REPRODUCTIVE BIOLOGY

External and internal modulators of sheep reproduction

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SUMMARY

Several factors such as season, genetics, social interaction and metabolic status control or modulate the reproductive capacity of sheep. In addition to these well-studied factors in sheep, the influence of emotional reactivity on the reproductive success of sheep has started to be investigated over the last two decades. In this paper, after briefly reviewing the impact of classical factors affecting reproduction in sheep, we define emotional reactivity and the expression of its inter-individual variability, named temperament. Then, following a description of the protocol to measure temperament in sheep and discussion on the heritability of temperament traits, we illustrate how this selection affects the reproductive biology of sheep. We will be mainly using results obtained from a unique flock of sheep selected for low or high emotional reactivity. In conclusion, we propose that energy partitioning could be one of the mechanisms by which selection for temperament

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INTRODUCTION

Several external and internal factors can affect the reproductive capacity of both males and females (fig. 1). External factors affecting reproduction such as photoperiod, nutrition, and social sexual cues have been extensively studied in sheep because of the economic importance of sheep and also because of the value of sheep as an experimental animal [6]. Many of the responses of the reproductive system to inputs such as nutritional and socio-sexual signals depend on the degree to which the genotype under consideration responds to photoperiod [10]. In breeds of sheep from tem-

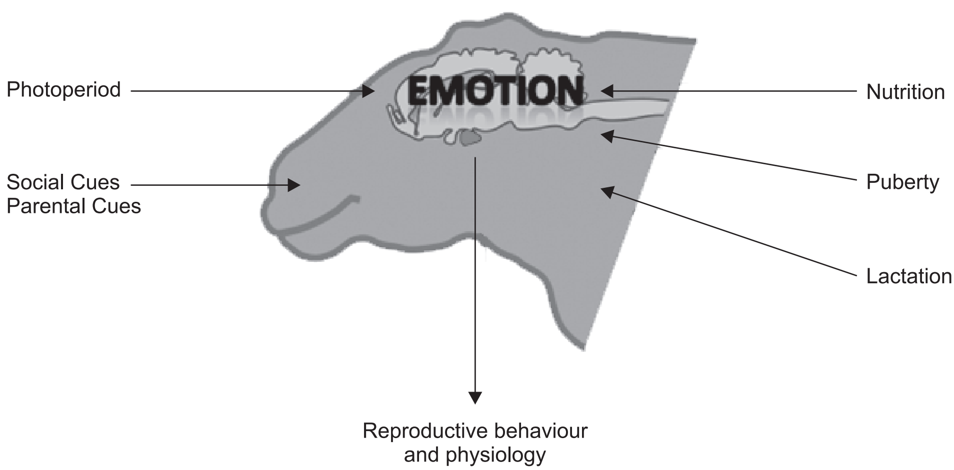

Figure 1. Factors affecting the reproductive biology of sheep. Environmental factors (or external factors) can either trigger or modulate both the behavioural and the physiological events of the reproductive cycle in both males and females. The brain is responsible for the integration between those factors. Emotion, and emotional reactivity, can also modulate the effect of the environmental factors of the reproductive biology.

perate regions, photoperiod is a very strong driver of reproductive activity in both males and females, while photoperiod does not affect the reproductive activity of breeds from more equatorial or semi-arid regions [40]. In breeds of sheep that evolved in semi-arid climates, such as the Merino, nutrition has a significant effect on the reproductive capacity of both sexes (for review see [15, 42, 7, 10]). In Merino sheep, increasing the plane of nutrition increases fertility by increasing sperm production in the male and ovulation rate in the female (for review see [31]). However, nutrition can modulate almost any stage of the reproductive cycle; affecting embryo survival, changing the genetic make-up of the offspring through fetal programming and enhancing offspring survival by stimulating colostrum and milk production (for review see [31]). In sheep, sexual-social signals play an important role in the regulation of the activity of the hypothalamo-pituitary-gonadal axis in both males and females. Following segregation of the sexes, re-establishing contact between the male and female sheep triggers ovarian cyclicity in females and re-activation of libido in males (for review see [18]). Interestingly, this phenomenon was originally described and thought to be efficient only during the non-breeding season, but lately the effect of male presence on female cyclicity has been observed even during the breeding season in sheep [28].

