Application of a scoring system for nasal injuries caused by nose flaps in beef calves

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ABSTRACT

The weaning period is often regarded as one of the most challenging times in a beef calf's life where they must establish independence from their dam. Rather than abruptly severing the maternal-offspring bond, alternate weaning methods were developed to make this process more gradual. In Chapter 1, I introduced and compared different approaches for weaning beef calves in the United States. Two-stage methods, such as fence line and nose flap were developed for reducing the behavioral and physiological stress response that calves undergo by providing a physical barrier, either a fence or nose flap, that allows the calf to be weaned from milk prior to separation from the dam. Although these are both thought to have a net positive effect on calf welfare, the objective of the current study was to explore both positive and negative consequences. In Chapter 2, one potential tradeoff made when utilizing a nose flap for weaning – injuries was investigated. While a nose flap has been shown to be beneficial in minimizing the behavioral distress response, there are several types commercially available, and recent findings showed that they cause injuries on the calf's nasal septum. The objectives were to (1) identify if a nose flap created injuries on the nasal septum, (2) explore whether factors like calf body weight or septum size could be used to predict the incidence of injuries or flap loss; and (3) create a binomial scoring system that could use to reliably score characteristics observed. While no injuries were observed in calves that had not worn the nose flap, it was found that after 7 d of wearing them, injury was ubiquitous at the time of removal and still visible at least 6 d afterwards. Injuries and flap loss were widespread; therefore, body weight or septum size of the calf had no effect. Wound descriptions were scored in duplicate by a trained observer (damage, impression, and blood; 97%, 91%, and 100% agreement between first and second evaluations, respectively), indicating that scoring system proposed is repeatable. The outcomes of this

research have the potential to improve beef calf welfare and provide a valuable basis for industry recommendations regarding weaning methods for calves. Injuries inflicted from a nose flap may counteract the benefits to this method, making it less advantageous than other two-stage alternatives, like fence line weaning.

CHAPTER 1

Introduction

The maternal-offspring bond in cattle

Like many young animals, calves have the capacity to forge a strong, specific bond with their dam and heavily depend on them for receiving resources and protection (Newberry and Swanson, 2008). This bond is established at birth when changes in hormone concentrations (von Keyserlingk and Weary, 2007) trigger maternal behaviors in the dam (Orihuela et al., 2021). After birth, the dam licks amniotic fluid off the calf to stimulate them to stand and search for the udder (Jensen, 2012), while the calf simultaneously becomes familiar with their dam's vocalizations (Marchant-Forde et al., 2002). The effect of suckling on the establishment of a maternal-offspring bond is unknown. Calves still show preferences for their dam even when suckling was prohibited (Johnsen et al., 2015) and cows are motivated to reunite with their calves after being separated, especially if suckling is allowed (Wenker et al., 2020). A cow acts as the primary social contact to her offspring (Jensen et al., 2017). Although the formation of a strong maternal-offspring bond is prevented in most dairy systems, Stěhulová et al., (2008) found that dairy calves show preferences for their dams' vocalizations after 24 h together in a maternity pen. The behavioral responses to separation are more intense and last longer if the cow and calf are kept together for a longer period of time (e.g. Stěhulová et al., 2008, Johnsen et al., 2018). Beef cattle are typically raised in extensive systems, so establishment of a maternal-offspring bond is important for offspring survival. Calves heavily rely on their dams for nutrition (Johnsen et al., 2018), immunity (von Keyserlingk and Weary, 2007), protection (Kluever et al., 2008), and social learning (Jensen et al., 2017).

Natural weaning considerations

In natural or feral settings, cows and calves remain together as a herd and are not separated. Natural weaning involves a gradual decrease of the offspring's milk intake and a gradual increase of social independence over several months (Martin, 1984; von Keyserlingk and Weary, 2007; Weary et al., 2008). This process is not well understood in domestic cattle, but the maternal-offspring conflict theory (Trivers, 1974) suggests that the dam invests high levels of maternal care when the offspring is young and fully dependent for resources. Maternal investment decreases overtime, as the offspring can obtain their own resources (Trivers, 1974). Cows allow fewer suckling attempts as the calf ages (Reinhardt and Reinhardt, 1981; Vitale et al., 1986) and refuses suckling attempts when her next calf is born (Reinhardt and Reinhardt, 1981; Veissier and le Neindre, 1990). The age at which a cow weans her calf is highly variable. It has been observed between 7-14 mo, averaging around 10 mo (Reinhardt and Reinhardt, 1981). Calf sex also appears to effect weaning age. Reinhardt and Reinhart, (1981) reported that female calves were weaned by their mothers approximately 2.5 mo earlier than males of the same age.

Caretaker imposed weaning/ Traditional weaning

Natural weaning is not typically utilized in the United States because herd managers adjust their protocols for a variety of reasons such as forage availability or cow condition (USDA, 2020). Additionally, calves are often weaned to promote and maximize reproductive success of the cows (USDA, 2020) and allows for specialized feeding and marketing of the calves (Myers et al., 1999).

The beef calf weaning process imposed by caretakers occurs sooner than it would naturally (von Keyserlingk and Weary, 2007). This process often combines nutritional, social, psychological, and physical stressors (Weary et al., 2008). Beef calves are often subjected to multiple husbandry practices during weaning, such as frequent handing by humans, vaccinations, mixing of unfamiliar cattle, castration, dehorning, and transportation to new environments. In commercial settings, this can be anywhere from 4-8 mo of age (USDA, 2020). At this stage, calves may still be nutritionally and socially dependent on the cow (Weary et al., 2008) and have intense behavioral responses to separation.

The most intense response occurs in the first 48 to 72 h after separation (Price et al., 2003; Haley et al., 2005; Veissier and le Neindre, 1989), but can last several days (Haley et al., 2005). Calves will perform reinstatement behaviors, such as vocalization and walking, which can be indicative of distress (Haley et al., 2005; Latham and Mason, 2008; Enríquez et al., 2010) but may also help them reunite with their dam (Watts and Stookey, 2000; Newberry and Swanson, 2008; Weary et al., 2008). Vocalizations may also indicate that the calf is hungry, as illustrated by 5 – 6 wk old dairy calves vocalizing intensely when weaned from milk (Thomas et al., 2001). An increase in activity, typically described as walking (Haley et al., 2005; Solano et al., 2007), pacing (Price et al., 2003; Ungerfeld et al., 2009; Enríquez et al., 2010), seeking (Enríquez et al., 2010), as well as restlessness in beef (Price et al., 2003; Haley et al., 2005) and dairy calves (Loberg et al., 2008) are used to identify distress associated with weaning.

