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Biological stimulation techniques and pheromonal modulation of reproduction in animals

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The exposure of male and female animals has been reported to influence the reproductive function in opposite sex of the conspecifics. Biological stimulation or biostimulation is a term mainly used to describe the stimulatory effect of male animal on female animal reproduction mainly through genital stimulation, priming pheromones, or other well defined external cues. Biostimulation description can also be applied to the stimulatory and behavioural response in male animals after the exposure to female animals of the same species. Biostimulatory interventions has been used successfully in many farm animal species including cattle, sheep, goats, and pigs. It is known that pheromones play an important role in mammalian behaviour and reproductive processes and male animal exposure and pheromonal communication has been reported to influence reproductive function in females. Various studies in insects, sheep, pigs, goats and cattle have established the significance of pheromones in the strong influence exerted by male on reproduction in the females. Pheromones can act through olfaction, visual, auditory or tactile stimuli and can elicit both behavioural and endocrine responses in animals of the same species. Effects of pheromones and biostimulation can be beneficial in improving breeding efficiency and overall reproductive performance in farm animals reared in tropical countries like India. Over the past few decades, numerous advances have also been made regarding the applications of biostimulation and pheromonal agents like aerosol nano-drug delivery systems and holds promise for enhancing fertility in livestock. This article aims to provide an up-to-date overview of the current state of knowledge regarding various methods of biostimulation and pheromonal use, their applications and prospects in reproductive management of farm animals.

What is Biostimulation in animals?

Biostimulation in animals can be defined as physiological effect of male animal exposure and pheromonal communication on female animals mediated mainly through genital stimulation, pheromonal agents, or other well defined external cues. Animal biostimulatory techniques refers to the use of various interventions involving animal exposure and associated signals or cues to positively influence physiological and reproductive processes of the opposite sex members of the same species. The exposure of males has been reported to influence reproductive functions in females of many animal species. Stimulatory action triggered by the male exposure and conspecific chemical signals can act through different pathways including olfactory, visual, and auditory signal pathways. Chemical messengers, mostly called as pheromones, have a great potential and pheromonal communication and interactions play important roles in animal behavior and reproductive processes. Biostimulatory interventions can influence the reproductive efficiency by advancing sexual maturity,

inducing ovulation, reducing of postpartum anestrus and improving mating outcomes in cattle (Burns and Spitzer, 1992). Sexually mature male exposure can stimulate the onset of ovarian activity and estrus in anoestrus sheep, goat, pig, cattle and buffalo (Burns and Spitzer, 1992; Gokuldas et al., 2010).

Pheromones and their role in biological stimulation

Pheromones are volatile and complex chemical signals released or secreted in the urine, faeces, saliva or gland secretions of animals that are perceived by the olfactory system and elicit specific behavioural reactions and hormonal responses in conspecifics (Rekwot et al., 2001). The first conclusive evidence of pheromonal communication derived through the experiments performed in the 1870s by the French scientist Jean-Henri Fabre in the peacock female moth (*Saturnia pyri*) and the first pheromonal agent identified was the sex-attractant pheromone of commercial silkmoth (*Bombyx mori*). Unlike hormones, pheromones are secreted externally, involved extensively in socio-sexual behaviour and these signals can regulate conspecific behavior and physiology. Extensive studies in mice have established the importance of pheromones as powerful regulators of reproduction and strong influence exerted by the male pheromones on reproductive activity in the female mice. There are different types of pheromones in mammals. Two major groups are priming and signaling pheromones, which are thought to act through olfaction, visual, auditory or tactile stimuli. Signaling pheromones are substances that elicit an instant, short-term behavioral response and stimulation, invoking a stimulus-response pathway mostly for communication and mediated by the central nervous system (Izard, 1983). These pheromones can act as both attractants and repellents, depending on the context and the species involved. In contrast, priming pheromones are substances that cause physiological events and comparatively longer-term effects either through inhibition or stimulation wherein hormonal, reproductive and other systems could be altered (Izard, 1983). Priming pheromones have been shown to be associated with the mammalian reproduction like in rodents. In domestic mammals, priming pheromones from the male have potential influence on induction of puberty, shortening of seasonal anoestrus and resumption of postpartum ovarian activity and estrus. Priming pheromones from male opossum can stimulate ovarian follicular development and body growth in juvenile females (Harder and Jackson, 2003). Similarly, pheromones seem to be involved in the boar effect and ram effect. The accessory olfactory system detects priming pheromones through the specialized vomeronasal organ (VNO) which is well-developed in species like cattle and buffalo. Most of their effects on reproduction are mediated via Hypothalamic-Pituitary-Gonadal axis.

