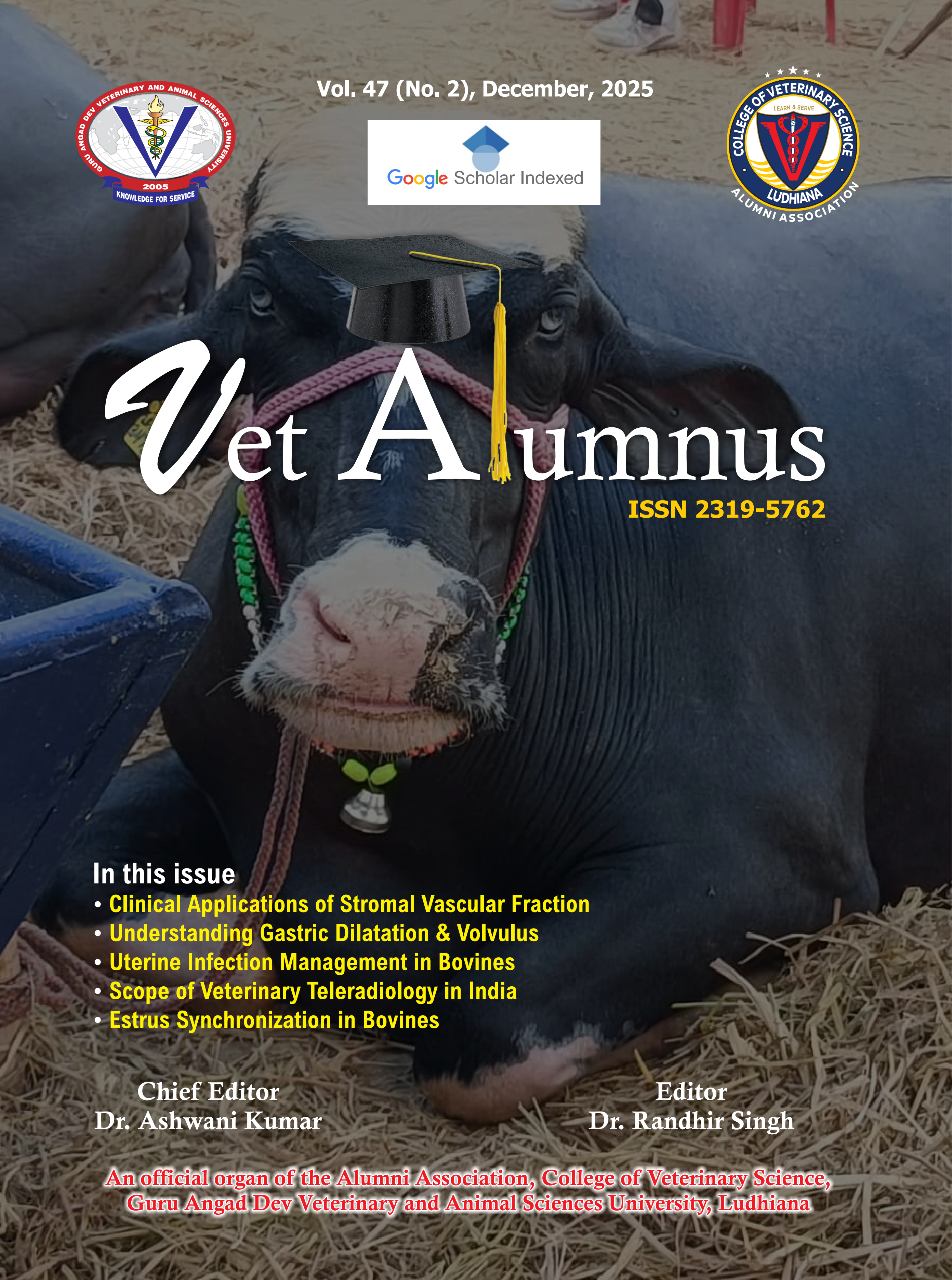

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Short Communication

Scope of Veterinary Teleradiology in India: Unlocking New Frontiers in Diagnostic Imaging

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Abstract

Veterinary teleradiology has emerged as a transformative tool in modern animal healthcare, enabling veterinarians—especially those in resource-limited settings—to access expert radiological interpretation remotely. This article explores the evolution, applications, and scope of veterinary teleradiology in the Indian context. It highlights the integration of cloud-based PACS systems, mobile-compatible DICOM viewers, and real-time reporting platforms. Despite its potential, adoption in India remains limited due to infrastructural gaps, a shortage of trained radiologists, and a lack of regulatory frameworks. However, with increasing digital penetration and the growth of veterinary telemedicine platforms, India is well-positioned to harness the full potential of teleradiology. The article emphasizes its multifaceted benefits—ranging from expert second opinions and round of clock emergency support to teaching, research, and rural outreach—while calling for structured implementation, legal reform, and capacity building to establish veterinary teleradiology as a mainstream diagnostic modality.

Keywords: *Diagnostic imaging, Picture archiving and communication system, Teleradiology, Veterinary practice*

During the author's visit to the University of Dublin, Ireland, in June 2023 for the IVRA–EVDI Joint Scientific Conference, an insightful and rather unusual observation was made. The event brought together leading veterinary radiologists from across the globe, particularly from Europe and North America. During the conference sessions, the author noticed several veterinary radiologists engrossed in interpreting diagnostic images—ranging from radiographs and ultrasound scans to CT and MRI studies—on their laptops and tablets.

Upon inquiry, it was revealed that these radiologists were engaged in real-time remote interpretation of diagnostic images via well-established teleradiology platforms such as Antech Imaging Services (AIS), VET.CT, IDEXX Telemedicine, AcuroVet, Panacea Vet, and IVR Teleradiology. These platforms offer structured, secure, and highly professional frameworks wherein veterinary radiologists receive imaging cases, provide expert interpretations, and are remunerated accordingly. Unlike informal exchanges via platforms like WhatsApp—common in many developing regions—these systems maintain robust reporting standards, medico-legal traceability, and diagnostic accuracy. This experience highlighted how veterinary teleradiology has evolved into a sophisticated, well-integrated global service, offering both clinical support and career opportunities for radiologists.

Teleradiology, a subset of telemedicine, has revolutionized human healthcare by allowing electronic transmission of radiographic images (X-rays, ultrasound, CT, MRI,) to be interpreted at a distance. Veterinary medicine is now embracing this transformation (Burute and Jankharia, 2009; Walden, 2017). In India, the growing access to digital radiography, broadband internet, and smartphone technology has opened up promising avenues for veterinary teleradiology—particularly vital in a country where geographical disparities, diverse animal population, and a shortage of imaging specialists challenge timely animal care.

What is Veterinary Teleradiology?

Veterinary teleradiology refers to the transmission of radiographic, ultrasonographic, CT, or MRI images from one location to another for remote interpretation by a veterinary radiologist (Poteet, 2008; Akash et al., 2021). It enables primary practitioners, especially in resource-limited or rural areas, to access expert opinions—improving diagnostic accuracy and clinical decision-making.

Veterinary Teleradiology: From Concept to Reality

The first commercial veterinary teleradiology system was introduced in 1994 in the U.S. by Professional Software Inc., but it saw limited adoption due to technological constraints (Poteet, 2008). Today, faster broadband, PACS integration, and mobile cloud based DICOM viewers make real-time and asynchronous consultations not only feasible but efficient and cost-effective. In India, where access to specialists is sparse in many states, the direct transmission of images to experienced veterinary radiologists is increasingly seen as a practical solution (Akash et al., 2021).

India's veterinary infrastructure is expanding digitally, yet teleradiology is still in its infancy. While several human teleradiology companies operate successfully from India, veterinary teleradiology services are sparse and often linked to urban referral centers. However, with rising demand for advanced diagnostic imaging in small animal and livestock practice, the potential for growth is enormous.

Digital radiography systems that support DICOM standards are increasingly being used, particularly in veterinary colleges and corporate hospitals. Cloud-based platforms, PACS (Picture Archiving and Communication Systems), and mobile-friendly DICOM viewers are now available, but adoption among private clinics remains limited.

Benefits and Applications

1. **Expert Second Opinions**: General Veterinary practitioners and/or pet parents can access and consult experienced radiologists for complex or equivocal cases without needing patient transfer. This is particularly beneficial for second opinions, or preoperative evaluations (Johnson, 2011; Walden, 2017)
2. **24/7 Availability**: On-call radiologists provide timely interpretations without physical presence. Emergency and after-hours reporting (night hawking) is possible through asynchronous image transmission. This is ideal for emergency cases, enabling rapid decision-making during off-hours (Poteet, 2008).
3. **Teaching and Teleconsultation**: Teleradiology also enhances veterinary continuing education. Image archives and collaborative platforms improve student learning and promote discussion among institutions and professionals across geographies (Johnson, 2011).
4. **Cost-effective Extension of Services**: Clinics without full-time radiologists can still provide specialized interpretation, boosting client confidence.
5. **Rural Outreach**: India's rural veterinarians and farmers can leverage teleradiology to seek expert interpretation without logistical hurdles; ensuring animals in remote areas receive timely and appropriate care, minimizing unnecessary animal transport.
6. **Digital Image Archival and Legal Records:** Proper PACS-based storage helps maintain structured databases, useful for clinical audits, teaching, and medico-legal documentation.

Global Experiences and Lessons

In the USA and Europe, veterinary teleradiology is a mainstream service with companies offering rapid reads, board-certified consultations, and mobile app interfaces. The COVID-19 pandemic significantly boosted the adoption of teleconsultation and teleradiology services across sectors, including veterinary medicine (Papageorges et al., 2001; Becker et al., 2023).

Notably, teleradiology has also helped to bridge the gap in advanced imaging interpretation, such as CT/MRI, where trained personnel are scarce even in referral hospitals. The projected rise in telemedicine usage in veterinary care globally—from 350,000 users in 2013 to 7 million in 2018—shows the direction, veterinary healthcare is heading (Becker et al., 2023).

Challenges in India

- **Lack of Trained Radiologists**: Veterinary diagnostic imaging as a specialty is still developing. Unlike western world, currently veterinary radiology has not been recognized as a separate speciality in India (Akash et al., 2021). Recently, a few institutions in India have started offering postgraduate diploma training in veterinary diagnostic imaging.
- **Inconsistent Digital Infrastructure**: Many clinics often lack high-resolution scanners, PACS, DICOM compliance, or stable internet connectivity.
- **Legal and Regulatory Gaps**: There is no unified framework or telemedicine guideline for veterinarians in India, unlike in human medicine.
- **Quality Assurance**: Image compression, improper acquisition techniques, and reliance on non-specialist opinions can compromise interpretation quality (Poteet, 2008).
- **Lack of Advanced Surgical Facilities:** Although many veterinary practitioners in India currently manage cases conservatively—often due to cost constraints, lack of pet parents or, livestock farmers awareness, owner reluctance or limited availability of referral centers—there is a growing trend toward advanced diagnostics and treatment. As awareness, emotional attachment among pet parents or, livestock farmers rises and access to referral hospitals improves, so does the demand for specialized surgical interventions such as spinal stabilization, intracranial procedures, and oncologic surgeries.

However, in many parts of India, veterinary clinics and hospitals still lack advanced surgical setups like neurosurgery units, dedicated oncology centers, or radiotherapy facilities. This makes it extremely difficult to manage complex cases such as spinal cord trauma, brain disorders, or neoplasia. In such situations, obtaining an expert opinion on radiographic images becomes vital to make informed decisions—whether to treat conservatively, refer to a specialized center, or, in unfortunate scenarios, consider humane euthanasia.

This is where veterinary teleradiology proves invaluable. It allows veterinarians, particularly in rural or semi-urban areas, to share X-rays and ultrasound scans with experienced radiologists across India or abroad for accurate interpretation. Although CT and MRI access remains limited at present, their adoption is gradually increasing, making advanced teleradiologic support even more relevant.

Timely input from radiology experts can be lifesaving in cases such as vertebral fractures, suspected spinal compression, or metastatic tumors. Even in scenarios where curative treatment options like radiotherapy are unavailable, early and precise radiologic interpretation can help chart a plan for palliative care or surgery. In this way, teleradiology not only bridges the gap left by the absence of high-end infrastructure but also ensures equitable access to expert diagnostic support, enhancing clinical decision-making across all tiers of veterinary practice.

