JAST (J Anim Sci and Technology) TITLE PAGE

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Review article
Article Title (within 20 words without abbreviations)	The Male Effect and Pheromones in Bovines: Neuroendocrine Mechanisms and Strategic Applications for Cattle and Buffalo Production
Running Title (within 10 words)	Male Effect and Pheromones in Bovine Reproduction
Author	Junyoung Kim1, Minjung Yoon1,2,3,*
Affiliation	1 Department of Animal Science and Biotechnology, Kyungpook National University, Sangju, 37224, Republic of Korea 2 Research Institute for Innovative Animal Science, Kyungpook National University, Sangju, 37224, Republic of Korea 3 Department of Horse, Companion and Wild Animal Science, Kyungpook National University, Sangju, 37224, Republic of Korea
ORCID (for more information, please visit https://orcid.org)	Junyoung Kim (https://orcid.org/0000-0003-0711-5479) Minjung Yoon (https://orcid.org/0000-0001-9112-1796)
Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources	This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (RS-2024-00400429).
Acknowledgements	The authors acknowledge the members of the horse science lab for their assistance with this work.
Availability of data and material	Upon reasonable request, the datasets of this study can be made available from the corresponding author.
Authors' contributions	Conceptualization: Kim JY Funding acquisition: Yoon MJ Project administration: Yoon MJ Resources: Yoon MJ Supervision: Yoon MJ Writing - original draft: Kim JY Writing - review & editing: Kim JY, Yoon MJ
Ethics approval and consent to participate	This article did not require IRB/IACUC approval because there were no human or animal participants.

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Dr. Minjung Yoon
Email address – this is where your proofs will be sent	mjyoonemail@gmail.com
Secondary Email address	

Address	Department of Animal Science and Biotechnology, Kyungpook National University, Sangju 37224, Republic of Korea
Cell phone number	+82-10-5285-9850
Office phone number	Tel.: +82-54-530-1233
Fax number	Fax: +82-54-530-1959

ABSTRACT

Livestock reproductive efficiency is a cornerstone of global food security; however, conditions such as postpartum anestrus and silent ovulation remain significant challenges in cattle and buffalo production. Although conventional hormonal interventions are effective, increasing demand for “clean, green, and ethical” production has shifted the focus toward biostimulation as a non-invasive alternative, particularly through the male effect. This review provides a comprehensive analysis of male-specific pheromones in bovines, specifically cattle (*Bos taurus* and *Bos indicus*) and buffalo (*Bubalus bubalis*). The neuroendocrine pathways through which male-derived volatile compounds activate the hypothalamic–pituitary–gonadal axis are discussed, with emphasis on the primary role of the vomeronasal organ in bovine species. Pheromonal signals are shown to target KNDy neurons (kisspeptin, neurokinin B, and dynorphin neurons) in the arcuate nucleus, thereby resetting the gonadotropin-releasing hormone pulse generator and inducing ovulation. Furthermore, physiological and metabolic constraints, such as negative energy balance and heat stress, are evaluated for their effects on increasing the activation threshold of the KNDy network and limiting the efficacy of biostimulation. By integrating management strategies such as optimizing bull-to-cow ratios and ensuring environmental and social well-being of females, the strategic potential of pheromonal tools for managing reproductive disorders is highlighted. Overall, establishing a comprehensive management framework that balances biostimulatory cues with metabolic and environmental optimization offers a scientifically grounded and sustainable pathway for enhancing reproductive productivity in cattle and buffalo production systems.

Keywords:

Male effect, Pheromones, Biostimulation, Cattle, Buffalo, Postpartum anestrus

INTRODUCTION

Reproductive efficiency plays a crucial role in improving livestock production, particularly in cattle. Reproductive challenges, such as prolonged postpartum anestrus, significantly limit the overall productivity of dairy and beef herds. Among the various strategies employed to address these challenges, biostimulation, in which exposure to a male, particularly a bull, influences the female reproductive system, has garnered attention for its potential benefits.

Biostimulation involves the use of sensory cues, particularly chemical signals (pheromones), to stimulate hormonal and behavioral responses in female livestock [1]. Male pheromones, which are chemical signals released by males that influence female reproductive behaviors, play a critical role in livestock reproduction [2]. These pheromones are emitted through urine, feces, and skin glands [3] and can influence female hormonal activity, ovulation, and the synchronization of estrous cycles [4, 5]. In bovines, the “bull effect” serves as a primary biostimulatory tool, capable of inducing ovulation and shortening the interval between calving and first estrus [6]. Such biotechnical interventions are essential for addressing anestrus, which remains a significant challenge that decreases productivity in the livestock industry [7, 8].

To optimize lifetime productivity, modern breeding programs employ two critical strategies: advancing the timing of the first mating to reduce age at first calving [9-11] and ensuring rebreeding within 80–85 d postpartum to maintain a strict 365 d calving interval [12]. Although conventional hormonal treatments have effectively addressed these goals [13, 14], the livestock sector is experiencing a transformative shift toward “clean, green, and ethical” (CGE) management [15, 16]. This shift aims to minimize reliance on exogenous hormones, favoring non-invasive pheromonal tools that align with consumer demands for sustainable production.

Although the male effect is a multisensory phenomenon involving visual, auditory, and tactile cues, these non-pheromonal stimuli are generally considered supplementary factors that enhance, rather than initiate, the primary endocrine response [5, 17]. Evidence from olfactory-bulbectomized animals indicates that, while alternative sensory pathways may partially modulate luteinizing hormone (LH) release, they lack the specificity and potency required for effective estrous synchronization or the resumption of ovarian activity [18]. Thus, identifying specific pheromonal compounds in bovines is essential for the development of reliable biostimulatory techniques. Although biostimulation is widely utilized in swine and sheep, its application in cattle and buffalo remains understudied, highlighting the need to move beyond rodent-based models and establish a comprehensive understanding of neuroanatomical pathways unique to ungulates [19]. To address this gap, this review provides a focused analysis of male-specific scents in bovines, exploring the mechanisms underlying the bull effect to address prevalent reproductive disorders using a sustainable and scientifically rigorous framework.

THE MALE EFFECT IN BOVINE REPRODUCTION

The concept of the “male effect” in bovine reproduction refers to the stimulatory influence exerted by the male on female reproductive physiology and behavior through pheromonal and sensory cues. This biostimulatory effect has been well-documented in various livestock species, particularly in cattle. The presence of a bull can induce estrus and accelerate the resumption of ovarian activity in postpartum cows, thereby reducing the length of the anestrous period and improving reproductive performance [2].

Pheromonal signaling plays a crucial role in the male effect. Specific chemical cues, often found in bull urine and other body fluids, stimulate the female’s olfactory and endocrine systems, triggering changes that influence the estrous cycle [6]. Research indicates that exposure to bulls, particularly during the early postpartum period, can accelerate the onset of estrus and enhance fertility rates by modulating the hypothalamic–pituitary–gonadal (HPG) axis [3]. Furthermore, these effects are not solely limited to direct physical contact; the exposure to bulls or their excretory products can elicit similar hormonal responses, even in the absence of mating [5].

These biostimulatory effects have been successfully utilized in breeding management. Understanding the mechanisms underlying the male effect allows for the optimization of breeding strategies, particularly in livestock systems that rely on artificial insemination (AI). Therefore, harnessing the male effect offers a viable solution for improving reproductive efficiency in cattle, ultimately contributing to enhanced productivity in the livestock industry [20].

Beef cattle

The male effect in beef cattle has been extensively studied to determine how bull exposure influences the resumption of ovarian activity in postpartum cows. This phenomenon plays an important role in enhancing reproductive efficiency, particularly in herds where calving intervals need to be shortened. Several studies have shown that bull exposure can effectively reduce the interval between calving and the onset of estrus, which is crucial for improving conception rates and fertility in beef herds [6, 20-24].

In beef cattle, the presence of a bull induces estrus behavior and accelerates the resumption of ovarian cycling. Exposure to bulls has been linked to hormonal changes that stimulate the release of gonadotropin-releasing hormone (GnRH), which promotes the secretion of LH and follicle-stimulating hormone, thus initiating ovarian activity [2]. Zalesky et al. demonstrated that postpartum beef cows exposed to bulls showed an earlier onset of estrus and a higher proportion of cows exhibiting estrus within a shorter postpartum interval, ultimately contributing to improved fertility outcomes [20].

Further research has demonstrated that the effectiveness of bull exposure is highly dependent on the duration and consistency of the stimulus. Berardinelli and Joshi [25] reported that 12 h of daily exposure to bull excretory products, such as urine, significantly shortened anestrus intervals compared to non-exposed controls. This was further elaborated by Tauck et al. [24], who found that although both 6 and 12 h of daily exposure significantly reduced postpartum anestrus, the 12 h duration resulted in a greater cumulative proportion of cows resuming cyclic activity. These findings suggest a dose–response relationship where a sufficient daily threshold of pheromonal stimulation is required to activate the HPG axis.

However, the necessity of prolonged, uninterrupted exposure remains a subject of debate. Notably, Tauck et al. [26] observed that 24 h continuous exposure to bull urine had no significant effect on cyclic activity. To explain this paradox, Tauck et al. [24] proposed that alternating periods of "stimulation" and "relaxation" may be necessary for eliciting a positive neuroendocrine response. This implies that constant saturation of pheromones may lead to sensory adaptation or a lack of response, whereas strategic timing and frequency are crucial for optimizing biostimulatory outcomes.

In contrast, highly infrequent exposure has been shown to be insufficient to provide the necessary biological cue. Fernandez et al. [27] demonstrated that exposing primiparous suckled beef cows to bulls for only 2 h every 3 d (beginning at 30 d postpartum) had no significant effect on the resumption of ovarian cyclicity compared to isolated controls and, in some cases, even prolonged the postpartum anestrus interval. Collectively, these studies indicate that although daily and consistent exposure is superior to intermittent methods, the most effective strategy may involve a structured rhythm of exposure that avoids continuous saturation while maintaining a meaningful daily threshold.

In addition to the timing and duration of exposure, the physiological and environmental context of the female animal also significantly modulates the efficacy of the male effect. Among physiological factors, parity and nutritional status are recognized as particularly important, as they determine how energy is allocated within the body. Parity plays a distinct role, with primiparous cows often exhibiting more consistent sensitivity to male stimuli later in the postpartum period than multiparous cows [28]. This variation is primarily attributed to differences in energy partitioning; primiparous animals must balance the biological demands of continued physical growth with those of lactation, whereas multiparous cows may prioritize milk production [29].

Furthermore, environmental stress experienced by the herd can significantly influence the success of biostimulation. Bull exposure has been associated with reduced cortisol pulse frequency in cows, which may facilitate the neuroendocrine processes required for estrus expression [30]. This reduction in cortisol pulse frequency was correlated with an increase in LH pulse frequency, supporting the notion that bull exposure can modulate stress hormones, thereby enhancing estrus expression and ovarian cyclicity [31]. Therefore, minimizing stress through appropriate management of bull exposure is crucial for maximizing the effectiveness of biostimulation in beef cattle.

In summary, the male effect represents a powerful biological tool for enhancing reproductive performance in cattle. However, its successful application requires a comprehensive management approach. Effective bull exposure protocols must consider factors such as parity, nutritional status, and stress management to maximize reproductive outcomes. By understanding these multifaceted interactions, bull exposure can be effectively synergized with AI and other reproductive technologies to optimize the productivity and sustainability of bovine production systems.