Reproductive processes are either triggered or modulated by the interaction of external factors with internal factors. Using a model, based on our work, on the relationship between nutrition and reproduction, we have categorized the relationship between internal and external factors into four interdependent 'dimensions': genetic, structural, communicational and temporal [11]. The genetic dimension accounts for the differences between species, breeds and individuals. The structural dimension encompasses all organs involved in the regulation of the reproductive axis, amongst them the brain having a special integrative role. The communication dimension includes physiological systems that can generate either endocrine, neural or nutrient-based signals or/and sense those same signals. The temporal dimension illustrates the time dependent regulation of the reproductive axis, such as changes in response to particular nutrients or responses of regulatory hormones associated with previous changes in body condition. Of the four dimensions, domestication and classical or modern genetic selection have dramatically modified the genetic dimension. Domestication has primarily increased the capacity of animals to “adapt to man and the environment he provides” [38], while genetic selection has enhanced production traits such as wool or milk quality and quantity [47]. In this paper, we argue that domestication can be “pushed further” using genetic selection for temperament traits. We give examples showing that temperament can affect reproductive capacity by modifying the physiology and biology of the animal. We base our arguments mainly on data obtained using a unique resource of sheep selected for differences in temperament, and where possible, other ruminants.

EMOTION AND TEMPERAMENT IN ANIMALS

Darwin recognized the existence of emotion in animals and suggested that animals can experience both negative (fear, frustration) and positive (pleasure) emotions [17]. Amusingly, Darwin commented that ruminants “displayed in so slight a degree their emotions or sensations” (Darwin, 1872; page 131). Before Darwin and ever since, the existence of emotion in animals has been widely debated. Recently, using the definition of emotion from the appraisal theories developed in psychology, Dr Boissy’s group has been able to demonstrate that animals can experience emotions in similar ways to humans [13]. The French group measured the behavioural and physiological response of sheep and suggested that sheep can sequentially evaluate a triggering situation based on elementary characteristics (suddenness, unfamiliarity and pleasantness of the situation and an individual’s ability to predict and to control it). The use of the same elementary characteristics as humans to evaluate their environment, strongly suggests that ruminants have emotional experiences [12] as suggested by Darwin in other species [17]. For example, exposing sheep to an unfamiliar and unpleasant situation elicits either fear, if the individual cannot control it, or anger if the situation is under its control [23].

Emotional reactivity can vary greatly between animals and this variability has been named temperament. Temperament has been defined for humans as ‘nature controlling the way he behaves, feels and thinks’ [14].

Many attempts have been made to apply similar criteria to animals based on our perceptions of their 'temperament'. It has been strongly suggested that animals have personality, temperament or individual behaviours, amongst which fearfulness plays an important role in an individual's behavioural response to threatening situations [13]. In fact, the level of emotional reactivity of an individual has a significant impact on its relationship with its environment. In addition, emotions can bias cognitive functions such as judgement and decision-making, which explains how temperament traits can be strongly expressed. It is only very recently that the nature of emotions in animals and the way to assess temperament have been investigated [13]. Investigations have been conducted to select for temperament with the objective to either decrease risks associated with handling in cattle or improving the survival of the newborn lambs [21]. However, the effect of temperament on the behaviour or physiology of animals can only be studied if a reliable and discriminative measure of temperament is available.

HOW TO MEASURE AND SELECT FOR TEMPERAMENT

Several methods have been designed to measure either fear reactions or temperament in domesticated species such as sheep, cattle, chickens, dogs and horses (see reviews [30]). For cattle, such assessments have included subjective measures, such as observations of crush behaviour, or objective measures of escape and/or avoidance behaviours, such as flight-time [44, 20]. Flight-time, measured as the speed of escape from a crushing crate or a scale, assesses the reaction to both isolation and human contact. Flight-time has been extensively used as a measure of temperament in animals because it is a very rapid test and it has been linked to production traits, such as meat quality [29]. In contrast, most validated tests developed for sheep, that have some degree of reliability, are time consuming (from 1 to 15 min) and use complex test designs [21].

For the past 18 years a flock of Merino sheep (UWA sheep) have been selected for either a high or low reactivity to humans and social isolation [33, 32]. The selection process involves measuring the behavioural reactivity of lambs after weaning (at approximately 16 weeks of age) using two behavioural tests described by Murphy [32] and Blache & Ferguson [8]. These two tests can select animals that are less socially motivated, less fearful of humans and less reactive to isolation, characteristics that are beneficial for the establishment of the ewe-lamb bond and lamb survival.