Future Prospects in India

1. **Integration into Telemedicine Hubs**: Future veterinary telehealth platforms may offer teleradiology as an embedded service.
2. **Artificial Intelliegence and Decision Support**: Machine learning tools are being developed to assist in preliminary reads—especially in identifying fractures, cardiomegaly, or nephropathies (Becker et al., 2023).
3. **Cloud-based, Mobile-compatible Systems:** These will empower field veterinarians to connect instantly with radiologists, even from remote livestock camps.
4. **Government Support and Public Health**: In livestock and zoonoses surveillance, teleradiology could aid in remote diagnostics linked to animal health systems like National Animal Disease Referral Expert System (NADRES).
5. **Start-up Ecosystem**: A potential niche for veterinary start-ups offering teleradiology services, especially for small animal clinics in tier-2 cities.

Recommendations for Practitioners

- Invest in DICOM-compliant imaging systems and basic internet infrastructure.
- Partner with academic institutions or freelance radiologists for interpretation services.
- Store images systematically using PACS for legal, educational, and clinical audit purposes.
- Follow good imaging practices—correct positioning, exposure, and annotation—to ensure interpretable images.

In summary, veterinary teleradiology in India is more than a digital tool—it is a bridge to equitable, expert, and expedited care. Whether it's guiding a rural veterinarian managing a dystocia case or assisting a city-based clinician with orthopedic post-op evaluations, teleradiology promises to democratize veterinary imaging expertise. As the veterinary community embraces digital transformation, the collaborative spirit of teleradiology—linking practitioners, radiologists, educators, and AI—must be nurtured through ethical implementation, capacity building, and infrastructural investment.

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Short Communication

Stromal Vascular Fraction in Veterinary Regenerative Medicine: Isolation, Characterization, and Applications

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Abstract

The Stromal Vascular Fraction (SVF) is a heterogeneous cell population derived from adipose tissue, which has gained significant traction in veterinary regenerative medicine over the past ten years. As a point-of-care, SVF autologous therapy is rich in adipose-derived mesenchymal stem cells (MSCs), endothelial progenitor cells, pericytes, and immunomodulatory cells, which exert powerful paracrine effects, promoting tissue regeneration, angiogenesis, and potent immunomodulation. This mini review summarizes the recent therapeutic applications of SVF in veterinary medicine, with a focus on canine and equine species.

Keywords*: Dog, Horse, Regenerative medicine, Stem cells, Stromal Vascular Fraction*

In modern canine practice, managing chronic and degenerative diseases remains one of the most significant clinical challenges. Conditions like surgical wounds, osteoarthritis, chronic joint disease, and complex non-healing wounds often leave both the veterinarian and the pet owner seeking more effective, long-term solutions and management. Against this backdrop, regenerative medicine is a promising and efficient tool. SVF is the pellet of non-adipocyte cells isolated from adipose (fat) tissue, typically through enzymatic digestion (e.g., with collagenase) and centrifugation (Wang et al., 2025).

Unlike cultured mesenchymal stem cells (MSCs), which require weeks of *in vitro* expansion, SVF can be harvested, processed, and re-administered to the patient, either autologously or even allogeneically to other patients. This “point-of-care” advantage makes it a practical and increasingly cost-effective option for clinicians (Sharun et al., 2022). SVF is not just a source of adipose-derived MSCs (ADSCs), but a rich, biological cocktail that includes endothelial progenitor cells (EPCs), pericytes, T-regulatory cells, and M2-phenotype macrophages, which are potentlly anti-inflammatory and pro-reparative (Wang et al., 2025).

For the practicing veterinarian, SVF is rapidly transitioning from a novel research concept to a clinical reality. This article provides a practical overview of the current applications of canine SVF in key canine diseases, with a focus on its emerging applications in enhancing surgical wound healing, osteoarthritis, and its role in soft tissue repair. Furthermore, it will examine the clinical efficacy and the practical considerations required for the successful implementation of this regenerative modality in a clinical setting. This mini-review focuses on the isolation, characterization, and key clinical applications of SVF in canine and equine species.

Isolation of SVF from Adipose Tissue

Adipose tissue from dogs can be harvested from the periovarian or abdominal fat during surgery of either ovariohysterectomy or cesarean section during delivering the pup or surgical excision of fat. Yield, viability, and percentage of stem cell fraction in SVF vary with many factors. Readers are encouraged to read the mini-review " Understanding and controlling the variables for stromal vascular fraction therapy" (Jeyaramane et al., 2024) and its commentary article (Choudhary, 2025). The following is the sequential protocol of SVF isolation (Fig. 1).

- Obtain adipose tissue
- Wash the tissue: Wash the fat tissue with PBS
- Mechanical disruption using a sharp sterile blade, followed by washing.
- Digest with collagenase: 37 °C for approximately 40-60 minutes
- Filter the solution using a cell strainer (40 micron)
- Centrifuge to pellet the SVF: 200×g for 10 min at room temperature.
- Collect the SVF pellet from the bottom of the centrifuge tube
- Resuspend the SVF in a suitable medium
- Assess cell viability using a hemocytometer or a cell counter

The key equipment required for SVF harvest and isolation includes a sterile biosafety cabinet, refrigerated centrifuge, and cell counter, CO2 incubator, microscopy, water bath and other basic requirements of a cell culture lab.

Characterization of SVF

The stromal vascular fraction (SVF) is a highly valuable, heterogeneous cell population isolated from adipose tissue. It contains various cell types critical for tissue regeneration and repair. The morphology of freshly harvested canine SVF can range from small to large cells, indicating heterogeneity within the cell population. Loss of cell heterogeneity may indicate that cells are either differentiated and may not represent a representative SVF. Canine SVF is a non-homogeneous collection of predominantly viable nucleated cells, mainly appearing round, with some background debris and lipid droplets (Fig. 2). While in culture, SVF cells can be fibrous or spindle-like, with both small and large cell types.

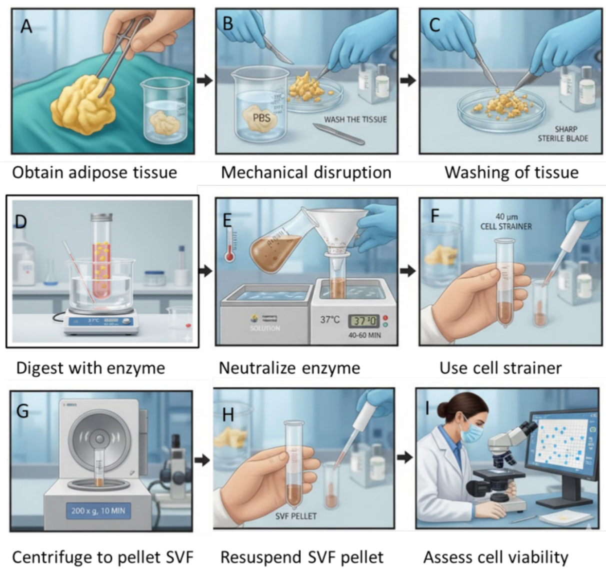

Fig. 1. Step-by-step schematic representative image of SVF isolation from canine fat tissue. *(Credit: Image generated using an AI tool)*.

Key metrics for SVF characterization are cell count and yield, cell viability, and cell phenotyping. Immunophenotyping can be performed using fluorescently labeled antibodies specific for cell-surface markers of mesenchymal stem cells (MSCs). Flow cytometry is the gold standard method for characterizing stem cells. MSCs are identified by a panel of positive markers (e.g., CD73+, CD90+, CD105+) and the absence of hematopoietic markers (e.g., CD45, CD31) (Ghaneialvar et al., 2018; Kresic et al., 2021).

Key Applications in Veterinary Medicine

Canine Orthopedic Disease

The most extensively studied application of SVF in veterinary medicine is the management of canine osteoarthritis (OA). Numerous clinical studies have demonstrated that a dose of 12 ± 3.2 million allogeneic SVF intra-articularly injected led to significant improvements in comfort and mobility (Wang et al., 2025). The primary mechanisms of therapeutic response of SVF in OA involve modulating the inflammatory joint environment by reducing pro-inflammatory cytokines (such as TNF-α and IL-1β) while increasing anti-inflammatory cytokines (such as IL-10). It also appears to shift synovial macrophages from a destructive M1 (pro-inflammatory) phenotype to a reparative M2 (anti-inflammatory) phenotype (Wang et al., 2025). Clinical studies have shown significant improvements in owner-assessed pain scores and objective gait analysis (force plate) in dogs with elbow and hip OA following SVF treatment (Harman et al., 2016).

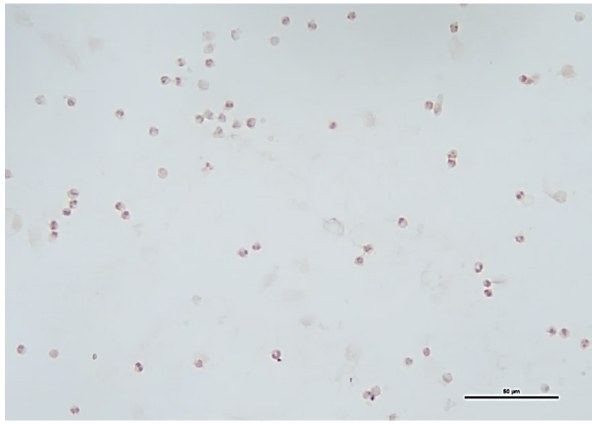

Fig. 2. Hematoxylin staining of canine SVF immediately after the harvest from peri-ovarian adipose tissue. (Photo credit: Hitesh Rana)

Canine Soft Tissue Repair and Wound Healing

Studies conducted at Guru Angad Dev Veterinary and Animal Sciences University on canine SVF and their secretory products include Immunomodulatory potential of SVF, as established through *in vivo* and *in vitro* studies using PBMC and clinical cases, respectively (Rana, 2023), and *in vitro* and *in vivo* models (Choudhary, 2025). They reported that SVF showed better and faster surgical wound healing in dogs (Fig. 3).

Recent work on canine adipose tissue-derived SVF extends beyond characterization and focuses on immunomodulatory activities using *in vitro* coculture models of SVF with PBMC (Rana, 2023), which are suitable for conditions like lymphoma or immune-mediated diseases in pre-clinical and clinical settings.

The pro-angiogenic and immunomodulatory properties of SVF make it an excellent therapy for complex or non-healing wounds. The endothelial progenitor cells in the SVF promote neovascularization by delivering oxygen and nutrients to the wound bed. In contrast, the ADSCs and immune cells manage inflammation and secrete growth factors that accelerate epithelialization and granulation tissue formation (Sharun et al., 2022). It has shown promise in treating conditions like acral lick dermatitis in dogs and large degloving injuries in both small and large animals.

In *an vivo* study on immunomodulation and therapeutic applications of allogenic SVF on canine surgical wound (Grewal et al., 2025) evaluated the efficacy of allogeneic canine stromal vascular fraction (SVF) and xanthosine (Xs), individually and combined, for enhancing surgical wound healing in client-owned dogs compared to standard antibiotic treatment. Dogs received subcutaneous injections post-suture, and healing was assessed via photographic monitoring, hair regrowth, wound redness, and serum levels of

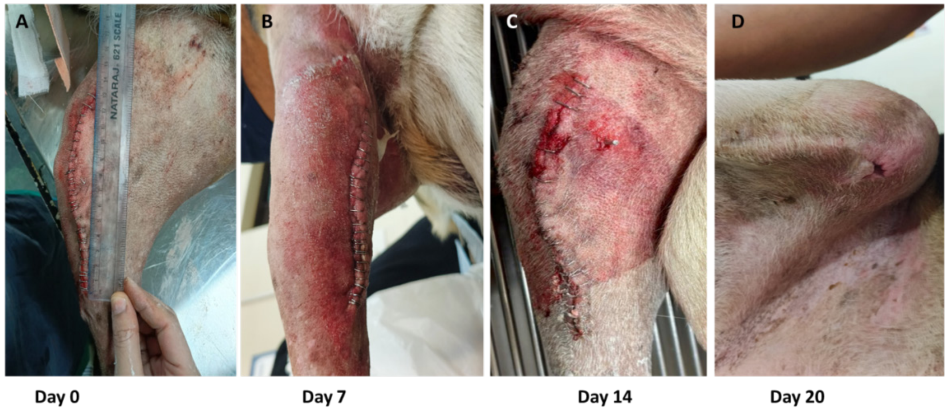

Fig. 3: Hind leg fractured surgical wound healing of a dog using two doses of SVF was able to show complete healing by the 20th day after surgery. (Photo credit: Narinder Kaur Grewal)

VEGF and TGF-β on Days 0, 7, and 14. The SVF group showed the highest hair regrowth scores by Day 14 (1.6 ± 0.16 vs. control 0.6 ± 0.09; $p < 0.05$), indicating accelerated tissue regeneration. The Xs group significantly reduced wound redness and elevated VEGF and TGF-β levels, reflecting decreased inflammation and enhanced angiogenesis/remodeling, respectively. Both SVF and Xs significantly improved surgical wound healing by promoting distinct yet complementary regenerative mechanisms, highlighting their potential in veterinary medicine. These results demonstrate that both allogeneic SVF and xanthosine significantly improve surgical wound healing in dogs by promoting tissue regeneration, reducing inflammation, and enhancing angiogenesis through distinct but complementary mechanisms, highlighting their potential as promising regenerative therapies in veterinary medicine.