Dairy cattle

Bull exposure during the postpartum period has long been recognized as a potential tool for improving reproductive performance in cattle. In beef cattle, the presence of a bull is known to accelerate the resumption of ovarian activity and shorten the postpartum anestrus period. However, in high-producing dairy cows, the effects of male exposure are relatively poorly understood and often contradictory. This phenomenon, referred to as the “high-producer paradox,” occurs because, despite the availability of potent male cues, high-yielding dairy cows often remain reproductively “blind” to the male effect [32].

This paradox is not a consequence of an absence of male pheromonal signals but is more likely linked to a metabolic blockade that hinders the cow’s responsiveness. In dairy cattle, particularly those with high milk production, metabolic factors, such as negative energy balance (NEB), play a critical role in reproductive physiology [33]. The substantial energy demands of lactation can suppress the hypothalamic–pituitary–ovarian (HPO) axis, thereby inhibiting the physiological mechanisms necessary for the bull’s pheromonal signals to elicit a response [34].

In these cows, hormonal responses that are critical for ovarian function, such as LH secretion, are inhibited due to metabolic imbalances. Decreased circulating levels of insulin and insulin-like growth factor 1 (IGF-1) act as inhibitory signals that decrease ovarian sensitivity to LH [35]. Consequently, this leads to gonadotropin insensitivity at the ovarian level, which limits the animal’s ability to respond to bull exposure. In such cases, the expected LH surge, which is a necessary response for successful insemination, fails to occur, and pregnancy rates remain unchanged [36]. The reproductive axis in high-producing dairy cows appears to be suppressed by lactational demands, leaving the reproductive system in a dormant state despite exposure to males.

This metabolic blockade highlights the importance of nutritional management in determining the effectiveness of biostimulation in dairy herds. Heifers and cows receiving higher levels of nutrition are more likely to exhibit a robust response to bull stimuli because their metabolic state signals that the environment is favorable for reproduction. Conversely, cows experiencing NEB or poor nutritional status often fail to respond to male cues [37]. In these cows, survival is prioritized, leading to a reallocation of resources away from reproduction and toward basic maintenance. This metabolic prioritization effectively reduces hormonal responsiveness to the male effect, reinforcing the idea that biostimulatory cues are effective only when metabolic conditions are favorable for reproduction.

The timing of bull exposure also plays a critical role in its effectiveness. Strategic application of the male effect in dairy systems may only be viable during the late-lactation period, when cows are in a more favorable energy balance and can potentially respond to biostimulatory cues [38]. In systems where nutritional management minimizes the depth of NEB, male exposure may be more effective in inducing estrous behavior and promoting ovarian reactivation. However, during early lactation or when cows are in severe NEB, bull exposure is less likely to yield positive results. Therefore, understanding the metabolic state of the dairy cow is essential when considering the application of bull exposure to improve reproductive performance.

In addition to nutritional status, other factors, such as parity, also influence the response to bull exposure [39]. Primiparous cows, which are still growing, must balance the biological demands of physical growth with those of lactation. Consequently, their reproductive systems may be less responsive to male cues than those of multiparous cows, which prioritize milk production. This difference in energy partitioning may explain the variability in response to biostimulation between first-calf heifers and mature cows.

Overall, while male exposure has shown some positive effects on ovarian activity and reproductive performance in dairy cattle, its effectiveness is significantly influenced by the cow’s metabolic state, particularly during lactation. High-yielding dairy cows, particularly those experiencing NEB, show a reduced capacity to respond to bull exposure, highlighting the critical role of nutritional management. Thus, the timing of bull exposure and the cow’s nutritional status must be carefully considered to maximize the effectiveness of biostimulation in dairy herds. Future studies should further investigate how metabolic and hormonal factors interact with biostimulatory cues to improve fertility outcomes in dairy cows.

Buffaloes

Buffalo reproduction is often limited by delayed ovarian reactivation and subtle estrous behavior, frequently accompanied by silent ovulations. These factors contribute to prolonged calving intervals and reduced reproductive efficiency. These challenges are particularly acute in tropical and subtropical regions, where high environmental temperatures and poor nutrition further constrain performance [40]. To overcome these limitations, biostimulation through bull exposure has gained attention as a valuable management tool.

The primary benefit of bull exposure is the enhancement of estrus expression, which is crucial for successful AI, given the subtlety of estrus signs in buffaloes. A study by Barman et al. [41] reported that continuous or close proximity to a bull significantly increased the proportion of buffaloes showing overt signs of estrus. This stimulation reduces the incidence of silent ovulation, a condition where the animal ovulates without external signs, by improving the female's responsiveness to reproductive cues. Gokuldas et al. [42] further confirmed that exposure to a vasectomized bull significantly shortens the interval to ovarian cyclicity and helps ensure that ovulations are accompanied by detectable heat behavior. This exposure also reduced the incidence of silent ovulation, which is common in buffaloes under natural conditions.

Bull exposure significantly improves fertility outcomes, even under challenging environmental conditions. Recent evidence from Kayani et al. [43] indicates that these benefits extend to the low breeding season (summer). In their study of Nili Ravi buffaloes synchronized with a 7-d controlled internal drug release (CIDR) protocol, animals continuously exposed to a bull exhibited a significant increase in conception rates. Specifically, the group receiving GnRH in addition to bull exposure exhibited a conception rate of 87.5%, compared to only 37.5% in the group receiving GnRH without exposure. The independent efficacy of the male effect is further supported by Zaidi and Anwar [44], who, without the use of CIDR or other hormonal interventions, recorded a 40% pregnancy rate in bull-exposed Nili-Ravi buffaloes compared to 0% in non-exposed controls. Collectively, these findings demonstrate that while hormonal protocols are beneficial, the biostimulatory influence of a male is a fundamental driver of reproductive success.

The effectiveness of biostimulation is highly dependent on timing and intensity. Choudhary et al. [45] reported that bull exposure is most effective during the late postpartum period, after completion of uterine involution and attainment of a favorable nutritional state. Their finding indicates that continuous exposure during these later stages is most effective in reducing the duration of the anestrus period and improving first-service conception rates. Furthermore, consistent and prolonged exposure is generally associated with more robust reproductive outcomes than intermittent contact.

In conclusion, bull exposure is a powerful management strategy for mitigating the challenges of silent ovulation and prolonged postpartum anestrus in buffaloes. By integrating bull exposure with other reproductive management strategies, such as estrous synchronization protocols, producers can significantly optimize fertility and productivity. The distinct reproductive challenges, physiological mechanisms, and optimized application strategies for beef cattle, dairy cattle, and buffaloes are summarized for comparative reference (Table 1).

NEUROENDOCRINE MECHANISMS OF MALE PHEROMONES IN RUMINANTS

Building on the observed phenomena of the male effect, this section examines the underlying neuroendocrine mechanisms driven by male pheromones. These potent chemical signals are perceived via the main olfactory epithelium (MOE) and the vomeronasal organ (VNO), directly stimulating hypothalamic centers [46]. This activation triggers a rapid increase in the pulsatility of GnRH and LH, which is essential for synchronizing estrus in cattle and buffalo. While these pathways are well established in small ruminants, they are not well characterized in bovine species. Thus, this section utilizes small ruminant models to elucidate conserved mechanisms that translate pheromonal cues into reproductive success.

Chemosensory reception: Dual pathways via the MOE and VNO

In ruminants, the detection of male pheromones and the subsequent neuroendocrine effects are fundamental to reproductive processes, including estrus synchronization and the induction of ovulation. This biostimulatory phenomenon, referred to as the male effect, is mediated by two anatomically and functionally distinct chemosensory systems within the nasal cavity: the main olfactory system (MOS) and the accessory olfactory system (AOS) [19]. These systems serve as the primary pathways for the perception of socio-sexual signals in female ruminants such as cattle, buffalo, sheep, and goats.

The MOS uses the MOE to detect volatile, airborne chemical signals. The MOE is composed of bipolar olfactory sensory neurons whose long cilia extend into the nasal mucus, providing an extensive surface area for interaction with odorant molecules. These neurons transmit sensory information to the main olfactory bulb and subsequently to brain regions involved in regulating reproductive behavior [47]. In contrast to rodents, ruminants appear to rely more heavily on the MOS for the male effect. Evidence indicates that disruption of the olfactory epithelium through zinc sulfate irrigation completely blocks the endocrine response to male odors in anestrous females, whereas VNO lesions often do not inhibit the luteinizing hormone (LH) response [48].

The AOS relies on the VNO, a paired tubular structure located on both sides of the base of the nasal septum. The VNO specializes in detecting non-volatile or low-volatility compounds present in biological fluids such as urine, saliva, or vaginal secretions [49]. In ruminants, the VNO communicates with the oral and nasal cavities through the nasopalatine (incisive) ducts [50]. Access to these chemical stimuli is facilitated by flehmen behavior, in which the female adopts a characteristic posture to pump fluid from the oral cavity into the sensory epithelium of the VNO

[51]. Vomeronasal sensory neurons possess microvilli instead of cilia and express specific G-protein-coupled receptors, namely V1Rs and V2Rs, which transduce pheromonal signals [52, 53].

Recent studies using live-cell calcium imaging have demonstrated that MOE and VNO neurons play distinct yet complementary roles in pheromone reception. In female goats, hair compounds from sexually active bucks activate a higher proportion of sensory cells than those from castrated males, regardless of the extraction method. Notably, the reproductive status of the female influences these responses. MOE cells from anestrous does show significantly higher activation levels in response to buck hair extracts than cells from does during the breeding season, suggesting that the MOS plays a more critical role in the male effect during anestrus [54]. However, in bovine species, the VNO remains the indispensable primary pathway for pheromone-induced GnRH activation [2], highlighting a distinct neuroendocrine architecture compared to small ruminants.

In conclusion, ruminants employ a sophisticated dual chemosensory system that integrates inputs from both the MOE and the VNO to facilitate reproductive biostimulation. While the main olfactory system serves as the primary mediator of the male effect in small ruminants, experimental evidence indicates that MOE sensitivity is significantly enhanced during anestrus to improve detection of volatile male cues. This sensory dominance is not universal across all ruminant species. In bovine species, the VNO remains the indispensable primary pathway for pheromone-induced activation of the GnRH pulse generator, reflecting a distinct neuroendocrine architecture compared to sheep and goats. Ultimately, this integrated sensory system ensures that diverse pheromonal signals are accurately processed by the hypothalamus, triggering the hormonal cascades necessary for successful estrus synchronization and enhanced fertility.

Hypothalamic integration of pheromonal signals via KNDy networks

The regulation of reproductive physiology in cattle is primarily centered in the hypothalamus, where various external and internal signals converge to control GnRH secretion [55]. A key component of this system is the network of neurons that co-express kisspeptin, neurokinin B (NKB), and dynorphin A (Dyn), collectively referred to as KNDy neurons. These neurons, predominantly located in the arcuate nucleus (ARC) of the hypothalamus, serve as a crucial hub that integrates hormonal feedback and pheromonal cues to drive the pulsatile secretion of GnRH. This rhythmic release is essential for maintaining important reproductive functions, such as follicular development and spermatogenesis, in mammals [56]. The integrated neuroendocrine pathways mediating the bull effect on the bovine reproductive axis are schematically illustrated in Figure 1.