Arena test

The first behavioural test is an arena test, which exposes sheep to an approach-avoidance situation. In this test a single sheep is introduced into a rectangular arena (fig. 2a). At one end of the arena, a small group of sheep (approach stimulus) is kept in a pen behind a barrier, with a human (avoidance stimulus) standing in front of it. Because the test sheep cannot simultaneously be far

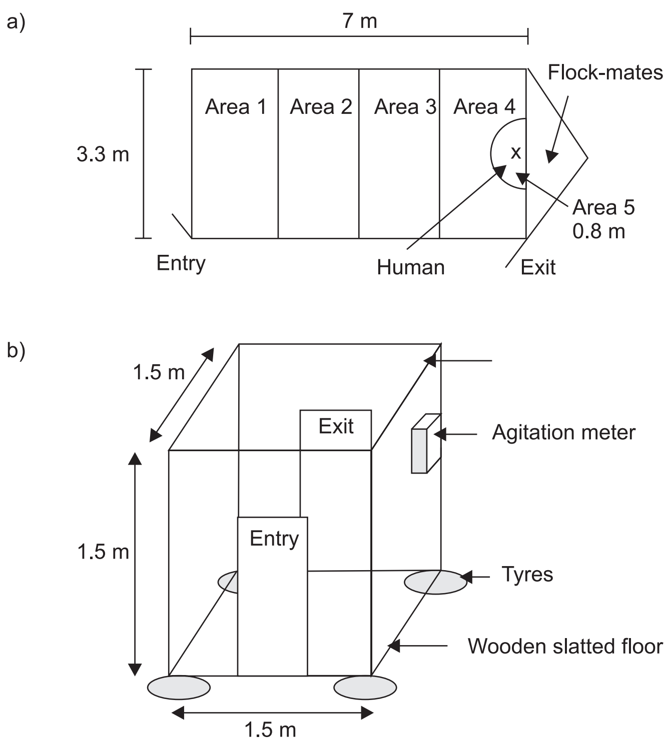

Figure 2. Schematic diagram of a) the arena test and b) the isolation box used to test the temperament of sheep. See text for test procedures.

away from the human and close to its companions it has to find a distance between the human and its companions that reflects a trade-off between their conflicting motivations. The number of zones crossed during the arena test (TOTAL CROSS) is taken as an indicator of the locomotor activity of the individual. High or low locomotor and vocal activity in the arena test is taken to reflect a high or low degree of fear, respectively. It has been argued that immobilization may reflect quietness of the animal and an absence of fear in one situation but it can also reflect a high degree of disturbance and nervousness in another situation [43]. In the presence of a human, like in the arena test, inhibition of locomotor and vocal behaviour can be interpreted as a reaction to towards a predator [39] and thus represent a higher degree of fear. However, in the arena test a high locomotor activity could also be a search to rejoin flock mates and thus the behaviour represents strong social motivation [39]. Due to the ambiguous interpretation of the behaviour in the arena test, the UWA sheep are also tested in a second test, the isolation box test.

Isolation box test

The second behavioural test is an isolation test in an enclosed box. A single sheep is isolated in a 3.4 m^3 enclosed box. An agitation score (BOX), based on the animal movements and vocalisations, is measured for each individual. The agitation reflects the animal's inherent fear of isolation but also its capacity to adapt to the isolation challenge [8]. The differences in the behavioural responses of sheep to social isolation are thought to reflect variation in the propensity of individual sheep to react fearfully [39, 43].

Selection score

All animals are assigned a selection score based on their behaviour in the two tests according to the following equation where i = individual score, x = flock mean, sd = standard deviation of mean [33, 32]:

$$\text{Selection score} = 100 + \frac{[\text{BOX}i - \text{BOX}x]}{\text{BOX}sd} + \frac{[\text{TOTAL CROSS}i - \text{TOTAL CROSS}x]}{\text{TOTAL CROSS}sd}$$

Higher levels of locomotor and vocal activity have been reported to indicate higher levels of fear in sheep when exposed to various fear eliciting situations [39, 43]. Accordingly, the UWA Merino sheep expressing high or low levels of locomotion or vocalisation in the two selection tests have been classified as having a high or low, respectively, reactivity and are labelled, respectively, ‘nervous’ or ‘calm’. Nervous animals generate high selection scores while calm animals generate low scores. The benefit of selecting the sheep on these components of temperament is that it is also moderately heritable. Heritability estimates range from low for the trait CROSS (0.11; [33, 32]) to moderate for BOX (0.41; [8]).