Other neurological studies utilize stem cell-based therapies, including bone marrow-derived cells or cultured SVF (not just the raw SVF fraction). Rehabilitation of spinal cord injury with autogenous SVF, ranging between 1-10 million cells administered intrathecally, restored lost motor functions by repairing spinal cord injury (Raghuvanshi et al., 2021).

Equine Regenerative Medicine: Orthopedic Injuries

In equine practice, SVF is a cornerstone of regenerative therapy for sports-related injuries, particularly tendonitis and suspensory desmitis (inflammation of ligament). The goal of treatment is not only to heal the injury but also to promote functional tissue regeneration and minimize the formation of restrictive scar tissue, which is prone to re-injury. The combination of ADSCs and EPCs in SVF promoted superior angiogenic and tenogenic responses within the lesion (Sharun et al., 2022). Studies have shown that horses with tendonitis treated with SVF, along with plasma-rich protein (PRP), when administered early after injury with optimal and repeated doses of cells, can provide optimal benefits for tendon injury (Connard et al., 2025).

The Indian veterinary research community has made significant contributions to the field of SVF therapy. A comprehensive review by Sharun et al. (2022) detailed the various clinical applications of SVF, highlighting its potential for treating orthopedic conditions and enhancing wound healing in veterinary practice. This work highlighted the benefits of SVF, including the ability to preclude the need for culturing and reduce costs, making it an ideal option for clinical use in India. Furthermore, researchers are exploring protocols for isolating and characterizing SVF from rabbit models for bone tissue engineering applications (Sharun et al., 2021) and for isolating the SVF secretome from canine models (Choudhary et al., 2024).

Challenges and Future Directions

Despite its successes, SVF therapy faces challenges. The primary issue is a lack of standardization in isolation protocols (enzymatic vs. mechanical), cell quantification, viability assessment, and dosing. Moreover , the cellular composition of SVF can vary significantly depending on age, harvest site, and processing technique, resulting in variable clinical outcomes (Wang et al., 2025). Adipose tissue extraction is critical as the choice of separation of tissue by liposuction or excision of fat tissue; the anatomical sites from which the tissue is harvested significantly impact the quality of the starting material (Jeyaraman et al., 2024). Immediate and appropriate processing after extraction is essential to maintain the functionality of the cells within the SVF. The subsequent step, enzymatic digestion, is pivotal. Researchers must fine-tune enzyme concentrations—such as collagenase—to achieve maximum cell yield while avoiding damage to the delicate cell population. Following digestion, precise filtration and centrifugation are required to effectively separate and purify SVF from lipid and aqueous components. The necessity for standardized protocols across all these steps is highlighted to ensure reproducibility and successful clinical application.

The future of SVF therapy lies in:

1. Standardized protocols: Establishing consensus guidelines for SVF processing to ensure consistent and comparable results.
2. Allogeneic (Off-the-Shelf) products: Developing and testing cryopreserved, allogeneic SVF from healthy donors to eliminate the need for adipose harvest from sick or geriatric patients.
3. Secretome-based therapy: Moving towards cell-free therapies that use the potent cocktail of growth factors, cytokines, and exosomes (the "secretome") secreted by SVF cells, which could be easier to standardize and store (Choudhary et al., 2024).

4. Information on SVF isolation and characteristics in other animals, like goat, sheep, bovine, and camel, is lacking till the time this minireview was written.

In summary, clinical applications of SVF are rapidly growing, but challenges remain, including a lack of a standardized isolation protocol, variable proportions of the true stem cell population, and dosing. So, establishing consensus guidelines, developing stable allogeneic ("off-the-shelf") SVF products, and exploring secretome-based, cell-free therapies that offer easier standardization and storage should be future endeavours that ultimately enhance the reliability and accessibility of this promising regenerative modality.

Currently, commercial preparation of canine and equine SVF is unavailable, making widespread commercial therapy infeasible. Furthermore, the Department of Biotechnology (DBT) has not yet issued formal guidelines for animal stem cell therapy, which remain under development.

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Mini Review

Reproductive Management of Dairy Animals through Estrus Synchronization

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Abstract

Assisted reproductive technologies, primarily estrus synchronization and artificial insemination (AI), are fundamental tools for accelerating genetic gain in dairy herds. Estrus synchronization enhances reproductive efficiency by deliberately manipulating the estrous cycle or inducing estrus to synchronize a large proportion of females within a short, predetermined period. This is achieved by administering specific reproductive hormones to bring a large number of females into estrus (heat) within a narrow, predetermined window. The primary hormones used in synchronization protocol include: progesterone, prostaglandin $F_2\alpha$*, gonadotropin-releasing hormone, follicle-stimulating hormone and luteinizing hormone. Synchronization protocols remain among the most widely adopted & effective reproductive interventions in modern dairy production systems. The success of any synchronization and AI program depends on sound herd management, selection of cows with adequate body condition, and strict adherence to protocols. Therefore, the selection of an evidence-based and practically feasible synchronization protocol is vital. This approach, tailored to the specific herd's management practices, nutritional status, and genetic goals, is essential for achieving optimal conception rates and sustaining long-term genetic progress.*

Keywords: *Anoestrus, Corpus luteum, Estrus synchronization, Fixed Time Artificial Insemination, Hormone*

Estrus synchronization is a reproductive management practice that intentionally regulates the estrous cycle or stimulates estrus so that a majority of females in a herd express heat within a specific and shortened time frame. This technique functions as an advanced tool to improve reproductive efficiency, reduce human intervention errors, and lower overall management expenses, particularly in large-scale operations. Ovulation synchronization, a more targeted method, uses specific hormonal combinations to control ovulation, allowing insemination to occur at a predetermined and fixed time. These hormonal protocols are commonly referred to as Fixed-Time Artificial Insemination (FTAI) systems. Although not every estrus synchronization approach is classified as FTAI, each FTAI protocol incorporates estrus synchronization as a fundamental component. By concentrating breeding activities within a limited period, this practice allows controlled scheduling of calving to coincide with seasons favourable for animal health, feed availability, and environmental conditions, thereby enhancing offspring survival and growth. Economically, synchronization contributes to greater efficiency and profitability. While natural mating occurs over an approximately 21-day cycle, synchronization techniques can shorten this duration to fewer than five days, depending on the hormonal treatment employed. These systems enable precise regulation of the ovarian cycle, allowing FTAI to be performed without labour-intensive detection of estrus behaviour.

Bovine Estrous Cycle

In cattle, the average duration of the estrous cycle is approximately 21 days in cows and 20 days in heifers and is broadly divided into two phases: the follicular phase, dominated by estrogen (E_2) and characterized by Graafian follicle development, and the luteal phase, dominated by progesterone (P_4) with the corpus luteum (CL) as the principal ovarian structure. These phases are further classified into four distinct stages: proestrus, estrus, metestrus, and diestrus. During proestrus, CL regression lowers progesterone, while follicular growth elevates estradiol. Estrus, lasting 6–30 hours (average 20 h), is marked by behavioural receptivity, increased mucus discharge, low progesterone, and an LH surge triggering ovulation. Metestrus follows for 3–5 days, during which ovulation occurs, CL formation begins, and progesterone gradually rises. Diestrus, lasting approximately 12 days, represents the functional phase of the CL, characterized by maximal progesterone secretion essential for maintaining pregnancy (Bridges, 2010).

Detection of Estrus

Accurate estrus detection is vital for optimizing insemination timing and enhancing herd fertility. Effective programs depend on proper identification, reliable recordkeeping, standardized protocols, and skilled personnel. Visual observation two to three times daily for about 30 minutes should focus on behavioural cues such as mounting and increased activity. Hormonal treatments and monitoring returns to estrus at 18–24-day intervals further improve detection efficiency. Managing lameness is essential, as affected cows exhibit reduced mounting behaviour, lowering detection precision (Britt et al., 2007). Modern aids integrating behavioural, physiological, and sensor-based data now enhance accuracy and consistency in reproductive management.

Modern estrus detection technologies enhance reproductive efficiency in dairy animals through behavioural, physiological, and digital monitoring approaches.

- **Mounting-based systems** (e.g., tail paint, heat mount detectors, Estrotect, HeatWatch) identify mounting activity via devices applied on the tailhead.
- **Activity monitors** (Pedometers, AfiAct, CowScout, HR Tag, IceQube,

CowManager) track movement, lying, and feeding patterns with 80–90% detection accuracy (Mayo et al., 2019).

- **Computer vision systems** like CowXNet and YOLOv11 use AI to analyze posture and interactions, achieving up to 83% accuracy (Lodkaew et al., 2023).
- **Odor and physiological sensors** including electronic nose (E-nose) devices, detect volatile organic compounds from the perineal area for precise estrus classification (Ali et al., 2022).
- **Infrared thermography (IRT)** measures vulvar temperature elevations linked to increased blood flow and hormonal changes during estrus (Rajput et al., 2024).
- **Hormone-based kits** such as Ovacheck™, analyze progesterone and estrogen levels, offering near-perfect accuracy for estrus prediction and fixed-time insemination scheduling (Rajput et al., 2024).

Choice of Selection of Estrus Synchronization Methods

A comprehensive understanding of the hormonal dynamics and ovarian structures across various stages of the estrous cycle is crucial for the precise design and successful implementation of estrus synchronization protocols Nevertheless, the success of estrus synchronization programs remains highly dependent on the careful selection and precise execution of protocols. The efficacy of these protocols is modulated by several factors, including the cow's physiological status, stage of lactation, body condition score, cyclicity, and the specific phase of the estrous cycle at the time of treatment (Julanov et al., 2024). Extensive scientific efforts have been directed toward the development of estrus synchronization protocols, which can be broadly categorized into following approaches.

APPROACHES FOR ESTRUS SYNCHRONIZATION

There are two major approaches for estrus and ovulation synchronization in dairy animals which are either shortening or extending the luteal phase of estrus.

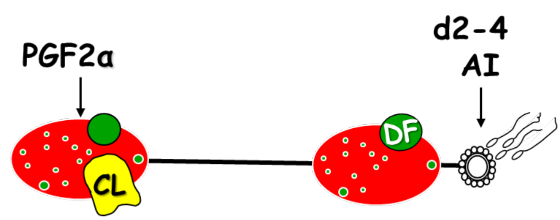
1. **By Shortening of Luteal Phase:** This depend upon the principle of luteolysis whereby, the use of Prostaglandin F2α (PGF2α) which causes lysis of an active corpus luteum (CL). This approach can have only use of PGF2α or in a combination of PGF2α with other hormones like GnRH, estradiol etc. This approach only works in the cyclic animals. PGF2α induces luteolysis, facilitating follicular growth, elevated estradiol secretion, estrus expression, and ovulation (Madureira et al. 2019). When combined with GnRH, it optimizes neuroendocrine regulation, thereby enhancing synchronization and ovulatory response (Cedeño et al. 2021).

A. Prostaglandin based Protocols:

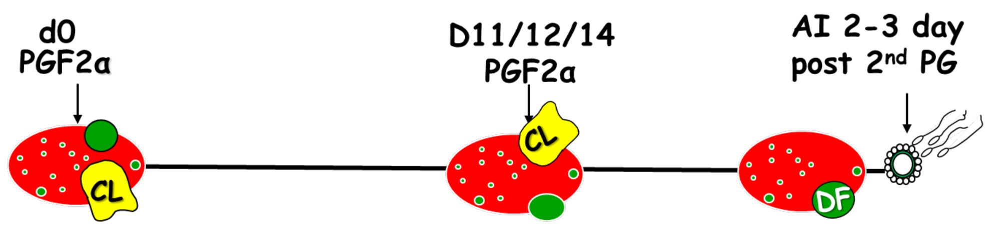
a. **Single PG based Protocol:** This protocol involves the administration of a single PGF2α injection and is effective only in animals bearing a functional CL between 6th and 16th day of the estrous cycle. Estrus typically occurs within 2–4 days post-injection, with ovulation observed approximately 60–120 hours thereafter, yielding a response rate of about 55–60%.

b. **Double PG based Protocol:** It involves the administration of two PGF2α injections given 11–14 days apart, thereby targeting nearly all cyclic animals irrespective of the stage of corpus luteum development. Estrus is typically observed within 2–4 days following the second injection, and the overall response rate is superior to that achieved with a single-injection protocol.

a. Single PG protocol: CL in d6-16 of cycle



b. Double PG protocol: CL stage unknown



Limitations: Alone PG based protocols fail to synchronize ovulation, unpredictable timing of insemination, and effective only in cyclic animals.

