rumin Res* 2006; 65: 223–229.
34. Martin GB. Ram-induced ovulation in seasonally anovular Merino ewes: effect of oestradiol on the frequency of ovulation oestrus and short cycles. *Theriogenology* 1979; 12: 283–287.
35. Claus R, Over R, Dehnhard M. Effect of male odour on LH secretion and the induction of ovulation in seasonally anoestrous goats. *Anim Reprod Sci* 1990; 22: 27–38.
36. Walkden-Brown SW, Restall BJ, Henniawati. The male effect in Australian cashmere goats. 2. Role of olfactory cues from the male. *Anim Reprod Sci* 1993; 32: 55–67.
37. Cohen-Tannoudji J, Einhorn J, Signoret JP. Ram sexual pheromone: first approach of chemical identification. *Physiol Behav* 1994; 56: 955–961.
38. Gelez H, Archer E, Chesneau D, Campan R, Fabre-Nys C. Importance of learning in the response of ewes to male odor. *Chem Senses* 2004; 29: 555–563.
39. Perkins A, Fitzgerald JA. The behavioral component of the ram effect: the influence of ram sexual behavior on the induction of estrus in anovulatory ewes. *J Anim Sci* 1994; 72: 51–55.
40. Poindron P, Lévy F, Keller M. Maternal responsiveness and maternal selectivity in domestic sheep and goats: the two facets of maternal attachment. *Dev Psychobiol* 2007; 49: 54–70.
41. Boissy A, Dumont B. Interactions between social and feeding motivations on the grazing behaviour of herbivores: sheep more easily split into subgroups with familiar peers. *Appl Anim Behav Sci* 2002; 79: 233–245.
42. Kendrick KM, Da Costa AP, Leigh AE, Hinton MR, Peirce JW. Sheep don't forget a face. *Nature (Lond)* 2001; 414: 165–166.
43. Winfield CG, Syme GJ, Pearson AJ. Effect of familiarity with each other and breed on the spatial behaviour of sheep in an open field. *Appl Anim Ethol* 1981; 7: 67–75.
44. Morgan PD, Arnold GW, Lindsay DR. A note on the mating behaviour of ewes with various senses impaired. *J Reprod Fertil* 1972; 30: 151–152.
45. Knight TW, Lynch PR. Source of ram pheromones that stimulate ovulation in the ewe. *Anim Reprod Sci* 1980; 3: 133–136.
46. Knight TW, Tervit HR, Lynch PR. Effect of boar pheromones, ram's wool and presence of bucks on ovarian activity in anovular ewes early in the breeding season. *Anim Reprod Sci* 1983; 6: 129–134.
47. Rosa HJD, Bryant MJ. The 'ram effect' as a way of modifying the reproductive activity in the ewe. *Small Rumin Res* 2002; 45: 1–16.
48. Gelez H, Fabre-Nys C. The "male effect" in sheep and goats: a review of the respective roles of the two olfactory systems. *Horm Behav* 2004; 46: 257–271.
49. Delgadillo JA, Flores JA, Veliz FG, Duarte G, Vielma J, Hernández H, Fernández IG. Importance of the signals provided by the buck for the success of the male effect in goats. *Reprod Nutr Dev* 2006; 46: 391–400.
50. Hawken PAR, Esmaili T, Scanlan V, Blache D, Martin GB. Can audio-visual or visual stimuli from a prospective mate stimulate a reproductive neuroendocrine response in sheep? *Animal* 2009; 3: 690–696.
51. Hurst JL. Female recognition and assessment of males through scent. *Behav Brain Res* 2009; 200: 295–303.
52. Sanchez-Andrade G, Kendrick KM. The main olfactory system and social learning in mammals. *Behav Brain Res* 2009; 200: 323–335.
53. Uriarte N, Agrati D, Banchero G, González A, Zuluaga MJ, Cawen E, Olivera V, Alsina M, Poindron P, Ferreira A. Different chemical fractions of fetal fluids account for their attractiveness at parturition and their repulsiveness during late-gestation in the ewe. *Physiol Behav* 2012; 107: 45–49.
54. Murata K, Tamogami S, Ito M, Ohkubo Y, Wakabayashi Y, Watanabe H, Okamura H, Takeuchi Y, Mori Y. Identification of an olfactory signal molecule that activates the central regulator of reproduction in goats. *Curr Biol* 2014; 24: 681–686.

55. Iwata K, Kikusui T, Takeuchi T, Mori Y. Substances derived from 4-ethyl octanoic acid account for primer pheromone activity for the “male effect” in goats. *J Vet Med Sci* 2003; 65: 1019–1021.
56. Kendrick KM. How the sheep’s brain controls the visual recognition of animals and humans. *J Anim Sci* 1991; 69: 5008–5016.