CHARACTERISTICS OF THE TWO LINES SELECTED FOR DIFFERENCE IN TEMPERAMENT

In sheep, such as in cattle and quail, temperament has a strong genetic basis. The temperament of young lambs, which were the cross-fostered progeny of calm and nervous ewes, was assessed by measuring locomotor activity on two occasions, 1) one week after birth during an open-field test and 2) at weaning (16 weeks of age) during an arena test and agitation score measured during an isolation box test [3]. At both assessments of temperament, there was a genotype effect but no maternal or fostering effect on the lamb’s behaviour, suggesting that temperament in Merino sheep is mainly determined by genetic transmission of the trait rather than from behaviour learned from the mother [3].

ANIMAL TEMPERAMENT AND REPRODUCTIVE BIOLOGY

The reproductive endocrine axis can be profoundly influenced by stress [45] and the selection for different temperament affects the stress response of sheep. We have collected data showing that nervous ewes have a greater stress response than calm ewes, as measured by their agitation and plasma cortisol levels, when exposed to a novel stressor or when they anticipated a stressful event (tab. 1). These results suggest that the nervous ewes not only have a difference in stress responses but have also a different level of "anxiety". Furthermore, selection for temperament could be compared to domestication which also decreases an animal's fear of humans and affects reproduction [38]. In sheep, so far, we have shown that the selection for temperament affects the entire reproductive cycle from mating behavior to the survival of newborn lambs.

The sexual behaviour of calm and nervous sires is similar, a result not surprising considering the intensity of the sex-drive in rams [18]. However, the ovulatory response of anoestrous ewes to the introduction of a male, a phenomenon known as the 'ram effect' [18], is affected by temperament. A higher proportion of nervous ewes were cycling after ram introduction than calm ewes, but only in the ewes that were sexually inexperienced [16]. The lack of effect of temperament on the ram effect in the sexually experienced ewes suggests that this effect of temperament could be neutralized by sexual experience [16]. In addition, in the nervous inexperienced ewes that responded to the introduction to the male: 1/ a lower proportion had delayed cycles than calm inexperienced ewes, and 2/ more exhibited more cycles (short and normal cycles) than the calm inexperienced ewes [16]. The causes and mechanisms of the delayed onset and the maintenance of ovarian activity still need to be investigated. As well as influencing the ewe's response to the male effect,

Table 1. Mean plasma concentrations of cortisol (mean±SEM) in calm (n=8) and nervous (n=8) sheep during social isolation with or without an additional stressor

	Calm sheep	**Nervous sheep**
	ng/ml	
Isolation only (Control – pooled data)	20.1 ± 4.5^{a}	32.3 ± 4.4^{b}
Isolation + novel object (Day 1)	19.5 ± 3.4^{a}	23.4 ± 3.6^{a}
Isolation + novel object (Day 2)	17.4 ± 3.7^{a}	30.7 ± 4.7^{b}
Isolation only (Day 3)	13.8 ± 2.8^{a}	27.2 ± 4.9^{b}

Ewes (n=8) of each temperament were either exposed to either 10 minutes of social isolation each day over 3 days (Control) or to social isolation and an additional stressor (novel object) on Days 1 and 2 and to social isolation only on Day 3 (Treatment group, 10 min each day). Social isolation was achieved by keeping the ewe for 10 minutes in the enclosed box used for the isolation box test (see main test). The novel object was 100 cm height and an electric fan at the base blew air into a white soft plastic tube (20 cm in diameter, 50 cm in length) so that the tube moved at random and made moderate noise. Different superscripts indicate difference in the same row $p<0.05$. Data comparisons were made using a repeated measures analysis of variance.

temperament also modified the expression of reproductive behaviour in sexually inexperienced females, leading to an increase in proceptivity (search for the male) but not receptivity (acceptance of mating) in the calm temperament ewes [22]. Overall, the selection of sheep for temperament affects the expression of reproductive behaviour and physiology but it is still difficult to predict which temperament, calm or nervous, will affect a given items of behaviour or physiology in a given situation, as further illustrated in the next set of results.