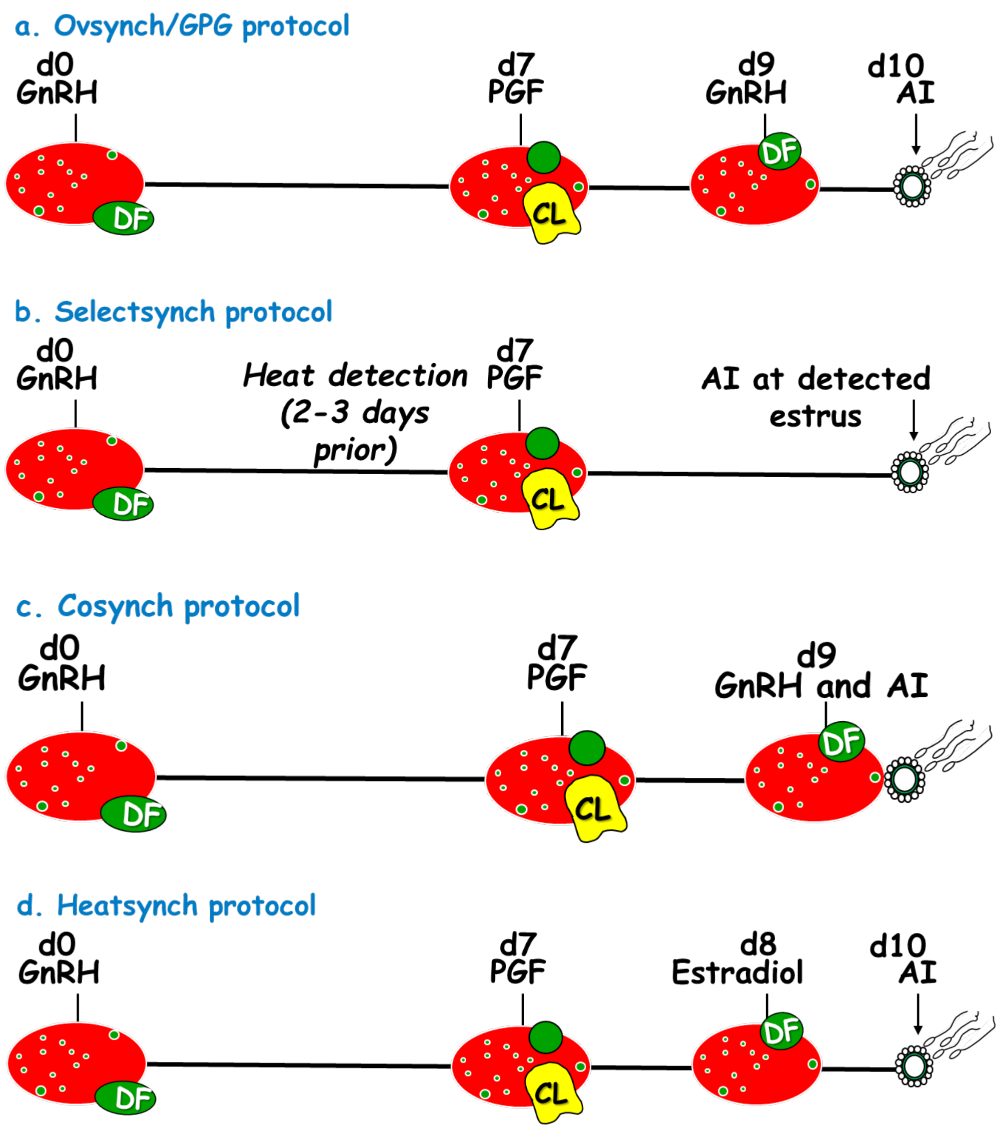
B. GnRH and Prostaglandin based Protocols:

a. **Ovsynch/GPG Protocol:** It involves initial intramuscular administration of a GnRH analogue (Buserelin acetate, 10 µg), followed by a PGF2α injection 7 days later, and a second GnRH injection administered 56 hours after PGF2α. AI is then performed 16–24 hours after the second GnRH injection. Reported conception rates with this protocol generally range between 30% and 40%.

b. **Selectsynch**: It involves administration of GnRH analogue Buserelin acetate 10µg followed by PGF2α injection 7 days later. Heat detection is initiated 2–3 days before PGF2α administration, and AI is carried out at the observed estrus.

c. **Cosynch**: This protocol mirrors the Ovsynch scheme but differs in timing. It involves administration of GnRH analogue Buserelin acetate 10µg followed by PGF2α injection 7 days later and administration of second GnRH injection 48-56h after PGF2α injection with AI along with second GnRH.

d. **Heatsynch**: It involves the administration of a GnRH analogue (Buserelin acetate, 10 µg), followed by a prostaglandin F2α (PGF2α) injection seven days later. Estradiol benzoate (1 mg) is administered 24 hours after PGF2α, and artificial insemination is performed 48–56 hours after estradiol benzoate administration.



Presynch Protocols: These estrus synchronization/ FTAI protocols are designed to improve the efficiency of estrus synchronization & fixed-time artificial insemination by administering one or more hormonal treatments prior to the initiation of the main synchronization protocol. This approach aligns animals to a similar stage of the estrous cycle, thereby optimizing conception rates, although it generally incurs higher costs compared to basic protocols.

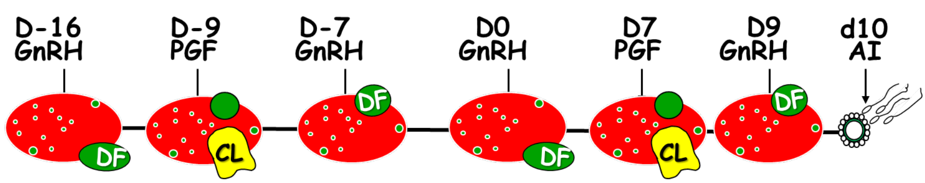
a. **Doubleovsynch:** It involves two consecutive Ovsynch programs separated by a 7-day interval, with AI performed 16–24 hours after completion of the second Ovsynch sequence.

b. **Pre-synch 11/12/14:** It involves two PGF2α injections administered at 11-, 12-, or 14-day intervals, initiated twelve days before the start of the Ovsynch protocol.

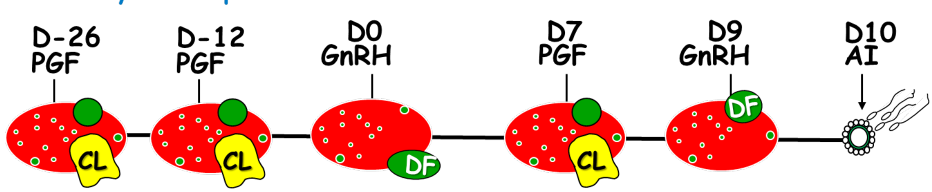
c. **Doublesynch:** It involves a single PGF2α injection administered 48 hours prior to the commencement of the Ovsynch protocol.

d. **Estradoublesynch:** It involves administration of PGF2α injection 48h before the actual start of heatsynch protocol.

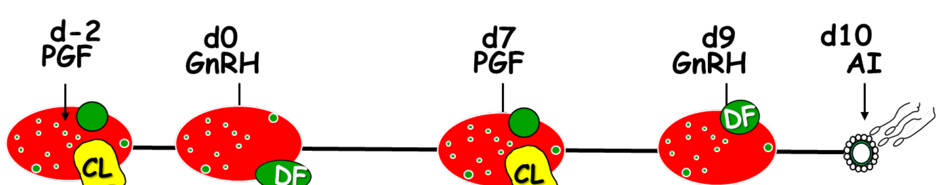
a. Double Ovsynch protocol



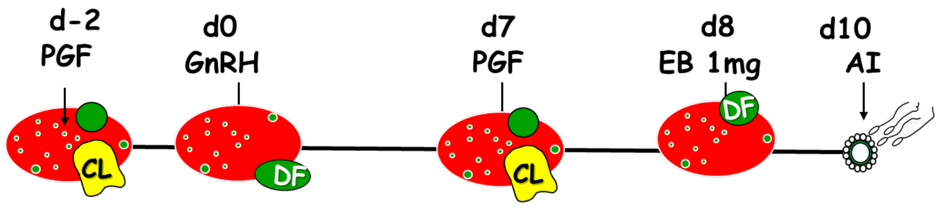
b. Presynch-14 protocol



c. Doublesynch protocol

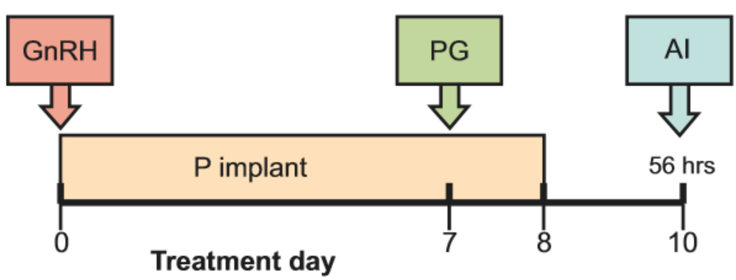


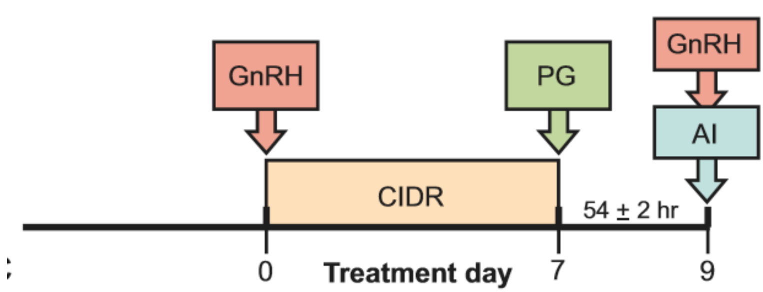
d. Estradoublesynch protocol



2. **By Extending the Luteal Phase:** This approach depend upon the principle of use of progesterone or its analogue to increase the circulating progesterone concentrations by supplementation (daily oral, slow releasing injectable (ear implant), or vaginal inserts) creating a temporary CL or source of progesterone and sudden withdrawal of source of progesterone shall lead to initiation of follicular activity and induction of estrus in the animals. This approach only works in both cyclic and non-cyclic animals (Kasimanickam, et al. 2015).

Progesterone based and combination of Progesterone and GnRH, Prostaglandins based protocols: Progesterone can be administered over a period of 7–12 days through various delivery methods, including oral formulations, injectable preparations, ear implants, and intravaginal depot devices. These treatments mimic the luteal phase, suppress ovulation, and synchronize follicular turnover, allowing precise timing of estrus and artificial insemination. The different progesterone preparations are:





Category	Examples / Preparations	Route of Administration	Dose / Content	Duration	Key Features / Advantages	References
Oral Progesterone	Megestrol acetate (MGA), Altrenogest	Oral (mixed with feed)	MGA: 0.5 mg/animal/day (typical) Altrenogest: 0.044 mg/kg BW/day	7–12 days	Simple administration, cost-effective, suppresses estrus until withdrawal; useful for group synchronization	Haridas et al. (2025)
Injectable Preparations	Hydroxyprogesterone caproate, Medroxyprogesterone acetate (MPA), Proligestone	I/M injection	Dose varies by product and animal weight	Single injection, effective for 7–12 days	Provides sustained progesterone release; useful where repeated handling is difficult.	Morotti et al. (2018)
Depot (Intravaginal) Preparations	CIDR (Controlled Internal Drug Release), PRID (with or without Estradiol), TRIU-B, PRID-Delta	Intravaginal device	CIDR: 1.38 g P4 PRID: 1.55 g P4 + 10 mg estradiol benzoate TRIU-B: 3×186 mg + 1×400 mg P4 PRID-Delta: 1.55 g P4	7–12 days	Maintains consistent P4 levels, easy insertion/removal, highly reliable; enhance follicular synchronization with estradiol.	Frenkel et al. (2025)
Ear Implants	Crestar, Synchromate-B	S/C ear implant (+ injection for Crestar)	Crestar: 3 mg norgestomet implant + 2 mL inj. (3 mg norgestomet + 5 mg estradiol valerate) Synchromate - B: 6 mg norgestomet implant	7–12 days	Long-acting, ensures strong luteal mimicry, combined estrogen promotes follicular turnover and estrus synchronization	Yizengaw (2017)

Future Research Perspectives on Estrus Synchronization

Despite the considerable progress achieved through hormonal interventions to enhance the reproductive efficiency of female cattle, further refinements remain necessary. Advances in genetic engineering have enabled the production of recombinant hormones, providing an ethical and sustainable alternative to animal-derived products. Among these, equine chorionic gonadotropin (eCG), traditionally extracted from the endometrial cups of pregnant mares, continues to play a pivotal role in timed artificial insemination (TAI) protocols for *Bos indicus* cows. Recent studies have reported comparable fertility outcomes between conventional eCG and its recombinant form. Similarly, the use of recombinant follicle-stimulating hormone (FSH) and recombinant bovine somatotropin (rbST) has shown encouraging effects on reproductive performance. Electrospun pullulan nanofibers impregnated with progesterone have been investigated as an intravaginal drug delivery system designed for sustained hormone release over seven days (Lavanya, et al., 2024). This system not only enables prolonged progesterone administration but also contributes to the prevention of vaginitis. Looking ahead, the integration of nanotechnology for the controlled, organ-specific release of reproductive drugs may further enhance synchronization efficiency, reduce animal handling and stress, and optimize overall cattle production systems.