The journey of male pheromonal signals begins with their detection by the MOE and the VNO, as described in previous sections. Once these chemical cues are perceived, sensory information is transmitted through the olfactory bulbs to the corticomedial amygdala. The amygdala acts as the primary relay station, channeling socio-sexual information to the hypothalamus [46]. Neural projections from the amygdala then reach the preoptic area (POA) and the ARC. While the POA is significantly involved in the surge mode of GnRH secretion necessary for ovulation, the ARC is the well-established site of the GnRH pulse generator. In ruminants, the male effect is characterized by an immediate increase in GnRH pulse frequency, indicating that pheromonal signals directly stimulate the pulse-generating circuitry within the ARC [57].

The KNDy hypothesis proposes that the synchronized activity of neurons co-expressing kisspeptin, NKB, and Dyn within the ARC serves as the primary pacemaker for pulsatile GnRH release. Although foundational research was initially established in small ruminants, recent immunohistochemical studies have confirmed that this neuroendocrine architecture is highly conserved in bovine species, with the co-expression of these peptides verified in the ARC of heifers [58]. Functional evidence supporting the role of the ARC kisspeptin system in bovine GnRH/LH regulation has also been reported. In Holstein steers, immunohistochemistry revealed the presence of kisspeptin-immunoreactive cell bodies in the ARC, with fibers projecting densely to the median eminence in close association with GnRH terminals [59]. Intravenous administration of kisspeptin-10 significantly increased LH secretion in a dose-dependent manner, supporting the functional capacity of kisspeptin to stimulate GnRH/LH secretion in bovine species [59]. Further mapping of the bovine hypothalamus across the estrous cycle in Japanese Black cows showed that ARC kisspeptin and dynorphin immunoreactivity varies according to progesterone status [55, 58]. During diestrus, higher circulating progesterone is associated with reduced kisspeptin and increased dynorphin immunoreactivity in the ARC, whereas kisspeptin expression in the POA increases during the follicular phase under positive estrogenic feedback [55, 58]. These observations support a functional distinction between ARC KNDy neurons, which are primarily associated with pulsatile GnRH/LH secretion, and POA kisspeptin populations, which are more closely associated with estrogen-positive feedback and the preovulatory surge mechanism. In addition to this anatomical and functional evidence, maturation of this network is driven by a complex molecular cascade involving phospholipase C beta 1 (PLCβ1), which acts as a critical intracellular mediator in the Gq-coupled signaling pathway of the kisspeptin receptor (KISS1R) [60]. Upon kisspeptin binding, PLCβ1 facilitates neuronal depolarization by catabolizing phosphatidylinositol 4,5-bisphosphate and potentiating transient receptor potential canonical 4 (TRPC4) channel activity, a process essential for network self-activation and subsequent GnRH output

[61]. This proteomic evidence highlights significant shifts in hypothalamic signaling proteins during the transition from prepubertal to postpubertal stages, marking the maturation of the KNDy network's responsiveness to external stimuli such as male pheromones.

The generation of rhythmic neural bursts within the KNDy network relies on a delicate balance of excitatory and inhibitory signals. Neurokinin B serves as the primary "start" signal for each pulse; by binding to neurokinin 3 receptors on neighboring KNDy neurons, it initiates a recurrent excitatory loop that triggers a synchronized burst of activity [62, 63]. Conversely, Dyn acts as the "stop" signal through kappa-opioid receptors, providing inhibitory feedback that terminates the burst and defines the inter-pulse interval [64]. Recent updates to the KNDy model also emphasize the role of glutamate in enhancing rapid communication and synchronization within this network [63]. This multi-layered regulatory system ensures that the pulse generator remains highly adaptable to external modulation, allowing male pheromones to increase GnRH pulse frequency, potentially by enhancing excitatory NKB drive or by alleviating inhibitory dynorphin tone [57].

The functional integration of these neuropeptide signals is empirically supported by electrophysiological studies in ruminants. Multiple-unit activity (MUA) recordings from the arcuate nucleus, the gold standard for monitoring the GnRH pulse generator, reveal periodic "volleys" of neural firing that are tightly synchronized with LH pulses [64]. In ovariectomized goats, these MUA volleys typically occur at a low frequency; however, brief exposure to male pheromones induces an immediate increase in MUA in close proximity to ARC kisspeptin neurons [65]. This immediate neural response provides direct evidence that pheromonal signals are effectively transmitted to the ARC KNDy network, overriding the suppressive state of anestrus by inducing a burst of activity that generates pulsatile GnRH release. Furthermore, the timing-dependent nature of this pheromonal action indicates that the GnRH pulse generator possesses a specific refractory period for pheromonal signals, which is likely involved in regulating intermittent bursts when animals in natural environments are continuously exposed to pheromones [66]. By ensuring a sustained and rhythmic endocrine response rather than a single exhausted output, this sophisticated neural integration prevents desensitization and serves as a fundamental driver of the male effect observed in livestock management.

As the final output signal of the KNDy network, kisspeptin serves as the critical neuroendocrine bridge that transmits rhythmic oscillations to GnRH neurons. While NKB and dynorphin coordinate the internal rhythm within the arcuate nucleus, kisspeptin provides the final stimulus required to trigger GnRH release. At the cellular level, the binding of kisspeptin to KISS1R modulates key ionic conductance, specifically through the activation of nonselective cation (TRPC) currents and the inhibition of inwardly rectifying potassium currents. This synergistic ionic modulation results in sustained depolarization and increased action potential firing, which effectively drives the synchronized release of GnRH into the hypophyseal portal system [67]. The high sensitivity of the bovine reproductive axis to this peptide is evidenced by the rapid and significant surge in LH following the peripheral administration of kisspeptin [68]. This functional potency is supported by neuroanatomical studies in heifers, which confirm that kisspeptin neurons in the ARC uniquely co-express NKB and Dyn, whereas those in the POA population do not [58]. This distinction confirms that ARC-based KNDy neurons serve as the primary pacemaker for pulsatile GnRH secretion, whereas the POA population is primarily associated with the surge mode of release. Within the framework of the male effect, the ARC KNDy network acts as the central integrator of external pheromonal signals [69]. Consequently, male-induced acceleration of reproductive cycles is primarily mediated by activation of these ARC neurons, which provide the essential stimulatory input to GnRH terminals in the median eminence, thereby increasing pulse frequency.

KNDy neurons do not function in isolation; instead, they act as both sensors and effectors that integrate a wide array of internal and external signals. They are highly sensitive to feedback from gonadal steroids, as well as to metabolic signals and environmental stressors. For instance, in female lambs and mature ewes, undernutrition or malnutrition can induce a state of metabolic insufficiency that impairs reproductive performance by reducing the expression of KNDy neuropeptides in the ARC [70, 71]. This metabolic inhibition is further associated with low circulating levels of insulin and IGF-1, which serve as indirect modulators of KNDy neuron excitability [72]. Consequently, diminished metabolic signaling reduces the sensitivity of KNDy neurons to kisspeptin or pheromonal inputs, thereby creating a physiological barrier to reproductive activation. In contrast, the biostimulatory effect of a male is particularly potent because it provides a direct excitatory input that can bypass certain inhibitory metabolic pathways. By acting as a central integrator, the KNDy network fine-tunes the timing of GnRH secretion based on the balance between external opportunities, such as the presence of a male, and internal readiness, such as nutritional status [63, 69].

Collectively, hypothalamic integration of pheromonal signals through the KNDy network represents a cornerstone of reproductive control in ruminants. This specialized population of neurons in the ARC processes chemical cues from the environment and translates them into rhythmic GnRH pulses through the coordinated actions of NKB, dynorphin, and kisspeptin. Confirmation of this system in bovine species, supported by proteomic evidence of molecular maturation such as the upregulation of PLCβ1, highlights its universal importance in livestock reproduction [60]. Male pheromones act as a critical external modulator that stimulates this pulse generator to accelerate reproductive cycles, providing a robust biological basis for using biostimulation in managing postpartum anestrus. Furthermore, the capacity of the KNDy network to integrate metabolic signals, such as insulin and IGF-1, underscores its role as a central sensor that balances internal physiological readiness with external reproductive opportunities [69, 72]. Overall, the molecular and electrophysiological fine-tuning of this network is essential for navigating the complex interplay between environmental cues and the reproductive axis, ensuring successful production outcomes in cattle and buffalo.

Stress-induced suppression of the reproductive axis

The reproductive success of cows is profoundly influenced by their ability to maintain homeostatic balance in the face of environmental and physiological stressors. Exposure to stressors such as extreme temperatures, handling, or disease activates the hypothalamic-pituitary-adrenal (HPA) axis, initiating a cascade in which the hypothalamus releases corticotropin-releasing hormone and arginine vasopressin. This stimulates the pituitary to secrete adrenocorticotropic hormone, leading to elevated circulating glucocorticoids, predominantly cortisol [73]. Although this endocrine response prioritizes immediate survival by mobilizing energy resources, it simultaneously induces a well-characterized suppression of the HPG axis in females [74, 75].

Glucocorticoids serve as central regulators that integrate an individual's inflammatory and metabolic status with the reproductive system [76]. High cortisol levels exert potent inhibitory effects on the GnRH pulse generator. Specifically, KNDy neurons in the ARC are primary targets; stress signals reduce the expression of kisspeptin mRNA and protein, thereby diminishing the stimulatory output required for pulsatile GnRH secretion [69]. Furthermore, glucocorticoids are essential for stress-induced inhibition of GnRH receptor synthesis in the pituitary, which reduces pituitary sensitivity to GnRH and leads to a decline in LH pulse frequency and amplitude [77]. Chronic stress also shifts the immune response from cellular to humoral immunity, weakening overall defense mechanisms and increasing susceptibility to infections that further impair fertility [78].

Heat stress (HS) represents a major barrier to successful reproduction in cattle, primarily by disrupting the endocrine and physiological environments necessary for fertility [79]. This disruption involves complex modulation of LH secretion; although responses vary across studies, the prevailing evidence indicates attenuation of LH pulse amplitude and suppression of the pre-ovulatory surge [80]. This neuroendocrine suppression is frequently driven by the synergistic effects of elevated cortisol levels and diminished estrogenic support, a phenomenon particularly evident in thermosensitive animals [80, 81]. In such individuals, abnormal estradiol secretion fails to provide the positive feedback necessary to sustain a normal LH surge, which directly leads to suboptimal follicular maturation and a higher incidence of silent ovulation [81, 82]. These internal endocrine failures manifest behaviorally as “silent heats,” characterized by the significantly reduced duration and intensity of estrus, which severely complicates detection and reproductive management in dairy herds [83]. Mechanistically, HS further impairs follicular development and oocyte competence by inducing systemic oxidative stress and altering the secretion of other key hormones, such as progesterone and prolactin [78, 84]. To accurately evaluate the climatic conditions related to animal comfort, the temperature-humidity index (THI) is widely used as a standard measure of environmental heat load. According to this index, ruminants are generally considered to be within the comfort zone at THI values below 68. As the index increases, animals experience mild stress (THI 69–78), whereas values between 79 and 89 indicate moderate stress. THI levels exceeding 90 reflect severe stress, prompting significant physiological and metabolic adaptations to mitigate strain [80, 85]. Ultimately, high THI values are strongly correlated with decreased conception rates and increased embryonic mortality in both beef and dairy cattle [86, 87].

