Can accelerometry and proximity loggers determine the onset of puberty in sheep?

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Abstract

The global demand for sheep products has increased over the past decade, which presents both opportunities and challenges in sheep production. One strategy to enhance productivity is to mate ewes as lambs, reducing the generational interval and increasing the number of lambs weaned per year. However, this approach requires the accurate detection of puberty attainment, a crucial period marked by physiological and behavioural changes. Puberty can be defined as the first oestrus followed by elevated blood progesterone concentrations above 1.0 ng/mL. Traditional methods for detecting the first ovulatory oestrus of ewe lambs include teaser rams, hormonal analysis and ultrasound examinations. This thesis explored the potential of collar-mounted accelerometers as a novel, non-invasive method for detecting behavioural and activity changes associated with puberty in ewe lambs. Eight prepubertal ewe lambs (seven Dorset Down x Romney crosses and one Wiltshire x Romney cross) sourced as orphans or mismothered lambs from Massey University's sheep farms were used for this study. The ewe lambs were fitted with ActiGraph® accelerometers and continuous (30 Hz) acceleration and proximity data were collected throughout the trial. Two teaser rams were alternated every 7 – 10 days within the flock. Live weight was recorded and blood samples were collected twice weekly. Ultrasound examinations were conducted weekly to measure follicular growth and the presence of a corpus luteum. Over the 73 day study period, multiple measures of puberty attainment were assessed, including serum progesterone and oestradiol concentrations, tupping data, proximity data, ultrasound examinations and activity data. Ewe lambs were removed from the study after receiving a Grade 3 tup mark or when the presence of a corpus luteum was confirmed. While hormonal analysis and ultrasound examinations were reliable methods for the accurate detection of puberty attainment in the ewe lambs, their high costs and the need for experienced professionals to perform them make them impractical for routine farm management. Tupping was not a reliable method for determining puberty attainment, as the ewe lambs were marked weeks before their first ovulation. The early tupping was likely associated with anovulatory prepubertal follicular growth. The results from this study revealed that as lambs approach puberty, there was an increase in the time spent in close proximity to the ram. This was likely related to oestrus and mating behaviours by the increase in oestradiol concentrations. There was also a consistent decline in the daily activity of the ewe lambs following their first ovulation, which was correlated with the development of their first CL and elevated progesterone concentration. Thus, these accelerometer devices show potential for the detection of a ewe lamb's first ovulatory oestrus and subsequent luteal phase. However, further research is required to validate their reliability and accuracy for detecting the onset of puberty in ewe lambs.

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ADG	Average daily gain
ANOVA	Analysis of variance
CL	Corpus luteum
CP	Crude protein
DBA	Dynamic body acceleration
E_2	Oestradiol
eNDF	Effective neutral detergent fibre
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
GPS	Global positioning system
HPG	Hypothalamic-pituitary-gonadal
HSD	Honest significant difference
LH	Luteinizng hormone
LW	Live weight
ME	Metabolisable energy
MP	Metabolisable protein
NDF	Neutral detergent fibre
ODBA	Overall dynamic body acceleration
P_4	Progesterone
pODBA	Proportional overall dynamic body acceleration

Chapter 1. Introduction

The global demand for sheep products has experienced a significant increase over the last ten years (Rosales Nieto et al., 2018). This growth, while beneficial, also poses challenges such as increased ethical concerns and environmental impacts associated with producing more livestock (Rosales Nieto et al., 2018). One method to mitigate these issues in sheep production is mating ewes as lambs, at seven to nine months, rather than the traditional 18 months (Kenyon et al., 2014). This strategy could potentially shorten the generational interval and increase the number of lambs weaned per year (Kenyon et al., 2014). By reducing the generational interval and increasing productivity, this method can also maximise both the environmental and economic benefits (Edwards et al., 2015). However, breeding ewes younger than 18 months of age requires the ability to accurately determine when they reach puberty.

Puberty plays a crucial role in managing breeding cycles in livestock, influencing overall productivity and efficiency on farms (Edwards & Juengel, 2017). This period, marked by the development of sexual characteristics, maturation of reproductive organs and changes in behaviour, signifies when ewes become capable of producing offspring (Rosales Nieto et al., 2018). The timing of puberty is a complex process influenced by various factors including hormonal changes, nutrition, body weight, photoperiod and social interactions (Valasi et al., 2012). One of the main challenges in managing breeding cycles is the ability to accurately determine when a lamb reaches puberty (Edwards & Juengel, 2017; Valasi et al., 2012).

Visible signs of puberty can be tough to detect, particularly in female lambs. For ewe lambs, the first oestrus is usually silent, meaning they do not exhibit the typical behaviours associated with oestrus such as tail fanning, increased vocalisation and seeking out the ram (Fabre-Nys & Gelez, 2007). Traditional methods for detecting first oestrus, and thus puberty, include the use of teaser rams with harnesses and crayons, or blood sampling and hormonal analysis (Kenyon et al., 2012). However, these methods may be subjective, costly or have a negative effect on animal welfare, contributing to the increasing consumer awareness of the treatment of livestock (Alhamada et al., 2017).

Alternative methods could offer a solution to these challenges by using behavioural analysis to determine when a lamb reaches puberty. For instance, the use of accelerometers has proven to be an effective and reliable way to detect oestrus in cattle, however there has been no data on puberty attainment (Galvão & Santos, 2008). These monitors can track certain behaviours from the animal for extended periods of time, allowing farmers to see changes in behavioural patterns (Hlimi et al., 2024). The use of accelerometers in sheep farming is a novel approach for the purpose of oestrus detection that has not been extensively studied. Furthermore, there is a paucity of literature on the use of this technology for puberty attainment in any production species.

This research aims to evaluate the reliability of collar-mounted accelerometers in identifying behavioural and/or activity pattern changes associated with puberty attainment in ewe lambs, potentially providing a more accurate and non-invasive method for determining this important stage. However, it is first necessary to conduct a comprehensive review of the current literature addressing the physiological changes associated with puberty and the existing methods for measuring puberty attainment, along with their limitations.

Chapter 2. Literature review

2.1 Physiology of puberty in ewes

The oestrous cycle in ewes is an essential aspect of their reproductive physiology, lasting an average of 17 days, ending with a 24 - 36 hour period of oestrus followed by spontaneous ovulation (Delano et al., 2002). This 17 day cycle is divided into four phases: proestrus, oestrus, metoestrus and dioestrus (Hasbi & Gustina, 2020). During proestrus, which lasts two to three days, ovarian follicles grow and estrogen production rises (Hasbi & Gustina, 2020). Oestrus, the period of sexual receptivity, lasts 24 - 36 hours and is when ovulation occurs (Bartlewski et al., 2011). Metoestrus follows for three to four days, marked by the development of the corpus luteum (CL) and an increase in progesterone concentrations (Hasbi & Gustina, 2020). Dioestrus is the final stage, lasting approximately 10 - 12 days, during which the CL is fully functional and progesterone peaks to prepare the uterus for a possible pregnancy (Bartlewski et al., 2011). If fertilisation is unsuccessful, the cycle will repeat within the breeding season.

A significant element affecting the lifelong production of ewes and the profitability of sheep farming is the age at which females reach puberty (Quirke, 1981). The definition of puberty differs among authors, but most consider it to be the first oestrus that is followed by elevated blood progesterone concentrations (above 1 ng/mL) for at least seven days (Fitzgerald & Butler, 1982; Lozano et al., 2020; Valasi et al., 2012). During each oestrous cycle in ewes, follicular growth occurs in a wave-like pattern (Figure 2.1; Evans et al., 2000). These follicular waves are characterised by the formation of a cluster of small antral follicles, from which one to two grow to a diameter of at least 5 mm (Menchaca & Rubianes, 2004). In mature sheep, the number of follicular waves in a single oestrous cycle can vary from two to four, with three waves being the most common (Evans et al., 2000). Over the peripubertal period, there is an increase in the frequency and amplitude of gonadotropin pulses, which supports the development and selection of dominant follicles, leading up to the first ovulation (Rawlings et al., 2003). Puberty in both ewes and rams is traditionally reached around seven to eight months of age (Delano et al., 2002). In ewe lambs, the attainment of puberty is controlled by both internal and external cues, mainly depending on endocrine changes, photoperiod, nutrition and social interaction (Foster et al., 1985).

Figure 2.1 Overview of antral follicle growth during a three and four wave oestrous cycle in ewes. Over the 17 day interovulatory interval, follicles will reach 5 mm before regressing or ovulating. OV indicates ovulation. The typical durations of the growing (G), static (S) and regressing (R) phases of the largest follicles in each wave are shown (Bartlewski et al., 2011).

2.1.1 Changes to the HPG axis over the peripubertal period

Puberty attainment is regulated by both genetics and environmental factors. The development of hypothalamic kisspeptin and gonadotropin-releasing hormone (GnRH) neurons during embryogenesis is considered one of the more important factors for puberty attainment, as puberty involves the activation of GnRH secretion (Duittoz & Kenny, 2023; Ebling, 2005). Kisspeptin is a peptide hormone crucial for initiating puberty by regulating the hypothalamic-pituitary-gonadal (HPG) axis (Smith & Clarke, 2010). It binds to the GPR54 receptor on GnRH neurons in the hypothalamus, stimulating the release of GnRH, which then prompts the anterior pituitary gland to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH), two gonadotropins (Kauffman et al., 2007). These pituitary gonadotropins cause the ovaries to produce progesterone and oestrogen, promoting the development of ovarian follicles and eventually ovulation (Brogni & De Souza, 2021). In prepubertal lambs, GnRH is active and able to produce LH secretions on an hourly basis (Foster et al., 1985). Circulating concentrations of LH, however, remain low because the hypothalamus and pituitary gland are sensitive to the inhibitory effects of oestradiol (Kinder et al., 2019). Although a low LH pulse frequency can cause a brief increase in oestradiol, it is insufficient to support the growth of preovulatory follicles, as their development in later stages is primarily driven by LH rather than FSH (Foster et al., 1985). As females reach puberty the tonic centre becomes more active and the pulse frequency of GnRH increases, which is linked to an increase in the number of kisspeptin neurons, thus enabling the positive feedback of oestrogen on GnRH through the kisspeptin pathway (Duittoz & Kenny, 2023). As LH pulse frequency increases, oestrogen production rises, stimulating uterine growth and development (Kinder et al., 2019). Elevated concentrations of oestrogen are vital in triggering the onset of sexual behaviours in ewe lambs, including increased proximity to and receptiveness towards the ram (Fabre-Nys & Gelez, 2007; Brogni & De Souza, 2021). This increase helps the ewe maintain reproductive readiness, ensuring that other hormones that control the reproductive cycle are produced regularly (Senger, 2012). Prior to puberty, the reproductive axis is still maturing, so the positive feedback pathways for oestrogen are not fully established (Evans et al., 2000). Low oestrogen receptor concentrations in the brain, due to the lack of progesterone, reduce the sensitivity to oestradiol, preventing the LH surge necessary for ovulation to occur (Xie et al., 2022). As follicles grow, they produce more oestrogen, which eventually leads to a significant surge in the release of gonadotrophins, triggering ovulation (Kinder et al., 2019).

There are two distinct increases in progesterone concentrations around the time of puberty (Kinder et al., 2019). Fitzgerald and Butler (1982) found that, following first ovulation, the circulating progesterone concentrations of lambs increased to over 1.0 ng/mL in two or more consecutive blood samples, which were collected every three to four days (Fitzgerald & Butler, 1982). This increase in progesterone was preceded by a rise in oestradiol above 7 pg/mL (Fitzgerald & Butler, 1982), which is a much lower concentration than for a mature ewe in oestrus, which can range from approximately 44.1 – 72.2 pg/mL, depending on breed and day of the oestrous cycle (Scaramuzzi & Land, 1978). There were several rises in oestradiol prior to the first ovulation, however, they were from anovulatory follicular waves. Thus, the rise in progesterone only occurred following the first ovulation. These findings suggest that progesterone plays an important role in the hormonal and ovarian development of ewe lambs. However, there are many factors that can determine when the follicular waves of prepubertal lambs can finally become ovulatory, and thus when puberty is attained.

2.1.2 Photoperiod/season

Seasonally breeding species can be termed short or long-day breeders (Malpaux et al., 1999). In sheep, reproductive activity is initiated when there is a decrease in the length of daylight, making them short-day breeders (Gündoğan et al., 2003). The seasonal pattern of reproduction helps ensure that births take place at the best time of the year, which is often spring, allowing the offspring to develop under ideal temperatures and food availability before winter (Forcada & Abecia, 2006). Normally, ewes cease to show signs of oestrus when they become pregnant or the breeding season ends due to photo refractoriness (Gündoğan et al., 2003; Robinson & Karsch, 1984). Melatonin is primarily responsible for transmitting photoperiodic information to the neuroendocrine reproductive axis, regulating the secretion of GnRH (Malpaux et al., 1999). The physiological pathways by which this occurs is beyond the scope of this thesis, but a comprehensive review has been published by Malpaux et al. (1999).

The melatonin signal associated with the breeding seasonality in ewes leads to an increase in ovarian weight and activity (i.e., the number of follicles present), as well as the presence of corpora lutea and the establishment of a complete oestrous cycle (Gündoğan et al., 2003). There are also changes in rams during the breeding season, with testicular diameter and testosterone concentrations increasing, as the total daily sperm production increases (Gündoğan et al., 2003). Lambs are normally born in late winter to spring and develop quickly (Foster et al., 1985). Due to the seasonal nature of sheep breeding, lambs will reach puberty around the same time, even though they can be born several months apart. However, since spring-born lambs are born later, they will reach puberty at a younger age compared to winter-born lambs (Duittoz & Kenny, 2023). Eventually, being born later in the year no longer benefits the lamb and puberty might even be delayed until the next breeding season, when they are over a year old (Foster et al., 1985). Foster et al. (1985) found that autumn-born lambs raised under natural conditions experienced a delay in puberty because the anoestrous season fell during the time when the lambs would normally reach puberty. They discovered this delay could be prevented by rearing the ewes in an annually reversed artificial photoperiod (Foster et al., 1985). These findings indicate that photoperiod can have a direct impact on the mechanisms involved with puberty attainment. If lambs reach the typical age or weight for puberty during the non-breeding season, puberty will be delayed until the next breeding season.

2.1.3 Nutrition

Since puberty occurs while lambs are still growing, nutrition plays a vital role in puberty attainment (Dýrmundsson, 1973). Feeding regimes and food composition can influence lamb growth during the pre-weaning phase, which can have a significant impact on farm profitability (Herath et al., 2022). It has been widely reported that optimising nutrition to improve growth rates has been linked to ewes reaching puberty at a younger age (Allen & Lamming, 1961; Foster et al., 1985; Rosales Nieto et al., 2018). If growth is inhibited in the early stages of life, the young ewe will stay prepubertal until they attain the necessary percentage of their mature body adiposity (Rosales Nieto et al., 2013). Nutrition is one of the main variables influencing ovulation rate (i.e., the number of ovulations per oestrus event), having a greater impact on ovulation than on reproductive behaviour (Foster et al., 1985). Research has shown that malnutrition in ewes can lead to acyclicity, even during the breeding season (Forcada & Abecia, 2006). A faster increase in body weight during rearing will favour an earlier onset of the first oestrus in ewe lambs (Dýrmundsson, 1973). Thus, ewe lambs reared under unfavourable conditions will typically grow at a slower rate and may even fail to reach the stage of physiological and endocrinological development that is necessary for the onset of normal reproductive function (Dýrmundsson, 1973). Another factor that can influence ewe live weight (LW) and possibly puberty attainment is the lamb's birth rank. Lambs with higher birth ranks generally have lower birth weights, and slower growth rates compared to single-born lambs, which can delay their onset of puberty (Dýrmundsson, 1973).

For small ruminants, the start of puberty is associated with LW and condition rather than age; it is estimated to be between 40 - 60% of their mature ewe LW (Kenyon et al., 2014; Wall et al., 2018). Differences in growth rates have been found to cause changes in the physiological development of ewe lambs, and therefore affect their age at puberty (Allen & Lamming, 1961). When different breeds of ewes were raised under the same conditions, there were no significant breed-related differences in the onset of puberty (Table 2.1; Lozano et al., 2020). Creole ewes had a smaller adult body size, but still achieved puberty at similar ages to larger breeds when they reached a higher body development (Table 2.1; Lozano et al., 2020). Increased weight gain and body development are dependent on the nutrition provided to the ewes, therefore nutrition can have a significant impact on when ewes attain puberty.