Abrupt weaning

The traditional method of weaning calves, commonly referred to as abrupt weaning, is to separate calves and move them to a new location where visual or auditory communication is not possible between mother and offspring. With this method, the calf is likely challenged with an

abrupt loss of milk as a dietary component (Haley et al., 2005), a change in social structure (Veissier and le Neindre, 1989), and a novel environment (Weary et al., 2008) all at once. Abrupt weaning is well documented as a stressful event for both the cow (Lynch et al., 2010a) and the calf (Price et al., 2003; Enríquez et al., 2010; Lynch et al., 2010b), although the behavioral response is reported to last longer and be more intense in calves (Price et al., 2003).

Abrupt weaning is known to cause distress in calves that experience it, illustrated by intense behavioral responses, decreased growth rate, and physiological parameters that are affected. When compared to un-weaned calves or those kept with their dam, abruptly weaned beef (Price et al., 2003) and water buffalos (de la Cruz-Cruz et al., 2021) spent more time walking and more time vocalizing. Price et al. (2003) observed vocalizations in weaned calves that were 2000-4000 times greater than un-weaned controls. Abrupt weaning also reduced play behaviors in yak calves after weaning compared to un-weaned (Liu et al., 2018). Additionally, abruptly weaned calves gained less weight daily following separation (Freeman et al., 2021), spent less time eating (Price et al., 2003) than their un-weaned counterparts. Abruptly weaned calves have markedly greater physiological indicators of stress, such as a blood cortisol concentration (de la Cruz-Cruz et al., 2021), noradrenaline concentrations (Hickey et al., 2003), blood glucose or blood lactate levels (de la Cruz-Cruz et al., 2020) compared to un-weaned calves. Lastly, abrupt weaning can impair immune function. This has been illustrated by greater neutrophil counts in weaned compared to un-weaned calves (Hickey et al., 2003; Lynch et al., 2010b; O'Loughlin et al., 2014; Lynch et al., 2019).

Fence line Weaning

Two alternative methods of weaning intended to make it a more gradual and less stressful process than abrupt, fence line and nose flap weaning. Unlike abrupt weaning, the two-stage

approach aims to prevent suckling between cow-calf pairs in stage 1 and then physically separate them in stage 2 (Price et al., 2003; Haley et al., 2005). By spacing out these stages, it is thought to have a beneficial effect on calves during the transition to being fully independent (Weary et al., 2008).

Fence line weaning involves separating the calves from their dams with a fence and housing them in an adjacent pen. The calf cannot suckle, but visual and auditory communication can be maintained (Stookey et al., 1997) through the fence. Price et al. (2003) and Stookey et al. (1997) both reported that fence line weaned calves vocalized and paced less than their abruptly weaned counterparts. Calves weaned with a fence line also showed greater weight gain overall following weaning (Price et al., 2003; Taylor et al., 2020) and had to be treated less than those abruptly weaned (Boyles et al., 2007). This method has been shown to have little effect on the markers of oxidative stress (Burke et al., 2009). One challenge with this type of weaning is that fencing structure is necessary and this may not be feasible for all herds. Fence line weaning has received relatively little scientific attention, perhaps because of physical requirements and replication necessary.

Nose flap weaning method

The other two-stage method of weaning is the utilization of an anti-suckling device, such as a nose flap. In the first stage, a plastic (Haley et al., 2005; Lambertz et al., 2015) or metal (Lambertz et al., 2015) flap is inserted into the nose and is held in place by the calf's septum. The flap acts as a barrier that prevents the calf from suckling while physical, visual, and auditory contact with the cow is maintained (Haley et al., 2005; Orihuela et al., 2020). The second stage is to remove the flap, and then physically separate the cow and calf. The flap can be removed as early as 4 d after insertion (Haley et al., 2005) and removal is recommended on or before 7 d

(QuietWean.com). Nose flaps have been fitted onto the calf septum for 5 d (Valente et al., 2022), 6 d (Taylor et al., 2020), and 7 d (Burke et al., 2009; Lambertz et al., 2015; de la Cruz-Cruz et al., 2020; Freeman et al., 2021). Although not recommended, periods longer than 7 d have also been evaluated (Enríquez et al., 2010; Alvez et al., 2016; Lippolis et al., 2016) to align with other aspects of weaning procedures, such as vaccination schedules.

Previous research reported that calves weaned with nose flaps performed less vocalizations compared to abruptly weaned (Enríquez et al., 2010; Alvez et al., 2016; Freeman et al., 2021). Additionally, calves weaned with a nose flap showed less walking behaviors compared to abruptly weaned calves (Haley et al., 2005; Enríquez et al., 2010; Alvez et al., 2016). Haley et al. (2005) reported more resting behavior overall in nose flap weaned calves, although activity varied between trials. Compared to the preweaning observations, walking increased in the first 2 d after flap placement (Lambertz et al., 2015) but returned to baseline thereafter. Calves with nasal flap spent more time in close proximity to their dams in the first 2 d after flap insertion compared to those that were fence line weaned (Enríquez et al., 2010).

Nose flap weaning literature is mixed regarding weight gain and physiological parameters. Firstly, daily weight gain of the calves that experience it has been reported to vary across studies. Enríquez et al. (2010), Lippolis et al. (2016), Freeman et al. (2021) found that nose-flap weaned calves had a greater weight gain throughout the time that the flaps were inserted but had less overall average daily gain (ADG) compared to abrupt and fence line weaned calves. Alternatively, calves had less daily weight gain when the nose-flap was in place, but showed greater (Haley et al., 2005; Valente et al., 2022) or similar (Burke et al., 2009) weight gains compared to abruptly weaned calves when weighed after separation. Some studies report that the flap has no effect on overall weight gain (Haley et al., 2005; Burke et al., 2009;

Lambertz et al., 2015; Alvez et al., 2016) between abrupt and nose flap. Nose flap weaning is not associated with physiological parameters, such as cortisol and haptoglobin levels, beyond the normal range in beef (Freeman et al., 2021) and dairy calves (Loberg et al., 2008).