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Mini Review

In Vivo Oocyte Maturation: An Alternative to *In Vitro* Oocyte Maturation for Producing Quality Bovine Embryos

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Abstract

The capacity of the bovine oocytes to successfully fertilize and sustain healthy embryonic development, is greatly impacted by the maturation environment. Oocyte maturation involves both the nuclear as well as cytoplasmic maturation. In vivo maturation occurs within the ovarian follicle whereas in vitro maturation involves extracting immature oocytes from antral follicles and maturing them in artificial media. Superstimulation protocol for in vivo oocyte maturation requires the administration of gonadotropic hormone before the ovum pick. In vivo maturation could become a good alternative to in vitro maturation as the results of in vitro maturation are compromised. The present review aimed to discuss the protocol for in vivo maturation of oocytes and their developmental competence in comparison to in vitro maturation of oocytes.

Keywords: *Bovine, Embryo, In vitro, In vivo, Maturation, Oocyte*

In vitro and *in vivo* generated bovine embryos have been utilized for various biotechnological applications from a long time. Currently, the number of *in vitro* produced bovine embryos that are transferred into recipients are more than that of *in vivo* produced embryos. Ovum Pick-up *In Vitro* Embryo Production, or OPU-IVEP, has resulted in a reduction in the generation interval and an increase in selection intensity, indicating that it is a dependable substitute for Multiple Ovulation Embryo Transfer (MOET) in the effective genetic improvement of cattle (Merton et al., 2003). However, the amount of embryo production is actually lower in OPU-IVEP, than the MOET procedures (Lonergan and Fair 2016). The most pivotal step in IVEP is *in vitro* maturation (IVM) of oocytes as a major proportion of oocytes fail to attain complete maturation (nuclear and cytoplasmic) and further progress to the embryo stage. Therefore, the present review aimed to discuss the protocol for *in vivo* maturation of oocytes and their developmental competence in comparison to *in vitro* maturation of oocytes.

In vivo and *In vitro* Maturation of Cumulus Oocytes Complexes (COC's)

Nuclear and cytoplasmic maturation processes, which can take place *in vitro* (in a lab culture) or *in vivo* (in the ovarian follicular environment), have a significant impact on oocyte competence. While cytoplasmic maturation refers to the restructuring of organelles, accumulation of mRNAs and proteins, and other metabolic changes required for early embryogenesis, nuclear maturation entails the restart and completion of meiosis. To produce high-quality embryos, these two processes must be balanced and coordinated. The differences between *in vivo* and *in vitro* maturation greatly affect oocyte quality, fertilization outcomes, and subsequent embryonic development.

In vivo maturation of bovine oocytes takes place within the ovarian follicle under the regulation of the hypothalamic-pituitary-gonadal axis. The surge of LH (Luteinizing Hormone) plays a pivotal role by initiating the resumption of meiosis, cumulus cell expansion, and synchronization of nuclear and cytoplasmic events. Endocrine, paracrine and autocrine factors are among the complex signals provided by the follicular environment that regulate the maturation process. *In vitro* maturation, entails removing immature oocytes from antral follicles and cultivating them in synthetic media. To replicate the *in vivo* environment, these media are typically supplemented with growth factors, serum and gonadotropins.

Protocol for Retrieval of *in vivo* Matured Oocytes (Fig. 1)

- In cows, at the random stage of the estrous cycle (day=0), CIDR (Controlled Internal Drug Release Device; Progesterone = 1.38 gram) is inserted intravaginally and are administered estradiol-17β (2 mg, i.m.).
- A total dose of 200 mg of FSH (follicle-stimulating hormone), administered in 8 separate doses at 12-hour intervals, is used to superstimulate cows starting at day 4.
- At 84 and 96 hours following the initiation of superstimulatory treatment, the superstimulated cows receive two injections of PGF2α analogue (Prostaglandin; cloprostenol 500 µg; i/m), and CIDR is removed at the time of the second prostaglandin injection.
- GnRH (Gonadotropin Releasing Hormone) is administered (5 ml; i/m) (0.0042mg buserlin acetate per ml) 12 hrs after the end of superstimulatory treatment.
- The COC's are collected after 24 hours of GnRH administration.
- COC's are aspirated from >6 mm follicles only (Fig. 2).

Role of Estradiol Administration Prior to Stimulation

The emergence of follicular wave can be controlled in bovines through the administration of estradiol-17β at a random stage of the estrous cycle which could facilitate recruitment of a pool of growing follicles of uniform size on stimulation (Honparkhe et al., 2014). The administered estradiol-17β at a random day along with exogenous luteal

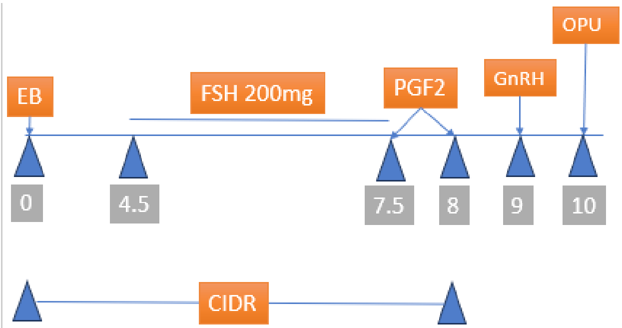

Fig. 1: Superstimulation protocol for in vivo maturation of oocytes

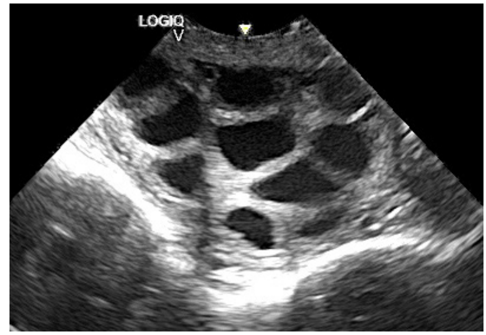

Fig. 2: Ultrsonographic image of superstimulated follicles

support prior initiating stimulation develops a greater number of small and medium antral follicles for stimulation and aspiration. A fresh follicular wave, four days after estradiol-17β was administered may have contributed to the higher number of follicles (≥8 mm) by making a significantly greater number of smaller, uniformly sized follicles available for stimulation. Therefore, it is thought that priming with estradiol before an FSH stimulation procedure will enhance the number of follicles that are available for stimulation.

Role of FSH Administration during Superstimulation

FSH influences recruitment, growth and development of pre-antral follicles, therefore, during stimulation FSH acts by increasing the recruitment and growth of antral follicles (≥ 2 mm), besides preventing the rate of follicular atresia (Sharma et al., 2011). Oocytes retrieved from medium to large-sized follicles exhibit superior developmental competence *in vitro*, and FSH stimulation enhances oocyte-cumulus cell connections, boosts mitochondrial reorganization, and increases ATP production, all of which contribute to increased developmental competence in bovines (Sugimura et al., 2017). Therefore, ovarian stimulation with FSH increases the proportion of medium to large sized follicles available for the OPU procedure and improves the blastocyst rates and embryo yield per OPU-IVEP. Hence, it has widely been used to improve the efficiency of the technique in cattle and buffalo (Vieira et al., 2014).

Duration and dose of FSH used for stimulation has significant effect on stimulatory response. According to Blondin et al. (2002) administration of FSH for longer duration (300 mg divided into 6 equal dosage, 12 hr interval each), had better stimulatory response as reflected by higher number of smaller follicles reaching ovulatory size in cattle. Similar finding was observed by Singh et al. (2017) in buffalo. Subsequently, single administration of 200 mg FSH diluted in 0.5 % Hyaluronan was also found to be effective in terms of higher recovery (84 % vs 81 %) and improved embryo production rates (30.3 vs 25.9) as compared to regular 2-day multiple FSH injections (Vieira et al., 2015).

Effect of $PGF_{2\alpha}$ Analogue on Following Superstimulation

Luteolytic treatment by PGF2α analogue during FSH stimulation is used in cows aiming to benefit the OPU procedures. Various stimulation regimen includes PGF2α on third or fourth day of simulation. An increased number of follicles were aspirated in cows post luteolytic treatment or with low circulating progesterone during FSH treatment (El-Sherry et al., 2010). Therefore, the absence of the CL (Corpus Luteum) may play a critical role in the stimulation response by controlling the number of growing follicles and additionally regression of CL reduces the proportion of ovarian parenchyma occupied with luteal tissue and consequently facilitating the identification and aspiration of small antral follicles present close to the CL.

Use of GnRH or LH (Luteinizing Hormone) to Retrieve *in vivo* Matured Oocytes

The stimulation protocols' kinetics include the production of numerous medium- to large-sized (also known as "dominant type") follicles, their starvation through the cessation of FSH support, and the completion of follicular and oocyte maturation through the administration of LH or GnRH before OPU. Sobti (2018) incorporated LH in stimulation treatment for buffaloes to recover *in vivo* matured oocytes and observed that *in vivo* matured oocytes had higher *in vitro* embryo developmental competence in terms of cleavage rate (44 % vs 32.2 %), 4-8 cell embryos (30 % vs 21.5 %) and blastocyst rate (16 % vs 7 %) as compared to *in vitro* matured oocytes of unstimulated buffaloes. Consequently, incorporating GnRH in stimulation is believed to have positive impact on oocyte recovery, oocyte quality and IVEP. Sakaguchi et al. (2019) in their stimulation attempt in riverine buffalo recovered more culturable grade oocytes than control (5.1 vs 4.8) and better blastocyst rate as compared to unstimulated (21.6% vs 9.1%).

Comparative Developmental Competence of Oocytes

The most important component influencing the outcome of the *in vitro* produced embryos is thought to be the quality of the oocytes maturated *in vivo* or *in vitro*. When combined with *in vitro* fertilization (IVF), ovum pick-up has been shown to be a more effective way to produce embryos than multiple ovulation embryo transfer as it also uses a smaller number of sperms than artificial insemination (Merton et al., 2003). Oocytes recovered from small follicles and matured *in vitro* usually have low blastocyst developmental rates (<30 to 40%; Lonergan et al., 2003). However, compared to *in vitro* matured oocytes, blastocyst development from *in vivo* matured oocytes is consistently higher (Rizos et al., 2003). The cytoplasmic maturation of *in vitro* matured oocytes, including the distribution of intracellular organelles and molecules and the storage of maternal mRNAs and proteins, is compromised, whereas *in vivo* matured oocytes developing under the ideal conditions in follicles exhibit fully achieved cytoplasmic maturation. This could be one reason for the disparity in developmental competence (Merton et al., 2003).

For effective embryo development, OPU-IVEP deems the use of *in vivo* matured oocytes to be appropriate. Therefore, one method of increasing the quantity of transferable embryos in applied reproductive programs in dairy cattle is the use of *in vivo* matured oocytes. In terms of lipid accumulation, oocytes matured by intrafollicular transfer of immature oocytes are comparable to those matured *in vivo*, indicating superior quality compared to those matured *in vitro* (Faria et al., 2021). Furthermore, compared to oocytes from unstimulated animals, those from superstimulated animals displayed a higher rate of development competence (Spricigo et al., 2015). *In vivo* developed oocytes obtained by OPU from superstimulated Japanese Black cows can be effectively used to produce high-quality embryos in terms of developmental kinetics (Egashira et al., 2019).

In vivo maturation still remains the gold standard for obtaining high-quality oocytes and ongoing research in optimizing *in vitro* maturation protocols continues to close the gap and enhance the efficiency of bovine *in vitro* embryo production.