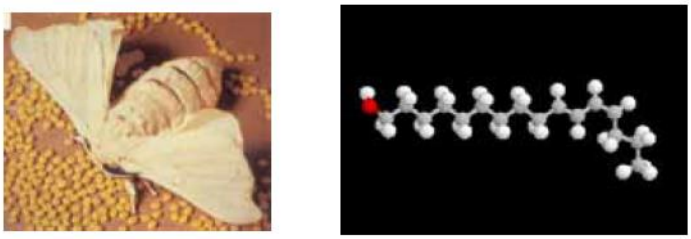

Fig. 1: First pheromone identified in silkmoth

Other types of pheromones include releaser and sex-attractant pheromones. Releaser pheromones are those chemical signals that cause an alteration in the behaviour of the recipient, and they produce short-term behavioural changes and act as attractants or repellents. Male pig stimulates the standing reflex of the female pig through sex pheromones. These pheromones are synthesized in the testes and released in boar saliva and were identified as steroids viz. 5 alpha-androst-16-en-3-one and 5 alpha- androst-16- en-3 beta-ol. Some organisms use powerful attractant molecules to attract mates from a distance and elicits a rapid response, but is quickly degraded. Sex-attractant pheromones are released when receptive female animals available for breeding and these pheromones can also transmit signals about their genotype and species. Sex pheromones are produced mostly by the females and few types of these pheromones are also produced by male counterparts. There are attractant pheromones which induce sex drive and arousal and mating

behavior of male in species like dogs. Estrus-specific pheromonal agents in urine of female dogs have been found to influence the pre-copulatory behavior in male dogs.

Mechanism of biostimulation and pheromonal action

Exact mechanisms of biostimulation in animals have not yet established and require more attention. However, it has been widely proposed that male stimuli could exert its effects on reproductive processes through pheromonal and other allelo-mimetic cues via Hypothalamic-Pituitary-Gonadal axis and complex neuro-endocrine cascade in animals. Pheromones can be transported from animals in faeces, urine and cutaneous gland excretions. They are released into the environment as hydrophobic bioactive chemical signals and are perceived through the vomeronasal organ of the recipient animal and subsequently, chemosensory neuron receptors are activated. Generated signals could directly stimulate the hypothalamus to elicit a suitable neuro-endocrine cascade unique to the specific subpopulation of neurons stimulated in VNO, which in turn can regulate endocrine, physiological and reproductive functions as well as characteristic social behaviours. These events exert significant influence on reproductive processes through the release of Gonadotropin releasing hormone (GnRH) from the hypothalamus (Rekwot et al., 2001). In addition to the GnRH and other reproductive hormones, Kisspeptin has been indicated and may also serve as a target for pheromones to modulate the reproductive axis (Keller et al., 2012). There is also an indication that the adrenal activation is involved in the response of female to male pheromonal stimuli. Studies have indicated possible association between resumption of ovarian activity and increased cortisol concentrations in bull-exposed cows and buffaloes (Tauck et al., 2007; Gokuldas, 2009).

Biostimulation and pheromonal modulation in animal reproductive management

Animal biostimulatory techniques and pheromonal modulation can yield potential applications in various fields ranging from agronomy, ecology and farm animal management. Manipulations of key biostimulatory factors and pathways linking environmental inputs to reproductive outcomes may effectively aid in regulating the reproductive performance of animals. Many studies have supported biostimulatory effect on reproductive processes like puberty induction, resumption of ovarian cyclicity and estrus expression and thus can be used advantageously to improve reproductive efficiency in farm animals. Some of these potential applications in animals are briefly summarized hereunder.