Unlike abrupt and fence line weaning, several studies mention the occurrence of injuries that are inflicted on the nasal septum from wearing a nose flap. These have been referred to as modest to moderate nasal injuries (Lambertz et al., 2015; Taylor et al., 2020), hemorrhage, ulceration, erosions (Taylor et al., 2020), abrasions (Lambertz et al., 2015), lesions (Freeman et al., 2021) or open wounds (Valente et al., 2022). Even more serious or life-threatening abscesses or infections (Fernandes et al., 2000; Loretti et al., 2003) have been reportedly caused by wearing of nose flaps.

Although many studies describe injuries or blood were noticed upon nose flap removal, few attempted to quantify or characterized them. Freeman et al. (2021) mentioned bloody sores or nasal lesions were observed in 12 of 38 calves with nose flaps. Taylor et al. (2020) reported that hemorrhages, ulcerations, and erosions were seen in many calves upon nose flap removal but did not detail these in the paper, aside from stating that there were no long-term complications. More serious implications include pituitary abscesses, neurological problems and even death. Loretti et al. (2003) and Fernandes et al. (2000) both described incidence of infection following the fitting of nose flaps that led to pituitary abscess syndrome. Although uncommon, a small percentage (>1.5%) suffered serious neurological symptoms and death due to infection that traveled to the brain (Fernandes et al., 2000; Loretti et. al., 2003).

Nasal injuries have only been characterized in 2 studies. Lambertz et al. (2015) evaluated nasal abrasions associated with the use of plastic and metal nose flaps held in place for 7 d and observed nasal abrasions in over 95% of calves at flap removal, and 58% of those had slight or

heavy bleeding. Lambetz et al. (2015) reported that metal nose flaps created pus or deep purulent wounds in the nasal septum for 25% of the calves that wore them, therefore plastic materials should be favored. One wk later, 45% of all calves that received a nose flap showed indication of injury (Lambertz et al., 2015). Recently, a case study by Valente et al. (2022) found that all calves (41 of 41) were identified as having an injured nasal septum with or without secretion at flap removal, including calves that lost them before they were removed on d 5.

The closest information, in terms of the functional aspects of attachment to the nasal septum, is how many flaps were lost before removal. Flap loss is a concern when using this weaning method because if the flap were to fall out before the calf is effectively weaned from milk, suckling can be resumed. Then, when the calf is separated from its dam, this process would resemble abrupt weaning. While Haley et al. (2005) and Taylor et al. (2020) reported less than 5% flap loss over multiple trials, others have reported 24% (Lambertz et al., 2015) or even 27% loss (Valente et al., 2022). At the time of the current study, the relationship between calf size or nostril septum width measurement and nose flap loss had not been evaluated.

Cattle producers have options when choosing which weaning method to use on their beef calves, but this choice is complex and seems to involve tradeoffs between a method that has a net positive welfare outcome, but negative drawbacks. Overall, two-stage methods are thought to have overall welfare benefits over abrupt weaning. With this in mind, fence line weaning requires costly infrastructure, such as a sturdy fence that can keep cows and calves separated. Nose flap weaning does not require any additional facilities to be built as long as a working chute is available and utilizes relatively inexpensive flaps, but the incidence of injury and flap loss are concerns to be explored further.

Objectives

To accurately and reliably describe nasal injuries inflicted by a nose flap, a common type of nose flap (QuietWean nose paddle) available in the United States, was utilized. In Chapter 2, the objectives were to identify if a nose flap created injuries on the nasal septum, (2) explore whether factors like calf body weight or septum size could be used to predict the incidence of injuries, or flap loss; and (3) create a scoring system that could use to reliably score characteristics observed. Chapter 2 is in preparation for Translational Animal Science and is formatted in accordance with their guidelines.

CHAPTER 2

Development and application of a scoring system for septum injuries in beef calves with and without a nose flap

ABSTRACT: The weaning period is a stressful time for beef calves because they must quickly gain nutritional and social independence from their dam. Gradual methods of weaning, such as when the calf is fitted with a nose flap to prevent suckling, are known to reduce the behavioral and physiological indicators of stress. Nose flaps are held in place by the nasal septum and are worn for 4 to 7 d. In the present study, the objectives were to 1) identify if a plastic nose flap worn for 7 d caused nasal injuries, (2) identify if factors like calf body weight or septum size predict injuries or flap loss, and (3) create a scoring system that could reliably score wound characteristics. Eighty-two (N = 82) Angus and Angus-Hereford crossbred beef calves were randomly assigned to 'Flap' or 'No Flap' treatments. Calves weighed 247 ± 29 kg and those with a flap had septums that were 39 ± 2 mm (mean ± SD). Images were taken of each nostril before flap insertion, on the day of removal, and 6 d after removal. Wounds were scored for the presence/absence of 3 characteristics in either nostril: damage (tissue where the flap rested was a different color than surrounding nostril), impression (edges of the wound were clearly raised or sunken), and blood (bright red liquid). One trained observer scored a subset of photos (N = 64) twice, in a consistent manner for all 3 characteristics (damage, impression, and blood; 97%, 91%, and 100% agreement between 1st and 2nd evaluations, respectively), indicating that our system is repeatable. Thirty-two percent of calves in the Flap treatment lost their flap before the day of removal. No calves in the No Flap treatment were injured. All animals that kept their flap in for 7 d had damage and impressions in at least 1 nostril and 86% of calves had blood present immediately after nose flap removal ($P \leq 0.001$ compared to No Flap) indicating that the flaps

altered the nasal tissue and created open wounds. Six d after flap removal, 100% still had visible damage, 64% had impressions, and 29% had blood, indicating that while damage is longer lasting, wounds can start to repair after the flap is removed. Injuries were prevalent in all calves, thus there was no relationship between calf size (body weight or septum width) on these wounds ($P \geq 0.374$). Body weight or septum size did not differ ($P \geq 0.489$) between calves that kept or lost their flap. Injuries inflicted from a nose flap may counteract the previously-documented benefits of this method, making it less advantageous than alternatives, like fence line weaning.