Table 2.1 Physiological parameters associated with puberty in lambs. Values are presented as mean ± SEM.

Average Age (days)	Breed	Liveweight (kg)	% of mature liveweight	Progesterone concentrations (ng/mL)	Maximum follicle diameter (mm)	Reference
188	Creole	28.8 ± 3.1	73.5 ± 8.3	2.04 ± 0.54	-	Lozano et al., 2020
225	Creole	30.3 ± 2.4	77.2 ± 6.1	2.19 ± 0.44	-	Lozano et al., 2020
164	Romney Marsh	31.3 ± 3.7	56.2 ± 7.4	1.95 ± 0.33	-	Lozano et al., 2020
249	Romney Marsh	37.8 ± 5.7	71.5 ± 10.8	2.16 ± 0.24	-	Lozano et al., 2020
209	Hampshire	30.4 ± 5.4	58.8 ± 10.4	2.08 ± 0.20	-	Lozano et al., 2020
261	Hampshire	35.8 ± 3.3	69.3 ± 7.9	2.20 ± 0.32	-	Lozano et al., 2020
194	Corriedale	30.5 ± 4.1	57.3 ± 8.0	2.11 ± 0.11	-	Lozano et al., 2020
228	Corriedale	32.7 ± 6.8	62.0 ± 12.9	2.21 ± 0.13	-	Lozano et al., 2020
233	Merino	37.8 ± 0.2	-	-	-	Rosales Nieto et al., 2015
219 ± 3	Merino	39.4 ± 0.5	63.7 ± 0.7	-	-	Rosales Nieto et al., 2013
195	Suffolk x Western White Face	-	-	1.42 ± 0.37	5.2 ± 0.5	Bartlewski et al., 2002

As with ewes, heifers that had a faster average daily gain during the prepubertal period reached puberty approximately 108 days earlier than those with a slower growth rate (Drackley, 2008). The heifers that grew quickly also reached the same LW at a younger age. This suggests that proper nutrition is needed for optimal growth and reproductive function in ruminants (Valasi et al., 2012). To accelerate the onset of puberty, many farmers will increase the amount of daily feed being consumed by the ruminant. When calves were fed 20% of their body weight of milk replacer, they had a significantly higher weight than calves only fed 10%, however, at slaughter, both groups had similar onset of puberty (Röttgen et al., 2023). The onset of puberty was determined by the proportion of animals having a CL at slaughter, however, a corpus albican was not observed histologically, so there may have been periods throughout the oestrous cycle when the CL was not visible (Röttgen et al., 2023). The onset of puberty is regulated by a complicated physiological process that is still not fully understood, but there is evidence to suggest that adequate nutrition plays a crucial role for these animals to reach reproductive maturity (Duittoz & Kenny, 2023).

2.1.4 Social interaction

Social interactions play a crucial role in the attainment of puberty in ewe lambs. Behaviours exhibited during the prepubertal period can be significantly influenced by the presence of rams (Dýrmundsson, 1973). Studies have shown that the presence of a ram can have a profound effect on the reproductive development of ewe lambs. Fence-line contact with a ram for seven days was shown to increase antral follicle size by at least 3 mm and stimulate the formation of luteal structures, indicating that contact with a ram can induce follicular development in prepubertal ewes (Bartlewski et al., 2002). This suggests that the presence of a ram can accelerate the onset of puberty by promoting ovarian activity.

Furthermore, the introduction of a teaser ram before the breeding period has been found to increase the number of ewe lambs displaying oestrus (Kenyon et al., 2005). It is evident that exposure to a teaser ram can accelerate the onset of puberty by stimulating follicle growth and enhancing reproductive performance (Kenyon et al., 2005, 2006, 2012). Therefore, the timing of ram exposure can induce earlier puberty in ewe lambs and improve overall reproductive performance. Understanding mechanisms through which rams influence reproductive maturation can provide valuable insights into managing and optimising breeding programs to enhance reproductive performance in sheep.

2.2 Current techniques for determining puberty attainment in sheep

Assessing puberty attainment in livestock is crucial as it directly influences farm productivity and reproductive efficiency (Nafziger et al., 2021). There are several techniques that can be used to determine when an animal reaches puberty. In sheep production, these methods include the use of a teaser ram to detect oestrus, blood samples to look at progesterone concentration, and ultrasonography to observe the development of follicles and luteal structures (Kenyon et al., 2012). This section will discuss the advantages and disadvantages of each of these methods.

2.2.1 Teaser rams

The use of teaser rams is a common practice among farmers in New Zealand to enhance breeding efficiency. Teaser rams are sterile males that still produce testosterone, exhibiting normal ram behaviour (e.g., mounting the ewes), while being azoospermic (Crilly et al., 2022). These teasers can be either naturally sterile or surgically induced, and can provide a non-invasive method to detect the onset of the reproductive cycle in ewes (Cave et al., 2012). Using vasectomised rams as teasers is particularly effective for increasing hogget lambing efficiency because they produce all the hormones associated with mating without the risk of reproducing (Edwards & Juengel, 2017). An alternative to vasectomised rams is the use of short-scrotum rams, which are commonly found on New Zealand farms and may offer a less expensive option (Kenyon et al., 2008). This can occur naturally or through the application of a rubber ring distal to the testes of ram lambs, which shortens the scrotum and forces the testes toward the abdomen, reducing fertility while preserving male growth characteristics (Kenyon et al., 2008). Exposure to a teaser ram helps synchronise and stimulate the ewes' reproductive system and has been shown to improve pregnancy rates. It was found that teaser rams increased the number of ewe lambs conceived and the conception rate within the first 17 days of breeding (Kenyon et al., 2005). Further research highlights that using teaser rams with two-year-old and mature ewes before the breeding season can initiate breeding activity earlier than those not exposed to a teaser (Kenyon et al., 2005). The use of teaser rams, however, extends further than just improving the reproductive efficiency of ewes.

Teaser rams, when fitted with a harness and crayon, can be used to detect oestrus in ewes through the observation of tupping marks (i.e., crayon marks left on the ewes rump; Alhamada et al., 2017). Crayon colours can be changed every cycle to accurately identify which ewes have been marked and when. In this context, tupping checks can be used to assess conception rates and as an earlier indicator of pregnancy through the assessment of non-return rates from one cycle to the next (Kenyon et al., 2012). However, the use of tupping data to monitor oestrus events in ewes is time-consuming; observations must be made continuously day and night for at least one complete oestrous cycle, which lasts around 17 days. Additionally, these methods require experienced observers to accurately determine if oestrus has been displayed, making this measuring technique subjective (Alhamada et al., 2017). Despite this, teasers are widely used for determining when ewes are ready to be mated (Kenyon et al., 2012).

While teaser rams fitted with a mating harness can be used to detect first oestrus (i.e., puberty), there is a lack of evidence on how accurately a teaser can identify when an ewe lamb reaches puberty. By the time ewes are receptive to the teaser, they may already be on their second cycle and displaying signs of oestrus (Kenyon et al., 2006). Hoggets that were not mated until the second 17 day period had a smaller LW compared to hoggets that were mated in the first period, indicating that they did not reach puberty before introduction to the ram (Kenyon et al., 2006). Further investigation is required on the effectiveness of using teaser rams to determine puberty attainment in ewe lambs.

2.2.2 Hormonal analysis

Measuring serum progesterone concentrations is an accurate and reliable method for detecting puberty in lambs (Al-Ali & Rahawy, 2022). As detailed in section 2.1.1, there is a direct relationship between progesterone concentrations and puberty attainment in ewes. Progesterone concentrations exceeded 1.0 ng/mL in two or more consecutive blood samples collected three to four days apart, coinciding with the ewe lamb's first ovulation (Fitzgerald & Butler, 1982). Progesterone is produced by the CL after ovulation, indicating that the lamb has reached puberty (Auletta & Flint, 1988). This method is accurate and reliable, however, obtaining progesterone to measure puberty requires blood sampling, which is invasive and stressful for the animals involved (Bartlewski et al., 2002). Progesterone levels fluctuate during the oestrous cycle, so accurate representation requires multiple blood samples over time to confirm the patterns indicative of puberty (Bartlewski et al., 2011). Regular blood sampling and hormone assays, while highly accurate for detecting puberty, are labour-intensive and costly procedures that do not provide real-time results (Brown et al., 2015). They require specialised equipment, trained personnel, and significant analytical costs, making them impractical for routine use on farms.

2.2.3 Ultrasonography

The understanding of ovarian physiology has advanced due to ultrasonography (Menchaca & Rubianes, 2004). This imaging technique allows for a non-invasive, repeated visual assessment of changes in the ovarian structures over time (Toosi et al., 2009). High-frequency ultrasonic pulses reflect off tissues and scatter, generating an ultrasound image (Brogni & De Souza, 2021). Ultrasonography is an accurate method of estimating the size and number of both follicles and CL. The validity of the images produced by ultrasounds to determine the status of ovarian follicles in domestic species has been well-researched in cattle (Toosi et al., 2009). Ultrasonography produces real-time results, allowing researchers to better understand ewe's follicular waves and the differences between the breeding and non-breeding seasons (Brogni & De Souza, 2021). Transrectal ultrasonography is a technique that involves the use of an ultrasound probe inserted into the rectum to visualise and evaluate the reproductive organs, including the ovaries, uterus, and ovarian follicles. When follicles grow to around 1 - 2 mm, ultrasonography is useful to track ovarian development, ovulation and the formation of the CL (Figure 2.2; Bartlewski et al., 2011).

Figure 2.2 Ultrasonographic images of ovaries in cycling ewe displaying small follicles during anoestrus (F; image A), preovulatory follicles (F; image B) and a corpora lutea (CL; image C) (Gonzalez-Bulnes et al., 2010).

This method for measuring puberty would also have to be done multiple times throughout the cycle to determine when the formation of a CL occurs. Preovulatory follicles can reach 6 - 7 mm in ewes and are mainly detected when the follicle is larger than 4 mm (Gonzalez-Bulnes et al., 2010). Three to four days after ovulation, the CL can reach 6 – 8 mm in diameter, to a maximum diameter of 11 – 14 mm around six days later (Bartlewski et al., 2011). On the ultrasound image, the CL will appear as a grey structure (Figure 2.2, C) and there could be cavities within the CL (Gonzalez-Bulnes et al., 2010). Ultrasonography provides accurate and reliable real-time imaging of follicular structures, making it a highly effective tool for reproductive management and assessing puberty attainment. However, units capable of detecting these structures are costly and typically not affordable for routine practice on farms

(Bagley et al., 2023). Both methods of ultrasonography require a professional to perform this procedure and can be stressful for the animal, impacting their welfare (Brogni & De Souza, 2021). Having a trained technician perform the procedure on-farm can typically cost up to $10 USD per animal, in addition to a visitation fee (Bagley et al., 2023). These expenses can accumulate significantly, particularly for larger flocks, making it a considerable financial investment for routine reproductive management practices.

2.2.4 Limitations of the current methods of determining puberty attainment

Understanding and optimising puberty attainment in the agricultural industry can significantly enhance reproductive efficiency and overall productivity. Early puberty can improve the production and lifetime performance of lambs, leading to greater economic benefits for farmers (Edwards et al., 2015). The attainment of puberty in ewes is influenced by a combination of factors including nutrition, environmental conditions, and hormonal signals (Valasi et al., 2012). However, detecting puberty in lambs is challenging due to the first oestrus often going undetected. While there are several methods to detect oestrus, there is limited research on determining the onset of puberty in ewes, with most studies concentrating on cattle (Galvão & Santos, 2008). Current methods for detecting puberty include the use of teaser rams, hormonal assays and ultrasound examinations (Kenyon et al., 2012). While these methods are useful, they can be unreliable for detecting first oestrus, negatively impact animal welfare, are costly or require expensive equipment and a trained technician to operate (Alhamada et al., 2017). Exploring new methods can provide valuable insights into more effective and non-invasive ways to detect puberty attainment, potentially leading to significant improvements in flock productivity and reproductive efficiency.

2.3 Can accelerometry or proximity loggers be used to determine the onset of puberty in sheep?

Visual observation is not always sufficient for detecting changes in animal behaviour because continuous monitoring is impractical, not all animals can be observed simultaneously, the process can influence the animal's behaviour and it is subjective (Alhamada et al., 2017). However, new technological developments may make it possible to remotely monitor livestock and alert management to significant changes in behaviours (Gurule et al., 2021). Monitoring devices such as global positioning system (GPS) trackers, location sensors, proximity loggers and accelerometers have become more common for tracking animal behaviour (Vázquez Diosdado et al., 2015). This increase has created a more efficient and accurate method for analysing many movements and behavioural changes. In particular, accelerometer data has been widely used to monitor, classify and understand behaviour in cattle (Vázquez Diosdado et al., 2015). Activity monitors are widely used to detect oestrus in the dairy industry, as they are key to managing reproduction and maintaining steady milk production (Marquez et al., 2023). When investigating the efficiency of accelerometers on oestrus detection in cattle, results found that activity behaviours increased following a decline in milk progesterone preceding the oestrus period (Marquez et al., 2023). These devices, such as collars and leg bands equipped with accelerometers and GPS trackers, monitor increased physical activity and mounting behaviour that typically occurs during oestrus. In recent years, accelerometry data has been used to predict distinct markers associated with sheep activities such as resting, grazing and walking (Ikurior et al., 2021).

Tri-axial accelerometers are devices that measure acceleration in three directions and detect changes in velocity by measuring the acceleration due to gravity and other forces (Gurule et al., 2021). These devices, when attached to animals, provide data on a wide range of behaviours that are not easily observed, as they capture both animal orientation and movement dynamics (Shephard et al., 2008). Studies have shown their effectiveness in detecting grazing activity, with the potential to assist in identifying various types of activity in sheep (Ikurior et al., 2021). By logging data continuously, accelerometers can detect small changes in activity, providing insights into animal behaviour and physiology beyond human observation, which is limited to shorter periods and can miss part of the animal's daily activities (Gurule et al., 2021). Tri-axial acceleration data can be used to identify a variety of behaviours, including locomotion, feeding and social interactions (Shephard et al., 2008). With minimal human intervention, sensor technologies like accelerometers can identify significant behavioural changes related to the health and welfare of livestock, improving production efficiency (Gurule et al., 2021). Since each species exhibits a unique signature in tri-axial data, this data must be processed using a specialised algorithm designed to classify the activities and behaviours specific to the species being monitored (Ikurior et al., 2021). Although these monitoring devices have not been extensively researched with puberty attainment in ewe lambs, their ability to detect normal sheep activities and oestrus in cattle shows promise that they can detect the behavioural and activity changes associated with puberty.

2.4 Thesis aims and objectives

The primary aim of this thesis is to evaluate the effectiveness of accelerometry and proximity loggers in detecting the onset of puberty in ewe lambs. This research seeks to determine if these technologies can reliably indicate behavioural and physiological changes associated with puberty, providing an efficient and non-invasive method for monitoring reproductive development in ewe lambs. The hypothesis for this thesis is that the use of accelerometers or proximity devices can detect specific behavioural and activity changes associated with the onset of puberty in ewe lambs.

Chapter 3. Materials and methods

All experimental procedures were carried out with the approval of the Massey University Animal Ethics Committee MUAEC 23/35. This study was conducted from 12 February to 25 April 2024 (73 days; late summer to mid-autumn) at Massey University in Palmerston North, New Zealand (Lat: 175.6˚ E, Long: 40.4˚ S).

3.1 Animals and animal husbandry

Eight healthy prepubertal ewe lambs (seven Dorset Down x Romney cross and one Wiltshire x Romney cross) were used for the study. Lambs were sourced as orphans or mismothered from Massey's sheep farms and all were born from multiple birth sets. The ewe lambs were 172 ± 3.5 days of age (range: 156 - 180 days) and weighed 32.7 ± 1.65 kg (range: 26.9 - 38.8 kg) at the start of the study. The percentage of mature LW for the ewe lambs was estimated to be 80 kg (P. Back & J. Kerslake, personal communication, 17 June 2024). Two teaser rams were used: one ram was a bilateral cryptorchid (101.5 kg and 18 months of age) and the other ram had a shortened scrotum (71.4 kg and 18 months of age), as we did not have access to vasectomised rams. The LW of all ewe lambs was recorded twice a week (Monday and Thursday) throughout the duration of the study. Lambs had *ad libitum* access to pasture and fresh water throughout the study, except when blood samples and ultrasound evaluations took place. In addition, lambs were supplemented with approximately 500 g of pellets (Table 3.1) per animal daily.

Table 3.1 Ingredients and nutritional value of the pellets fed to the ewe lambs daily.