1. Hastening of puberty and sexual maturity: The exposure to male pheromonal stimuli can hasten the onset of puberty in prepubertal females of various species including sheep, goat, pig, and cattle and this is advantageous for optimal reproductive performance and overall productivity (Rekwot et al., 2001). Boar exposure has been found to accelerate puberty by about 30 days in female pigs. Additionally, introduction of boar to gilts at about 190 days of age causes marked synchrony in attainment of pubertal estrus. Exposure of vasectomised bulls can be used an effective management tool to decrease age at puberty in cattle.

2. *Estrus induction, synchronization and resumption of cyclicity in anestrus females:* Exposure of male priming pheromones to a batch of anestrus females during the non-breeding season results in activation of gonadotropin release, termination of anestrus and ovulation synchronization in goat and sheep. The "ram effect" has been reported to accelerate the onset of estrus activity and aid in varying degrees of estrus synchronization in sheep. Positive effects of biostimulation on estrous cyclicity have been demonstrated with bulls, androgenized steer and androgenized cows (Berardinelli and Tauck, 2007; Fiol et al., 2010). It can be an effective management strategy to increase the proportion of animals resuming cyclicity and to increase the effectiveness of A.I. and estrus synchronization programs. The efficacy of estrus synchronization protocols can be enhanced by bull exposure after AI (Berardinelli et al., 2001). Sterile bulls, bull urine, direct and fence-line exposure to bulls, and exposure

to testosterone treated female cattle were found to be effective biostimulants (Berardinelli and Joshi, 2005; Gokuldas et al., 2010). Daily exposure of vasectomised bull has been demonstrated to improve conception rate in postpartum cattle (Izard, 1983).

3. Enhancing the intensity and expression of Estrus: Pheromones from the male can be helpful in enhancing the estrus expression and duration of estrus in animals. Studies have reported higher duration of estrus and more intense estrus when female cattle were exposed to bulls. The presence of a boar has been shown to increase the expression of estrus and ovulation in sows (Langendijk et al., 2000). Bull exposure significantly increased both incidence of overt estrus and the intensity of estrus, while decreasing the incidence of silent estrus in buffaloes (Gokuldas, 2010). Additionally, Barman (2008) reported higher conception rates in biostimulated buffaloes compared to non-exposed buffaloes.

4. Improving male animal libido and mating behaviour: Male sexual performance can be improved by various sexual stimulation technique and exposure to females in various farm animal species (Bailey et al., 2005). Pheromones are released by females during estrus, thus signalling the stage of the cycle, and stimulating sexual behavior and endocrine functions of males. Exposure time of females and change of females is also responsible for producing effect in males. It was also demonstrated that the bulls show improved sexual behaviour after application of cervical mucus. Rapid introduction of estrus ewes in a group of rams induces changes in the behavioural and endocrinological systems in the rams.

5. Stimulatory effect on semen quality: Introduction of new female stimuli to breeding male can influence various semen attributes like ejaculate volume and sperm motility. It was reported that semen volume was notably increased in goats by changing the doe stimulus after the first ejaculation (Prado et al., 2003). These effects were nonetheless diminished in subsequent days and thus the overall benefits on male libido and semen quality were minimal. In pigs, males in contact with the new female groups produced semen with the better quality and quantity. Ejaculates after sow exposure had larger volumes, sperm motility, live sperm, total sperm per ejaculate and normal acrosome integrity (Umesiobi, 2010).

Recent trends and prospects in Biostimulatory techniques and pheromonal therapy

Development of newer technologies and knowledge in the area of pheromonal agents and advent of nanotechnology in animal science points to novel and innovative strategies for managing animal reproduction. Using nanotechnology, many biological molecules and synthetic analogues can acquire novel physicochemical properties like bioavailability and controlled release. Further, development of aerosol nano-drug delivery system may facilitate drug delivery through olfactory pathways or nose-to-brain route. This opens way for pheromone-based protocols for biological control of reproductive processes like such as the male effect, estrus detection, anestrous, puberty, pregnancy diagnosis, sexual activity of males and holds promise for enhancing fertility in farm animals (Hashem and Gonzalez-Bulnes,