The efficacy of biostimulation, particularly the male effect, relies on the precise activation of the KNDy network by external pheromonal cues, which accelerates GnRH pulse frequency [66]. However, environmental and physiological stressors can significantly diminish this biostimulatory process by reducing the sensitivity of the ARC to these stimulatory inputs [69]. Under chronic stress or high thermal load, elevated glucocorticoid levels create an inhibitory neuroendocrine environment that counteracts the excitatory drive induced by male pheromones [76]. The specific inhibitory pathway through which this interference suppresses the ability of the KNDy network to translate pheromonal signals into synchronized firing is schematically represented in Figure 2. Within this framework, high cortisol levels increase the threshold required for pheromonal induction of the LH surge by decreasing the responsiveness of kisspeptin neurons to external stimuli [76, 80]. In heat-stressed cattle, the synergistic effects of hypercortisolemia and reduced estrogenic support create a refractory state in which even strong male stimuli fail to trigger the transition from anestrus to cyclicity [79, 84]. Consequently, the inability of the KNDy network to effectively integrate chemical cues under stressful conditions explains the inconsistent results of biostimulation in challenging environments, making stress mitigation a prerequisite for maximizing the reproductive potential of the male effect.

In summary, stress-induced suppression of the reproductive axis is a multi-layered process initiated by activation of the HPA axis and the subsequent rise in systemic cortisol. This hormonal shift inhibits the HPG axis by reducing kisspeptin expression in KNDy neurons and diminishing pituitary sensitivity to GnRH pulses. Under heat stress conditions, quantified by the THI, these neuroendocrine disruptions lead to suppressed LH surges, silent ovulations, and impaired oocyte competence. Importantly, elevated cortisol levels increase the activation threshold of the KNDy network, thereby attenuating the efficacy of pheromonal biostimulation such as the male effect. Therefore, mitigating environmental and physiological stressors to reduce cortisol tone is a fundamental prerequisite for maximizing the success of reproductive technologies and biostimulatory interventions in ruminant production.

Behavioral and hormonal responses: Integration of internal and external modulators

The reproductive efficiency of ruminants is determined by the precise integration of internal endocrine signals and external environmental stimuli, representing a fundamental adaptive strategy to maintain homeostasis under stress [85]. This integration involves a complex, bidirectional mechanism in which hormonal shifts drive behavioral changes, and altered behaviors, in turn, provide feedback to the endocrine environment to prioritize immediate survival over reproductive functions.

At the physiological level, these hormonal fluctuations directly regulate an animal's activity levels and nutritional priorities. Systemic cortisol promotes the redistribution of nutrients for maintenance, whereas exposure to stressors also suppresses thyroid gland activity, leading to a decrease in plasma concentrations of triiodothyronine and thyroxine [88]. This reduction in thyroid hormones lowers the basal metabolic rate and minimizes net body heat production, shifts that are behaviorally reflected in a marked decline in physical activity and feed intake [89]. Furthermore, in excitable or aggressive individuals, chronic distress reduces feed intake and leads to a subsequent NEB, which severely depletes body condition score and impairs the physiological processes required for reproduction [90].

Beyond metabolic adjustments, activation of the HPA axis induces hormonal cascades that alter the animal's psychological state. Individual temperament plays a critical role, as excitable heifers exhibit significantly higher levels of cortisol, substance P, and haptoglobin compared to calmer counterparts [91]. Elevated cortisol suppresses the secretion of GnRH and LH, thereby delaying puberty or prolonging postpartum anestrus. Although these integrated responses function as protective mechanisms against potential threats, heightened vigilance and anxiety in excitable dams often disrupt the social stability and synchronization necessary for the expression of reproductive behaviors.

These behavioral outcomes are further modulated by the social hierarchy within the herd, which serves as a primary driver of adaptive strategies. Cattle society is highly hierarchical, influencing social behavior, relationships, and access to resources such as feed and handling chutes [92, 93]. For instance, low-ranking dams may adopt passive behavioral strategies to reduce stress during handling; however, they often experience suppressed LH production as a result of their lower social status [94]. This social suppression hampers normal ovulation and estrus behavior, illustrating how hierarchy interacts with the HPG axis to regulate fertility. Consequently, stable social environments and calmer temperaments are as critical as environmental cooling for maintaining reproductive success.

Ultimately, this relationship forms an inextricably linked feedback loop in which hormones and behavior continuously interact under various stressors. Chronic distress, whether arising from social conflict, aggressive temperament, or environmental challenges, directly impacts the brain’s reproductive centers by reducing estradiol support, which manifests behaviorally as silent heats or the complete absence of estrus [91]. Conversely, the success of adaptive behaviors, such as establishing stable social relationships or mitigating environmental load, plays a crucial role in lowering systemic cortisol levels and stabilizing the internal endocrine environment. Thus, the harmonious integration of behavioral responses to external stimuli and internal hormonal states is essential for sustaining the reproductive potential of ruminants in challenging environments.

STRATEGIC APPLICATIONS OF THE MALE EFFECT FOR REPRODUCTIVE DISORDERS IN BOVINES

Mitigation of postpartum anestrus

Postpartum anestrus remains one of the most significant challenges in the reproductive management of both beef cattle and water buffalo, directly affecting the economic viability of the livestock industry by prolonging the intercalving interval. The resumption of ovarian cyclicity after parturition is governed by the restoration of LH pulse frequency, which is typically suppressed by the combined inhibitory effects of suckling-induced neuroendocrine signals and NEB [12, 95]. In suckled beef cows, this neuroendocrine suppression is primarily characterized by a significant reduction in LH pulsatility, the key endocrine factor determining whether a dominant follicle ovulates, which remains inhibited regardless of postpartum energy intake [96]. Importantly, this blockade of LH secretion is sustained only if the cow recognizes the offspring as her own through visual or olfactory cues, as the maternal bond plays a more significant role than the physical act of suckling itself in maintaining this inhibitory tone [96, 97]. Because these sensory-mediated pathways rooted in calf recognition actively maintain the anestrous state, the strategic application of the male effect provides a logical non-hormonal method to overcome this inhibition. Empirical evidence confirms that, even under conditions involving adequate nutrition and permanent suckling, biostimulation exerts a significant positive effect on early reproductive performance in postpartum Angus cows [6]. By providing potent pheromonal cues that target the same neuroendocrine centers, the male effect can effectively re-establish endogenous gonadotropin pulsatility despite ongoing suckling-mediated inhibition.

The efficacy of biostimulation is evident across various postpartum stages, demonstrating that bull pheromones can elicit a robust response regardless of the timing of exposure during the anestrous period. For instance, bull exposure initiated as early as one week postpartum in well-fed Angus cows significantly shortened the interval to the resumption of estrus by 13 to 21 d compared to control groups [6]. Similarly, interventions started at a later stage, such as at approximately 50 d postpartum, confirmed that 12 h of daily exposure effectively reduced the interval to ovulatory activity and increased the proportion of cows cycling within 45 d [24]. These findings are further supported by observations in Murrah buffaloes, where exposure beginning on day 40 postpartum significantly increased the proportion of animals resuming cyclicity [42]. Previous studies examining the dose-dependent relationship between exposure duration and ovulatory response have shown that maintaining a minimum daily threshold of 12 h of exposure is necessary to effectively override the inhibitory effects of suckling and energy deficits [24, 25]. This practical threshold offers significant management flexibility, allowing for intermittent exposure protocols, such as nighttime introduction of bulls, without compromising the acceleration of cyclicity. Ultimately, when integrated with optimal nutrition, these strategic biostimulatory protocols can maximize the natural reproductive potential of the herd while minimizing the need for pharmacological interventions [95, 98].

Induction of puberty in pre-pubertal heifers

The timing of puberty onset in replacement heifers is a critical determinant of the economic efficiency and sustainability of beef and dairy production systems. To optimize lifetime productivity, producers generally target an age at first calving of approximately 24 months, which requires heifers to attain sexual maturity and conceive by 15 months of age [99, 100]. Delayed puberty prolongs the non-productive rearing period, significantly increasing cumulative feed and maintenance costs while also slowing the rate of genetic gain within the herd [99]. In this context, biostimulation via the male effect represents a sustainable alternative to exogenous hormonal treatments, as it can accelerate the onset of puberty and help synchronize reproductive cycles in young females [101].

The efficacy of the male effect is closely correlated with the body weight and nutritional status of the heifers, with biostimulation serving as a catalyst for activation of the HPG axis. Evidence indicates that bull exposure is most effective when heifers are approaching their target weight for puberty [102]. For instance, heifers with higher initial body weights typically respond more rapidly to biostimulation, exhibiting a shorter interval to the formation of the first corpus luteum compared to lighter counterparts [102]. This response is thought to occur because male pheromonal cues interact with metabolic signals to stimulate the hypothalamic GnRH pulse generator, subsequently increasing LH pulse frequency and promoting follicular development [12]. Notably, biostimulated heifers exhibit an upward trend in growth hormone and leptin concentrations; these changes suggest that the male effect may provide a supplementary synergy that supports the nutritional ‘switching on’ of the reproductive axis [45].

Physiological differences between breeds, particularly between *B. indicus* (Zebu) and *B. taurus* (temperate) cattle, are important considerations when implementing biostimulation protocols. *Bos indicus* breeds, such as Nelore, are typically characterized by a later age at puberty and a higher sensitivity to environmental and social stressors compared to *B. taurus* counterparts [103]. In tropical environments, studies on Nelore heifers have shown that bull exposure can significantly advance the age at puberty, particularly when integrated with strategic nutritional supplementation [104]. Similarly, in Sahiwal heifers, bull biostimulation advanced the onset of puberty by approximately 5 months compared to non-exposed controls, with treated groups reaching puberty at approximately 19 months of age compared to 24 months in the isolated group [105]. Beyond advancing the mean age at puberty, the male effect serves as a potent synchronizing agent that triggers the HPG axis at lower physiological thresholds. Comparative studies in Bunaji and Friesian crossbred heifers demonstrated that bull exposure reduced age at puberty by over 3 months and significantly increased the proportion of heifers attaining puberty between 17 and 24 months of age (70.8% vs. 18.3% in non-exposed controls). Furthermore, biostimulated heifers attained puberty at significantly lower live weights (224.4 kg vs. 255.8 kg), indicating that the male effect can effectively override the traditional reliance on body growth to initiate cyclicity across diverse genotypes and climatic regions [106].

The intensity and modality of male exposure are critical variables influencing the success of biostimulatory interventions. While fence-line contact alone can initiate a reproductive response, neuroendocrine activation in heifers appears to be significantly enhanced when fence-line exposure is combined with periods of direct physical contact [45, 105]. Direct contact provides a more comprehensive array of sensory inputs, including visual, auditory, olfactory, and tactile stimuli, which collectively exert a stronger excitatory influence on the HPG axis. Furthermore, biostimulation has been shown to decrease the incidence of “silent estrus,” in which ovulation occurs without behavioral symptoms, and to increase the intensity of overt estrous behavior [107]. This improvement in behavioral expression significantly enhances the efficiency and accuracy of heat detection in AI programs. Consequently, a management protocol that strategically introduces bulls as heifers approach their developmental threshold represents a logical and cost-effective approach to maximize the natural reproductive potential and long-term herd efficiency [100, 101].