Key words: beef, calf, injury, nose flap, two-stage, wean

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INTRODUCTION

The weaning period is a particularly stressful time for beef calves because they must quickly establish nutritional and social independence from their dam (Weary et al., 2008). In the United States, beef calves are typically weaned from their dams between 4 to 8 mo of age (USDA, 2020). At this time, calves may be abruptly separated and experience changes in their diet, social structure, and their environment all at once. When separated, abruptly weaned calves responded with more behaviors that are indicative of distress like vocalization and walking compared to calves who underwent a gradual weaning method (Price et al., 2003; Haley et al., 2005). Calves who were abruptly weaned also gained less weight after separation compared to calves when the process was gradual (Price et al., 2003; Taylor et al., 2020). Biomarkers indicative of stress, such as increased blood cortisol or noradrenaline were markedly greater in abruptly weaned calves compared to gradually weaned counterparts (Hickey et al., 2003; de la Cruz-Cruz et al., 2021). In addition, an altered immune response and neutrophil concentrations was found in calves that were abruptly weaned compared to gradual methods (O'Loughlin et al., 2014; Lippolis et al., 2016; Lynch et al., 2019).

A purposed method to mitigate the distress that calves experience at weaning is the utilization of an anti-suckling device, such as a nose flap. In this two-stage method of weaning, a plastic or metal flap is inserted into the nose and is held in place by the calf's septum in the first stage (Haley et al. 2005, Lambertz et al., 2015; Taylor et al., 2020). This provides a physical barrier that prevents the calf from suckling while maternal-offspring contact is maintained to simulate a more naturalistic weaning process (Haley et al., 2005; Weary et al., 2008). After a given time period, the second stage involves the removal of flap and then physical separation from their dam. Calves weaned with a nose flap demonstrated fewer behaviors indicative of

distress such as vocalization and locomotion than abruptly weaned calves (Haley et al., 2005; Loberg et al., 2008; Lambertz et al., 2015). When compared to abrupt weaning, calf weight gain was not affected by the nose flap (Burke et al., 2009; Lambertz et al., 2015; Alvez et al., 2016). Some researchers have reported that calves had a decrease in daily weight gain when the nose flap was in place, but similar overall gains compared to abruptly weaned calves (Haley et al., 2005; Burke et al., 2009; Valente et al., 2022). Plasma cortisol concentrations remained in normal range 0.3 μg/dL (Hopster et al., 1999) to 5.5 μg/dL (Doornenbal et al., 1988) for calves that were weaned with a nose flap (Freeman et al., 2021) suggesting that this method did not cause additional stress to the calves. Overall, there are behavioral advantages to nose flap weaning, and even if the physiological indicators may be initially negative calves are expected to recover from the stress relatively quickly.

Although weaning with a nose flap is generally seen as having net gains for animal welfare, there have been concerns about injuries inflicted on the nasal septum during the process. Modest to moderate nasal injuries (Lambertz et al., 2015; Taylor et al., 2020), hemorrhage, ulceration, erosions (Taylor et al., 2020), abrasions (Lambertz et al., 2015), lesions (Freeman et al., 2021) or open wounds (Valente et al., 2022) have been observed at the time of flap removal. Although uncommon, more serious, or life-threatening abscesses (Fernandes et al., 2000; Loretti et al., 2003) have also been reportedly caused by wearing of plastic nose flaps.

To date, nose flap induced injuries have only been characterized in 2 studies. Lambertz et al. (2015) and Valente et al. (2022) both developed scoring systems that assigned a score or grade to the injury to classify its severity. Lambertz et al. (2015) evaluated nasal abrasions associated with the use of plastic and metal nose flaps held in place for 7 d and observed nasal abrasions in over 95% of calves at flap removal. Of these animals, 27% had slight irritation, 58%

of those had slight or heavy bleeding, about 7% had pus, and 4% had deep purulent wounds (Lambertz et al., 2015). Similarly, Valente et al. (2022) identified presence of injury and different types of secretion; including blood, and translucent or purulent secretion, and found that all calves (41 of 41) all fell into one of the 'injured with or without secretion' categories at flap removal. Both studies had mutually exclusive descriptions for wounds, scored live, and did not have controls where calves were kept in the same environmental conditions to ensure that injuries were solely caused by the nose flap. All these factors may have affected external validity and repeatability of the scoring systems.

Multiple types of nose flaps are commercially available and have been studied in a range of calf ages and sizes. Throughout these different designs and dimensions the goal of the nose flap is the same: to stay in place while the calf if being weaned. To do this, the flap must have a narrow gap to hold it in place, but it is common for these methodological details to be omitted in the description of the flap design. The loss of nose flaps has been indicated as concern for weaning *Bos taurus* (Lambertz et al,. 2015) and *Bos indicus* (Valente et al., 2022) breeds. The closest information in terms of the functional aspects of attachment to the nasal septum is how many flaps were lost before removal. While Haley et al. (2005) reported less than 5% flap loss, others have reported 24% (Lambertz et al., 2015) or even 27% (Valente et al., 2022) loss of the flap before calves had them manually removed. At the time of the current study, nose flap loss has not been evaluated in relationship to calf size or nostril septum width measurement.

The present study investigated nasal injuries inflicted by nose flaps and examined the relationship between calf weight or nasal septum size and these wounds. The objective was to develop a reliable scoring system to characterize any wounds observed. It was hypothesized that

heavier calves or those with wider nasal septum measurements would have more injuries inflicted by the nose flap.

MATERIALS AND METHODS

Animals and housing

All procedures involving animals were approved by the University of California-Davis Institutional Animal Care and Use Committee (Protocol #22254). This study was conducted at the Sierra Foothill Research and Extension Center (Browns Valley, CA) facility from May 2021 through June 2021. Eighty-two (N = 82) Angus and Angus-Hereford crossbred beef heifer (n = 37) and steer (n = 45) calves were utilized. Sample size was determined based on availability of calves and feasibility of restraint and photography in the time we had allotted for this study. The mean age of calves enrolled in this study was 205 ± 19 d old and weighed 247 ± 29 kg, ranging from 186 to 306 kg (mean ± SD, min to max values). Regardless of treatment, all calves were weaned with the fence line method, where they were placed in an adjacent pasture that separated them from their dams with a fence, as part of another project. On the day of weaning, calves were handled in a squeeze chute, weighed, and flaps were inserted. Calves were housed on rangeland pasture for the entirety of the study, all under the same conditions, allowing us to equalize the environmental risk of injury from non-flap sources.