In sheep, the number of ovulations can be affected by the level of nutrition [41] and the genotype of the sheep, such as sheep that carry the FecB (Booroola) gene [5]. When fed with the same diet, ewes of a calm temperament have a greater ovulation rate than nervous ewes [25]. At the beginning of the breeding season, the same proportion of calm and nervous ewes were spontaneously cyclic (about 30%), a proportion consistent for the Merino genotype in this environment [37]. However, calm ewes had a higher spontaneous ovulation rate compared to nervous ewes (1.63 vs. 1.26; $p=0.003$) and a higher ovulation rate than nervous ewes (1.83 vs. 1.57; $p=0.03$) in response to synchronization with intravaginal progestagen and an injection of eCG. More multiple gestations were observed in calm ewes than nervous ewes on Day 74 (Calm 1.60 vs. Nervous 1.35; $p=0.03$) but the pregnancy rate was similar in both lines [25]. Ewes of calm temperament also carried more twin embryos than nervous ewes (1.39 embryos, $n=472$: 1.29 embryos, $n=302$; $p<0.001$), and this has been observed over multiple years while the two temperament lines were managed as one flock except at mating and lambing. A possible mechanisms behind the differences in ovulation rate and twin bearing percentage could be that energy partitioning could be affected by temperament, however this hypothesis needs to be investigated further [9].

Early studies conducted at The University of Western Australia between 1990 and 1995 found that lambs born from calm mothers had a higher chance of survival between birth and weaning [33, 32]. It was suggested that the calm ewes were better mothers than nervous ewes and therefore lamb mortality was reduced. In fact, early studies showed that calm ewes spent more time with their lamb(s) and, when disturbed, had a shorter flight distance and returned to their lamb(s) sooner [33, 32]. In the early studies by Murphy et al. [33, 32], the incidence of lamb mortality, from birth to weaning, was about half for calm ewes compared to nervous ewes, independent of the physical status of the lambs. More recently, we have conducted a number of trials to closely examine the behavioural and physiological basis of the differences between the two selected lines. We did not record a difference in the maternal behaviour of the calm and nervous ewes under indoor conditions and using a smaller number of animals than used by Murphy [4, 32]. These results were supported by the fact that hormonal profiles of steroids known to influence maternal behaviour [19] were only slightly different between the two genotypes [4]. We concluded that the indoor conditions were not challenging enough for the differences in maternal behaviour to be expressed (for details see [4]). In outdoor conditions and with minimal human disturbance, temperament does influence the early post partum behaviour of ewes and lambs [4]. Calm ewes licked their lambs more and tended to stay longer on the birth site than the nervous ewes (tab. 2), as previously reported by [32]. In line with the high level of activity of the nervous lambs during behavioural tests at one week (see above, [2]), nervous lambs stood up earlier and were quicker to start performing exploratory behaviour than calm lambs (tab. 2). As a result, nervous lambs started suckling earlier than calm lambs (tab. 2). However, under outdoors conditions and with minimal human disturbance, temperament did not influence the incidence of lamb mortality [4]. Lamb mortality rates from birth to weaning were similar between the calm (15%) and nervous (18%) temperament lines when

Table 2. Behavioural and physiological differences between maternal ewes and lambs of calm or nervous temperament

Parameter	**Units**	**Calm line**	**Nervous line**
Immunoglobulin (IgG)[1]	(mg/ml)	35.7± 2.5	30.3±1.5
Latency to stand[2]	(min)	56.0±11.0	34.0±6.0
Latency to suck[3]	(min)	23.0± 5.0	10.0±1.0
Time spend licking[4]	(min)	55.0± 5.0	42.0±4.0
Agitation at one week of age[5]	(arbitrary unit)	21.0± 3.5	50.0±5.3

[1]Concentration of IgG in the colostrum of single bearing ewes, [2]latency of the lamb to stand after birth, [3]latency of the lamb to suck after birth,[4]time spend licking the newborn lamb during the first 2 hours after parturition and [5]agitation of one week old lambs in sheep from lines selected for calm temperament and nervous temperament. Data adapted from [3, 4, 26].

ewes and lambs were left undisturbed during the critical bonding period [4], in contrast to the conditions used in the early studies by Murphy *et al.* [32, 33].

The survival of the lamb is strongly influenced by the rapid establishment of a strong mother-lamb bond soon after birth (for review see [34]). We investigated the impact of the selection for temperament on mother-young recognition using a standardized two-choice test [2]. Calm and nervous multiparous ewes and their lambs showed a similar preference for their familiar kin at 6- and18-h after birth. Our results suggest that temperament does not affect the early process of ewe-lamb bonding. However, during the same experiment, we found that nervous lambs showed higher vocal and locomotor activity than calm lambs and that differences in temperament, measured as their agitation during behavioural tests, were already detectable at 1 week of age (tab. 2; [2]). This high level of motor activity of the nervous lambs could explain, in part, the high incidence of mortality in nervous lambs [32, 33] because it might increase the chance of mother-young separation in a field situation or increase the metabolic cost of survival.