Ingredients	Composition (%)
Barley	39
Wheat	0
Broll	35.1
Soy bean meal	21.8
Molasses	3
Limestone	1
Soybean oil	0
Premix	0.1
Per kg as fed	**Nutritional value**
CP (g)	174.9
MP (g)	128.3
ME (MJ)	11
eNDF (g)	121.2
NDF (g)	184.8
CP/ME (MJ/g)	16

3.2 Experimental design

At the start of the study, the prepubertal ewe lambs were weighed, blood samples taken, and collars fitted with an attached Actigraph® accelerometer. One teaser ram was introduced to the flock of ewe lambs after first being fitted with a mating harness, crayon and an accelerometer. To address battery life limitations with the accelerometers, the teaser rams (alternated between the two) were rotated every 7 – 10 days. Over the 10 weeks, multiple measures of puberty attainment were assessed at regular intervals (serum progesterone and oestradiol, tupping data, proximity data, ultrasound examinations, and activity data). Ewe lambs were removed from the study after receiving a Grade 3 tup mark, which was subsequently confirmed by the presence of a CL via ultrasound.

3.3 Methods for confirming the onset of puberty

3.3.1 Tupping

Crayon marks on the ewe lambs were recorded daily at approximately 9 am and the colour was changed to mark different oestrous cycles. The marks were scored as follows: no markings on the ewe lamb (none), one crayon line on the ewe lamb (Grade 1), at least two crayon lines on the ewe lamb around the rump (Grade 2), or circular crayon mark at the centre of the rump (Grade 3).

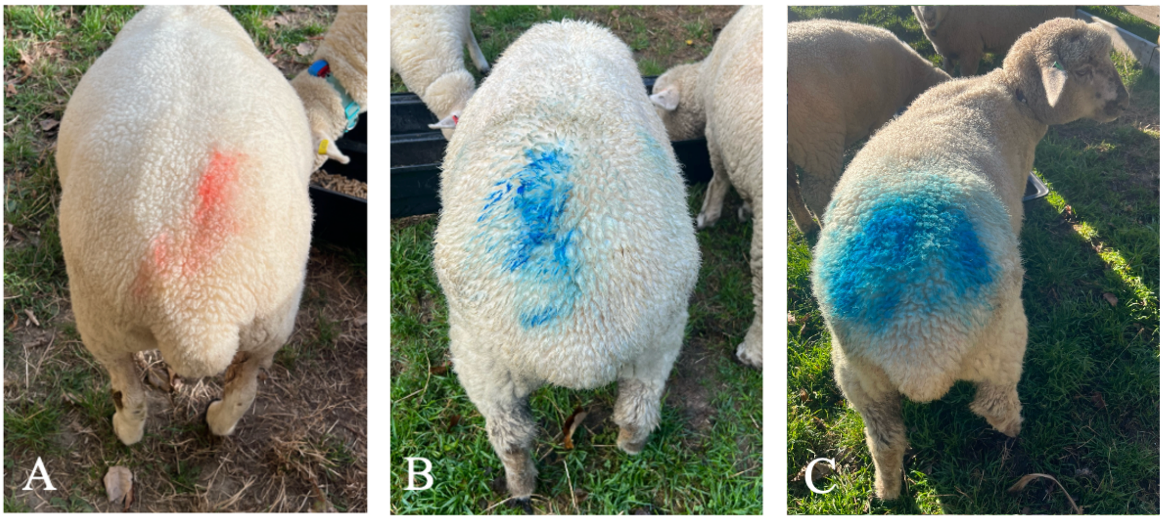

Figure 3.1 Crayon markings on the ewe lambs were used to track tupping activity by the teaser ram. Marks were assessed daily and assigned a grade: none (no marks on the ewe lamb), Grade 1 (one crayon line on the ewe lamb; A), Grade 2 (at least two crayon lines on the ewe lamb around the rump; B) or Grade 3 (circular crayon mark at the centre of the rump C). Crayon colours were changed to differentiate between oestrous cycles.

3.3.2 Blood samples for progesterone and oestradiol

Blood samples were taken from the ewe lambs twice a week (approximately 10 am on Monday and Thursday) throughout the study period. All blood samples (5 mL) were collected using jugular venepuncture and serum vacutainers (BD Vacutainer® REF 367895; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with a 20 G needle. The samples were then left for approximately 2 h to coagulate and subsequently the sample was centrifuged for 20 minutes at 2000x g for serum extraction. The serum samples were then placed in Eppendorf tubes (2 mL) and stored at -80˚C until analysis. At the end of the study, all samples were sent overnight to the Liggins Institute (University of Auckland, Auckland, New Zealand) for progesterone and oestrogen analysis.

Serum oestradiol-17β concentrations were measured using the Cobas® e601 analyser (Hitachi Ltd., Tokyo, Japan) and a commercially available electrochemiluminescence immunoassay, Cobas® Estradiol III (Roche Diagnostics New Zealand, Auckland, New Zealand). Cross-reactivity was reported to be < 1.0% for all steroids tested (n = 35) except for 6-α-hydroxy-oestradiol, which had a cross reactivity of 74.1%. This high reactivity for 6-α-hydroxy-oestradiol is not a concern, as we are primarily interested in total estrogen, as it is also produced by the ovaries (Kinder et al., 2019). The detection range was 5.0 – 3000.0 pg/mL. Values below the detection threshold were reported as 5.0 pg/mL. The mean intra-assay CV for the quality controls was 65.9%.

Serum progesterone concentrations were measured using the Cobas® e601 analyser (Hitachi Ltd., Tokyo, Japan) and a commercially available electrochemiluminescence immunoassay, Cobas® Progesterone III (Roche Diagnostics New Zealand, Auckland, New Zealand). Cross-reactivity was reported to be < 1.0% for all steroids tested (n = 27) except for 11-deoxycorticosterone, which had a cross reactivity of 3.9%. The detection range was 0.05 - 60.0 ng/mL. Values below the detection threshold were reported as 0.05 ng/mL. The mean intra-assay CV for the quality controls was 42.1%.

3.3.3 Ultrasound evaluation of ovarian structures

Ultrasonographic examinations were performed weekly (every Thursday from 8 am to 11 am) using a Mindray M9Vet (Mindray® Bio-Medical Electronics Co., Ltd., Shenzhen, China) and linear rectal transducer (Mindray® 6LE5Vs, 5 - 8.5 MHz; Mindray® Bio-Medical Electronics Co., Ltd., Shenzhen, China). The probe was lubricated and inserted into the rectum. The bladder was used as a reference point to locate the ovaries and uterus, which is located just cranial to the bladder (Al-Ali & Rahawy, 2022). Once the bladder was identified, the probe was slowly moved cranially and rotated in both directions until each ovary was found. A video (~ 150 frames at 30 - 60 Hz) of a continuous scan through each ovary was recorded and used for the retrospective analysis of ovarian structures. Measurements of ovarian size (average diameter and two perpendicular diameter measurements) and area at the widest point of the ovary were recorded for each ovary from the ultrasound videos (Figure 3.2). Ovarian volume was calculated as described (Raine-Fenning et al., 2008): $\text{Ovarian volume (cm}^3\text{)} = \left(\frac{4}{3}\right)\pi \times \text{radius}$

The number and size of all follicles (0.1 mm diameter) and corpora lutea were determined for each ovary. Follicles were classified as small (< 3 mm), medium (3 – 5 mm), and large (> 5 mm), according to Bjersing et al. (1972). The appearance of the first CL, alongside changes in serum progesterone, was used to determine the onset of puberty.

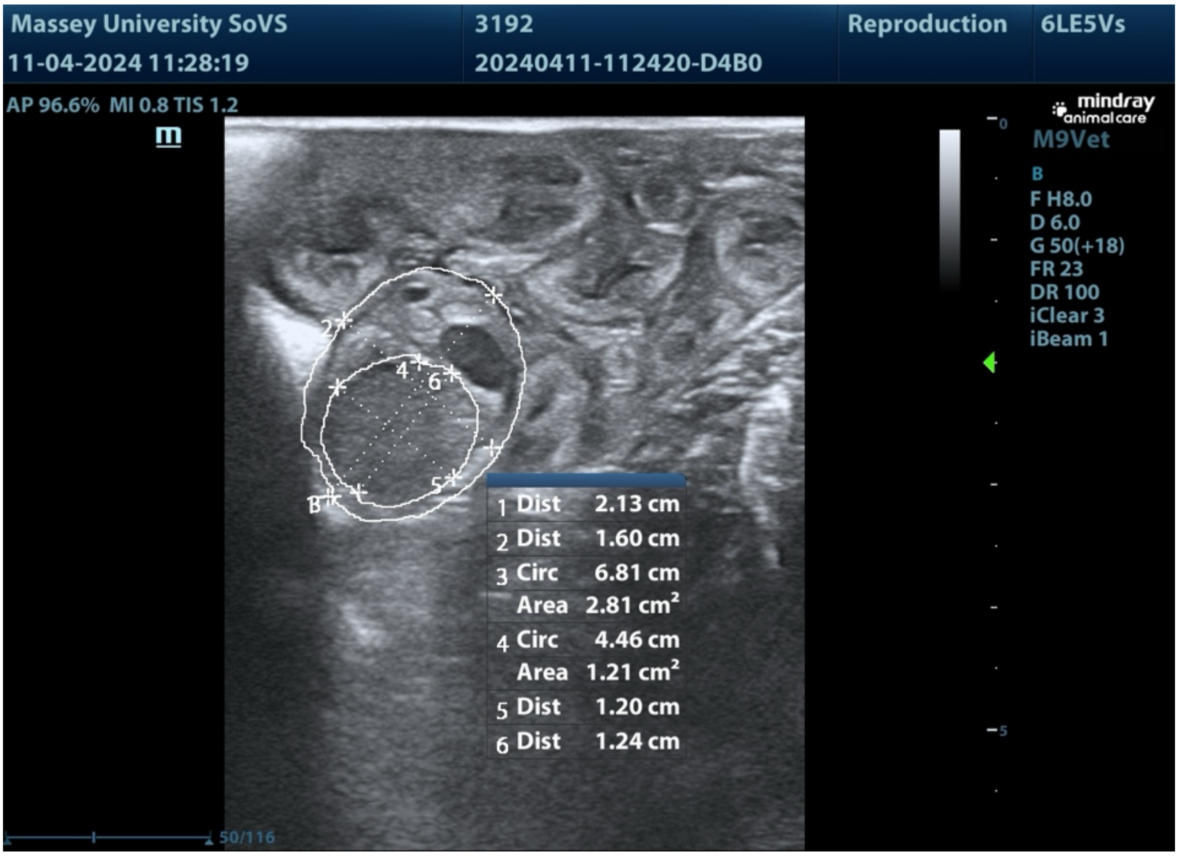

Figure 3.2 Ultrasound image of ovarian follicles in ewe lamb 3192. This image shows the measurements of the ovary, the follicles and a corpus luteum present within the ovary. Measurements taken included the diameter, area and circumference.

3.4 The collection of activity and proximity data

Ewe lambs and teasers were fitted with ActiGraph® wGT3X-BT accelerometers (ActiGraph®, LLC, Pensacola, FL, USA). Devices weighed 19 g and measured 33 mm x 46 mm x 15 mm, were attached to collars, and positioned ventrally on the ewe lamb's neck (Figure 3.3). Collar tightness was kept as consistent as possible to reduce residual movement of the devices. The ActiGraph® recorded raw acceleration (30 Hz; dynamic range ± 8.0 g; fitted by a commercial unit) along three independent axes: lateral (X), cranio-caudal (Y), and dorso-ventral (Z). The devices were also capable of recording proximity with other animals, with individual units being set as either a beacon or receiver. To evaluate ewe lamb interactions with the rams, the rams' Actigraphs were set as receivers and the ewe lamb's devices as beacons. The number of ewe lamb encounters within 1 m (determined to be a value of signal intensity, as recorded by the proximity sensor) of the ram was recorded. The Actigraph® was able to record continuous proximity and acceleration data for a maximum duration of 10 - 14 days. Thus, data were downloaded, and devices were charged every 7 - 10 days.

Figure 3.3 ActiGraph® accelerometer attached to the collar and placed ventrally on the neck of the ewe lamb.

The acceleration and proximity data were downloaded using the ActiLife 6® software (ActiGraph, Pensacola, FL, USA) and exported as 'csv' files. Using RStudio version 4.2.3 (R Foundation for Statistical Computing, Vienna, Austria), the overall dynamic body acceleration (ODBA) was calculated from the raw dynamic body acceleration (DBA) data of each axis as: $\mathrm{ODBA} = \sum_{i=1}^{n} |\mathrm{DBAX}| + |\mathrm{DBAY}| + |\mathrm{DBAZ}|$, where dynamic body acceleration was calculated for each axis as the cumulative acceleration count over 30 measurements (i.e., 1 second), minus the moving average of the raw acceleration data over the same period. These data were then summed to provide the total hourly and daily ODBA counts for each animal over the study period. The proximity data (i.e., the number of times each ewe lamb encountered each ram within 1 m) was summed to provide daily totals.

Previous research indicated considerable variability in average daily ODBA counts, suggesting that care had to be taken when comparing the activity of individual ewe lambs or when analysing the activity data of multiple lambs as a combined dataset (Barwick et al., 2018). To overcome this variability, the daily ODBA activity counts of each ewe lamb were standardised by converting them to a proportion of the average daily activity (i.e. the average daily counts for each animal over the entire study; Andrews et al., 2022). This was calculated for each animal using the following equation: $\text{Proportional daily ODBA activity count} = \frac{\text{Daily ODBA activity count}}{\text{Average daily ODBA activity count}}$

3.5 Statistical analyses

All statistical analyses were conducted using Rstudio version 4.2.3 (R Foundation for Statistical Computing, Vienna, Austria), and significance was set as $p < 0.05$, with $p < 0.10$ defined as a trend or tendency. A linear mixed effects model was fitted for the LW data to determine if there was a relationship between weeks of the trial while accounting for individual differences between the ewe lambs. A mixed effects model was used to evaluate changes in the CL size over time and to determine if there was a significant difference in CL sizes between ewe lambs. P-values were found using Satterthwaite's approximation for degrees of freedom. To analyse the time series, a linear regression analysis was conducted to evaluate the relationship between LW and weeks relative to the first luteal phase for each ewe lamb. Analysis of Variance (ANOVA) was performed to compare the proportional overall dynamic body acceleration (pODBA) values between ewe lambs at week 0, followed by Tukey's Honest Significant Difference (HSD) post-hoc test for pairwise comparisons. To determine if there were significant differences in the percentage of time spent interacting with the ram among individual ewe lambs, a one-way ANOVA was performed, followed by a post-hoc Tukey's HSD test to identify specific pairwise differences between ewe lambs.

Chapter 4. Results

4.1 Live weight

The average LW at birth was 4.78 ± 0.38 kg, respectively (Table 4.1). There was a linear increase ($p < 0.001$, $r^2 = 0.965$) in LW of the ewe lambs over the trial period (Figure 4.1). The average age at the first luteal phase, defined as serum progesterone concentrations over 1.0 ng/mL, was 228 ± 4.09 days, with a mean LW of 43.79 ± 1.52 kg (Table 4.1). This was an estimated 54.7% of mature body weight, which was defined in methods as 80 kg (Chapter 3 page 14; Figure 4.1). Average daily gain (ADG) from birth to puberty did not differ among animals with a mean of 0.169 ± 0.004 kg/day (Table 4.1).

Table 4.1 Age, live weight (LW), and average daily gain (ADG) of ewe lambs at first luteal phase (progesterone > 1.0 ng/mL)

Ewe lambs	Date of birth (dd/mm/yyyy)	Birth rank	Birth weight (kg)	Age (days) at first luteal phase	LW (kg) at first luteal phase	ADG (kg/day) at first luteal phase
14	16/08/2023	3	6.50	247	50.2	0.177
15	16/08/2023	3	5.15	237	43.5	0.162
24	17/08/2023	4	3.90	228	42.1	0.161
63	19/08/2023	2	4.80	226	44.5	0.176
139	25/08/2023	2	6.00	223	47.2	0.185
232[a]	3/09/2023	2	3.30	-	-	-
273	23/08/2023	3	4.50	225	40.9	0.162
3192	8/09/2023	3	4.10	213	38.1	0.160

[a] Ewe lamb 232 missing values at first luteal phase because serum progesterone concentrations never exceeded 1.0 ng/mL during the study period.