Experimental Design and Treatments

Calves (N = 82) were randomly assigned to one of two treatments, balanced for body weight, and checked that calf sex was balanced between treatments. On the day of flap insertion, all calves were gathered, restrained in a squeeze chute, then vaccinated, and weighed. Images of each nostril were taken before flap insertion with a Nikon D5300 camera with 18-55 mm

NIKKOR VR II lens kit attachment (Nikon Inc. Melville, NY). A ring light, ProMaster RL100 Macro LED Ring Flash (ProMaster; Fairfield, CT) was used to illuminate and provide consistent lighting. Images were taken of the left and right inner nostril and focused on the septum for all animals; all images were taken approximately 30 cm away. Then, calves who received the nose flap treatment (n = 41) had plastic nose flaps (Quiet Wean; JDA Livestock Innovations, Ltd., Saskatoon, Canada) inserted.

All calves were gathered 7 d after flap insertion (FLAP REMOVAL). The calves who received nose flaps and kept them for the entire 7 d (n = 28) had them removed. These calves and were noted as FLAP ENTIRE. Calves who lost the flap before the 7-d period ended (n = 13 or 32%) were noted as FLAP PARTIAL. Images of left and right inner nostrils were taken at flap removal with the same photography procedure.

A third set of images were taken a further 6 d after flap removal (6 D AFTER) for all calves that received nose flaps (FLAP ENTIRE and FLAP PARTIAL). Additionally, septum measurements were taken with a 150 mm MC1630EWRI Digital Caliper (Mahr GmbH; Göttingen, Germany). Measurements were the distance across the nose, between the nostrils as a proxy for the septum width.

Images were assessed and scored by a single trained observer using the nasal injury definitions outlined in Table 2.1. The observer scored a subset of photos (n = 64 nostrils or 32 calves) twice, in a consistent manner for all 3 characteristics (damage, impression and blood, 97%, 91% and 100% agreement between first and second evaluations, respectively). Then this observer (AAK) scored the photos from all 3 time points (before insertion, at removal, 6 d after removal). Images taken after removal of the nose flaps were compared to the same animal before

nose flap insertion to identify natural color variation in the nostril. If the injury was occluded by dirt, the image was scored "NA". The observer was not blinded to the treatments.

Statistical Analysis

Initially, 82 calves were included in this study and half (n = 41) received the flap treatment. The 13 FLAP PARTIAL calves were only included in data analysis comparing them to FLAP ENTIRE. Two of the calves in the NO FLAP treatment were excluded due to being missed during a handling event. A total of 3 characteristics (1 damage and 2 impression) were scored "NA" due to a blurry photo or dirt in the wound making it difficult to score.

Injury data were entered in Microsoft Excel (Redmond, WA; 2016) and it was noted if each calf had a given characteristic in at least 1 nostril. Analyses were conducted in R version 4.1.2 (R Core Team, 2021) via R Studio version 2021.09.02 (R Core Team, 2021). Presence of impression, damage, and blood in at least 1 nostril for FLAP ENTIRE and NO FLAP calves were analyzed with separate Fishers exact tests for each type of injury in the scoring system (*fisher.test* function, stats package version 4.1.2). Relationships between each injury characteristic and body weight, as well as each characteristic and septum size, were analyzed with a binomial regression (*glm* function, dplyr package version 1.0.9) with weight and septum size specified as a fixed effect and family entered as binomial (link = "logit"). Analyses comparing body weight and septum size between FLAP ENTIRE and FLAP PARTIAL calves was done with a two-sample T test (*t.test* function, base R version 4.1.2) with equal variance specified.

RESULTS

No calves (0 of 39) in the 'No Flap' treatment had damage, impression, or blood present on d 7. In contrast, all calves that kept their flap in for 7 d had at least 1 nostril with damage (28 of 28) and impression (28 of 28), and 86% (24 of 28) had blood present in at least 1 nostril immediately after flap removal ($P \leq 0.001$ compared to No Flap) for each characteristic (Table 2.2). A description of whether an injury characteristic occurred in one, or both nostrils is outlined in Table 2.3.

Wounds were scored 6 d after flap removal and 100% (28 of 28) of calves had at least 1 nostril that still had visible damage, 64% (18 of 28) of calves had impressions, and 29% (8 of 28) had blood present.

Body weight did not have an effect on damage, impression, or blood presence on the day of removal ($P \geq 0.824$) or 6 d afterwards ($P \geq 0.632$) (Fig. 2.1). Similarly, septum width did not have an effect on damage, impression, or blood on the day of flap removal ($P \geq 0.797$) or 6 d afterwards ($P \geq 0.374$) (Fig. 2.2).

Calf body weight or septum width did not have an effect on flap loss. Calves that kept their flap for the entire 7 d weighed 249 ± 6 kg and those that lost their flap prior to removal weighed 242 ± 7 kg (mean ± SE) ($P = 0.463$). Similarly, septums of calves that kept the flap measured 39 ± 0.4 mm and those that lost it were 39 ± 0.6 mm (mean ± SE) ($P = 0.677$).

DISCUSSION

The present study investigated if nose flaps that were worn by beef calves for 7 d caused nasal injuries. Injury characteristics (damage, impression, or blood) were absent in calves that did not receive a nose flap. However, a high occurrence of nasal injuries in all calves who retained the flap for 7 d both at flap removal and 6 d afterwards was observed. Additionally,

nasal injuries were present across all body weights and septum widths, therefore, these factors did not have an effect on nasal injuries within the range of sizes in the current study. Lastly, it was demonstrated that these wounds could be scored in a repeatable and consistent manner.

Although nose flaps used in weaning calves has have been regarded as a weaning method that can reduce behavioral signs of distress like vocalization and walking when compared to abruptly weaned calves (Haley et al., 2005; Enriquez et al., 2011; Alvez et al., 2016; Freeman et al., 2021), the incidence of nasal injury must be considered because it is a potential drawback to using this method. While several studies simply mentioned nasal injuries, Lambertz et al. (2015) and Valente et al. (2022) developed scoring systems to score or grade to the injury based on its severity. Lambetz et al. (2015) used an ordinal scale with scores 0 through 6. A score 0 meant no irritations while 6 indicated a perforated septum or fatal wound (Lambertz et al., 2015). Valente et al. (2022) identified presence of injury and different types of secretion; including blood, and translucent or purulent secretion. While these were instrumental in describing the incidence and merit of wounds, these scoring systems had mutually exclusive descriptions of each wound characteristic, were scored live, and had a no flap control in the same environment. In contrast, the current study captured high-definition images of nasal injuries and scored these for absence or presence of each wound characteristic, where each component was scored on its own right, and were not mutually exclusive. Authors in the current study also ensured that the images were reliably scored in duplicate to determine if this scoring system was repeatable over all time points. This approach also considered the natural variation of nostril color with our photos from before the flap was inserted. Additionally, No Flap calves acted as a control and allowed to rule out any external environmental factors that may have caused nasal injuries.