Lamb mortality during the first 3 days after birth, which represents 80% of lamb deaths before weaning, is also caused by starvation due to inadequate energy intake [1, 46]. A lamb's first meal after birth is of great importance for the lamb's survival because it facilitates the establishment of the ewe-lamb bond and provides energy and immune protection to the lamb [35]. The duration that ewes suckled their lambs in outdoor conditions did not differ between temperament lines [4] and the quantity of colostrum produced over the first 12 hours after parturition was similar between the two lines [26]. However, while the time that lambs spent sucking might have been similar, the quantity of nutrients ingested may have been different because the colostrum of calm ewes may have been easier to suck as it is less viscous than that produced by the nervous ewes [26]. Over the first 10 hours after parturition, the colostrum produced by calm ewes contained slightly more lactose and fat than that produced by nervous ewes (tab. 2; [26]). More importantly, the concentration of immunoglobulin-G (IgG), the most abundant Ig in ewe colostrum, was greater in the colostrum of calm ewes than in the colostrum of nervous ewes (tab. 2; [27]). At birth, the concentration of serum immunoglobulins (Ig) in the plasma of lambs is close to nil [36], thus lambs must absorb Ig from their mother's colostrum to acquire immunity and increase their chance of survival. Therefore, lambs born from calm ewes have access to a larger quantity of IgGs and passively acquire immune competency better than lambs born from nervous ewes.

CONCLUSIONS

The reproductive activity of sheep is controlled by a large number of external and internal factors. The mechanism involved in the action of external factors on the reproductive capacity of male and female sheep have been extensively studied. In the last 15 years, emotional reactivity has been unveiled as a new modulator of the reproductive capacity of sheep. Practically, we have demonstrated some effects of selection for temperament on the reproductive biology of Merino sheep (fig. 3). It is possible that dif-

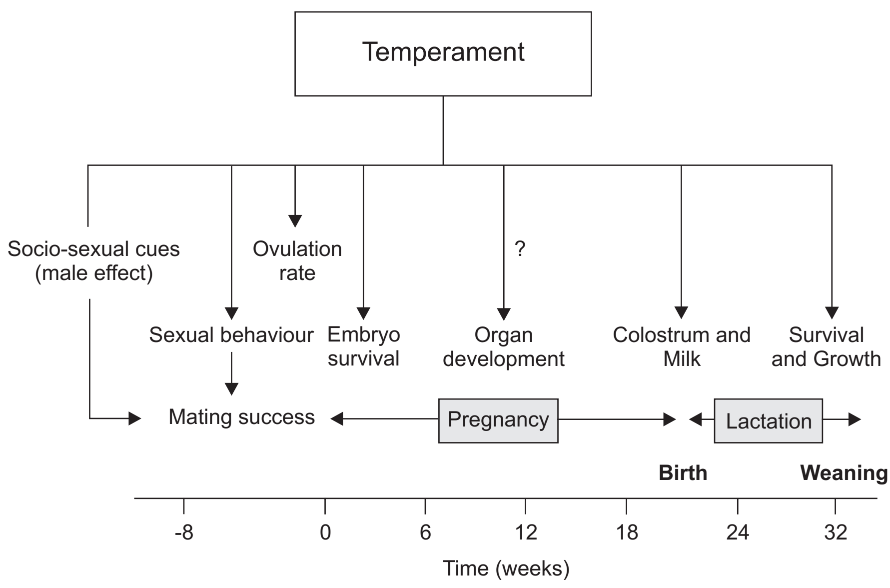

Figure 3. Diagram summarizing the effects of selection for calm or nervous temperament on the reproductive biology of Merino sheep. Question mark indicates an event of the reproductive cycle that is a potential target for temperament but has not been investigated yet. Time 0 corresponds to fertilisation.

ferences in energy requirement, nervous animals spending more energy to express their behaviours [24], and therefore energy partitioning could be responsible for specific effects such as the increase in ovulation rate [9]. The effect of temperament on the reproductive behaviour of both sexes and that of the offspring requires further investigation especially regarding the importance of the presence of stressors (fearful events such as human presence during the development of the ewe-lamb bond or nutritional stress). Moreover, other possible effects of temperament on, for example, embryo survival and organ development should be investigated because of the importance of stress on those intrauterine events.

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