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Mini Review

Cryopreservation of *In Vitro* Produced Embryos: A Tool for Conservation and Propagation of Elite Germplasm in Bovines

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Abstract

Embryo transfer plays a vital role in faster propagation of elite germplasm in cattle breeding programs. In vitro production (IVP) is generally considered more efficient than conventional superovulation-based embryo production methods. Successful cryopreservation of bovine embryos is the key for propagation of elite germplasm at field level especially in farm animals residing at distant areas. IVP embryos are more vulnerable to cryoinjury, limiting their widespread application. Slow freezing and vitrification are the two cryopreservation techniques utilized for embryos. The cooling pace is regulated in the slow-freezing procedure to keep the curve steady until the straws containing the embryos are submerged in liquid nitrogen. In vitrification samples are treated with cryoprotective agents in significantly higher quantities before being rapidly plunged into liquid nitrogen. Successful cryopreservation, revival of the frozen embryo after thawing and establishing pregnancy is the ultimate goal in bovine embryo cryopreservation. The process of cryopreserving in vitro produced bovine embryos has the potential to be a game-changing technique in the field of assisted reproductive technology.

Keywords: *Cryopreservation, Embryo, In vitro, Slow freezing, Vitrification*

Assisted reproductive technologies (ARTs) have emerged as powerful tools to accelerate genetic progress. Among these, *in vitro* embryo production (IVEP) followed by cryopreservation has gained prominence by enabling long-term storage and efficient dissemination of elite germplasm. An effective method for genetic improvement is the cryopreservation of *in vitro* produced bovine embryos, which maximizes the reproductive potential of genetically superior animals by selective use of embryos. This technique enables the long-term storage, transportation and global dissemination of high-value genetic material, facilitating genetic improvement and preservation. The most challenging part of embryo biotechnology is the cryopreservation technique, and even with recent improvements, the outcomes are still not always consistent (Sudano et al., 2013).

Methods of Embryo Production and Current Scenario

Two primary methods used for embryo production are *in vivo* embryo production and *in vitro* embryo production. *In vivo* embryo production involves the administration of exogenous gonadotrophin hormones to induce superovulation, followed by embryo collection from the uterus. Whereas, *in vitro* embryo production entails the retrieval of oocytes from slaughterhouse ovaries or via ovum pick-up from live animals. These oocytes then undergo maturation, fertilization, and culture in a laboratory setting to produce embryos.

In vitro embryo production is generally considered more efficient in terms of the number of embryos produced per unit time compared to traditional superovulation-based methods. However, *in vitro* embryos are more susceptible to cryopreservation stress compared to *in vivo*-derived embryos. According to Viana (2024), approximately 1.61 million bovine embryos were transferred globally in 2023, and out of which 80% were produced via *in vitro* embryo production. Out of the total embryos produced by *in vitro* method, 66.5% were transferred fresh, while 60% of *in vivo*-derived frozen embryos were transferred, highlighting the cryotolerance limitations of *in vitro* embryos. Successful cryopreservation is critical to enhance the field application of *in vitro* embryo production technology.

Mechanism and Types of Cryopreservation

Principle mechanism of cryopreservation is to pause cellular activity temporarily by cooling to sub-zero temperature and resume activity back successfully upon thawing without compromising cellular integrity and establishing pregnancy. So, the goal of cryopreservation is to retain the original stage of embryos after they have endured cooling and warming. The two cryopreservation methods used for embryos are slow freezing and vitrification. While vitrification has become the method of choice in human embryology, slow freezing remains the preferred and more practical method for cattle due to the ability of direct transfer after thawing.

Slow Freezing of Embryos

In vivo derived bovine embryos are commonly cryopreserved by slow freezing; worldwide, three-fifths of these embryos are frozen using this technique. Only one-third of bovine embryos created *in vitro* are cryopreserved. It is clear, therefore, that slow freezing is ineffective for cryopreserving bovine embryos created *in vitro*. The increased lipid content in these embryos' cytoplasm has been linked to their decreased cryotolerance (Abe et al., 2002; Mucci et al., 2006). The cryopreservation technique aims to minimizes the osmotic stress to the cells during embryo freezing by preventing the development of intracellular ice crystals and reducing the harmful effects of the cryoprotectant chemicals (Pryor et al., 2009).

Two factors determine cryopreservation protocols: cooling rates and the kind and concentration of cryoprotectant. Embryos are equilibrated in single or mix of cryoprotective agents at room temperature. The embryos individually mounted in straws are placed in the cryochamber of the freezer (Freeze Control, Cryologic; Fig 1), which is equilibrated at −6°C. The temperature is stabilized at −6°C for 8 min post-seeding and was then dropped to −32°C to −40°C at a slow cooling rate of @0.3-1.0°C/min (Cho et al., 2009: Idrissi et al., 2021). Growing ice crystals and increase in solute concentration in the extra cellular area leads to dehydration of the cell and it continues till it reaches to a stage when the outside liquid is so viscous that any further ice formation is not possible.

(a) Cryobath (b) Controller (c) Cryochamber

Fig. 1 Parts of Freeze Control by Cryologic for slow freezing of embryos

The primary benefit of this method is the use of modest quantities of cryoprotectants, as large concentrations are harmful to embryos. The best way to cryopreserve bovine embryos developed *in vitro* is to freeze them slowly at a rate of 0.5°C per minute (Assumpcao et al., 2008). Nevertheless, ice crystals have the potential to develop and harm the organelles' and membranes' structural integrity (Dode et al., 2013). Accordingly, the balance between the rate at which the cell dehydrates and the rate at which water turns into ice crystals is always necessary for the successful gradual freezing and direct transfer of embryos created *in vitro* (Visintin et al., 2002).

Practical Considerations during Slow Freezing

- Loading of the embryos should be done quickly.
- Loading of embryos should be done at room temperature.
- Seeding should be done properly.
- Label the marker sticks, goblets and the cryogenic container for proper traceability.

Vitrification of Embryos

The process of vitrifying embryos is easy, quick, and inexpensive (Sanches et al., 2013). Vitrification is achieved by combining high cooling rate and high concentration of cryoprotective agent to attain a high viscosity. At room temperature, samples are treated with cryoprotective chemicals in significantly higher quantities before being instantly cooled by submersion in liquid nitrogen at a rate of more than 100 °C per minute. Both inside and outside the cell, it causes the development of a non-crystalline amorphous solid known as the "glassy" layer. Despite being in a very viscous supercooled liquid phase, the intracellular molecular and ionic distributions are identical to those of the initial liquid state. When the temperature goes below glass transition temperature the highly cooled liquid converts to a glassy amorphous state. Both intracellular and extracellular ice production are avoided by vitrification.

Using a solution with a high osmolarity causes the intracellular water in the embryo to quickly exit, drying the cells and allowing the cryoprotectant to pass through. According to Vajta et al. (1998), the embryo may therefore tolerate direct immersion in liquid nitrogen (-196ºC) without developing ice crystals. However, even if exposed for even a brief time and a small volume of this solution, have been reported to promote considerable cellular toxicity (Vajta et al., 1998). Thus, different strategies (Fig, 2) have been developed for embryos to have rapid contact with liquid nitrogen and to reduce the volume of the cryoprotectant agent, such as the open pulled straw (Vajta et al., 1998), cryoloop (Lane et al., 1999) and cryotop techniques (Kuwayama et al., 2005).

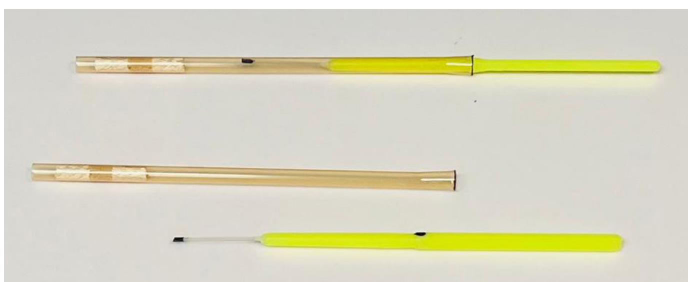

Fig. 2: Vitrification Device

Despite the advances in cryopreservation methods, embryos are exposed to physical and chemical damages during the cryopreservation process with damage comparing the apoptosis rate ranging from 1.7 to 3.7 folds (Park et al., 2006; Sudano et al., 2012).

Practical Considerations during Vitrification

- The process of vitrification should be performed fast to avoid over exposure to the high concentration of cryoprotectant.
- Strictly follow the time frame mentioned in the vitrification kit used.
- Loading of embryos should be done at room temperature.
- Minimal volume of cryoprotectant should be taken with embryos during vitrification.
- Warming or devitrification is also critical for embryo survival.

Factors Affecting Cryopreservation of *In vitro* Produced Embryos

The quality of the oocytes and semen, the culture medium composition (including additions, supplementation with or without fetal calf serum, pH, and osmolarity), and other parameters all affect embryonic survival during freezing or thawing (Feugang et al., 2009). The environment in which the embryos are grown, whether it has a lower or greater oxygen tension should also be taken into account. Low oxygen tension has been shown to enhance metabolism and reduce the generation of free radicals, which has been utilized extensively to reduce oxidative stress (Dode et al., 2013).

The lipid composition of bovine embryos was significantly influenced by the embryonic origin (*in vivo or in vitro*) (Idrissi et al., 2021). Compared with oocytes matured *in vivo*, oocytes matured *in vitro* have increased lipid content (Collado et al., 2017). Lipid accumulation may be due to the uptake of the culture medium itself or to the inefficient and unregulated metabolism of the embryonic mitochondria (Fimbres and Seidel, 2007). The increased lipid content in these embryos' cytoplasm has been linked to their decreased cryotolerance (Abe et al., 2002; Mucci et al., 2006). Lipid beta-oxidation seems to be crucial for the nuclear maturation of oocytes, the ejection of the first polar body, and meiotic maturation to metaphase II. The lipid droplets serve as essential energy reservoirs during oocyte maturation and also contribute to membrane biosynthesis (Sturmey et al., 2009)

Beta-oxidation also leads to the production of reactive oxygen species which affect competence of oocytes (Grupen, 2014). Naturally occurring during cellular metabolism, reactive oxygen species are controlled by both enzymatic and non-enzymatic antioxidants. Oxidative stress results when ROS generation exceeds a cell's antioxidant capability, causing lipid peroxidation, DNA damage, and protein degradation. Additionally, compared to embryos created *in vivo, in vitro* embryos exhibit lower transcript levels for genes linked to lipid metabolism (Gad et al., 2012). In addition to modifying the cryopreservation technique to increase the embryos' cryotolerance, it has been discovered that adding chemicals to the culture medium (lipid modulators) is advantageous (Dode et al., 2013).

Lastly, another problem restricting the use of cryopreserved embryos is the training of the field veterinarian or technician who will perform the embryo transfer into the recipient uterus. Embryo transfer must be performed in a careful, rapid and accurate manner. When taken into account collectively, these variables will directly represent pregnancy rates and could potentially benefit the widespread use of *in vitro* produced embryos at the field level.

Embryos Suitable for Cryopreservation

In vitro produced embryos are highly variable in terms of quality. This variability complicates the standardization of cryopreservation protocols, as embryos of different quality may respond differently to freezing and thawing procedure. Pregnancy rates with Grade I quality embryos were found to be higher compared with Grade II embryos (Edrem et al., 2020). Moreover, Grade I embryos re-expanded more rapidly (within 24 hours) than grade 2 embryos (Nunes et al., 2024) providing better results during cryopreservation also. The survival rates and total cell numbers of *in vitro* produced blastocyst stage embryos were 74.3% and 116 ± 8.7 and expanded blastocysts were 79.4% and 135 ± 9.1 following cryopreservation by controlled slow freezing (Cho et al., 2009). Moreover, fast developing embryos (expanded blastocyst and early hatching blastocyst stage) showed better freezability than delayed ones (early blastocyst stage) (Park et al., 1999)

Constraints in Bovine Embryo Transfer using Cryopreserved Embryos

- **Cryoinjury during freezing and thawing**: Formation of intracellular and extracellular ice crystals can damage blastomeres and zona pellucida. Additionally, osmotic stress caused by cryoprotectants leads to cell shrinkage or swelling, compromising viability.
- **Quality of embryos**: Grade 1 embryos survive well post-thaw, while lower-quality embryos often degenerate.
- **Cryopreservation method**: Both slow freezing and vitrification are used. Slow freezing requires controlled-rate freezers and reliable liquid nitrogen storage. Vitrification minimizes ice crystal formation but is technically demanding and requires high operator skill.
- **Post-thaw viability**: Cryopreserved embryos exhibit lower survival and pregnancy rates compared to fresh embryo transfer. Although some embryos re-expand after thawing, implantation success *in vivo* remains reduced.
- **Economic considerations**: Lower conception rates affect cost-effectiveness compared to artificial insemination.
- Future perspectives in cryopreservation of bovine embryos
- **Advances in cryoprotectant development**: Research should be focussed on less toxic, permeating cryoprotectants to reduce osmotic stress.
- **Improved vitrification techniques**: New ultra-rapid cooling methods and closed-system vitrification devices to be developed. Automation of vitrification protocols can reduce operator dependency.
- **Stage-specific optimization**: Protocols to be tailored for different developmental stages of embryos.
- **Field level innovations**: Portable cryopreservation kits, low-cost storage and training programs can enhance adoption of this technique.