Nasal injury characteristics

A 100% prevalence of nasal damage at removal and 6 d after removal was observed in calves that received a nasal flap. This characteristic included any alteration caused by the nose flap that damaged the epithelial cells of the septum skin barrier. Bovine nasal septum cartilage has been described to have viscoelastic properties (Correro-Shahgaldian et al., 2016) and be surrounded by collagen fibers (Verwoerd-Verhoef et al., 1998). The inner-nasal lining is made of highly vascularized tissue that is covered by a continuous layer of mucous (Harkema et al., 2006). Alterations or damage to this tissue may be caused by the relatively small gap that holds the nose flap in place creating inward pressure on the nasal septum. The European Pressure Ulcer Advisory Panel (1998) defines a pressure ulcer as a localized damage to the skin or underlying tissues, caused by pressure, shear, friction or a combination of these factors. Valente et al. (2022) also described abrasions caused by the rubbing the nose flap against the skin of the inner septum. Additional factors, such as moisture from mucous could also contribute to the formation of superficial skin injuries (Kottner et al., 2009) and pressure that is not relieved over longer periods of time can cause deeper tissue damage or necrosis (Kottner et al., 2009). In the present study, no perforated nasal septums were observed, but substantial injuries to the superficial layer of the nasal septum, like those reported by Valente et al. (2022) was reported. Because this characteristic was still prevalent 6 d after flap removal (100%), it suggests that damage is longer lasting, but it is unknown how long they take to resolve.

Impressions were present at removal (100%) and became less common 6 d afterwards (64%). This characteristic described the edges of the wound as being slightly raised or sunken compared to the surrounding nostril tissues. It was noticed that the flap made a circular shaped indentation into the nasal tissues where it rested, similar to the wounds pictured in Valente et al., (2022). Bisang et al. (2022) also mentioned round superficial ulceration upon nose ring removal

in dairy calves. General wound healing progression includes 3 stages: inflammation, new tissue formation, and remodeling (Watelet et al., 2002; Gurtner et al., 2008). After the tissues are damaged and skin barrier is broken, cells proliferate from the edges of the wound in an effort to restore the protective barrier (Watelet et al., 2002; Gurtner et al., 2008). After the flap was removed, the nasal mucosa may have been proliferating cells along the edge of the damaged tissue as a result of inflammation, or an attempt to form new undamaged tissues. This may have also caused impression to vary in one or both nostrils.

Presence of blood was observed in 85% of calves on the day of flap removal and was reduced to 28% 6 d afterward. This characteristic could be indicative of the breakage of protective barriers and rupture of blood vessels in nasal septum tissue. Presence of blood was observed in fewer calves and fewer nostrils 6 d after flap removal, suggesting that there were fewer open wounds at that time. Freeman et al. (2021) mentioned bloody sores were observed in 32% (12 of 38) of calves with nose flaps, but blood was not explored in the scope of the study. Results from the current study resembled findings from Lambertz et al., (2015) when approximately 58% of calves that received nose flaps had slight or heavy bleeding at the time of flap removal, and only 30% had slight or heavy bleeding when observed 1 wk later. Although Valente et al., (2022) only fitted calves with a flap for 5 d, they found that 7% of calves had bleeding present at removal.

Although not addressed within the scope of this study, future research could focus on quantifying how much these nasal injuries matter to the calf that experiences them. A high occurrence of nasal injury characteristics was observed on the day of removal, but impression and blood decreased over the next week. It would be beneficial to know how long these injuries

last before they are fully healed, and if there was any evoked pain associated with grazing or eating during that time.

Nose flap fit

The fit of the nose flap appears to be an important consideration for this method. The flap must fit securely to the calf's septum, so it will not fall out before the herd managers remove it. If flap loss does occur, the calves could resume suckling, and advantages of a gradual weaning process would be lost. In the present study, there was a 32% (13 of 41) loss of the flaps before d 7. These calves were excluded in the analysis because they were housed on rangeland pasture, so it was difficult to determine how or when these calves removed them. Haley et al. (2005), reported that less than 5% lost their flap, while Lambertz et al. (2015) and Valente et al. (2022) reported a 24% and 27% loss, respectively. It is unclear why the current study had the highest percent of flap loss, because calf size was similar to the previous studies and the national average (USDA, 2020). Future research could include observing calves for attempts to remove the nose flap or behaviors like head shaking to identify if flaps are uncomfortable. Additionally, calves kept with their dams would be motivated to nurse, creating potential rubbing or friction induced injuries that may warrant research.

In this study, calves wore the nose flap for 7 d. Manufacturer recommendations indicate that flaps should be kept in for 4-7 d before removing them and separating the calf from the cow (QuietWean.com). Previous studies (Lambertz et al., 2015; Freeman et al., 2021) have also kept the flap fitted to the nasal septum for 7 d and mentioned nasal injuries. However, some studies describe the occurrence of nasal injuries when the flap was fitted for only 5 d (Valente et al., 2022) or 6 d (Taylor et al., 2020). Although not recommended, periods longer than 7 d have also been evaluated such as 14 d (Loberg et al., 2008), 17 d (Enriquez et al., 2010) or 21 d (Alvez et

al., 2016; Lippolis et al., 2016) to align with other aspects of weaning procedures, such as vaccination schedules. Based on our results, we would not recommend keeping the flap in for longer time periods.

CONCLUSIONS

In conclusion, this study contributed to our knowledge about gradual weaning methods. Our results suggest that all calves who kept a nose flap in for the entire 7 d had injuries, and these persist at least 6 d after removal. We developed a reliable scoring system to describe characteristics visible in these injuries. Calf weight or septum size did not predict the occurrence of injury characteristics or flap loss. While nose flap weaning conveys animal welfare benefits over abrupt weaning, unforeseen consequences of injury created by these devices makes them less advantageous than other gradual methods, such as fence line weaning. Future research is necessary to determine if the tissue damage is painful and how much it matters to the calf in terms of their behavior, feedlot transition, weight gain, and morbidity.