Optimization of biostimulatory efficacy: Environmental and social considerations

The efficacy of biostimulation through the male effect is determined not only by the physical presence of pheromonal cues but also by the environmental and social context in which these stimuli are perceived. To maximize the biostimulatory response under practical conditions, it is essential to optimize management strategies that address external stressors and social dynamics.

The ability of male biostimulation to activate the HPG axis is significantly influenced by the level of environmental stress. Heat stress, in particular, acts as a potent external inhibitor that impairs follicular development, suppresses the expression of estrous behavior, and reduces the magnitude of the preovulatory LH surge [108]. As demonstrated by the capacity of the male effect to counteract the inhibitory influence of suckling [6], strategic biostimulation may offer a sustainable, non-hormonal alternative to mitigate reproductive suppression associated with high ambient temperatures. However, for this “override” to be effective, physical interventions such as cooling systems or shade provision must be implemented in tandem to ensure that environmental stress signals do not overwhelm excitatory pheromonal cues [108, 109].

Social hierarchy and inter-individual interactions within the herd are also critical variables determining the reception of biostimulatory signals. Cattle naturally establish dominant–subordinate relationships that influence access to resources and the frequency of exposure to male stimuli [110]. Subordinate individuals may be physically excluded from contact with the bull by more dominant animals. Notably, social stressors such as overstocking and frequent regrouping can lead to immune suppression and reduced reproductive efficiency [111]. To minimize these effects, management practices should include separate housing for primiparous and multiparous cows, as mixing age groups can exacerbate social stress and inhibit reproductive activation in younger females [111]. Maintaining social stability is therefore essential to ensuring that all females receive uniform pheromonal stimulation [112].

From a practical standpoint, the bull-to-cow ratio serves as a direct tool for modulating the biostimulatory “dose,” as maintaining an optimal ratio is essential to prevent dilution of pheromonal signals and to ensure improved synchronization and pregnancy rates [113]. The potency of this stimulus is further influenced by the bull’s age and sexual experience. Petherick [114] observed that older bulls exhibit superior libido and serving efficiency, primarily due to accumulated experience and social dominance rather than chronological age alone. Dominant mature bulls often maximize their sexual activity while potentially suppressing the libido of subordinate younger males, thereby ensuring a more consistent stimulus [114]. Consequently, optimizing herd reproductive performance requires a comprehensive approach that balances both the quantitative bull-to-cow ratio and the qualitative selection of mature, experienced bulls to ensure a robust and effective biostimulatory effect.

Overall, the success of reproductive management using the male effect depends on a synergistic balance between two key components. First, the potency of the male stimulus must be ensured through adequate pheromonal intensity, optimal bull-to-cow ratios, and the maturity of the bull. Second, the environmental and social comfort of receptive females must be prioritized. When these optimization strategies are integrated to minimize stress and address social imbalances, biostimulation can achieve its full potential. This approach offers a sustainable model that enhances both animal welfare and the economic efficiency of cattle and buffalo production systems.

CONCLUSION

Male pheromones represent a sustainable and promising biotechnical tool for enhancing reproductive efficiency in cattle and buffalo production systems. As a non-invasive, CGE alternative to conventional hormonal treatments, pheromone-based biostimulation effectively utilizes the male effect to modulate the HPG axis. The KNDy neuronal network in the ARC serves as the central neuroendocrine integrator, translating external pheromonal cues into the rhythmic GnRH pulses required to overcome reproductive challenges such as postpartum anestrus and silent ovulation. However, the efficacy of this biostimulatory response is not absolute; it is significantly influenced by the metabolic readiness and environmental stress levels of the female, which can increase the activation threshold of the GnRH pulse generator. Therefore, maximizing the potential of the male effect requires a comprehensive management framework that balances the potency of the male stimulus, optimized through strategic bull-to-cow ratios and the selection of mature, experienced bulls, with the mitigation of negative energy balance and heat stress. While current evidence supports the use of bull exposure to advance puberty and shorten anestrous intervals, future research should prioritize large-scale field trials across diverse breeds and environments. Integrating these biostimulatory interventions into a holistic management system provides a scientifically grounded and sustainable pathway for optimizing bovine reproduction.

ACKNOWLEDGEMENTS

The authors acknowledge the members of the horse science lab for their assistance with this work.

COMPETING INTERESTS

No potential conflict of interest relevant to this article was reported.

AUTHOR’S CONTRIBUTIONS

Conceptualization: Kim JY; Funding acquisition: Yoon MJ; Project administration: Yoon MJ; Resources: Yoon MJ; Supervision: Yoon MJ; Writing - original draft: Kim JY; Writing - review & editing: Kim JY, Yoon MJ

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article did not require IRB/IACUC approval because there were no human or animal participants.

REFERENCES

1. Eisenberg JF, Kleiman DG. Olfactory communication in mammals. Annual review of Ecology and Systematics. 1972:1-32. https://doi.org/10.1146/annurev.es.03.110172.000245
2. Rekwot P, Ogwu D, Oyedipe E, Sekoni V. The role of pheromones and biostimulation in animal reproduction. Animal reproduction science. 2001;65(3-4):157-70. https://doi.org/10.1016/s0378-4320(00)00223-2
3. Archunan G, Rajanarayanan S, Karthikeyan K. 16 Cattle pheromones. Neurobiology of Chemical Communication 1st edn CRC Press, New York. 2014:461-79. https://doi.org/10.1201/b16511-21
4. Doty R. Mammalian olfaction, reproductive processes, and behavior: Elsevier; 2012.
5. Izard MK. 9 - Pheromones and Reprod Domest Anim. In: Vandenbergh JG, editor. Pheromones and Reproduction in Mammals: Academic Press; 1983. p. 253-85. https://doi.org/10.1016/b978-0-12-710780-6.50015-7
6. Landaeta-Hernández A, Meléndez P, Bartolomé J, Rae O, Archbald LF. The effect of bull exposure on the early postpartum reproductive performance of suckling Angus cows. Revista Cientifica. 2008;18(6):682-91.
7. Peter A, Vos P, Ambrose D. Postpartum anestrus in dairy cattle. Theriogenology. 2009;71(9):1333-42. https://doi.org/10.1016/j.theriogenology.2008.11.012
8. Yavas Y, Walton J. Postpartum acyclicity in suckled beef cows: a review. Theriogenology. 2000;54(1):25-55. https://doi.org/10.1016/s0093-691x(00)00323-x
9. Day ML, Grum DE. Breeding strategies to optimize reproductive efficiency in beef herds. Veterinary Clinics: Food Animal Practice. 2005;21(2):367-81. https://doi.org/10.1016/j.cvfa.2005.02.003
10. Patterson D, Perry R, Kiracofe G, Bellows R, Staigmiller R, Corah L. Management considerations in heifer development and puberty. Journal of Animal Science. 1992;70(12):4018-35. https://doi.org/10.2527/1992.70124018x
11. Stevenson JS. Breeding Strategies to Optimize Reproductive Efficiency in Dairy Herds. Vet Clin North Am Food Anim Pract. 2005;21(2):349-65. https://doi.org/10.1016/j.cvfa.2005.02.006
12. Crowe M. Resumption of ovarian cyclicity in post-partum beef and dairy cows. Reprod Domest Anim. 2008;43:20-8. https://doi.org/10.1111/j.1439-0531.2008.01210.x

13. Diskin M, Austin E, Roche J. Exogenous hormonal manipulation of ovarian activity in cattle. Domest Anim Endocrino. 2002;23(1-2):211-28. https://doi.org/10.1016/s0739-7240(02)00158-3

14. Patterson D, Kojima F, Smith M. A review of methods to synchronize estrus in replacement beef heifers and postpartum cows. J Anim Sci. 2003;81(14_suppl_2):E166-E77. https://doi.org/10.2527/2003.8114_suppl_2E166x

15. Martin G, Milton J, Davidson R, Hunzicker GB, Lindsay D, Blache D. Natural methods for increasing reproductive efficiency in small ruminants. Anim Reprod Sci. 2004;82:231-45. https://doi.org/10.1016/j.anireprosci.2004.05.014

16. Martin GB, Ferasyi TR. 1. Clean, Green, Ethical (CGE) Management: What Research Do We Really Need? Int J Trop Vet Biomed Res. 2016;1(1):1-8. https://doi.org/10.21157/ijtvbr.v1i1.5066

17. Chenoweth P. Libido and mating behavior in bulls, boars and rams. A review. Theriogenology. 1981;16(2):155-77. https://doi.org/10.1016/0093-691x(81)90098-4

18. Cohen-Tannoudji J, Locatelli A, Signoret J. Non-pheromonal stimulation by the male of LH release in the anoestrous ewe. Physiol Behav. 1986;36(5):921-4. https://doi.org/10.1016/0031-9384(86)90453-1

19. Landaeta-Hernández AJ, Ungerfeld R, Chenoweth PJ. Biostimulation and pheromones in livestock: A review. Anim Reprod Sci. 2023;248:107154. https://doi.org/10.1016/j.anireprosci.2022.107154

20. Zalesky D, Day M, García-Winder M, Imakawa K, Kittok R, D'occhio M, et al. Influence of exposure to bulls on resumption of estrous cycles following parturition in beef cows. J Anim Sci. 1984;59(5):1135-9. https://doi.org/10.2527/jas1984.5951135x

21. Berardinelli J, Joshi P. Introduction of bulls at different days postpartum on resumption of ovarian cycling activity in primiparous beef cows. J Anim Sci. 2005;83(9):2106-10. https://doi.org/10.2527/2005.8392106x

22. Berardinelli J, Tauck S. Intensity of the biostimulatory effect of bulls on resumption of ovulatory activity in primiparous, suckled, beef cows. Anim Reprod Sci. 2007;99(1-2):24-33. https://doi.org/10.1016/j.anireprosci.2006.03.019

23. Tauck SA. Factors associated with the biostimulatory effect of bulls on resumption of ovarian cycling activity and breeding performance of first-calf suckled beef cows: [Ph.D. dissertation]. Montana State University-Bozeman, College of Agriculture; 2005.

24. Tauck SA, Olsen JR, Wilkinson JR, Berardinelli JG. Duration of daily bull exposure on resumption of ovulatory activity in postpartum, primiparous, suckled, beef cows. Anim Reprod Sci. 2010;118(1):13-8. https://doi.org/10.1016/j.anireprosci.2009.06.010

25. Berardinelli J, Joshi P. Initiation of postpartum luteal function in primiparous restricted-suckled beef cows exposed to a bull or excretory products of bulls or cows. J Anim Sci. 2005;83(11):2495-500. https://doi.org/10.2527/2005.83112495x

26. Tauck SA, Berardinelli JG, Geary TW, Johnson NJ. Resumption of postpartum luteal function of primiparous, suckled beef cows exposed continuously to bull urine. J Anim Sci. 2006;84(10):2708-13. https://doi.org/10.2527/jas.2006-181

27. Fernandez DL, Berardinelli JG, Short RE, Adair R. Acute and chronic changes in luteinizing hormone secretion and postpartum interval to estrus in first-calf suckled beef cows exposed continuously or intermittently to mature bulls. J Anim Sci. 1996;74(5):1098-103. https://doi.org/10.2527/1996.7451098x

28. Gifford D, D'occhio M, Sharpe P, Weatherly T, Pittar R, Reeve D. Return to cyclic ovarian activity following parturition in mature cows and first-calf beef heifers exposed to bulls. Anim Reprod Sci. 1989;19(3-4):209-16. https://doi.org/10.1016/0378-4320(89)90094-8

29. Ferreira MFL, Rennó LN, Rodrigues, II, Detmann E, Paulino MF, de Campos Valadares Filho S, et al. Effects of parity order on performance, metabolic, and hormonal parameters of grazing beef cows during pre-calving and lactation periods. BMC Vet Res. 2021;17(1):311. https://doi.org/10.1186/s12917-021-03019-0

30. Tauck SA. The biostimulatory effect of bulls on the hypothalamic-pituitary-adrenal and-ovarian axes and on temporal aspects of resumption of ovarian cycling activity in primiparous, postpartum, anestrous, suckled, beef cows: Montana State University; 2008.