Slow freezing remains a method of choice for *in vivo* produced embryos, largely due to its advantage of direct transfer after thawing. However, a consistently reliable cryopreservation method for *in vitro* produced embryos is still lacking, mainly due to variable quality of *in vitro* produced embryos. To improve outcomes, existing protocols must be tailored to specific embryo parameters, such as development stage, lipid content and production technique, particularly when applying direct transfer methods. In contrast, vitrification, although effective is cumbersome because it involves multistep thawing which is difficult to adopt under field conditions. The development of simplified direct transfer vitrification method would greatly benefit the application of the technology at field level. In future, advances in cryopreservation technique especially for *in vitro* produced bovine embryos are expected to make the process more reliable, cost effective and widely applicable, thereby accelerating livestock breeding and genetic conservation.

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Mini Review

Understanding Cystic Ovarian Follicles: Hormonal and Non-Hormonal Interventions in Dairy Cattle

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Abstract

Cystic ovarian follicles (COFs) can be defined as anovulatory structures with diameter of approximately 20mm or more and persisting for more than seven days with absence of luteal tissue. COFs are supposed to be one of the major causes of functional infertility in dairy cows with endocrine abnormalities having an incidence of 6-30%. COFs are classified into two major types as per the extent of luteinization of the follicular wall viz. follicular and luteal cyst whereby, follicular cyst is characterized by follicular wall thickness less than 3mm whereas, COFs with follicular wall thickness more than 3mm are termed as luteal cyst. Considering the adverse economic aspects of COFs in terms of increased inter-calving interval and increased number of inseminations per conception, treatment of COFs should be initiated at earliest possible after diagnosis without relying on spontaneous recovery. Various therapeutic measures have been implemented over the years including hormones like GnRH analogues, human chorionic gonadotropin (hCG), progesterone devices and non-hormonal approaches like manual rupture of COFs, ultrasound guided ablation, administration of naloxone etc. with variable success rate. This review highlights the incidence, associated factors, etio-pathogenesis and various therapeutic approaches for management of COFs in dairy animals.

Keywords: *Cystic Ovarian Follicles, Follicular cyst, Functional infertility, Naloxone*

The profitability of a dairy enterprise depends mainly upon reproductive performance of animals. It is desirable that calving interval at farm is maintained less than one year so that target of one calf crop per annum could be achieved. This would also ensure the better exploitation of the germplasm by obtaining good number of calves during complete reproductive life of an animal. The pre-requisite to accomplish this task is normal reproductive cyclicity and timely ovulation in the animal without any failure. However, this is affected by occurrence of various ovulatory defects including delayed ovulation, anovulation etc. and one of the major anovulatory conditions in dairy cattle is cystic ovarian follicles (COFs).

COFs can be defined as anovulatory structures with diameter of approximately 20mm or more and persisting for more than seven days with absence of luteal tissue

(Lima et al., 2019). The occurrence of COFs have been associated with multiple factors with well-established fact that there is a positive correlation between development of COFs with high milk yield and majority of cows are affected in their third lactation which is assumed as peak lactation.

Incidence of COFs

Many authors observed a large variation in incidence of COFs varying from 6-30% (Mimoune et al., 2017; Haldi et al., 2025). The majority of studies have focussed on the animals in their early postpartum period, which is assumed as peak of their lactation. It has been well established that negative energy balance in the animals during their peak milk yield is one of the major factor for occurrence of COFs. In the recent study conducted at Guru Angad dev Veterinary and Animal Sciences University, Ludhiana, Punjab, the incidence of COFs at organized and unorganized dairy farms of HF crossbred animals was found to be 13.37% and 22.95%, respectively (Kamboj, 2025).

Associated Factors for COFs Development

According to published literature, there is increased incidence of ovarian cysts in Dutch Holstein Friesian (HF) herds as compared to other cattle breeds. Holstein cows are the most susceptible to develop ovarian cystic condition compared to other cattle breeds like Jersey, Guernsey and Ayrshire and other indigenous dairy breeds. It has been seen that the risk of developing COFs during the current lactation is significantly increased for cows that had COFs during their previous lactation. The parity-wise distribution of disease prevalence reveals that animals are more susceptible in their third or above third parity, which are assumed as their peak lactation parity (Parkinson, 2018). In the recent study, highest disease occurrence was observed in animals of more than third parity (45.76%), which was significantly higher ($P<0.05$) compared to heifers and animals in their first, second and third parity indicating a clear trend of increasing disease prevalence with advancing parity (Kamboj, 2025).

Compared to the cows calved in the cold season, summer calved cows had a 2.6 folds higher risk of developing early cysts (Nelson et al., 2010). Reduced feed intake during heat stress seems to be reasonable as it could amplify the consequences of a negative energy balance during postpartum period and increases the duration of postpartum ovarian rebound leading to follicular growth and formation of COFs. Parity, BCS and milk output have been directly linked to the development of COFs. Similar finding has been observed in the current study conducted by Kamboj (2025) where animals having BCS more than three and high milk yield were affected.

Etio-pathogenesis of COFs

The formation of COFs may be influenced by high milk yield, hot weather stress, NEB and the metabolic adaptations made to maintain the animal's high level of production.

Studies conducted by Singh et al. (2009) showed that hormonal deficiency accounts for 28.42% of repeat breeding cases in cattle among which 37.1% animals have COFs. The underlying cause for occurrence of COFs was usually pre-ovulatory luteinizing hormone (LH) surge is either absent, insufficient in volume or occurs at the wrong time during the dominant follicle development leading to altered LH secretion from the pituitary and resulting in formation of COFs (Singh et al., 2009; 2017).

It has been documented that the development of COFs is due to defective LH surge which was in turn due to intermediate level of P_4 (0.1ng–1ng/ml) causing negative feedback on hypothalamus with resultant reduced LH pulse frequency and amplitude with failure of occurrence of LH surge and anovulation. The persistent dominant follicle increased in size and become cyst. Therefore, the development of COFs was attributed to dysfunction in hypothalamic-pituitary-gonadal axis. It has been seen that the primary cause of COFs was altered estrogen feedback on the hypothalamus-pituitary, which can lead to abnormal LH release and ultimately, COFs formation. It has been also opined that stress from negative energy balance led to reduced insulin, glucose, leptin and insulin like growth factor-1 (IGF-1) and high non-esterified fatty acids (NEFA) and β-hydroxy butyrate (BHB) which led to failure of release of estrogen from ovary with subsequent inhibition of LH surge and ovulation leading to formation of COFs (Parkinson, 2018).

Diagnosis of COFs

The clinical signs associated with COFs such as prolonged heat, short inter-estrus interval, persistent cervico-vaginal mucus discharge, nymphomania, sterility hump can be used to diagnose it but most often these signs are not exhibited and animal usually remains in anestrus with incidence of 62-85%. The rectal palpation of ovaries can be a reliable method for diagnosing COFs. Further, follicular cyst and luteal cyst can also be differentiated on rectal palpation by former being soft in consistency, which fluctuates due to large amount of fluid present in cyst whereas luteal cyst is comparative hard in consistency.

It has been seen that rectal palpation of ovaries was most commonly used method to diagnose COFs in field conditions but according to studies conducted differentiation between follicular and luteal cyst was successful in only 50% of cases of COFs. Although, in many studies conducted till date using trans-rectal ultrasonography (USG) and/or serum/follicular fluid steroid (Progesterone and estrogen) hormones concentrations, the prevalence of follicular cyst was found more than luteal cyst (Mimoune et al., 2017; Haldi et al., 2025). Also, there was an interesting finding in the study conducted by Mimoune et al. (2017) where diagnosis of COFs with combined use of per-rectal USG and serum P_4 level reversed with luteal cyst (55%) more common than follicular cyst (45%) than those observed by alone USG (51.66% follicular and 48.33% luteal cyst). These similar trends were also seen in the study by Kamboj (2025) where based on serum progesterone, the overall occurrence of luteal cyst (60.91%) was more ($p<0.05$) than follicular cysts (39.08%) while, trans-rectal USG revealed more occurrence of follicular (81.60%) than luteal cyst (18.39%; $p<0.05$). So, it is recommended to use both trans-rectal USG and endocrine profiling of animal to exactly identify the type of COFs.

Therapeutic Interventions of COFs

It has been seen that COFs developing during early postpartum period regress spontaneously with more than 50% spontaneous recovery rate but, COFs developing after 50 days post-partum have reduced chances (20%) of spontaneous recovery and requires subsequent treatment (Yimer et al. 2018). As occurrence of COFs cause economic losses to the dairy enterprises, therefore, it is recommended to treat the animals at earliest when diagnosed for occurrence of COFs.

Hormonal Approaches: The hormonal methods to treat COFs can be use of gonadotrophin-releasing hormone (GnRH) analogues, hCG, prostaglandin $F_2α$ ($PGF_2α$), progesterone, fixed timed artificial insemination/estrus synchronization protocols (FTAI) etc.

1. **Intramuscular GnRH with $PGF_2α$**: The combined use of use of GnRH and $PGF_2α$ results in a greater estrus rate, reduced persistence rate of COFs, increased ovulation rate and greater early response rate than those treated by GnRH alone. Silva et al. (2012) concluded that Lecirelin acetate (GnRH analogue) was effective in treating 75% COFs in dairy cattle. It has been seen that acute cases of follicular cysts can be successfully treated with GnRH in 80% of cases while, $PGF_2α$ was required along with or alone for the management of luteal cyst.
2. **Epidural GnRH:** Annalisa et al. (2011) conducted research on 220 HF cattle having COFs and found that single epidural administration of 50µg Lecirelin was more effective than 50µg intramuscular Lecirelin. Estrus rate was found to be 75 per cent in epidural group and 57 per cent in intramuscular group. Pregnancy rates were found to be 93 per cent and 76 per cent, respectively, from epidural and intramuscular groups.
3. **hCG:** Singh at al. (2012) conducted a study on 14 dairy cattle and compared the use of GnRH (20µg) and hCG (3000 IU) intramuscularly on day 0 with $PGF_2α$ (500µg) on day 7 followed by timed AI after 72 hours and found that complete luteinization of COFs occurred following GnRH and hCG administration with pregnancy rate of 43% in both groups.
4. **Progesterone:** A long term progesterone in form of intravaginal device (PRID), GnRH and hCG were found equally effective in terms of treatment efficacy, fertility rate, pregnancy rate and time duration between treatment and recovery (Mollo

et al. 2012). However, keeping in mind the effectiveness, affordability, ease of usage, GnRH was recommended as best possible medication for managing COFs in dairy cows. Slow releasing P_4 based protocols are more efficacious for treatment of COFs but their high cost, irregular market availability and development of vaginitis reduces their applicability at field level (Singh et al., 2017).

Non-Hormonal Approaches: Some non-hormonal therapeutic measures like transvaginal USG guided COFs ablation and naloxone have been used with variable success.

1. **Transvaginal USG guided COFs ablation:** In attempts to use non-hormonal approaches to treat COFs using aspiration technique, it was observed that aspiration alone was more efficacious than use of combination of GnRH and $PGF_2\alpha$ and combination of both aspiration and hormonal therapy resulted in 100% therapeutic efficacy (Amiridis, 2009). While, in another study by Singh et al. (2017) depicted that ultrasound guided follicular aspiration was similarly efficacious to CIDR (controlled internal drug releasing device) plus ovsynch protocol in terms of cyst dissolution and pregnancy rate. But it requires sophisticated instrumentation with more expertise therefore, limiting its use at field level.
2. **Naloxone:** Naloxone (competitive μ opioid receptor antagonist) has been used by some workers via intramuscular as well as epidural routes with optimum success rate (Fuentes-Hernandez et al., 2009); however, its usage and efficacy need to be explored more. Naloxone has a half-life of 30-80 minutes and can competitively inhibit the action of endogenous neuropeptides, thereby eliciting the LH surge and leading to either luteinization or regression of COFs. The drug when administered epidurally can reach to central nervous system via cerebrospinal fluid. Sympathetic nerve fibres innervate ovary which originate from neurons located in spinal cord nuclei and ovulation, steroid release, and follicular maturation are significantly influenced by the sympathetic innervation of the ovary.