OVERALL CONCLUSIONS

This thesis explored and summarized animal welfare implications between alternative, two-stage methods of weaning and the potential tradeoffs that make choosing between them complex. While it seems clear that abrupt weaning is not optimal for calves, illustrated by an intense behavioral response to separation, reduced weight gain and elevated physiological indications of stress, additional research is needed to determine best management practices for non-abrupt weaning methods. Nose-flap weaning requires less infrastructure than using the fence-line approach and therefore may be more practical overall but has the potential downside of extensive and serious nasal injuries. To date, studies have focused primarily on the beneficial aspects of nose-flap weaning. Assessing nasal injuries caused by the nose-flaps for severity or duration plays an important role in this comparison and allow producers to make more informed management decisions that are beneficial for the welfare of their calves. In Chapter 2, we found that all calves that kept the nose flap in for 7 d suffered nasal injuries, and these could be described with an innovative scoring system, designed to capture certain characteristics (damage, impression, and blood) that were not mutually exclusive in the wound. These characteristics were still seen about 1 wk later. Weight or septum size did not seem to be a good predictor for which animals developed injuries or lost the flap within our population of calves, which were the average size for weaning in the United States.

Future work in this area could identify how much these nasal injuries matter to the calf that experiences them. Many (32%) of the calves in the current study lost their flap before the handling event where they would have been manually removed (7 d after placed), suggesting that calves attempted to remove the flaps themselves. Observing calves for removal attempts could provide additional insight into the experience and if the flaps were uncomfortable. We also

observed that all animals presented nasal injury characteristics on the day of removal, but impression and blood decreased over 7 days after removal. Damage was longer lasting, but it would be beneficial to know how long these injuries last before they are fully healed, and if there was any evoked pain associated with grazing or eating during that time. Different designs of nose flaps that are adjustable or made with different materials could also be explored further to mitigate risk of injury.

Overall, this work may allow for weaning strategies to be viewed more holistically and help producers identify which method works best for them. It was reported that the injurious nature of nose flaps in this study may counteract the net positive animal welfare benefits of two-stage weaning, making it less advantageous than other gradual weaning methods.

TABLES

Table 2.1. List of features scored, definitions and examples of nose flap injuries in weaned beef calves. Features are not mutually exclusive.

Feature	Definition	Example
No injury	Absence of all injury characteristics	
Evidence of damage (yes/no)	The tissue in the site where the flap would rest is a different color than surrounding nostril; natural variation in nostril color[1] (e.g. spots) are not counted as wound-related difference	
Visible impression (yes/no)	Edges of the wound are clearly raised or sunken, distinct from surrounding tissue. May be an entire or partial circle or oval	
Visible blood (yes/no)	Bright red liquid present in the nostril area, either in or around the site where the flap would rest	

[1]as assessed in photos before flaps were inserted

Table 2.2. Percent of animals with damage, impression or blood present in calves that received a nose flap and kept it for the entire 7 d (FLAP ENTIRE) or did not receive a nose flap (NO FLAP). Data were collected before the flaps were inserted, at flap removal and 6 d after flap removal.

	Damage		Impression		Blood	
	FLAP ENTIRE	NO FLAP	FLAP ENTIRE	NO FLAP	FLAP ENTIRE	NO FLAP
Before flap	0	0	0	0	0	0
Flap removal	100	0	100	0	86	0
6 d after removal	100	N/A	64	N/A	28	N/A

Table 2.3. Percent of animals with damage, impression or blood present in one or both nostrils. (FLAP ENTIRE). Data were collected at flap removal and 6 d after flap removal.

	Damage		Impression		Blood	
	ONE	BOTH	ONE	BOTH	ONE	BOTH
Flap removal	1/28	27/28	1/28	27/28	5/24	19/24
6 d after removal	0/28	28/28	11/18	7/18	8/8	0/8

FIGURES

Figure 2.1. Proportion of FLAP ENTIRE (n = 28) calves with at least one nostril with nasal wound characteristics (A) Damage, (B) Impression and (C) Blood, in relationship to the animal's body weight (in kg) on the day of removal and 6 d afterward (D, E, F). There were no animals with damage, impressions, or blood present in the NO FLAP treatment.