57. Kendrick KM, Atkins K, Hinton MR, Broad KD, Fabre-Nys C, Keverne B. Facial and vocal discrimination in sheep. *Anim Behav* 1995; 49: 1665–1676.
58. Peirce JW, Leigh AE, Kendrick KM. Configurational coding familiarity and the right hemisphere advantage for face recognition in sheep. *Neuropsychologia* 2000; 38: 475–483.
59. Peirce JW, Leigh AE, Da Costa APC, Kendrick KM. Human face recognition in sheep: lack of configurational coding and right hemisphere advantage. *Behav Process* 2001; 55: 13–26.
60. Poindron P, Carrick MJ. Hearing recognition of the lamb by its mother. *Anim Behav* 1976; 24: 600–602.
61. Mak GK, Enwere EK, Gregg C, Pakarainen T, Poutanen M, Huhtaniemi I, Weiss S. Male pheromone–stimulated neurogenesis in the adult female brain: possible role in mating behavior. *Nature Neurosci* 2007; 10: 1003–1011.
62. Brus M, Meurisse M, Franceschini I, Keller M, Lévy F. Evidence for cell proliferation in the sheep brain and its down-regulation by parturition and interactions with the young. *Horm Behav* 2010; 58: 737–746.
63. Cohen-Tannoudji J, Lavenet C, Locatelli A, Tillet Y, Signoret JP. Non-involvement of the accessory olfactory system in the LH response of anoestrous ewes to male odour. *J Reprod Fertil* 1989; 86: 135–144.
64. Gelez H, Archer E, Chesneau D, Magallon T, Fabre-Nys C. Inactivation of the olfactory amygdala prevents endocrine response to male odour in anestrus ewes. *Eur J Neurosci* 2004; 19: 1581–1590.
65. Gelez H, Fabre-Nys C. Neural pathways involved in the endocrine response of anestrus ewes to the male or its odor. *Neuroscience* 2006; 140: 791–800.
66. Da Costa AP, Broad KD, Kendrick KM. Olfactory memory and maternal behaviour-induced changes in c-fos and zif/268 mRNA expression in the sheep brain. *Molec Brain Res* 1997; 46: 63–76.
67. Demas GE, Williams JM, Nelson RJ. Amygdala but not hippocampal lesions impair olfactory memory for mate in prairie voles (*Microtus ochrogaster*). *Am J Physiol (Regul Integr Comp Physiol)* 1997; 273 R1683–R1689.
68. Kippin TE, Cain SW, Pfaus JG. Estrous odors and sexually conditioned neutral odors activate separate neural pathways in the male rat. *Neuroscience* 2003; 117: 971–979.
69. Litaudon P, Mouly AM, Sullivan R, Gervais R, Cattarelli M. Learning-induced changes in rat piriform cortex activity mapped using multisite recording with voltage sensitive dye. *Eur J Neurosci* 1997; 9: 1593–1602.
70. Petrulis A, Eichenbaum H. The perirhinal-entorhinal cortex but not the hippocampus is critical for expression of individual recognition in the context of the Coolidge effect. *Neuroscience* 2003; 122: 599–607.
71. Keller M, Meurisse M, Lévy F. Mapping of brain networks involved in consolidation of lamb recognition memory. *Neuroscience* 2005; 133: 359–369.
72. Fulkerson WJ, Adams NR, Gherardi PB. Ability of castrate male sheep treated with oestrogen or testosterone to induce and detect oestrus in ewes. *Appl Anim Ethol* 1981; 7: 57–66.
73. Croker KB, Butler LG, Johns MA, McColm SC. Induction of ovulation and cyclic activity in anoestrous ewes with testosterone treated wethers and ewes. *Theriogenology* 1982; 17: 349–354.
74. Signoret JP, Fulkerson WJ, Lindsay DR. Effectiveness of testosterone-treated wethers and ewes as teasers. *Appl Anim Ethol* 1982; 9: 37–45.
75. Mellado M, Hernández JR. Ability of androgenized goat wethers and does to induce estrus in goats under extensive conditions during anestrus and breeding seasons. *Small Rumin Res* 1996; 23: 37–42.
76. Fabre-Nys C. Hormonal control of male and female sexual behavior in ewes: role of interactions between progesterone and estradiol. *C R Acad Sci (Paris)* 1983; 296: 961–964 (article in French with an abstract in English).
77. Sharma TP, Blache D, Blackberry MA, Martin GB. Role of peripheral and central aromatization in the control of gonadotrophin secretion in the male sheep. *Reprod Fertil Dev* 1999; 11: 293–302.
78. Sharma TP, Blache D, Roselli CE, Martin GB. Distribution of aromatase activity in brain and peripheral tissues of male sheep: effect of nutrition. *Reprod Fertil Dev* 2004; 16: 709–715.