31. Tauck SA, Olsen JR, Wilkinson JR, Wedlake RJ, Davis KC, Berardinelli JG. Characteristics of temporal patterns of cortisol and luteinizing hormone in primiparous, postpartum, anovular, suckled, beef cows exposed acutely to bulls. Reprod Biol Endocrinol. 2010;8:1-8. https://doi.org/10.1186/1477-7827-8-89

32. Shipka M, Ellis L. No effects of bull exposure on expression of estrous behavior in high-producing dairy cows. Appl Anim Behav Sci. 1998;57(1-2):1-7. https://doi.org/10.1016/s0168-1591(97)00132-9

33. Wathes D, Fenwick M, Cheng Z, Bourne N, Llewellyn S, Morris D, et al. Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. Theriogenology. 2007;68:S232-S41. https://doi.org/10.1016/j.theriogenology.2007.04.006

34. Mekuriaw Y. Negative energy balance and its implication on productive and reproductive performance of early lactating dairy cows: review paper. J Appl Anim Res. 2023;51(1):220-8. https://doi.org/10.1080/09712119.2023.2176859

35. Lucy M. Mechanisms linking nutrition and reproduction in postpartum cows. Reproduction (Cambridge, England) Supplement. 2003;61:415-27. https://doi.org/10.1530/biosciprocs.5.031

36. Ababneh M, Obeidat I, Husein M, Talafha A. Effect of acute bull exposure around the time of artificial insemination on serum oxytocin and progesterone concentrations and pregnancy rates in dairy cows. Reprod Domest Anim. 2013;48(2):223-30. https://doi.org/10.1111/j.1439-0531.2012.02136.x

37. Scaramuzzi RJ, Martin GB. The importance of interactions among nutrition, seasonality and socio-sexual factors in the development of hormone-free methods for controlling fertility. Reprod Domest Anim. 2008;43 Suppl 2:129-36. https://doi.org/10.1111/j.1439-0531.2008.01152.x

38. Shipka M, Ellis L. Effects of bull exposure on postpartum ovarian activity of dairy cows. Anim Reprod Sci. 1999;54(4):237-44. https://doi.org/10.1016/s0378-4320(98)00160-2

39. Adrien ML, Mattiauda DA, Artegoitia V, Carriquiry M, Motta G, Bentancur O, et al. Nutritional regulation of body condition score at the initiation of the transition period in primiparous and multiparous dairy cows under grazing conditions: milk production, resumption of post-partum ovarian cyclicity and metabolic parameters. Animal. 2012;6(2):292-9. https://doi.org/10.1017/s175173111100142x

40. Coman S, Berean DI, Cimpean R, Ciupe S, Coman I, Bogdan LM. Clinical modalities for enhancing reproductive efficiency in buffaloes: a review and practical aspects for veterinary practitioners. Animals. 2024;14(18):2642. https://doi.org/10.3390/ani14182642

41. Barman P, Yadav M, Kumar H, Meur S. Effect of bull exposure on ovarian cyclicity in postpartum buffaloes. Buffalo Bulletin. 2011;30(4):18-23.

42. Gokuldas P, Yadav M, Kumar H, Singh G, Mahmood S, Tomar A. Resumption of ovarian cyclicity and fertility response in bull-exposed postpartum buffaloes. Anim Reprod Sci. 2010;121(3-4):236-41. https://doi.org/10.1016/j.anireprosci.2010.06.005

43. Kayani M, Anwar M, Andrabi S, Ghaffar A. Conception rate after estrus synchronization and biostimulation during low breeding season in buffaloes. J. Anim. Plant Sci. 2017;27(1):337-339.

44. Zaidi N-e-S, Anwar M. Effect of Biostimulation on Estrus Expression, Resumption of Ovarian Activity and Conception Rate in Postpartum Anestrus Nili-Ravi Buffaloes during Low Breeding Season. Pak Vet J. 2018;38(1). https://doi.org/10.29261/pakvetj/2018.007

45. Choudhary S, Kamboj M, Sahu D, Dutt S, Magotra A, Singh P, et al. Effect of biostimulation on growth rate and reproductive development of Bos indicus dairy heifers. Trop Anim Health Prod. 2022;54(2):138. https://doi.org/10.1007/s11250-022-03129-3

46. Tirindelli R, Dibattista M, Pifferi S, Menini A. From pheromones to behavior. Physiol Rev. 2009. https://doi.org/10.1152/physrev.00037.2008

47. Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell. 1991;65(1):175-87. https://doi.org/10.1016/0092-8674(91)90418-x

48. Keller M, Lévy F. The main but not the accessory olfactory system is involved in the processing of socially relevant chemosignals in ungulates. Front Neuroanat. 2012;6:39. https://doi.org/10.3389/fnana.2012.00039

49. Torres MV, Ortiz-Leal I, Sanchez-Quinteiro P. Pheromone sensing in mammals: a review of the vomeronasal system. Anatomia. 2023;2(4):346-413. https://doi.org/10.3390/anatomia2040031

50. Moawad UK, Awaad AS, Abedellaah BA. Morphological, histochemical and computed tomography on the vomeronasal organ (Jacobson's organ) of Egyptian native breeds of goats (Capra hircus). Beni Suef Univ J Basic Appl Sci. 2017;6(2):174-83. https://doi.org/10.1016/j.bjbas.2017.03.003

51. Hart BL. Flehmen Behavior and Vomeronasal Organ Function. In: Müller-Schwarze D, Silverstein RM, editors. Chemical Signals in Vertebrates 3. Boston, MA: Springer US; 1983. p. 87-103. https://doi.org/10.1007/978-1-4757-9652-0_5

52. Höfer D, Shin D-W, Drenckhahn D. Identification of cytoskeletal markers for the different microvilli and cell types of the rat vomeronasal sensory epithelium. J Neurocytol. 2000;29(3):147-56. https://doi.org/10.1023/a:1026548020851

53. Munger SD, Leinders-Zufall T, Zufall F. Subsystem organization of the mammalian sense of smell. Annu Rev Physiol. 2009;71(1):115-40. https://doi.org/10.1146/annurev.physiol.70.113006.100608

54. Meunier MA, Trives E, Gerardin E, Porte C, Acquistapace A, Chemineau P, et al. Sensory responses of olfactory and vomeronasal neurons in does are influenced by buck odors and their reproductive status. Chem Senses. 2025;50:bjaf012. https://doi.org/10.1093/chemse/bjaf012

55. Tanco VM, Whitlock BK, Jones MA, Wilborn RR, Brandebourg TD, Foradori CD. Distribution and regulation of gonadotropin-releasing hormone, kisspeptin, RF-amide related peptide-3, and dynorphin in the bovine hypothalamus. PeerJ. 2016;4:e1833. https://doi.org/10.7717/peerj.1833

56. Uenoyama Y, Nagae M, Tsuchida H, Inoue N, Tsukamura H. Role of KNDy neurons expressing kisspeptin, neurokinin B, and dynorphin A as a GnRH pulse generator controlling mammalian reproduction. Front Endocrinol. 2021;12:724632. https://doi.org/10.3389/fendo.2021.724632

57. Okamura H, Tsukamura H, Ohkura S, Uenoyama Y, Wakabayashi Y, Maeda K-i. Kisspeptin and GnRH pulse generation. In: Kauffman, A., Smith, J. (eds) Kisspeptin Signaling in Reproductive Biology. Advances in Experimental Medicine and Biology, vol 784. Springer; 2013. p. 297-323. https://doi.org/10.1007/978-1-4614-6199-9_14

58. Hassaneen A, Naniwa Y, Suetomi Y, Matsuyama S, Kimura K, Ieda N, et al. Immunohistochemical characterization of the arcuate kisspeptin/neurokinin B/dynorphin (KNDy) and preoptic kisspeptin neuronal populations in the hypothalamus during the estrous cycle in heifers. J Reprod Dev. 2016;62(5):471-7. https://doi.org/10.1262/jrd.2016-075

59. Ohkura S, Takase K, Wakabayashi Y, Yayou K-i, Uenoyama Y, Tsukamura H, et al. Kisspeptin Neurons in the Hypothalamic Arcuate Nucleus Are Potent Stimulators of GnRH/LH Secretion in Holstein Steers. Biology of Reproduction. 2010;83(Suppl_1):576. https://doi.org/10.1093/biolreprod/83.s1.576

60. Nguyen LT, Lau LY, Fortes MRS. Proteomic analysis of hypothalamus and pituitary gland in pre and postpubertal brahman heifers. Front Genet. 2022;13:935433. https://doi.org/10.3389/fgene.2022.935433

61. Rønnekleiv OK, Qiu J, Kelly MJ, editors. Arcuate kisspeptin neurons coordinate reproductive activities with metabolism. Semin Reprod Med. 2019;37(03):131-140. https://doi.org/10.1055/s-0039-3400251

62. Goodman RL, Hileman SM, Nestor CC, Porter KL, Connors JM, Hardy SL, et al. Kisspeptin, neurokinin B, and dynorphin act in the arcuate nucleus to control activity of the GnRH pulse generator in ewes. Endocrinol. 2013;154(11):4259-69. https://doi.org/10.1210/en.2013-1331

63. Moore AM, Novak AG, Lehman MN. KNDy neurons of the hypothalamus and their role in GnRH pulse generation: an update. Endocrinol. 2024;165(2):bqad194. https://doi.org/10.1210/endocr/bqad194

64. Wakabayashi Y, Nakada T, Murata K, Ohkura S, Mogi K, Navarro VM, et al. Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. J Neurosci. 2010;30(8):3124-32. https://doi.org/10.1523/jneurosci.5848-09.2010

65. Murata K, Wakabayashi Y, Sakamoto K, Tanaka T, Takeuchi Y, Mori Y, et al. Effects of brief exposure of male pheromone on multiple-unit activity at close proximity to kisspeptin neurons in the goat arcuate nucleus. J Reprod Dev. 2011;57(2):197-202. https://doi.org/10.1262/jrd.10-070e

66. Okamura H, Murata K, Sakamoto K, Wakabayashi Y, Ohkura S, Takeuchi Y, et al. Male effect pheromone tickles the gonadotrophin-releasing hormone pulse generator. J Neuroendocrinol. 2010;22(7):825-32. https://doi.org/10.1111/j.1365-2826.2010.02037.x

67. Rønnekleiv OK, Kelly MJ. Kisspeptin excitation of GnRH neurons. In: Kauffman, A., Smith, J. (eds) Kisspeptin Signaling in Reproductive Biology. Advances in Experimental Medicine and Biology, vol 784. Springer; 2013:113-31. https://doi.org/10.1007/978-1-4614-6199-9_6