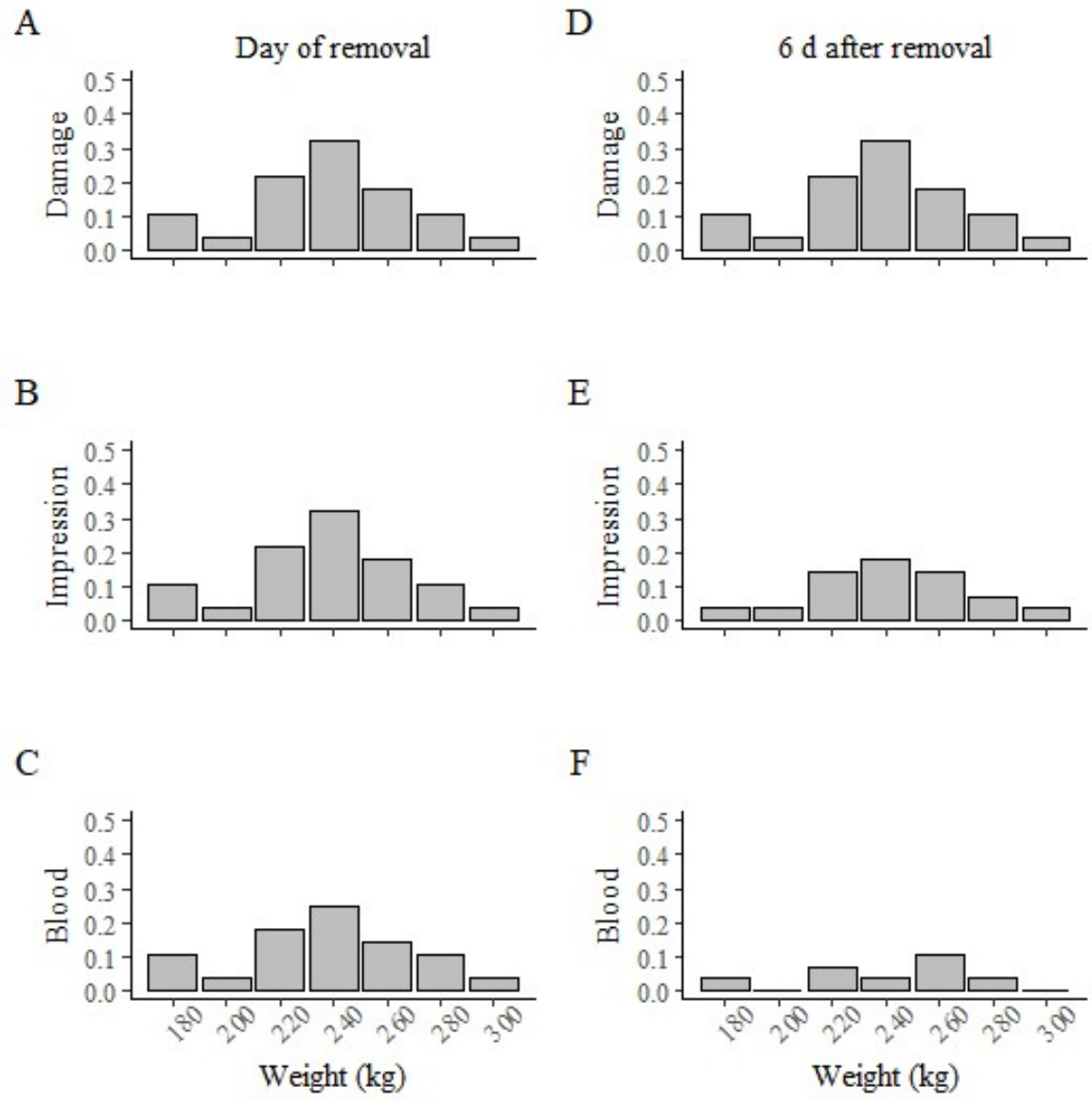
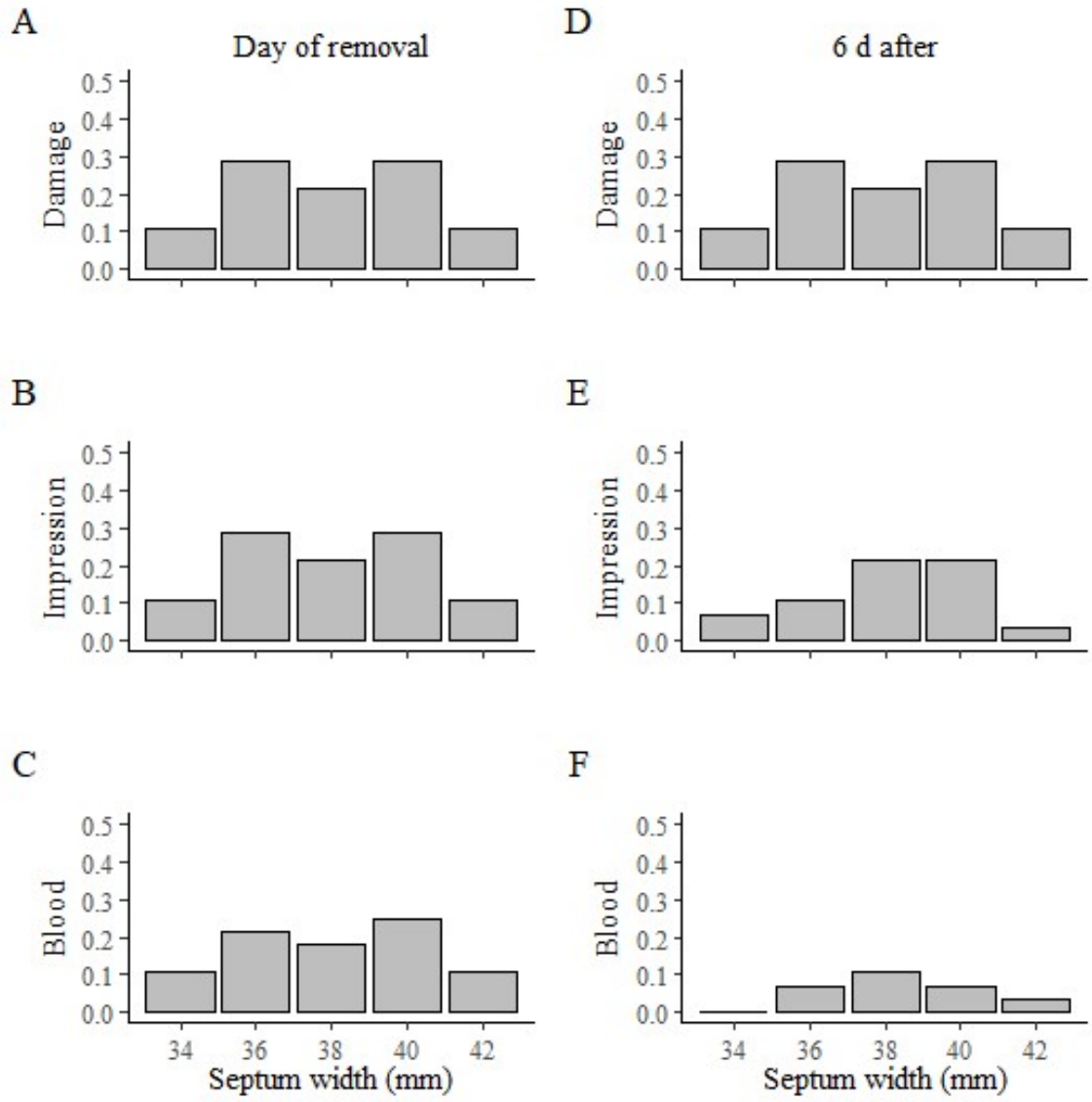


Figure 2.2. Proportion of FLAP ENTIRE (n = 28) calves with at least one nostril with nasal wound characteristics (A) Damage, (B) Impression and (C) Blood, in relationship to the animal's septum width (in mm) on the day of removal and 6 d afterward (D, E, F). There were no animals with damage, impressions, or blood present in the NO FLAP treatment.



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