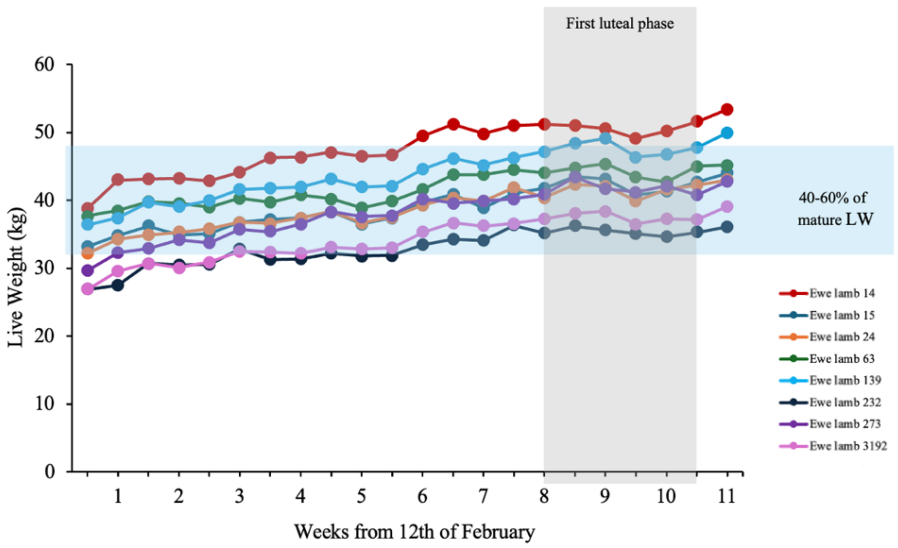

Figure 4.1 Live weight (LW) progression of ewe lambs over the trial period. The graph displays the live weight (kg) of ewe lambs from week 1 to week 11. The area shaded blue represents 40 - 60% of the mature live weight of the ewe lambs, which was estimated to be 80 kg. The area shaded grey indicates the period when serum progesterone concentrations exceeded 1.0 ng/mL, marking the first luteal phase.

4.2 Tupping data

Tupping marks above a grade 2 were observed during week 4, marking the start of the first 17 day oestrous cycle. The average number of ewe lambs tupped per day in each of the first two cycles was 0.375 ewe lambs. For the third cycle, the mean ewe lambs tupped per day by the ram was 0.25 (Figure 4.2). The third cycle ended on day 13, as all ewe lambs were tupped by that point in the study.

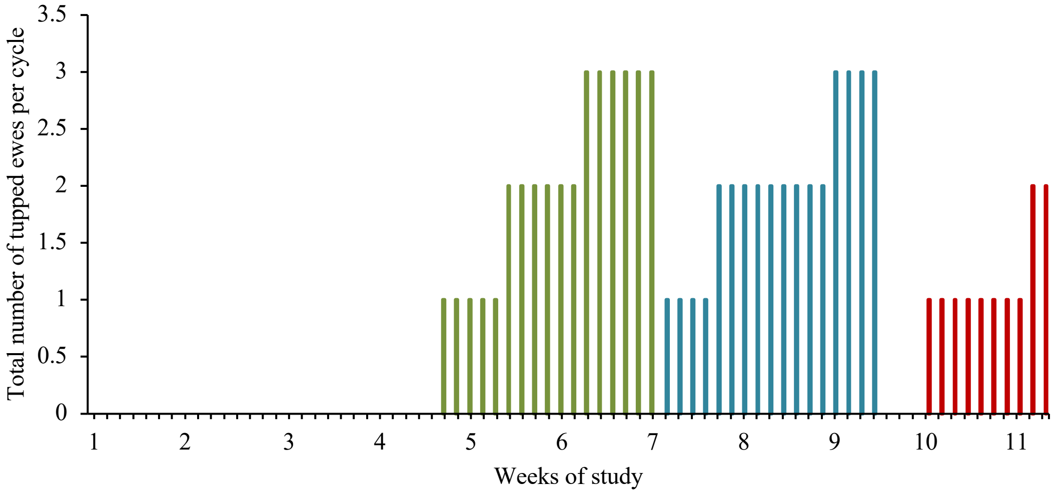

Figure 4.2 Number of ewes tupped per 17 day oestrous cycle. The marks were scored as follows: no markings on the ewe (none), one crayon line on the ewe (Grade 1), at least two crayon lines on the ewe around the rump (Grade 2), or circular crayon mark at the centre of the rump (Grade 3). Ewes were considered to be tupped if they had a tupping grade of 2 or higher. The start of the 17 day cycle occurred after the first ewe was tupped at a grade of at least 2. Colours were changed to represent a new 17 day period.

4.3 Blood samples for progesterone and oestradiol

Serum oestradiol rose to an average concentration of 33.04 ± 5.43 pg/mL during week 5, and concentrations differed between animals ($p < 0.001$; Figure 4.3). The average age of the animals at the serum oestradiol peak was 202.50 ± 3.82 days. Elevated progesterone concentrations were not observed in ewe lamb 232. Serum progesterone remained low (< 1.0 ng/mL) for the first eight weeks, after which the average concentration at the first rise in progesterone above 1.0 ng/mL was 2.17 ± 0.34 ng/mL (Figure 4.4). Progesterone concentrations did not differ between animals. Seven ewe lambs serum progesterone concentrations first rose above 1.0 ng/mL, which indicates a luteal phase (Fitzgerald & Butler, 1982), between weeks eight and ten in the study.

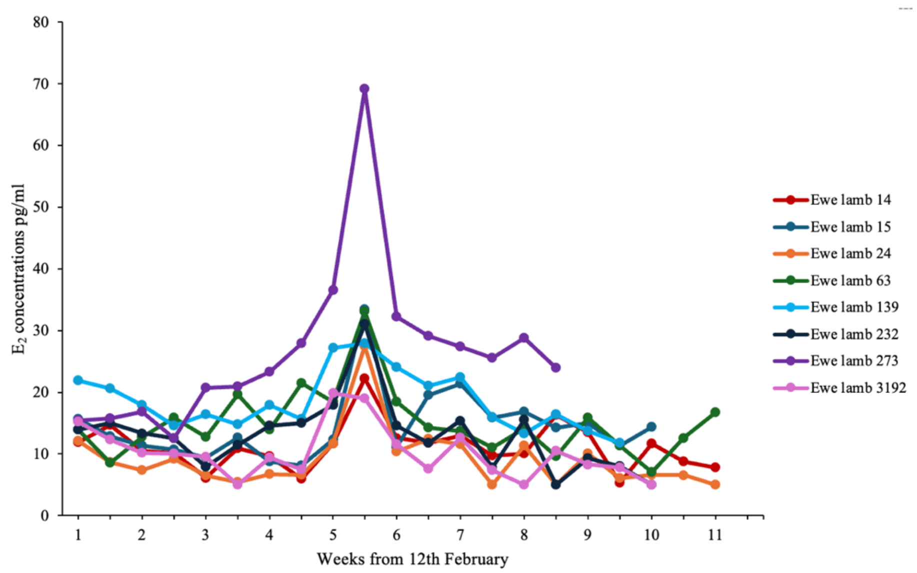

Figure 4.3 Serum oestradiol (E_2) concentrations (pg/mL) in ewe lambs over the study period. This figure shows concentrations of oestradiol from the 12th of February, with individual ewe lambs represented by different coloured lines.

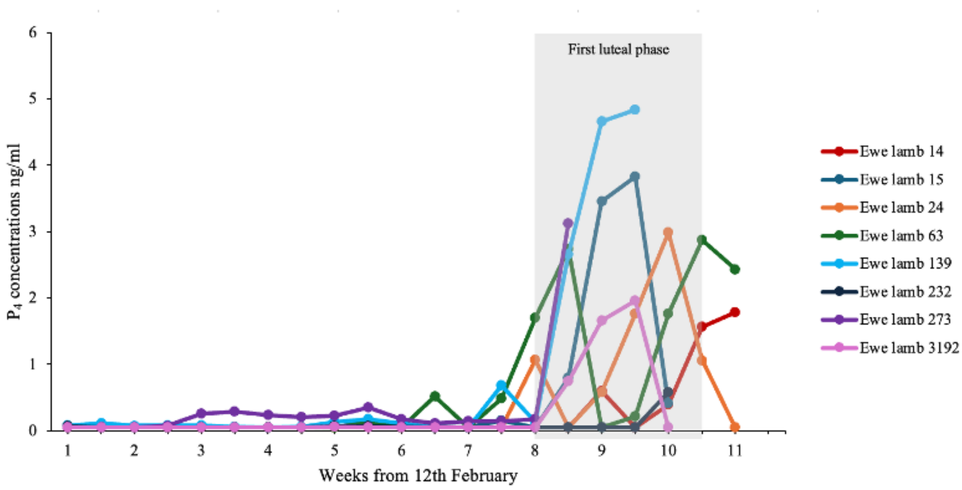

Figure 4.4 Serum progesterone (P_4) concentrations (ng/mL) in ewe lambs over the study period. The graph shows progesterone concentrations from the 12th of February, with individual ewe lambs represented by the different coloured lines. The shaded area represents the period that progesterone concentrations exceeded 1.0 ng/mL, indicating the onset of puberty.

4.4 Ultrasound evaluation of ovarian structures

During the onset of puberty, the average ovarian volume was 3.19 ± 0.11 cm^3 (Figure 4.5). The average CL size throughout the study period was 3.70 ± 0.52 mm (Figure 4.6). During the first luteal phase, the mean CL size was 10.87 ± 0.94 mm and average serum progesterone concentrations were 1.28 ± 0.36 ng/mL. Mean serum progesterone for all animals prior to puberty was 0.18 ± 0.05 ng/mL. The highest concentrations occurred during weeks 9 (1.70 ± 0.70), 10 (1.06 ± 0.37) and 11 (1.27 ± 0.58).

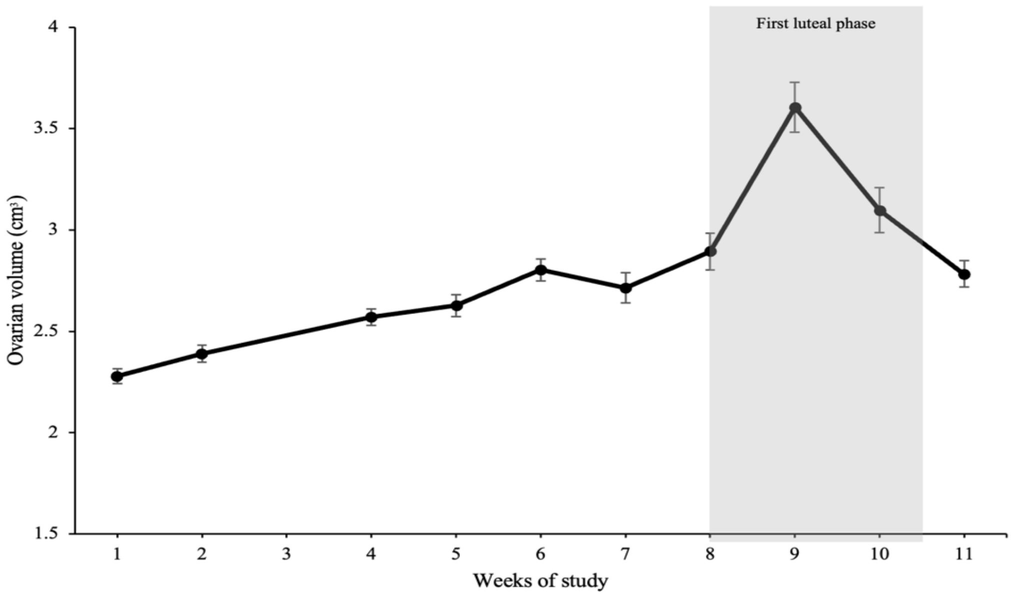

Figure 4.5 Average ovarian volume (cm^3) of the eight ewe lambs over the study period. Data are presented as mean ± SEM. The shaded area represents the first luteal phase, where serum progesterone concentrations were greater than 1.0 ng/mL. Weekly ultrasonographic examinations were performed to measure ovarian size. The area at the widest point of each ovary was recorded from the ultrasound videos. No ultrasound examinations were conducted during week 3, resulting in missing data for that week.

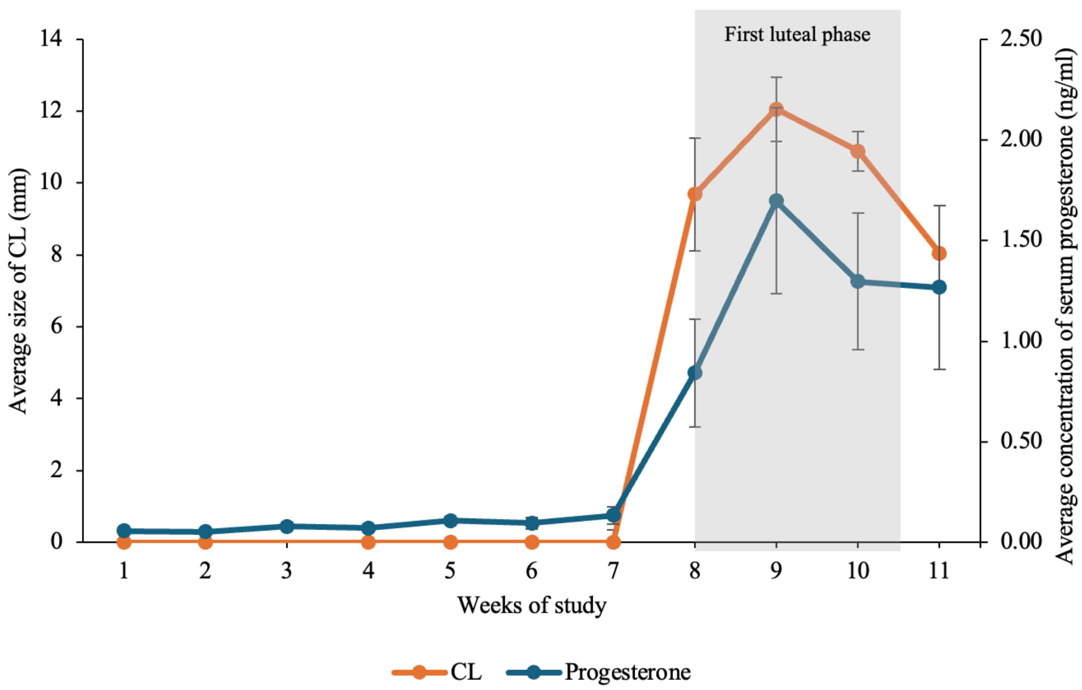

Figure 4.6 Average corpus luteum (CL) size (mm) and serum concentrations of progesterone (ng/mL) in the ewe lambs during the trial period. Data are presented as mean ± SEM. The shaded area indicates the first luteal phase, ranging from week 8 – 10. Ultrasonographic examinations were performed weekly, and blood samples were collected twice a week. Puberty was determined by the appearance of the first CL and rise in serum progesterone above 1.0 ng/mL. No ultrasound examinations were conducted during week 3, resulting in missing data for that week.

4.5 The collection of overall dynamic body acceleration and proximity data

The ODBA during the study period was 3.96 x10^5 ± 0.18x10^5 (Table 4.2). Ewe lamb 3192 demonstrated the highest average activity level at 4.95 x10^5. The average time the ewe lambs spent interacting with the ram over the study period was 25.6 ± 1.36% per day. Ewe lamb 3192 had the highest percentage of time spent interacting with the ram over the study period (Table 4.2).

Table 4.2 Daily average overall dynamic body acceleration (ODBA) and percentage of time the ewe lambs spent interacting with the ram. Values are presented as mean ± SEM.

Ewe lambs	Average daily ODBA (x10^5)	Percentage of time spent interacting with the ram per day
14	4.10 ± 1.24	24.8 ± 1.32
15	4.25 ± 1.05	24.2 ± 1.18
24	4.01 ± 1.37	27.4 ± 1.37
63	3.74 ± 1.25	24.0 ± 1.32
139	3.41 ± 1.08	26.4 ± 1.33
232	3.82 ± 1.07	22.8 ± 1.40
273	3.40 ± 1.48	24.8 ± 1.35
3192	4.95 ± 1.62	30.6 ± 1.59
Overall mean	3.96 ± 0.18	25.6 ± 1.36

4.6 Time series

All data were organised for further analyses based on the first rise in progesterone above 1.0 ng/mL. Since elevated progesterone concentrations above 1.0 ng/mL were not observed in ewe lamb 232, these data were not used for further analysis (Appendix A6).