68. Leonardi CE, Carrasco RA, Dias FC, Zwiefelhofer EM, Adams GP, Singh J. Mechanism of LH release after peripheral administration of kisspeptin in cattle. Plos one. 2022;17(12):e0278564. https://doi.org/10.1371/journal.pone.0278564

69. Scott CJ, Rose JL, Gunn AJ, McGrath BM. Kisspeptin and the regulation of the reproductive axis in domestic animals. J Endocrinol. 2019;240(1):R1-R16. https://doi.org/10.1530/joe-18-0485

70. Harlow K, Griesgraber MJ, Seman AD, Shuping SL, Sommer JR, Griffith EH, et al. The impact of undernutrition on KNDy (kisspeptin/neurokinin B/dynorphin) neurons in female lambs. J Neuroendocrinol. 2022;34(6):e13135. https://doi.org/10.1111/jne.13135

71. Thorson JF, Prezotto LD. Malnutrition alters protein expression of KNDy neuropeptides in the arcuate nucleus of mature ewes. Front Physiol. 2024;15:1372944. https://doi.org/10.3389/fphys.2024.1372944

72. Silva JdN, de Sousa LM, de Sousa ME, Vieira HR, Alves GA, Neifert NT, et al. KNDy neurons as an indirect target of insulin-like growth factor-1. J Neuroendocrinol. 2026;38(1):e70130. https://doi.org/10.1111/jne.70130

73. Mbiydzenyuy NE, Qulu L-A. Stress, hypothalamic-pituitary-adrenal axis, hypothalamic-pituitary-gonadal axis, and aggression. Metab Brain Dis. 2024;39(8):1613-36. https://doi.org/10.1007/s11011-024-01393-w

74. Li X, Knox A, O'byrne K. Corticotrophin-releasing factor and stress-induced inhibition of the gonadotrophin-releasing hormone pulse generator in the female. Brain Res. 2010;1364:153-63. https://doi.org/10.1016/j.brainres.2010.08.036

75. Whirledge S, Cidlowski JA. Glucocorticoids, stress, and fertility. Minerva Endocrinol. 2010;35(2):109-25.

76. Whirledge S, Cidlowski JA. A role for glucocorticoids in stress-impaired reproduction: beyond the hypothalamus and pituitary. Endocrinol. 2013;154(12):4450-68. https://doi.org/10.1210/en.2013-1652

77. Wagenmaker ER, Breen KM, Oakley AE, Tilbrook AJ, Karsch FJ. Psychosocial stress inhibits amplitude of gonadotropin-releasing hormone pulses independent of cortisol action on the type II glucocorticoid receptor. Endocrinol. 2009;150(2):762-9. https://doi.org/10.1210/en.2008-0757

78. Gupta S, Sharma A, Joy A, Dunshea FR, Chauhan SS. The impact of heat stress on immune status of dairy cattle and strategies to ameliorate the negative effects. Animals. 2022;13(1):107. https://doi.org/10.3390/ani13010107

79. Khan I, Mesalam A, Heo YS, Lee S-H, Nabi G, Kong I-K. Heat stress as a barrier to successful reproduction and potential alleviation strategies in cattle. Animals. 2023;13(14):2359. https://doi.org/10.3390/ani13142359

80. Dovolou E, Giannoulis T, Nanas I, Amiridis GS. Heat stress: A serious disruptor of the reproductive physiology of dairy cows. Animals. 2023;13(11):1846. https://doi.org/10.3390/ani13111846

81. Roth Z. PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM: Cellular and molecular mechanisms of heat stress related to bovine ovarian function1. J Anim Sci. 2015;93(5):2034-44. https://doi.org/10.2527/jas.2014-8625

82. Wolfenson D, Roth Z, Meidan R. Impaired reproduction in heat-stressed cattle: basic and applied aspects. Anim Reprod Sci. 2000;60:535-47. https://doi.org/10.1016/s0378-4320(00)00102-0

83. Țogoe D, Mincă NA. The impact of heat stress on the physiological, productive, and reproductive status of dairy cows. Agric. 2024;14(8):1241. https://doi.org/10.3390/agriculture14081241

84. Roth Z. Influence of heat stress on reproduction in dairy cows—Physiological and practical aspects. J Anim Sci. 2020;98(Supplement_1):S80-S7. https://doi.org/10.1093/jas/skaa139

85. Bernabucci U, Lacetera N, Baumgard LH, Rhoads RP, Ronchi B, Nardone A. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal. 2010;4(7):1167-83. https://doi.org/10.1017/s175173111000090x

86. Bova TL, Chiavaccini L, Cline GF, Hart CG, Matheny K, Muth AM, et al. Environmental stressors influencing hormones and systems physiology in cattle. Reprod Biol Endocrinol. 2014;12(1):58. https://doi.org/10.1186/1477-7827-12-58

87. Hansen P. Embryonic mortality in cattle from the embryo's perspective. J Anim Sci. 2002;80(E-suppl_2):E33-E44. https://doi.org/10.2527/animalsci2002.80e-suppl_2e33x

88. Li M, Hassan Fu, Tang Z, Guo Y, Liang X, Peng L, et al. Physiological, oxidative and metabolic responses of lactating water buffaloes to tropical climate of South China. Vet med sci. 2021;7(5):1696-706. https://doi.org/10.1002/vms3.570

89. Sejian V, Bhatta R, Gaughan J, Dunshea F, Lacetera N. Adaptation of animals to heat stress. Animal. 2018;12(s2):s431-s44. https://doi.org/10.1017/s1751731118001945

90. Kasimanickam R, Asay M, Schroeder S, Kasimanickam V, Gay J, Kastelic J, et al. Calm temperament improves reproductive performance of beef cows. Reprod Domest Anim. 2014;49(6):1063-7. https://doi.org/10.1111/rda.12436

91. Fernandez-Novo A, Pérez-Garnelo SS, Villagrá A, Pérez-Villalobos N, Astiz S. The effect of stress on reproduction and reproductive technologies in beef cattle—A review. Animals. 2020;10(11):2096. https://doi.org/10.3390/ani10112096

92. Šárová R, Špinka M, Panamá JLA, Šimeček P. Graded leadership by dominant animals in a herd of female beef cattle on pasture. Anim Behav. 2010;79(5):1037-45. https://doi.org/10.1016/j.anbehav.2010.01.019

93. Solano J, Galindo F, Orihuela A, Galina C. The effect of social rank on the physiological response during repeated stressful handling in Zebu cattle (Bos indicus). Physiol Behav. 2004;82(4):679-83. https://doi.org/10.1016/j.physbeh.2004.06.005

94. Squires E. Effects on animal behaviour, health and welfare. In: Applied Animal Endocrinology. CABI; 2010. p. 235-72. https://doi.org/10.1079/9781845937553.0235

95. Diskin M, Kenny D. Managing the reproductive performance of beef cows. Theriogenology. 2016;86(1):379-87. https://doi.org/10.1016/j.theriogenology.2016.04.052

96. Stagg K, Spicer LJ, Sreenan JM, Roche JF, Diskin MG. Effect of Calf Isolation on Follicular Wave Dynamics, Gonadotropin and Metabolic Hormone Changes, and Interval to First Ovulation in Beef Cows Fed Either of Two Energy Levels Postpartum1. Biol Reprod. 1998;59(4):777-83. https://doi.org/10.1095/biolreprod59.4.777

97. Griffith MK, Williams GL. Roles of Maternal Vision and Olfaction in Suckling-Mediated Inhibition of Luteinizing Hormone Secretion, Expression of Maternal Selectivity, and Lactational Performance of Beef Cows1. Biol Reprod. 1996;54(4):761-8. https://doi.org/10.1095/biolreprod54.4.761

98. Nava-Trujillo H, Valeris-Chacin R, Morgado-Osorio A, Zambrano-Salas S, Tovar-Breto L, Quintero-Moreno A. Reproductive performance of water buffalo cows: a review of affecting factors. J Buffalo Sci. 2020;9:133-51. https://doi.org/10.6000/1927-520x.2019.08.03.15

99. Wathes D, Pollott G, Johnson K, Richardson H, Cooke J. Heifer fertility and carry over consequences for life time production in dairy and beef cattle. Animal. 2014;8(s1):91-104. https://doi.org/10.1017/s1751731114000755

100. Day ML, Nogueira GP. Management of age at puberty in beef heifers to optimize efficiency of beef production. Anim Front. 2013;3(4):6-11. https://doi.org/10.2527/af.2013-0027

101. Fiol C, Ungerfeld R. Biostimulation in cattle: Stimulation pathways and mechanisms of response. Trop Subtrop Agroecosystems. 2012;15(1):S29-S45. https://doi.org/10.56369/tsaes.1342

102. Fiol C, Quintans G, Ungerfeld R. Response to biostimulation in peri-puberal beef heifers: influence of male-female proximity and heifer's initial body weight. Theriogenology. 2010;74(4):569-75. https://doi.org/10.1016/j.theriogenology.2010.03.015

103. Sartori R, Bastos M, Baruselli PS, Gimenes LU, Ereno RL, Barros C. Physiological differences and implications to reproductive management of Bos taurus and Bos indicus cattle in a tropical environment. Reprod Domest Rumin. 2011;7:357-75. https://doi.org/10.5661/rdr-vii-357

104. Oliveira C, Oliveira Filho B, Gambarini M, Viu M, Lopes D, Sousa A. Effects of biostimulation and nutritional supplementation on pubertal age and pregnancy rates of Nelore heifers (Bos indicus) in a tropical environment. Anim Reprod Sci. 2009;113(1-4):38-43. https://doi.org/10.1016/j.anireprosci.2008.08.006

105. Choudhary S, Kamboj M, Raheja N, Kumar N, Saini M, Lathwal S. Influence of bull biostimulation on age at puberty and reproductive performance of Sahiwal heifers. Indian J Anim Scis. 2020;90(1):28-34. https://doi.org/10.56093/ijans.v90i1.98172

106. Rekwot P, Ogwu D, Oyedipe E, Sekoni V. Effects of bull exposure and body growth on onset of puberty in Bunaji and Friesian x Bunaji heifers. Reprod Nutr Dev. 2000;40(4):359-67. https://doi.org/10.1051/rnd:2000104

107. Choudhary S, Kamboj ML. Effect of bull biostimulation on the oestrous behaviour of pubertal Sahiwal (Bos indicus) heifers. Anim Reprod Sci. 2019;209:106149. https://doi.org/10.1016/j.anireprosci.2019.106149

108. Dash S, Chakravarty A, Singh A, Upadhyay A, Singh M, Yousuf S. Effect of heat stress on reproductive performances of dairy cattle and buffaloes: A review. Vet World. 2016;9(3):235. https://doi.org/10.14202/vetworld.2016.235-244

109. Becker C, Collier R, Stone A. Invited review: Physiological and behavioral effects of heat stress in dairy cows. J Dairy Sci. 2020;103(8):6751-70. https://doi.org/10.3168/jds.2019-17929

110. Hubbard AJ, Foster MJ, Daigle CL. Social dominance in beef cattle—A scoping review. Appl Anim Behav Sci. 2021;241:105390. https://doi.org/10.1016/j.applanim.2021.105390