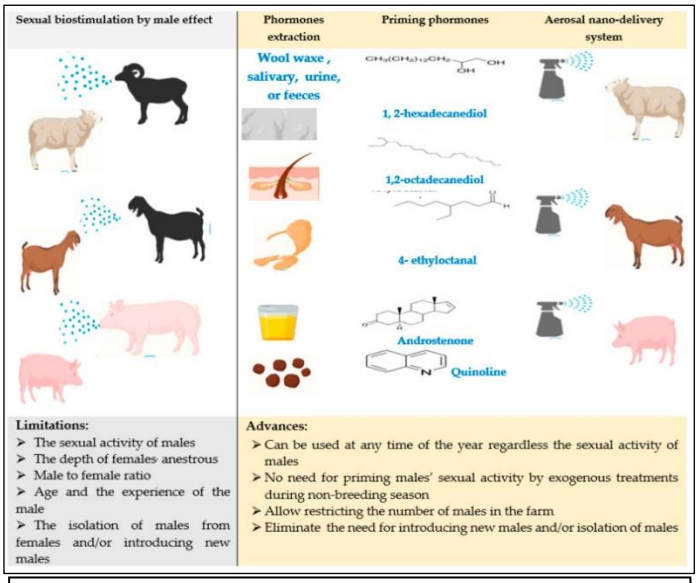

Fig. 2: Potential applications of an aerosol nano-delivery system in biostimulation in animals Source: (Hashem and Gonzalez-Bulnes, 2021)

2021). This could be achieved through developing synthetic analogues of priming pheromones or pheromone-based kits and preparations. There exists scope to develop novel nanoparticle-based bioassay kits for non-invasive detection of estrus or ovulation in buffaloes using urinary pheromonal compounds like p-Cresol and 9-octadecenoic acid (Rajanarayanan and Archunan, 2011). Synthetically produced pig pheromones like androstenone are now being used as active ingredients in commercial preparations of aerosol sprays for stimulation of estrus and standing reflex and improving success rate of AI. Moreover, this can also help to determine at the right stage to be insemination and in reduce the need for teaser males in the pig farms. Recently, unique mixture of boar salivary molecules (androstenone, androstenol and quinoline) has been found to be more powerful and effective in inducing sexual behavior in sows after weaning (McGlone et al., 2019). In pet dogs, commercially-available pheromonal agents which are synthetic analogues of calming or appeasing pheromones can reduce signs of stress, help the animal feel safe and prevent behaviors associated with fear, stress and anxiety. Estrus-specific pheromonal agents in urine of female dogs have been found to influence the pre-copulatory behavior in male dogs (Rajagopal et al., 2022). These specific volatile compounds appearing in estrus urine can also serve as non-invasive indicator of estrus. Albeit with significant level of advancements in the field, there are also some constraints and challenges associated with the pheromonal modulation of animal reproduction. Inconsistencies have been reported in pheromone response of various animals, which can limit the effectiveness of pheromone-based strategies in reproductive management. Moreover, there exist challenges in identifying and synthesizing specific pheromonal agents linked to different reproductive processes.

CONCLUSION

Biological stimulation and pheromonal stimuli can exert profound effects on reproductive processes through different neuro-endocrine pathways in members of the same animal species. Advances in our understanding of pheromones and their effects on both male and female animals have prompted researchers to intensely explore their use in animal reproduction. Manipulation of these factors and pathways connecting environmental inputs to reproductive outputs can potentially help in improving reproductive performance of animals. Different biostimulatory measures are demonstrated to accelerate the onset of puberty, manage seasonal and postpartum anestrus, improve estrus detection efficiency, libido and AI success rate. With largescale adoption of Artificial Insemination, male involvement or stimuli in reproductive processes have been significantly curtailed. This prolonged lack of male stimuli can negatively affect reproductive performance in females bred exclusively by AI. Biostimulation is a non-hormonal and non-invasive practical technique and thus holds promise in the context of animal-friendly, organic and sustainable livestock farming. It can be effectively adopted as a useful management tool for optimizing breeding programs and improving reproductive efficiency in farm animals.