There was a statistically significant positive relationship between the weeks relative to the first luteal phase and the average weekly LW of the ewe lambs ($p < 0.001$; Figure 4.7, A). Average LW over the study period was 39.46 ± 0.98 kg (Figure 4.7, A). The variability for each ewe lamb's daily ODBA activity counts was corrected by using proportional daily activity counts. The average weekly pODBA throughout the study period and for the week of the first luteal phase was 1.06 ± 0.03 and 0.80 ± 0.03 (Figure 4.7, B). The percentage of average weekly visits to the ram throughout the study period was 26.2 ± 1.53 % (Figure 4.7, C). At week 0, the percentage of visits to the ram had an average of 28.2 ± 1.48 % and all ewe lambs were tupped 3 weeks before the first luteal phase (Figure 4.7, C).

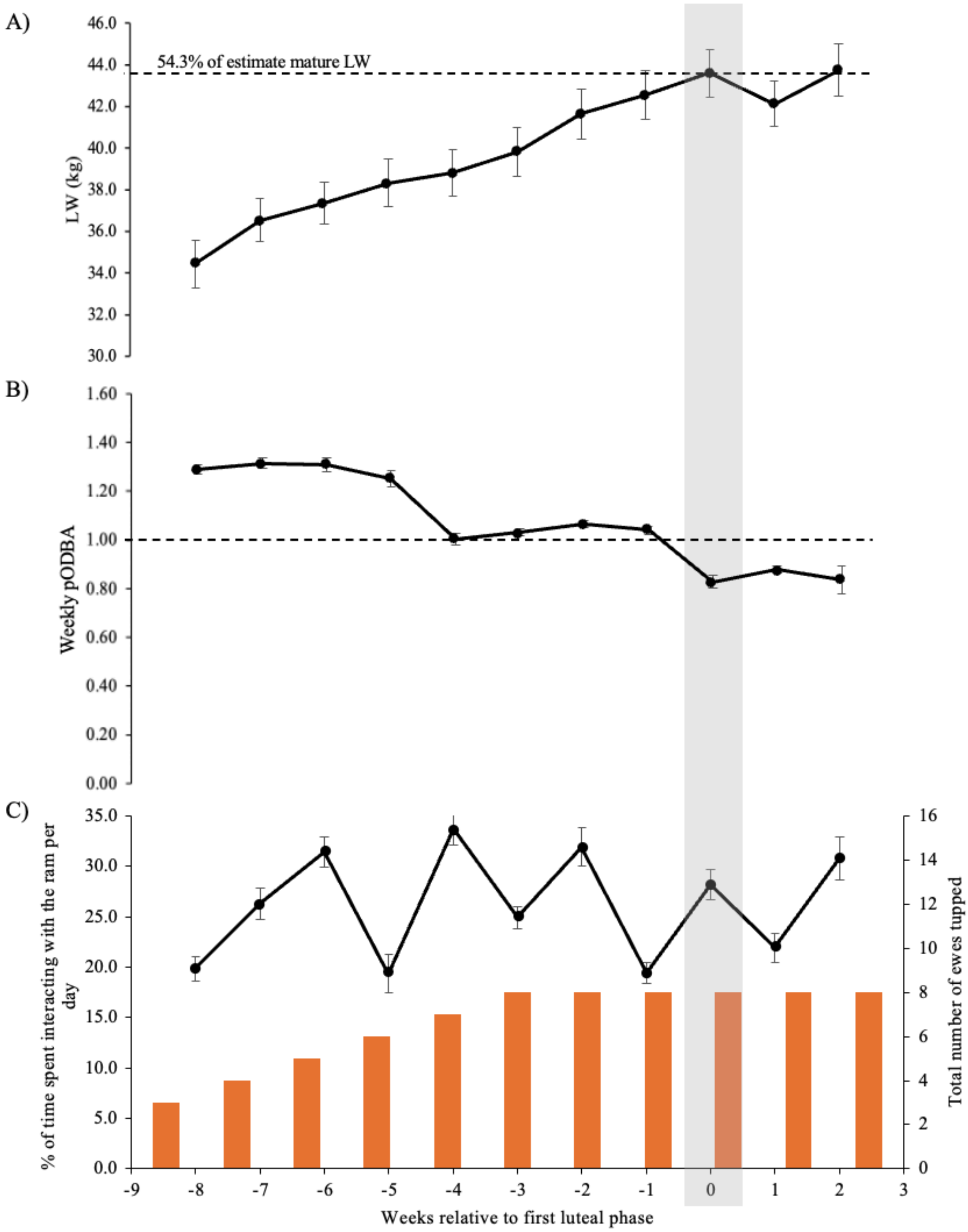

Figure 4.7 Time series on weekly changes in ovarian and behavioural parameters of ewe lambs relative to the first luteal phase. (A) Average live weight (LW) of ewe lambs across weeks, with the dashed line indicating the estimated percentage of mature live weight at puberty. (B) Weekly proportional overall dynamic body acceleration (pODBA) as a measure of activity levels across weeks, with the dashed line representing the baseline activity level. (C) Weekly percentage of time the ewe lambs spent interacting with the ram, with the bars representing the total number of ewe lambs tupped per week.

Since oestrus in sheep lasts from 24 to 36 hours, and the data presented in this study was collected on a weekly basis, it was challenging to detect any clear changes around the time of puberty. Therefore, to obtain a more accurate representation, the data was analysed on a daily basis.

Proportional ODBA tended to differ among ewe lambs in week 0 (p = 0.051; Figure 4.8). There are significant differences in the daily percentage of time the ewe lambs spent interacting with the ram (p = 0.007; Figure 4.9). The average percentage of ewe lamb interactions with the ram during the first luteal phase (week 0) was 28.2 ± 1.48 % (Figure 4.9). Ewe lamb 3192 had a significantly higher percentage of daily interactions with the ram compared to Ewe lamb 14 (p = 0.050), Ewe lamb 15 (p = 0.016), Ewe lamb 63 (p = 0.011) and Ewe lamb 273 (p = 0.050).

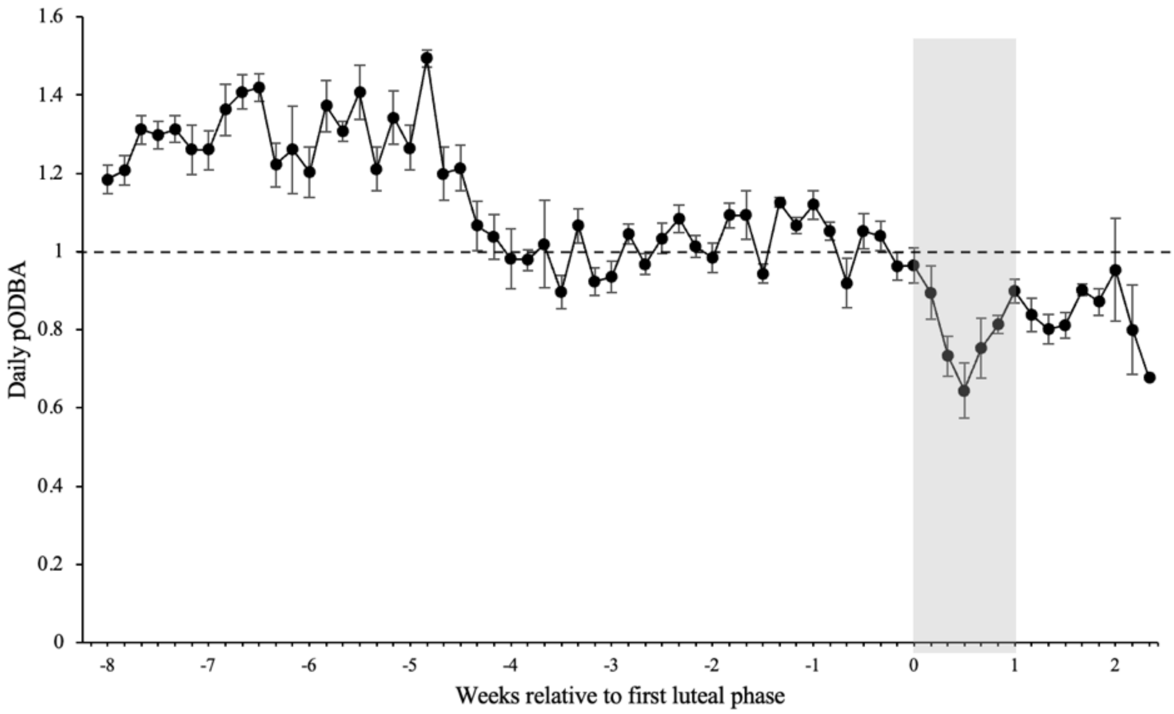

Figure 4.8 Mean daily proportional overall dynamic body acceleration (pODBA) of the seven ewe lambs over the study period. The dashed line represents the baseline activity level. The shaded area indicates the first luteal phase, with week 0 being the onset of puberty. Ewe lamb 232 was not included in this analysis since elevated progesterone concentrations above 1.0 ng/mL were not observed in the study.

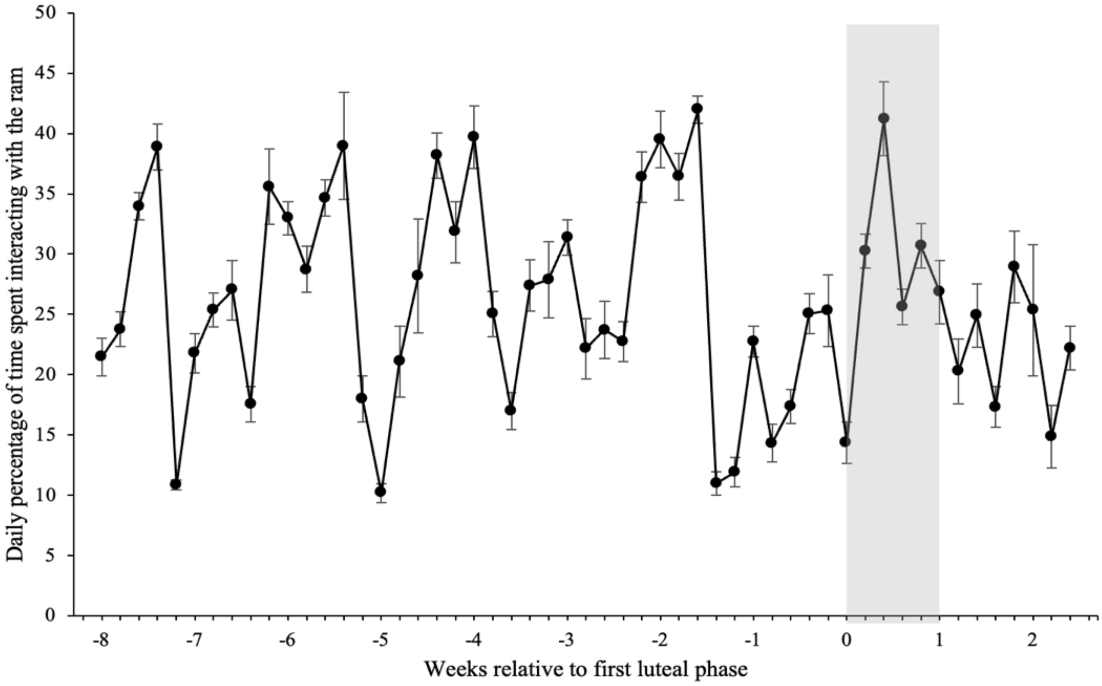

Figure 4.9 Mean daily percentage of time spent interacting with the ram relative to the first luteal phase. The total number of times each ewe lamb encountered each ram within 1 m was summed to provide daily totals. The shaded area represents the first luteal phase, with week 0 being the onset of puberty. Ewe lamb 232 was not included in this analysis since elevated progesterone concentrations above 1.0 ng/mL were not observed in the study.

Chapter 5. Discussion

This study aimed to evaluate and compare a range of methods for determining puberty attainment in ewe lambs. Serum progesterone concentrations and ovarian ultrasonography were used to accurately determine the onset of puberty, as defined as a ewes first ovulation and luteal phase. Interestingly, tupping observations were not reliable for puberty detection as the ewes were marked well before the ovulatory oestrus event. However, two novel methods, accelerometry and ram proximity sensors, showed promise for detecting puberty attainment. The results showed an overall decrease in ODBA following the first ovulation. Additionally, the percentage of daily visits with the ram increased as the ewe lambs approached their first luteal phase.

The linear increase in LW of the lambs over the study period is consistent with the growth and development of lambs essential for puberty attainment (Dýrmundsson, 1973). Past literature suggests that ewes reach puberty at 40-60% of their mature LW (Kenyon et al., 2014; Wall et al., 2018). In the present study, the first ovulation occurred when the sheep were approximately 54.7% of their estimated mature body weight. Live weight is a crucial factor that influences reproductive development and rapid weight gain can favour an earlier onset of the first oestrus in ewe lambs (Dýrmundsson, 1973). Indeed, there is a strong inverse relationship between LW and the age at puberty in sheep (Dýrmundsson, 1973). The physiological processes regulating this ensure that the ewe lambs have enough energy reserves and that their bodies are developed enough to support reproductive functions, in particular, pregnancy (Nestor et al., 2012). The onset of puberty largely depends on physiological factors such as body condition, with adipose tissue producing leptin, which plays a key role in stimulating the oestrous cycle, leading to a correlation between LW and puberty (Rosales Nieto et al., 2014). However, using LW to determine or estimate the exact onset of puberty can be challenging. Despite this, LW remains a useful indicator.

All ewe lambs in the present study reached puberty between the expected 40-60% mature LW, suggesting that LW can be a reliable tool for estimating when puberty should occur. Using LW as a general guide to determine puberty is a non-invasive method that can be easily and frequently measured using standard farm equipment, making it accessible and practical for most sheep farmers (Herath et al., 2021). However, when used exclusively, its accuracy is limited by variation between breeds, sexes and birth ranks (Dýrmundsson, 1973). Higher birth rank lambs may face more challenges that delay puberty such as nutritional competition, lower birth weight, and slower growth rates (Dýrmundsson, 1973). All ewe lambs from this study were from multiple birth sets, which could have impacted their growth rates and the age they reached puberty. To mitigate the effect of a variable feed intake on animal growth, all ewe lambs were artificially reared, had access to pasture, and were supplemented with pellets throughout the study period. Even with controlled feeding, there is variability in the estimated mature body weight. Therefore, if the onset of puberty needs to be accurately determined, LW alone is not sufficient due to individual variation.

A ram mating harness and crayons were used to determine the onset of puberty based on first tupping (i.e., first mating) in the ewe lambs, or first oestrus. This method is a common practice on farms as a way of measuring mating and synchronicity in ewes (Kenyon et al., 2005, 2006). When testing the effectiveness of teaser rams on hoggets, a higher percentage of hoggets were successfully mated in the teased group compared to the control (Mahmoud & Hussein, 2019; Kenyon et al., 2006). The presence of a teaser ram also induced breeding activity and achieved a degree of oestrus synchrony in ewes (Kenyon et al., 2006). However, the use of mating harnesses and tupping was not reliable as a marker of puberty attainment in the present study, as crayon marks from the teaser ram were observed several weeks prior to the first ovulation of the ewe lambs. At the time of these early tupping marks, the ewe lambs were all within 40 – 60% of mature LW and experienced their first spike in serum oestradiol concentrations. This suggests that tupping might be linked to the physiological changes related to anovulatory prepubertal follicular growth, but does not directly signify the onset of puberty itself (Mahmoud & Hussein, 2019). This also depends on what puberty is being defined as. While most farmers likely define puberty by the first behavioural oestrus, this is not necessarily a true indicator of puberty. Puberty is more accurately defined as the period when an ewe becomes capable of conceiving, which requires ovulation (Edwards & Juengel, 2017; Rosales Nieto et al., 2018).

As indicated by ultrasonography and serum oestradiol concentrations, several anovulatory waves of follicular growth were observed prior to the onset of puberty. However, since the HPG axis has not fully activated at this time, the preovulatory LH surge fails to occur and thus these follicles do not reach ovulation (Evans et al., 2000). In prepubertal lambs, the positive feedback pathways for oestrogen are not yet established because the reproductive axis is still maturing. Furthermore, the low concentrations of oestrogen receptors in the brain before puberty, due to the absence of progesterone, suppress the reproductive axis (Xie et al., 2022). This low receptor expression limits the brain's sensitivity to oestradiol, preventing the positive feedback response necessary for triggering the LH surge and ovulation (Evans et al., 2000). The number and activity of kisspeptin neurons play a critical role in activating this reproductive axis as the ewe lambs approach puberty (Decourt & Beltramo, 2018). The serum oestradiol peak observed in week 5 of the study may indicate this feedback, which delays the onset of puberty until the lambs are physiologically ready. Additionally, early peaks in oestradiol may help prepare the ewe lamb's reproductive system for the eventual onset of puberty (Bartlewski et al., 2011). Early increases in oestradiol can initiate physiological changes that prime the hypothalamus, pituitary and reproductive organs for the onset of puberty (Kinder et al., 2019). These early peaks in oestradiol might also explain the teaser ram's interest and the appearance of tupping marks around this time. However, while oestradiol is associated with these physiological changes, it alone cannot pinpoint when the lambs first ovulated. Thus, progesterone is likely a better marker for determining puberty.