111. Chebel RC, Silva PR, Endres MI, Ballou MA, Luchterhand KL. Social stressors and their effects on immunity and health of periparturient dairy cows. J Dairy Sci. 2016;99(4):3217-28. https://doi.org/10.3168/jds.2015-10369

112. Marina H, Ren K, Hansson I, Fikse F, Nielsen PP, Rönnegård L. New insight into social relationships in dairy cows and how time of birth, parity, and relatedness affect spatial interactions later in life. J Dairy Sci. 2024;107(2):1110-23. https://doi.org/10.3168/jds.2023-23483

113. Timlin CL, Dias NW, Hungerford L, Redifer T, Currin JF, Mercadante VR. A retrospective analysis of bull: cow ratio effects on pregnancy rates of beef cows previously enrolled in fixed-time artificial insemination protocols. Transl Anim Sci. 2021;5(3):txab129. https://doi.org/10.1093/tas/txab129

114. Petherick JC. A review of some factors affecting the expression of libido in beef cattle, and individual bull and herd fertility. Appl Anim Behav Sci. 2005;90(3-4):185-205. https://doi.org/10.1016/j.applanim.2004.08.021

Table

Table 1. Comparative analysis of the male effect in beef cattle, dairy cattle, and buffaloes

Category	Beef Cattle	Dairy Cattle	Buffaloes
Primary reproductive challenge	Prolonged postpartum anestrus exacerbated by suckling-mediated inhibition.	The “high-producer paradox” and reproductive “blindness” due to extreme metabolic demands.	High incidence of silent ovulation and seasonal anestrus in tropical/subtropical climates.
Key mechanism	Acceleration of GnRH/LH pulse frequency via direct pheromonal activation of the ARC KNDy network.	Metabolic gating of the HPO axis, wherein low insulin and IGF-1 levels reduce ovarian gonadotropin sensitivity.	Enhancement of estrus expression and synchronization of the LH surge through chemical biostimulation.
Critical Management Strategy	Implementation of a structured 12 h daily exposure rhythm to avoid sensory adaptation.	Strategic application during late lactation to align with positive energy balance and metabolic recovery.	Continuous or round-the-clock bull exposure to optimize heat detection and AI success.
Key Research Findings	Stress reduction via bull exposure leads to lower cortisol and increased LH pulse frequency.	High-yielding cows remain refractory to male cues during early lactation or severe NEB.	Synergy with CIDR/GnRH protocols achieves up to 87.5% conception during low breeding seasons.
Metabolic / hormonal insights	Influence of bull maturity and social dominance on serving efficiency and stimulus potency.	Nutritional management serves as a fundamental prerequisite for biostimulatory responsiveness.	Mitigation of environmental heat load (THI) is essential to maintain biostimulatory efficacy.
Optimal timing	Early postpartum period (beginning as early as 1-week postpartum).	Late-lactation or following the attainment of a favorable metabolic state.	Late postpartum or during the low breeding (summer) season.

Abbreviations: AI, artificial insemination; ARC, arcuate nucleus; CIDR, controlled internal drug release; GnRH, gonadotropin-releasing hormone; HPO, hypothalamic-pituitary-ovarian; IGF-1, insulin-like growth factor 1; KNDy, kisspeptin/neurokinin B/dynorphin; LH, luteinizing hormone; NEB, negative energy balance; THI, temperature-humidity index.

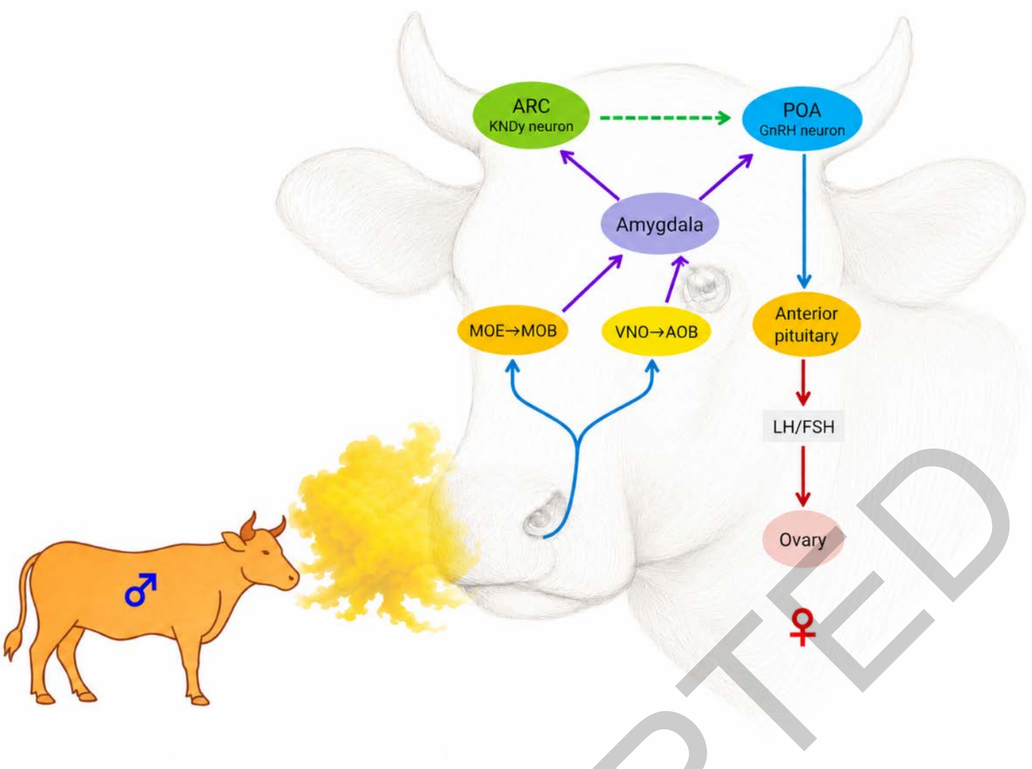

Figure 1. Integrated neuroendocrine pathways of pheromonal signaling in the bovine "male effect." Male-derived pheromonal cues emitted by bulls are detected by females through dual chemosensory pathways involving the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). These sensory signals are relayed through the main and accessory olfactory bulbs (MOB and AOB) to the amygdala, which serves as an intermediate relay station to hypothalamic reproductive nuclei. Within the hypothalamus, arcuate nucleus (ARC) KNDy neurons are proposed to modulate pulsatile GnRH/LH secretion, whereas preoptic area (POA)-associated GnRH neurons represent the principal GnRH output pathway to the anterior pituitary. This neuroendocrine activation promotes the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary and supports ovarian activity.

Abbreviations: AOB, accessory olfactory bulb; ARC, arcuate nucleus; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; KNDy neurons, kisspeptin/neurokinin B/dynorphin neurons; LH, luteinizing hormone; MOB, main olfactory bulb; MOE, main olfactory epithelium; POA, preoptic area; VNO, vomeronasal organ.

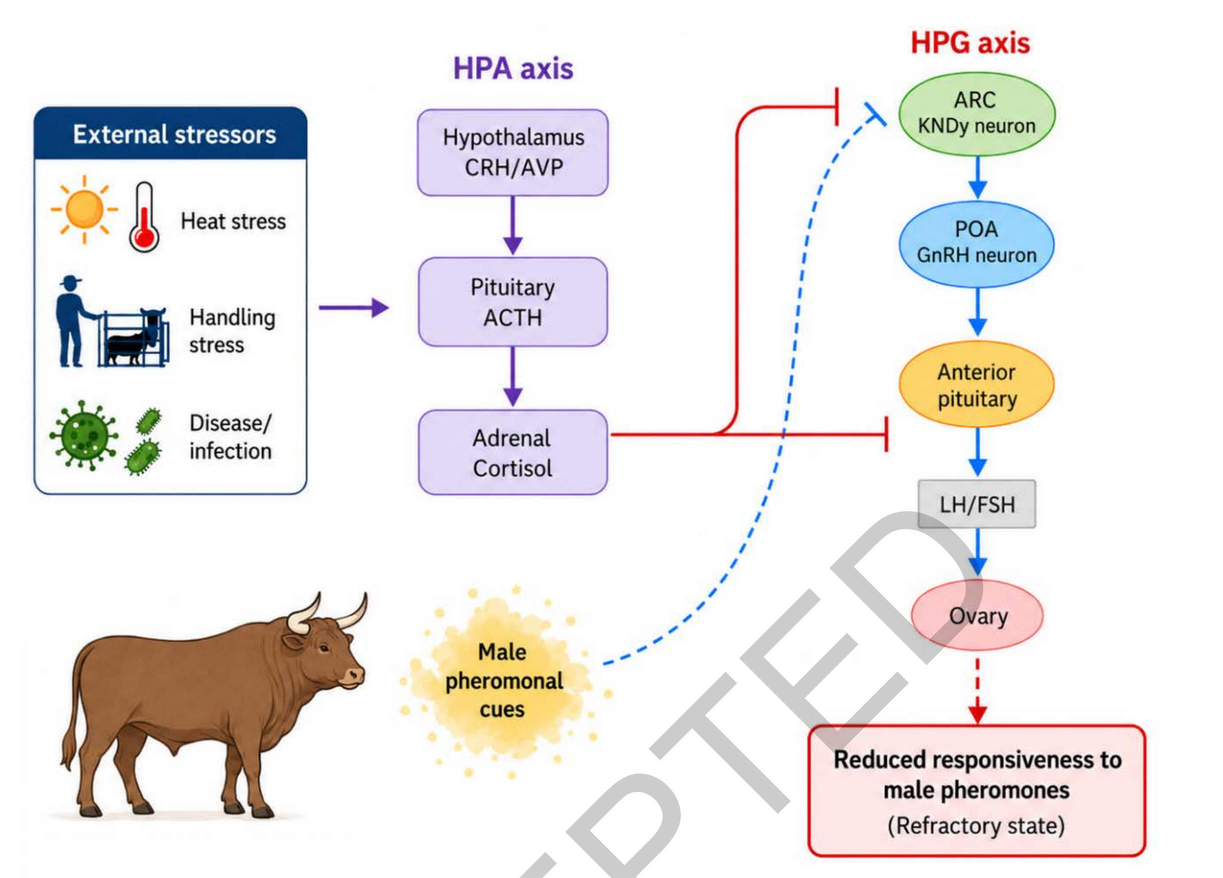

Figure 2. Neuroendocrine blockade of male pheromonal signal integration through HPA–HPG axis interactions in bovines. External stressors, including heat stress, handling stress, and disease/infection, activate the hypothalamic-pituitary-adrenal (HPA) axis and increase cortisol secretion. Elevated cortisol inhibits the hypothalamic-pituitary-gonadal (HPG) axis by suppressing the arcuate nucleus (ARC) KNDy neuronal network and reducing anterior pituitary responsiveness, thereby attenuating the integration of male pheromonal cues. Consequently, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion is reduced, ovarian responsiveness declines, and the animal enters a refractory state with reduced responsiveness to male pheromones.

Abbreviations: ACTH, adrenocorticotropic hormone; ARC, arcuate nucleus; AVP, arginine vasopressin; CRH, corticotropin-releasing hormone; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenal; HPG, hypothalamic-pituitary-gonadal; KNDy, kisspeptin/neurokinin B/dynorphin; LH, luteinizing hormone; POA, preoptic area.