79. Vielma J, Chemineau P, Poindron P, Malpoux B, Delgadillo JA. Male sexual behavior contributes to the maintenance of high LH pulsatility in anestrus female goats. *Horm Behav* 2009; 56: 444–449.
80. Martin GB, Oldham CM, Cognié Y, Pearce DT. The physiological responses of anovulatory ewes to the introduction of rams – a review. *Livest Prod Sci* 1986; 15: 219–247.
81. Sanford LM, Palmer WM, Howland BE. Influence of sexual activity on serum levels of LH and testosterone in the ram. *Can J Anim Sci* 1974; 54: 579–585.
82. Schanbacher BD, Lunstra DD. Seasonal changes in sexual activity and serum levels of LH and testosterone in Finnish Landrace and Suffolk rams. *J Anim Sci* 1976; 43: 644–650.
83. Lincoln GA, Davidson W. The relationship between sexual and aggressive behaviour and pituitary and testicular activity during the seasonal sexual cycle of rams and the influence of photoperiod. 1977; 49: 267–276.
84. Pelletier J, Garnier DH, de Reviers MM, Terqui M, Ortavant R. Seasonal variation in LH and testosterone release in rams of two breeds. *J Reprod Fertil* 1982; 64: 341–346.

85. Hötzel MJ, Walkden-Brown SW, Fisher JS, Martin GB. Determinants of the annual pattern of reproduction in mature male Merino and Suffolk sheep: responses to a nutritional stimulus in the breeding and non-breeding seasons. *Reprod Fertil Dev* 2003; 15: 1–9.
86. Meyer HH. Ewe and teaser breed effects on reproductive behaviour and performance. *Proc New Zeal Soc Anim Prod* 1979; 39: 68–76.
87. Tervit HR, Havik PG, Smith JF. Effect of breed of ram on the onset of the breeding season in Romney ewes. *Proc New Zeal Soc Anim Prod* 1977; 37: 142–148.
88. Knight TW, Lynch PR. The pheromone from rams that stimulates ovulation in the ewe. *Proc Aust Soc Anim Prod* 1980; 13: 74–76.
89. Nugent RA 3rd, Notter DR, McClure WH. Effects of ram preexposure and ram breed on fertility of ewes in summer breeding. *J Anim Sci* 1988; 66: 1622–1626.
90. Scott IC, Johnstone PD. Variation between years in the ram effect when Coopworth or Poll Dorset rams are introduced to seasonally anovular Coopworth ewes. *New Zeal J agric Res* 1994; 37: 187–193.
91. Delgadillo JA, Flores JA, Véliz FG, Hernández HF, Duarte G, Vielma J, Poindron P, Chemineau P, Malpaux B. Induction of sexual activity of lactating anovulatory female goats using male goats treated only with artificial long days. *J Anim Sci* 2002; 80: 2780–2786.
92. Rosa HJD, Juniper DT, Bryant MJ. Effects of recent sexual experience and melatonin treatment of rams on plasma testosterone concentration sexual behaviour and ability to induce ovulation in seasonally anoestrous ewes. *J Reprod Fertil* 2000; 120: 169–176.
93. Flores JA, Véliz FG, Pérez-Villanueva JA, Martínez de la Escalera G, Chemineau P, Poindron P, Malpaux B, Delgadillo JA. Male reproductive condition is the limiting factor of efficiency in the male effect during seasonal anestrus in female goats. *Biol Reprod* 2000; 62: 1409–1414.
94. Rosa HJD, Juniper DT, Bryant MJ. The effect of exposure to oestrous ewes on rams' sexual behaviour plasma testosterone concentration and ability to stimulate ovulation in seasonally anoestrous ewes. *Appl Anim Behav Sci* 2000; 67: 293–305.
95. Hillbrick GC, Tucker DJ. Effect of nutrition on lipid production and composition of Cashmere buck fleece. *Small Rumin Res* 1996; 22: 225–230.
96. Blache D, Zhang S, Martin GB. Fertility in male sheep: modulators of the acute effects of nutrition on the reproductive axis of male sheep. *Reproduction Suppl* 2003; 61: 387–402.
97. Estep DQ, Price EO, Wallach SJR, Dally MR. Social preferences of domestic ewes for rams (*Ovis aries*). *Appl Anim Behav Sci* 1989; 24: 287–300.
98. Chanvallon A, Sagot L, Pottier E, Debus N, François D, Fassier T, Scaramuzzi RJ, Fabre-Nys C. New insights into the influence of breed and time of the year on the response of ewes to the 'ram effect'. *Animal* 2011; 5: 1594–1604.
99. Bedos M, Flores JA, Fitz-Rodríguez G, Keller M, Malpaux B, Poindron P, Delgadillo JA. Four hours of daily contact with sexually active males is sufficient to induce fertile ovulation in anoestrous goats. *Horm Behav* 2010; 58: 473–477.