Progesterone receptors are found in various tissues including the uterus, ovaries, mammary glands and brain (Graham & Clarke, 1997). Before puberty, there is minimal expression of progesterone due to a lack of luteal activity (Bartlewski et al., 2006). However, serum progesterone concentrations increase with the formation and development of the CL following the ewe lamb's first ovulation, marking the onset of puberty (Brogni & De Souza, 2021). This rise in progesterone is essential for preparing the uterus for a potential pregnancy, maintaining the luteal phase and regulating the feedback mechanisms that control the oestrous cycle (Spencer & Bazer, 2004). Progesterone also plays a crucial role in priming the brain to respond to oestrogen, which is why the first behavioural oestrus often occurs after the first ovulation (Spencer & Bazer, 2004). Serum progesterone analysis has proven to be a reliable tool for determining the onset of puberty, however, its accuracy depends on the frequency of blood sampling (I'Anson & Legan, 1988). More frequent blood sampling could improve the precision of determining the exact day of ovulation. Another limitation is the cost of the analysis, making it not practical from a farm management perspective.

During the follicular phase of the oestrous cycle, FSH stimulates follicle growth in the ovary (Brogni & De Souza, 2021). In the current study, ovarian volume of the ewe lambs increased as they approached puberty, peaking during the luteal phase. The development of multiple follicles, with the emergence of a dominant follicle, is responsible for the increase in ovarian volume (Mahdi & Khallili, 2008). Following ovulation, the ruptured follicle becomes a CL, which is a large structure also contributing to the increased ovarian volume. These findings are similar to other studies that found increased ovarian weight was due to the increase in the number and size of follicles (Mahdi & Khallili, 2008). Following puberty, ovarian volume decreases as the CL regresses and a new follicular wave begins (Bartlewski et al., 2011). Transrectal ultrasound scanning proved to be effective for estimating follicle size and CL presence, aligning with results from a previous study (Viñoles et al., 2004). Ultrasonography allowed real-time visualisation of ovarian development, which, when combined with serum progesterone analysis, gave a reliable indication of when puberty was attained. Given that the CL produces progesterone to prepare the uterus for pregnancy (Auletta & Flint, 1988), it is logical that serum progesterone concentrations rise as CL size increases and decline as the CL regresses after ovulation. Oestrus in ewes typically lasts 24 – 36 hours (Bartlewski et al., 2011), therefore, only performing weekly ultrasounds in the present study reduced the accuracy of tracking the follicular changes and ovulation. Once the CL was detected on the ultrasound scan, ovulation had already occurred, since CLs may not be detected until day six after ovulation (Bartlewski et al., 2011). Depending on how accurate puberty detection needs to be, it is essential to perform ultrasounds more frequently than once a week to observe real-time changes in follicular activity and when ovulation occurs. Given that the oestrous cycle in ewes lasts approximately 17 days, conducting ultrasound examinations only once a week limits observations to just two or three times within each cycle. While ultrasonography has proven to be reliable in accurately detecting the physiological processes associated with puberty attainment in ewe lambs, it is costly and stressfull for the animal (Alhamada et al., 2017). Therefore, they are impractical for commercial use on farms, where more readily implemented, less expensive techniques are needed.

As proximity gradually increased leading up to the first luteal phase, it may indicate that the ewe lambs were becoming more receptive to the ram. In mature ewes, clear oestrous behaviours such as tail fanning, increased vocalisation and actively seeking out the ram are normally observed (Fabre-Nys & Gelez, 2007). However, as previously noted, the first oestrus in ewe lambs is often silent, with fewer visible signs of these behaviours. The lack of oestrous behaviours in ewe lambs can make the detection of puberty challenging when based on proximity alone. Even though they may not be approaching the ram themselves, they would accept the ram's advances during this time (Dýrmundsson, 1973). Since the monitoring devices cannot differentiate whether it was the ram or the ewe lambs initiating the interactions, it is unclear if the increase in proximity was due to the ewe lambs visiting the ram or the ram seeking out the ewe lambs. Based on the trends observed in the present study, proximity shows potential as an indicator that ewe lambs are approaching puberty. However, fluctuations in the proximity data suggest this method alone may not a consistently reliable indicator, especially given the lack of clear behavioural indicators during the first oestrus. Further research would be beneficial to determine whether mature ewes exhibit more visible ram-seeking behaviour and how it is reflected in the proximity data. This could provide insights into whether proximity data could be more reliable in detecting oestrous behaviour in mature animals.

The ewe lambs exhibited a decrease in overall activity during the first luteal phase. This drop in activity likely resulted from the rise in progesterone concentrations, as progesterone is known to suppress oestrous behaviour and activity (Fabre-Nys & Gelez, 2007). While this decrease was observed during the luteal phase, the activity data alone did not provide clear evidence of changes during oestrus or follicular growth. The peak in proximity occurred when pODBA was already declining, which may be related to the ewe lambs being less focused on grazing and more on interacting with the ram or accepting his advances. The drop in both proximity and pODBA may indicate the transition into the luteal phase, aligning with the rise in progesterone concentrations and the inhibitory effects on behaviours associated with oestrus (Axelson et al., 1981). However, while there was a notable overall decrease in activity, this pattern was not consistent enough across all ewe lambs to definitively identify ovulation based on the acceleration data alone (Appendix B). Without additional data on the hormone analysis or the ultrasound examinations, it remains unclear whether this activity decline is a reliable marker for ovulation. The variation in activity also suggests that accelerometers, while useful in identifying general trends associated with hormonal changes around puberty, may not be enough to determine the exact timing of puberty on their own. Given the variability in individual factors such as LW, nutrition, social interaction and environmental conditions (Valasi et al., 2012), future studies could focus on monitoring for longer periods to determine if the decrease in activity is a consistent pattern during oestrus in mature ewes. Additionally, they could target specific behaviours and compare them with ODBA data.

Accelerometers and proximity loggers have shown promise as non-invasive, practical tools for monitoring trends associated with puberty, but further research is needed to determine their reliability in accurately identifying the onset of puberty in ewe lambs.

5.1 Conclusion and future directions

This study provides valuable insights into the physiological and behavioural changes that occur in ewe lambs during the prepubertal period leading to puberty. Current methods that were used to measure puberty attainment included tupping checks with a teaser ram, blood sampling for hormonal analysis and ultrasound examinations. Both hormonal analysis and ultrasound examinations were reliable methods for determining puberty attainment when used exclusively. However, due to their cost and the need for an experienced professional to perform them, these methods are rarely used on farms. The more common practice on farms, tupping checks with a teaser ram, was less reliable when the timing of puberty attainment needed to be accurately determined.

Collar-mounted accelerometers were evaluated to determine their potential to detect behavioural changes associated with puberty in ewe lambs. This study exhibited an overall increase in proximity to the ram as the ewe lambs approached puberty, while their daily activity declined. These behavioural changes appear to be influenced by hormonal fluctuations, with oestradiol driving increased social behaviours in the prepubertal period and progesterone suppressing activity after ovulation. Although accelerometers successfully detected these changes in behaviour in the ewe lambs, further research is needed to establish their reliability and accuracy as a non-invasive method for determining the onset of puberty.

Future directions

- Longitudinal studies to examine the relationship between hormonal fluctuations and behavioural changes before, during and after puberty attainment.
- Using integrated tools that combine behavioural, hormonal and physiological monitoring to offer a more comprehensive understanding of puberty attainment in ewe lambs.
- Acceleration and proximity data during the normal oestrous cycle of mature ewes.
- Developing a behavioural algorithm to target specific oestrus behaviours in ewes compared to ODBA data.

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Appendices

Appendix A: Individual concentrations of serum progesterone (ng/mL) and oestradiol (pg/mL) of the ewe lambs over the study period.

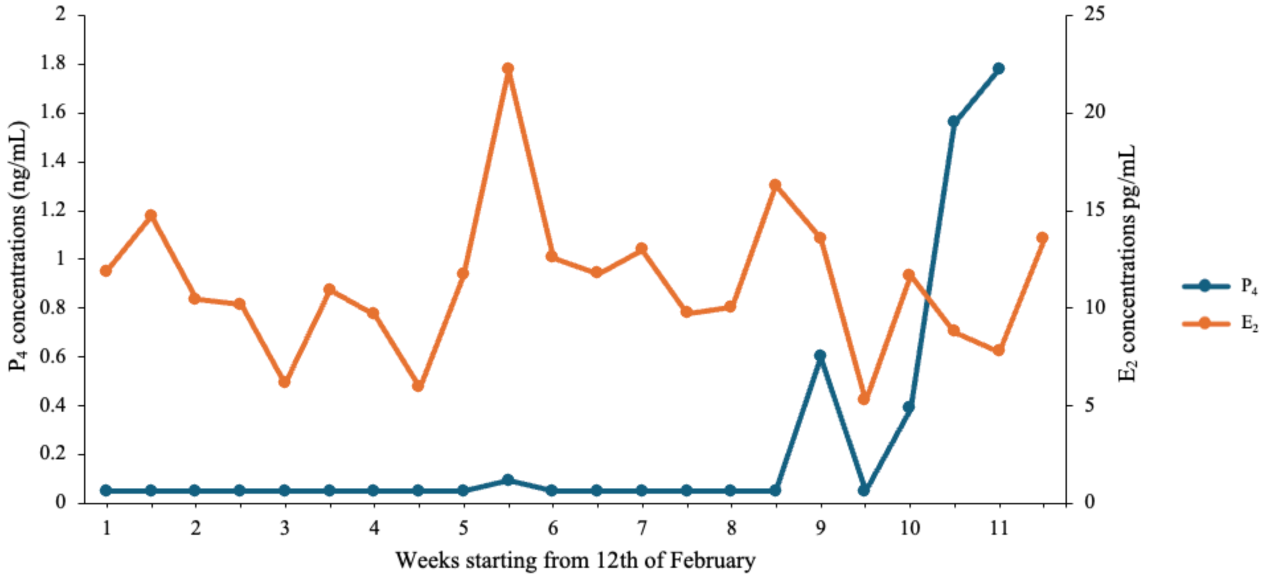

Figure A1 Serum progesterone and oestradiol concentrations for ewe lamb 14 over the study period.

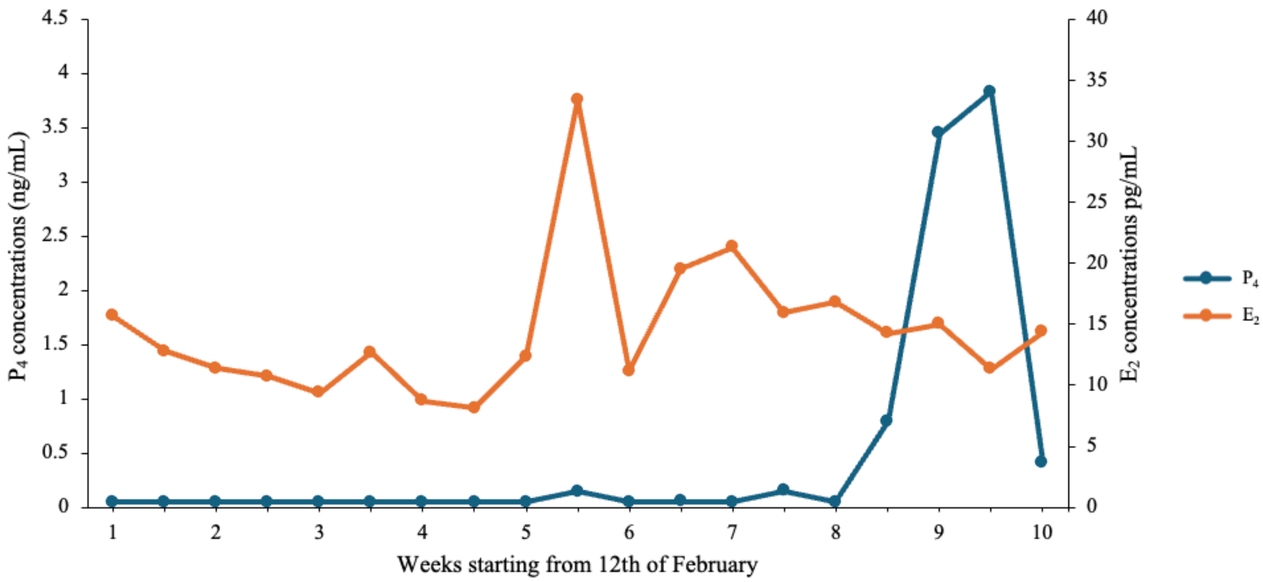

Figure A2 Serum progesterone and oestradiol concentrations for ewe lamb 15 over the study period.

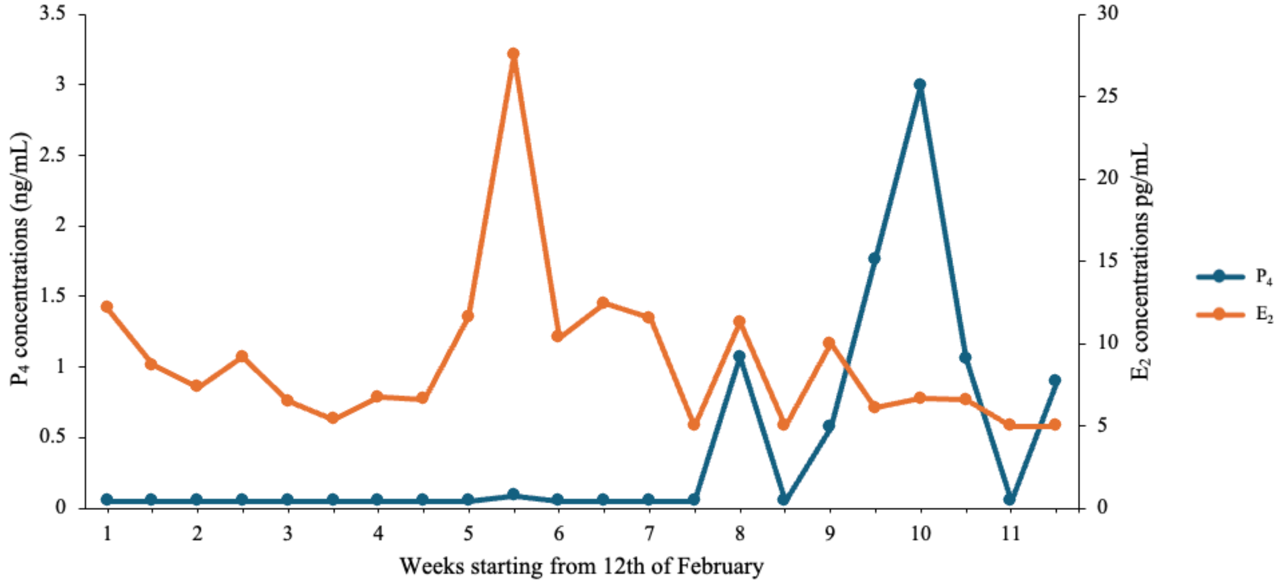

Figure A3 Serum progesterone and oestradiol concentrations for ewe lamb 24 over the study period.