REFERENCES

Bailey JD, Anderson LH and Schillo KK (2005). Effects of novel females and stage of the estrous cycle on sexual behaviour in mature beef bulls. J Anim. Sci., 83: 613-624.

Berardinelli JG, and Tauck SA (2007). Intensity of the biostimulatory effect of bulls on resumption of ovulatory activity in primiparous, suckled, beef cows. Anim. Reprod. Sci., 99: 24-33

Berardinelli JG, Anderson K, Robinson B and Adair R. (2001). Effect of presence of bulls and restricted suckling on breeding performance of first-calf suckled beef cow using the ovsynch protocol. Proceedings, Western Section, American Society of Anim. Sci., 52: 1-5.

Berardinelli JG and Joshi PS. (2005). 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., 83:2495–500.

Burns PD and Spitzer JC. (1992). Influence of biostimulation on reproduction in postpartum beef cows. Journal Animal Science., 70: 358–362.

Fiol C, Quintans G and Ungerfeld R. (2010). Response to biostimulation in peri-puberal beef heifers: influence of male-female proximity and heifer's initial body weight. Theriogenology, 74: 569-575.

Gokuldas PP. (2009). Studies on Resumption of Ovarian Cyclicity and Endocrine Profile in Bull Exposed Postpartum Buffaloes (MVSc Thesis submitted to Indian Veterinary Research Institute), ICAR-IVRI, Izatnagar, UP, India. pp: 1-126.

Gokuldas PP, Yadav MC, Kumar H, Singh G, Mahmood S and Tomar AKS. (2010). Resumption of ovarian cyclicity and fertility response in bull-exposed postpartum buffaloes. Anim. Reprod. Sci., 121(3): 236-241.

Hashem NM and Gonzalez-Bulnes A. (2021). Nanotechnology and Reproductive Management of Farm Animals: Challenges and Advances. Animals, 11(7):1932.

Izard MK (1983). Pheromones and reproduction in domestic animals. In: Vandenbergh, Journal G. (Ed.) Pheromones and Reproduction in Mammals. Academic Press, New York, pp. 253–285.

Keller M, Bedos M, Jouhanneau M, Martini M, Retana S and Delgadillo J. (2012). Pheromonal modulation of reproductive function in mammals. Tropical and Subtropical Agroecosystems. 15(1): S103-11.

Langendijk P, van den Brand H, Soede N M and Kemp B. (2000). Effect of boar contact on follicular development and on estrus expression after weaning in primiparous sows. Theriogenology, 54:1295–1303.

McGlone J, Devaraj SG and Garcia A. (2019). A Novel Boar Pheromone Mixture Induces Sow Estrus Behaviors and Reproductive Success. Appl. Anim. Behav. Sci., 219: 104832.

Prado V, Orihuela A, Lozano S and Perez-Leon I (2003). Effect on ejaculatory performance and semen parameters of sexually-satiated male goats (*Capra hircus*) after changing the stimulus female. Theriogenology, 60: 261–267.

Rajagopal T, Dhivya A, Ponmanickam P, Akbarsha MA, Archunan G. (2022). Identification of urinary sex pheromones in female Rajapalayam breed dog and their influence on stud male reproductive behaviour. J App. Biol. Biotech., pp: 1-9.

Rajanarayanan A and Archunan G (2011). Identification of urinary sex pheromones in female buffaloes and their influence on bull reproductive behaviour. Research in Veterinary Science, 91: 301–305.

Rekwot PI, Ogwu D and Oyedipe EO (2001). The role of pheromones and biostimulations in animal reproduction. Animal Reproduction Science, 65: 157–170.

Tauck SA, Olsen JR and Berardinelli JG. (2007). Adrenal involvement in the biostimulatory effect of bulls. Reproductive biology and endocrinology, 5(33): 1-7.

Umesiobi DO. (2010). Boar effects and their relations to fertility and litter size in sows. South African Journal of Animal Science, 40 (5) S1: 471-475.

Ungerfeld R. (2009). Short-term exposure of high body weight heifers to testosterone-treated steers increases pregnancy rate during early winter bull breeding. Animal Reproduction, 6(3): 446-449.