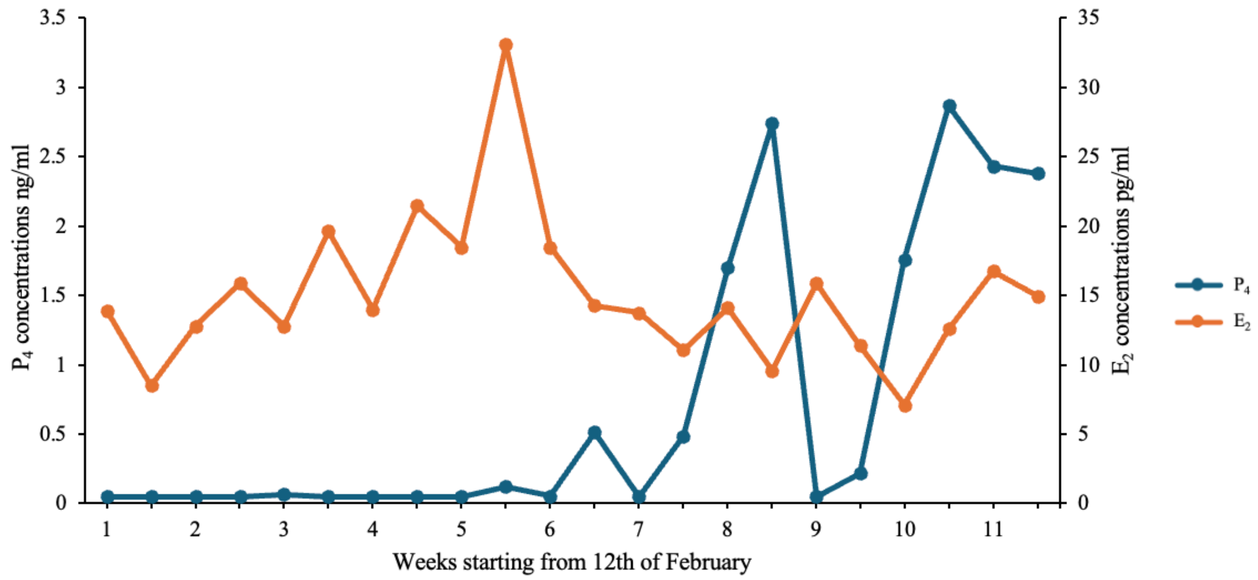

Figure A4 Serum progesterone and oestradiol concentrations for ewe lamb 63 over the study period.

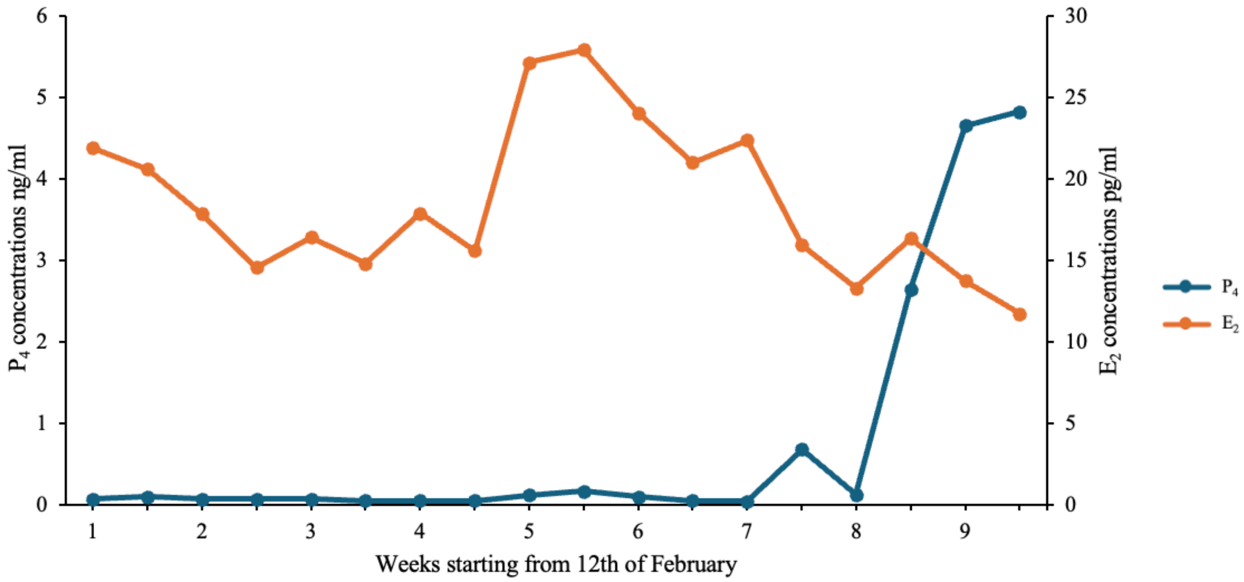

Figure A5 Serum progesterone and oestradiol concentrations for ewe lamb 139 over the study period.

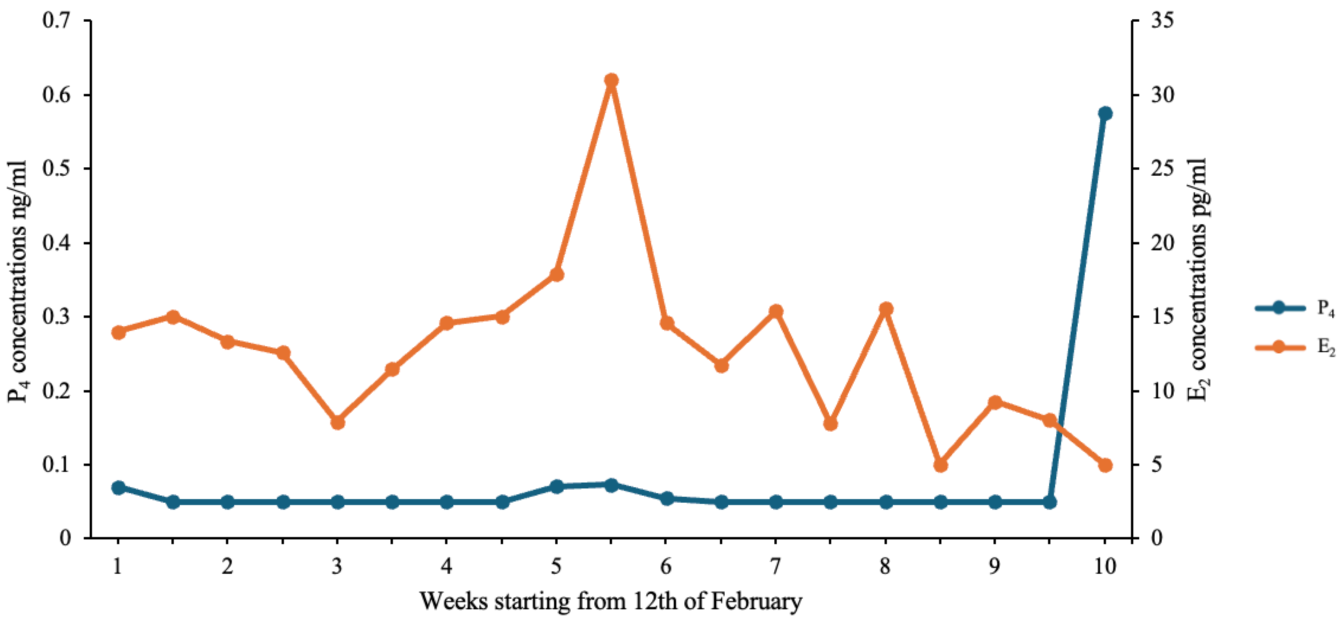

Figure A6 Serum progesterone and oestradiol concentrations for ewe lamb 232 over the study period.

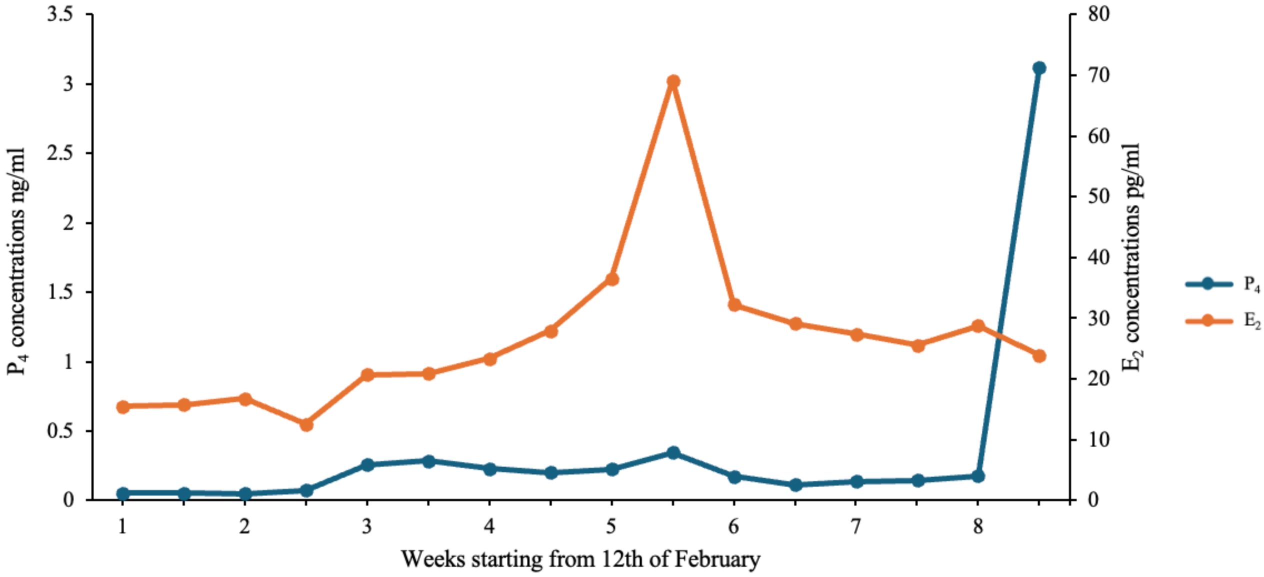

Figure A7 Serum progesterone and oestradiol concentrations for ewe lamb 273 over the study period.

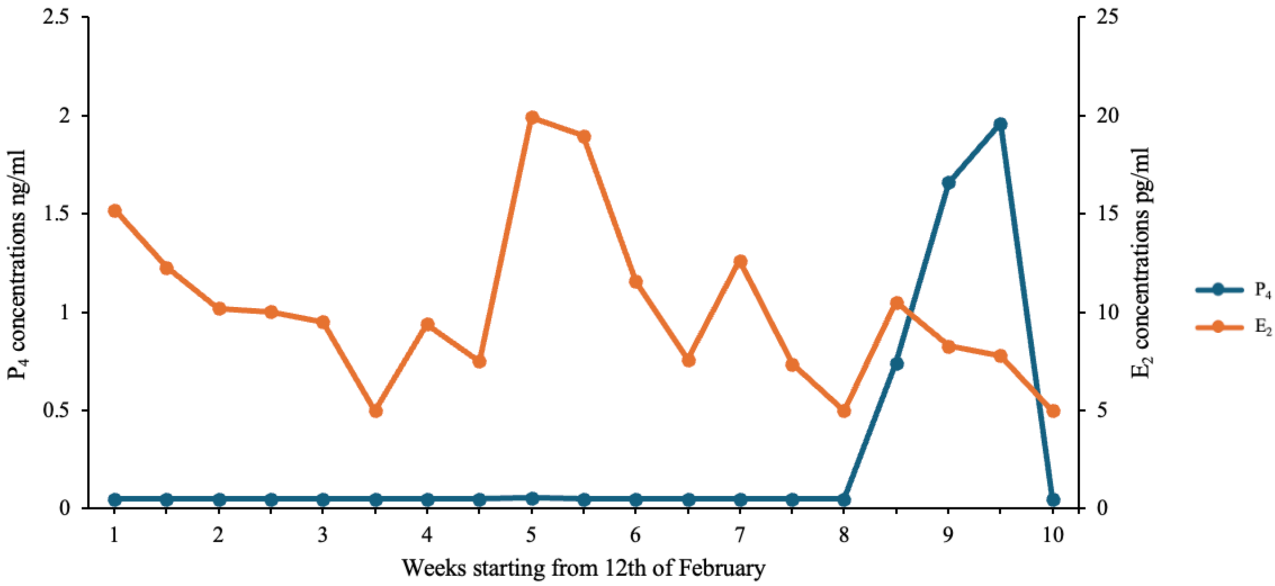

Figure A8 Serum progesterone and oestradiol concentrations for ewe lamb 3192 over the study period.

Appendix B: Individual proportional overall dynamic body accelertation (pODBA) of the ewe lambs over the study period.

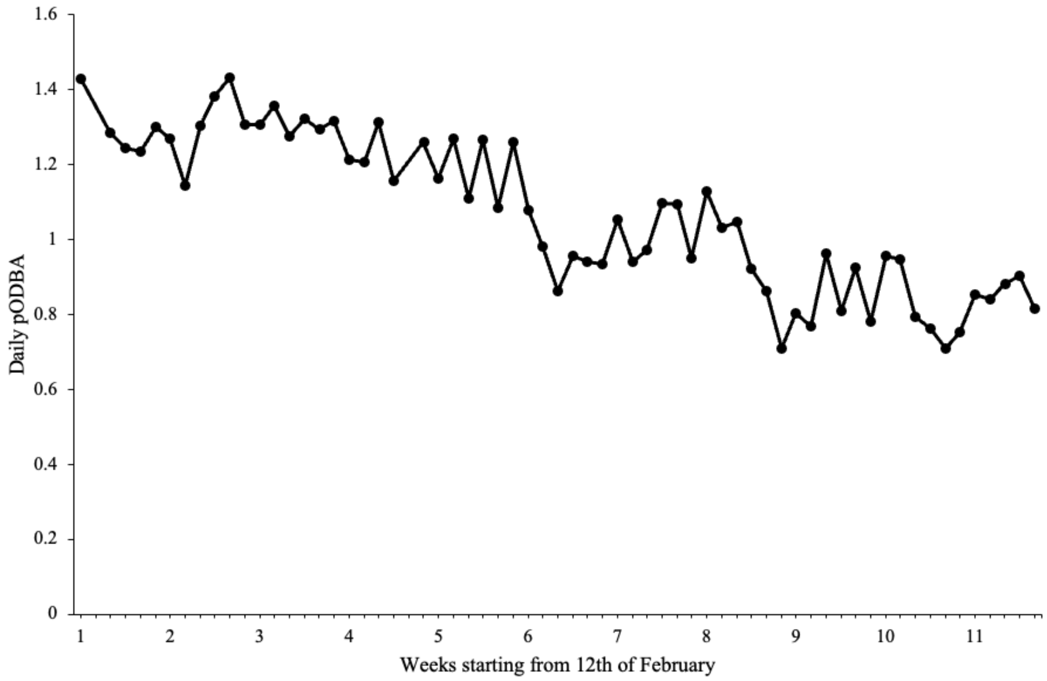

Figure B1 Proportional overall dynamic body accelertation (pODBA) of ewe lamb 14 over the study period.

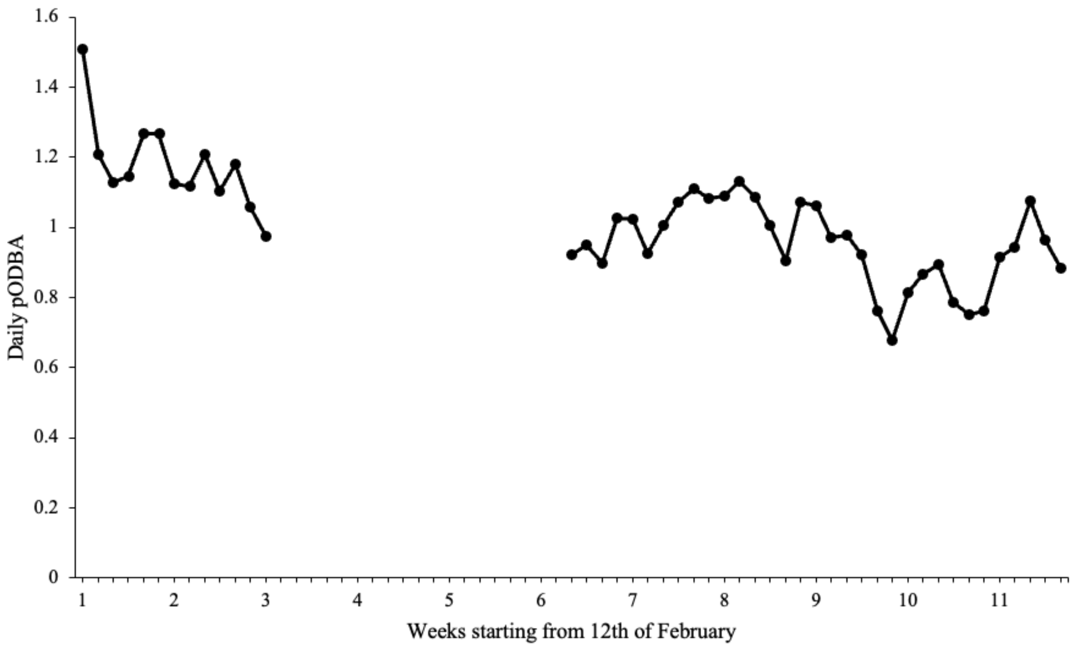

Figure B2 Proportional overall dynamic body accelertation (pODBA) of ewe lamb 15 over the study period. Due to water damage to the accelerometer, data was unavailable for the period between weeks 3 and 6.

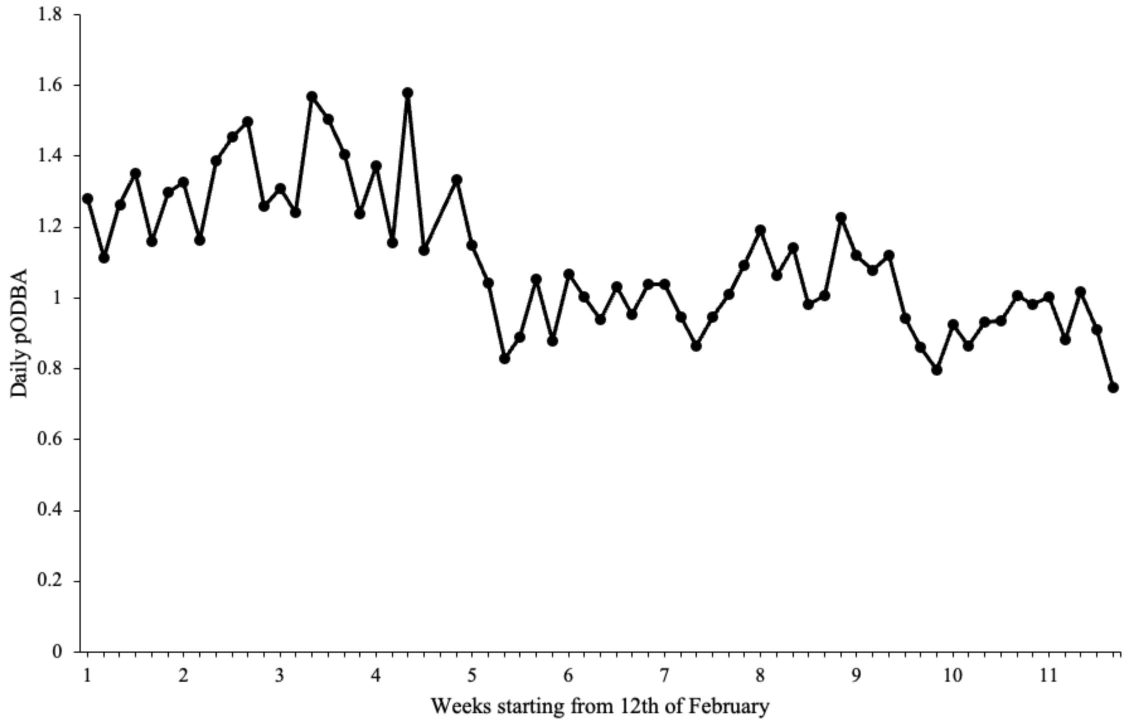

Figure B3 Proportional overall dynamic body accelertation (pODBA) of ewe lamb 24 over the study period.

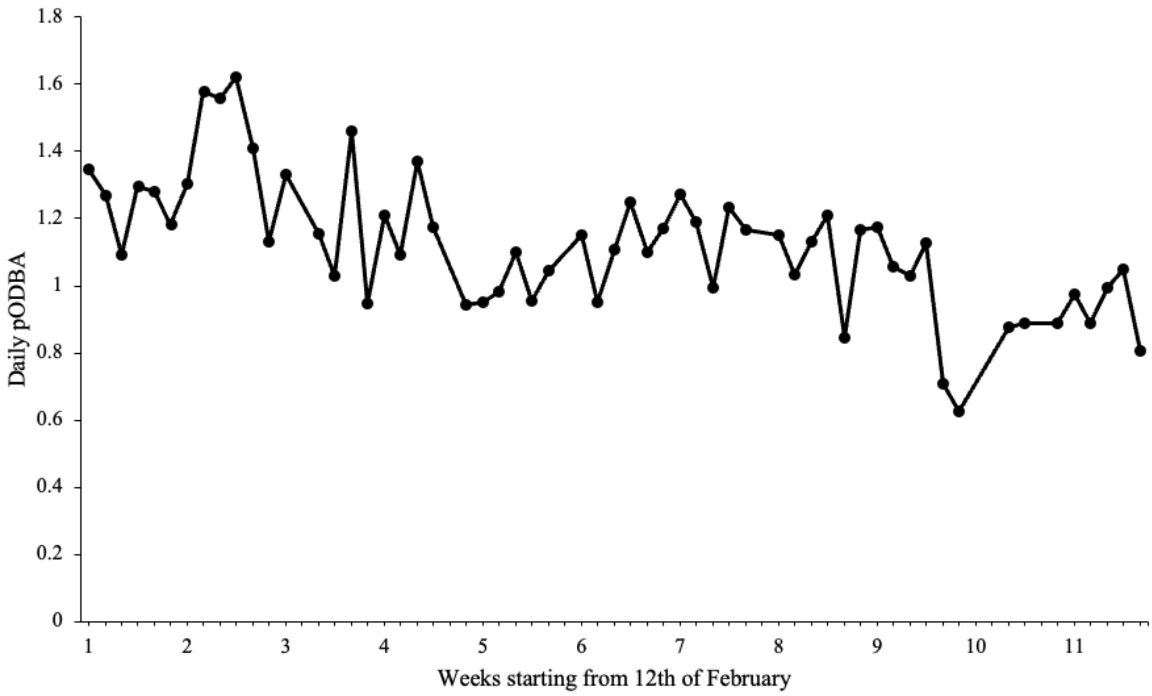

Figure B4 Proportional overall dynamic body accelertation (pODBA) of ewe lamb 63 over the study period.

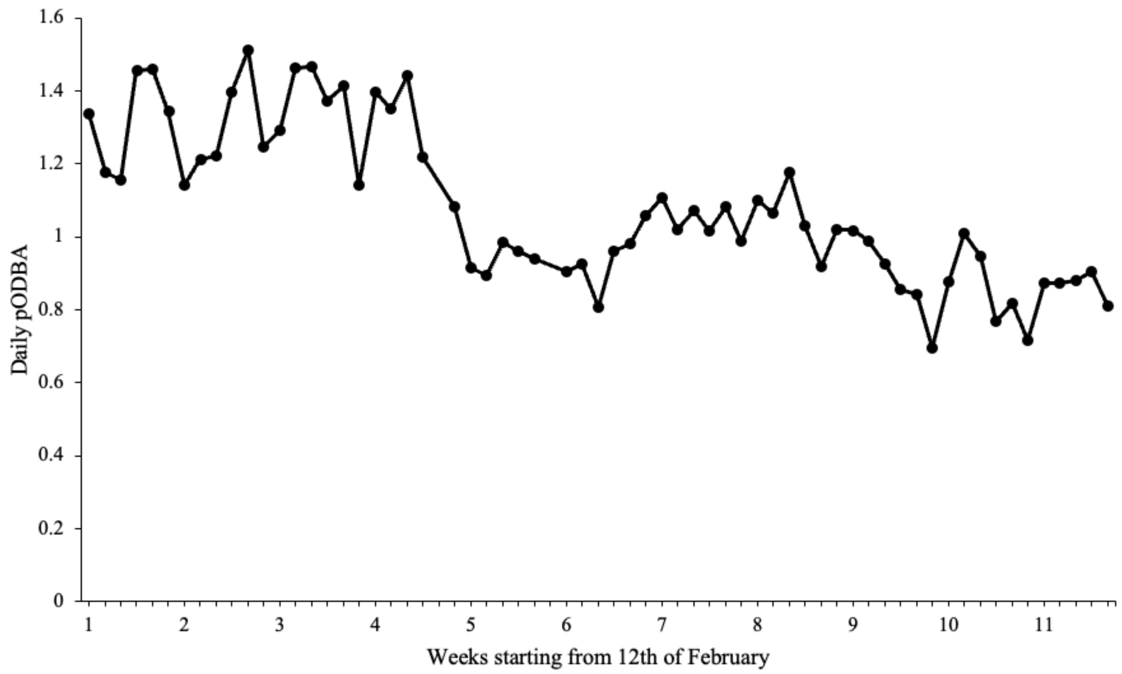

Figure B5 Proportional overall dynamic body accelertation (pODBA) of ewe lamb 139 over the study period.

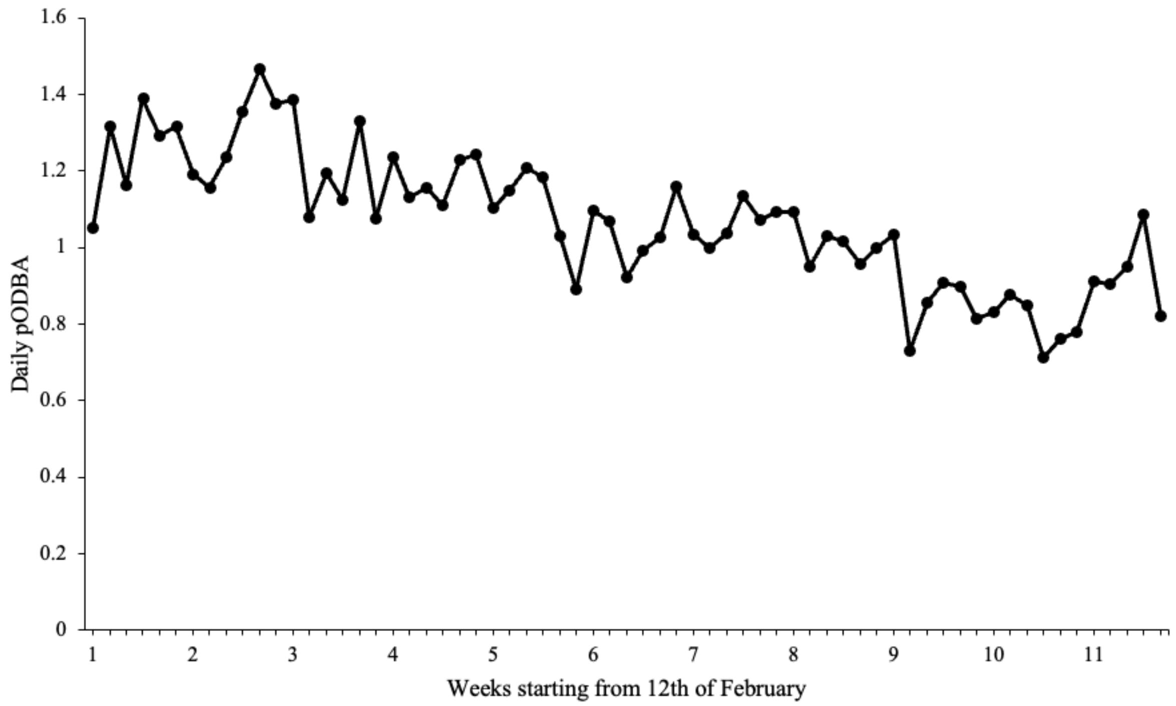

Figure B6 Proportional overall dynamic body accelertation (pODBA) of ewe lamb 232 over the study period.

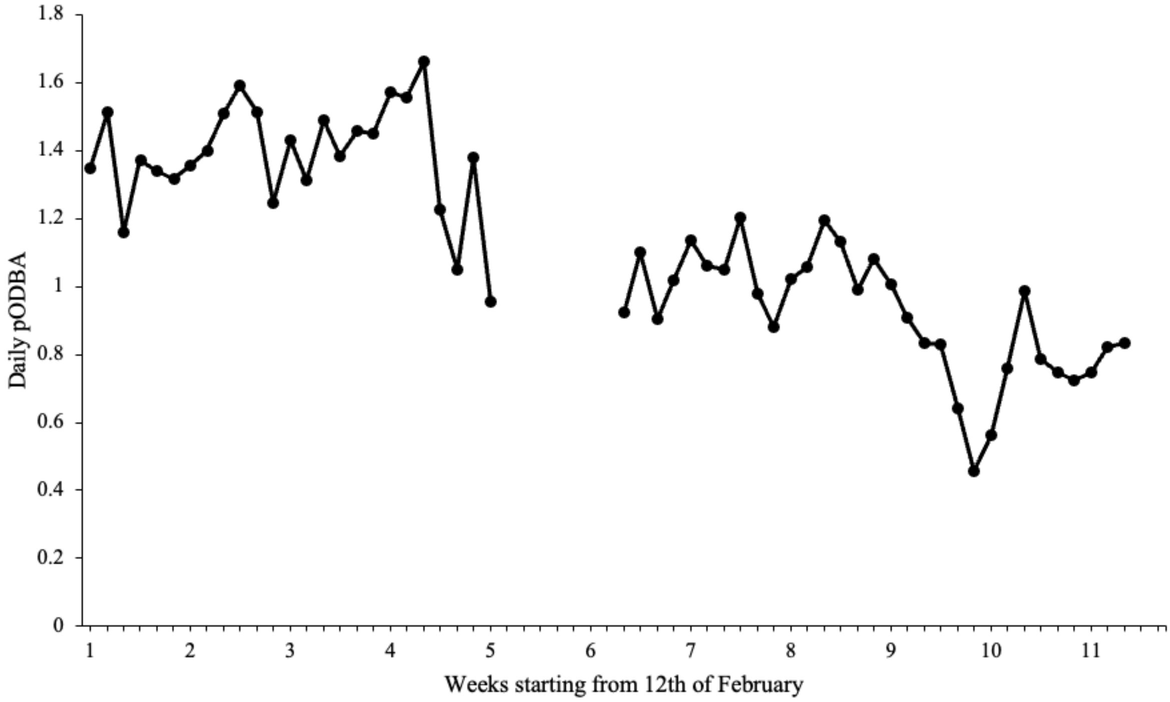

Figure B7 Proportional overall dynamic body accelertation (pODBA) of ewe lamb 273 over the study period. Due to water damage to the accelerometer, data was unavailable for the period between weeks 5 and 6.

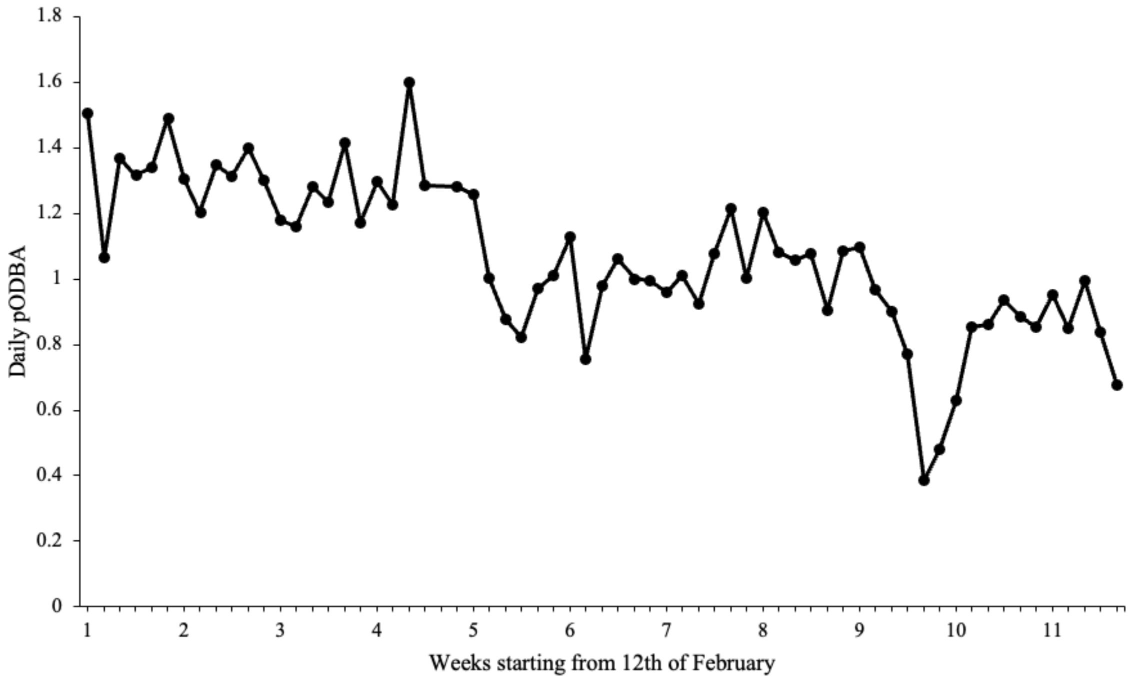

Figure B8 Proportional overall dynamic body accelertation (pODBA) of ewe lamb 3192 over the study period.