In a study conducted on usage of GnRH analogue lecirelin via epidural route it was postulated that epidural administration of GnRH analogue generated a neuronal influx of Ca^{2+} by acting on spinal GnRH receptors, which in turn prompted the sympathetic terminals of the neurons innervating the ovary to release norepinephrine (Schneider et al., 2006). In a study conducted by Palomar et al. (2008) it was concluded that single use of naloxone (0.6mg) plus buserelin acetate (8µg) epidurally led regression of COFs in 77.5% animals within 2-4 weeks while, in another study, Derakhshesh et al. (2016) concluded that epidural administration of 0.8mg naloxone was better.

Herbs and Plant Extracts

Karreman (2007) conducted research on Heat Seek, a botanical herb blend used to enhance the observable signs of estrus, may be given as 10 tablets orally every other day for twelve doses 24 days treatment for COFs. Safdarian et al. (2023) found specific complementary treatments for COFs in dairy cattle including the use of Vitex agnus-castus extract, commonly known as chaste berry, for its efficacy in regulating hormonal imbalances and reducing COFs size in dairy cows. Chasteberry contains bioactive compounds that act on the hypothalamic-pituitary axis, promoting the secretion of LH and FSH, thereby regulating ovarian function. Formulations containing Cimicifuga racemosa, also known as black cohosh, have notable anti-cystic properties. Research highlights the benefits of black cohosh in reducing ovarian cysts and improving reproductive outcomes in dairy cattle.

Homeopathic Drugs

Apis 30c or Lachesis 200c twice daily for 5 days, with either one being immediately followed by Natrum mur twice daily for 3 days are effective treatments for COFs. Apis mellifica is a common homeopathic medicine made from female honeybee. Lachesis is prepared from the fresh venom of the South American bushmaster snake. Natrum mur is made from sodium chloride or table salt (Karreman, 2007). However, homeopathy offers a non-invasive approach for treatment of COFs but limited research is available for its use.

Research Gaps and Future Prospects

Cystic ovarian follicles in dairy cattle are still not fully understood, and several gaps remain in research. We still do not know exactly how genetics, stress, nutrition, and hormone imbalances work together to cause these cysts. Diagnosis is also not consistent, as different farms and veterinarians use different methods, leading to missed or incorrect cases. The biological processes that make a follicle stop developing normally or fail to form a proper corpus luteum also need more study. In the future, research should focus on finding early and reliable markers for detection, understanding how management and environment influence cyst formation, and developing better treatment and prevention methods that fit modern dairy production systems.

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Mini Review

Uterine Torsion in Buffaloes: Current Perspectives on Management and Prognosis

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Abstract

Uterine torsion results from rotation of the gravid uterus around its longitudinal axis, leading to birth canal obstruction, impaired uterine blood flow and fetal hypoxia. The condition is multifactorial in origin, associated with weaker broad ligaments, reduced uterine tone, limited fetal fluids and excessive fetal movements. Incidence in buffaloes ranges from 67–83% of all dystocia cases, underlining its impact on dam health, calf survival and dairy herd profitability. Clinical manifestations vary with the degree and location of torsion with characteristic vaginal or rectal findings. Pathophysiological changes include impaired uterine perfusion, fetal asphyxia, uterine edema followed by necrosis, hematological alterations and compromised hepatic and renal functions, uterine adhesions often worsening prognosis. Management requires rapid intervention, with therapeutic options including per-vaginal fetal rotation, rolling techniques and modified Schaffer's method, while delayed or severe cases often necessitate caesarean section. Prognosis largely depends on the duration and severity of torsion, as well as timely referral and supportive therapy. Early diagnosis, aided by clinical examination and Doppler ultrasonography, combined with prompt corrective measures, remains key for improving maternal and fetal outcomes in uterine torsion of buffaloes.

Keywords: *Buffalo, Dystocia, Fetal distress, Manual correction, Obstetrics, Cesarean section, Uterine torsion.*

Dystocia, or difficult birth, is a major concern in bovine reproduction, impacting both the economic viability of dairy enterprises and animal welfare. Uterine torsion is most common cause of dystocia, particularly in late gestation and, at the onset of parturition. In this condition, the pregnant uterus becomes rotated around its longitudinal axis, obstructing the birth canal and compromising fetal blood supply (Mota-Rojas et al., 2020). According to reports, 67–83% of buffalo cases with dystocia had uterine torsion. The outcome has been suggested to depend on factors like the duration of the condition and the severity of the torsion (Amin et al., 2011; Deosi and Dhaliwal, 2015). Losses arise from calf death, diminished milk yield, and the need to address secondary conditions, including delayed uterine involution, endometritis, and infertility. This article provides a brief overview on uterine torsion in dairy buffaloes, addressing its multifaceted aspects from its underlying causes to the latest advancements in its diagnosis and management.

Etiology

The etiology of uterine torsion in buffaloes is multifactorial, involving a complex interplay of mechanical, physiological and anatomical factors. The uterine instability during a single-horn pregnancy and excessive fetal or dam motions seem to be the most plausible explanation for the rotation of a pregnant uterus on its axis (Ghuman, 2010). Rotational fetal movements occurring during the second stage of labor or in late gestation can induce uterine torsion, primarily due to the extensive fusion of the bubaline amnion with the surrounding allantois, which itself is anchored to the uterine wall. During mid to late gestation, the uterus resides on the abdominal floor in the absence of sufficient stabilizing attachments. Less fetal fluid, a smaller uterus, and a decreased uterine tone during the last stages of pregnancy all contribute to increased fetal discomfort, which in turn causes more fetal movements and a higher degree of torsion (Ghuman, 2010).

The deep capacious and pendulous abdomen of buffaloes, their wallowing behaviors and their innately weaker broad ligament muscles are also thought to contribute to the problem's increased incidence in these animals. Research showed that bovines affected by uterine torsion typically have narrow and less muscular broad ligaments, suggesting these anatomical differences may contribute to their increased susceptibility compared to other dystocia cases. Brar et al. (2008) reported that 11% of non-pregnant buffaloes and at least 25% of female progeny born to dams with a history of uterine torsion exhibit insufficient muscular development in their broad ligaments. In addition, the stability of the pregnant uterus in buffaloes is further compromised due to the suboptimal organization of broad ligament musculature.

Buffaloes have a higher predisposition to post-cervical uterine torsion, which is attributed to inadequate support by the broad ligament in the post-cervical area, along with the absence of dorsal attachments of the broad ligament during pregnancy (Brar et al., 2008; Deosi and Dhaliwal, 2015). Higher degree rotations typically do not resolve spontaneously, while slight rotations (less than 90°) are clinically asymptomatic and may be reversed on their own. In 83–85% of buffaloes with uterine torsion referrals, the gestation is often complete. Intestinal blockage, hemoperitoneum, uterine rupture, and the development of adhesions between the uterus and surrounding viscera can all be associated with uterine torsion (Amin et al., 2011).

Clinical Manifestations and Diagnosis of Uterine Torsion

A uterine horn may rotate during the middle to late stages of pregnancy, during a typical parturition, or occasionally after giving birth. The uterine horn can rotate either left (anticlockwise) or right (clockwise), with the degree of rotation varying between

90° and 720° (Purohit and Gaur, 2014). The point of rotation may occur either cranial to the cervix (pre-cervical) or caudal to it (post-cervical). In buffaloes, uterine torsion is most commonly observed during late pregnancy or at the time of parturition (Ali et al., 2011). Buffaloes are more prone to right side post cervical uterine torsion (87-99%) due to presence of rumen on left side and reduced muscular folds on right side broad ligament (Ali et al., 2011).

Clinical signs typically include the commencement of labor without fetal membranes or fetus delivery with mild discomfort. The animal may exhibit minor constipation and colic pain, as well as a rocking horse attitude (Purohit, 2006). There may be signs of dullness, depression and partial anorexia (Deosi and Dhaliwal, 2015). In post-cervical uterine torsion, the entire birth canal undergoes rotation, often causing one or both vulvar lips to be drawn inward (Ali et al., 2011). Vaginal examination reveals that the mucous membranes are twisted, and the anterior vagina, ending in a conical shape, is rotated by 180° or more, making it impossible to advance the hand further inside. However, in less severe cases, the fetus may occasionally be spotted. The evidence for the direction of torsion is thought to be the direction in which the vaginal fold twists. Rectal examination reveals a twisted uterine horn, a broad ligament on the torsion side that is rotated downward and occasionally palpable beneath the uterus, and a tense, stretched ligament on the opposite side (Ghuman, 2010).

Clinical Pathology and Hematological Changes

Uterine Changes: The middle uterine vein is compressed when the uterus rotates, disrupting venous circulation and raising the carbon dioxide level in the fetal blood. As a result, vigorous movements of fetus observed, which could further aggravate the degree of uterine torsion. As the severity of torsion intensifies, compression of the main uterine artery occurs, leading to a reduction in oxygen supply to the fetus (Schonfelder et al., 2005). The limited arterial perfusion and venous outflow result in ischemia, hypoxia, and cell death that harms the endometrium and myometrium irreversibly followed by death of the fetus. The uterine wall becomes delicate and susceptible to rupture when its blood supply is persistently disrupted, resulting in a loss of elasticity and tissue viability (Ghuman, 2010). The color of the uterine wall shifts from a rose-pink hue to blue-purple and eventually turns grey following rotation of the uterus, signifying the uterus's increasing metabolic degradation (Schonfelder et al., 2005).

Blood Components: Buffaloes affected by uterine torsion often develop normocytic normochromic anemia, characterized by reductions in red blood cell count, hemoglobin and packed cell volume, which results from the accumulation of metabolic waste products or relatively significant blood loss during abnormal parturition. These buffaloes' leukograms show eosinopenia along with lymphocytopenia, neutrophilia and monocytosis (Amer and Hashem, 2008). In surgically corrected cases of uterine torsion, these symptoms persist until the third day after delivery. Torsion-affected buffaloes are regularly found to have significantly lower levels of albumin and total plasma proteins. The hypoproteinemia and hypoalbuminemia in torsion-affected buffaloes could be associated with liver disease and a negative nitrogen balance, primarily due to reduced protein intake. Besides, hyperglycemia to the extent of 15-30% could be because of increased cortisol release (Amer and Hashem, 2008).

Liver and Renal Functions: Strong abdominal contractions during uterine torsion are thought to cause significant muscular exhaustion, which is responsible for the rise in plasma aspartate amino transferase and muscle-specific creatine phosphokinase (Hussein and Abd Ellah, 2008). A marked increase in plasma urea and creatinine levels during the initial presentation of uterine torsion cases suggests poor prognosis (Schonfelder et al., 2007; Amer and Hashem, 2008). Urine production decreases due to impaired renal functions (Schonfelder et al., 2007). Moreover, acute or chronic kidney failure may arise due to stress-induced reductions in renal blood flow, shock, dehydration, and nephropathy caused by toxins released from a deceased fetus. This will lower the elimination of urea and creatinine (Amer and Hashem, 2008).

Therapeutic Approaches

Uterine torsion cases should be treated as emergencies, and prompt treatment must be started quickly upon diagnosis. Prior to any handling attempts, a thorough assessment of the patient's overall health is essential. The animal is assessed for signs of toxemia and shock; for those cases presented to the obstetrician more than 36 hours after onset, treatment involves administration of copious fluids, corticosteroids, and antibiotics as needed to combat toxemia and shock prior to managing the delayed case. Toxemia, fetal mortality, fluid loss, and uterine inertia are likely to have developed in cases that were presented after 36 to 72 hours. As the duration of uterine torsion increases, the survival percentage for bovines impacted by torsion actually decreases linearly from 87 to 43% (Ghuman 2010). Before starting therapy, a uterine rupture examination should be performed because of the relatively high occurrence of this condition. Elevated histamine levels have been observed in animals with uterine torsion therefore, administration of antihistamines is recommended. The proposed techniques for detorsion management include:

Rotation of the Fetus Per-Vaginum: This method is feasible only in moderate torsion cases, where the obstetrician can reach the fetus with their hand and there is an adequate amount of uterine fluid present (Ghuman, 2010). During detorsion, the handler should grasp a prominent bony landmark of the fetus, such as the elbow, sternum, or thigh, and gently swing it side to side before rotating it in the direction opposite to the torsion.

If identifiable, both fetal limbs can be secured within the cuffs of a Kuenh's crutch or Caemmerer's torsion fork, allowing an assistant to aid with rotation. When the dam is standing, the cervix is sufficiently dilated to accommodate the fetus, and the fetus is alive, the likelihood of successful detorsion is high (Ghuman, 2010).

Rolling of Buffalo: This method is among the earliest and most straightforward techniques for correcting uterine torsion in buffaloes. The animal is placed in lateral recumbency on the side corresponding to the detected torsion. Both the forelegs and hind legs are securely bound separately with ropes (Ghuman, 2010). The animal is then swiftly rolled in the same direction as the uterine torsion. The buffalo's gravid uterus rotates more slowly than its fast revolving body. The animal's body must be returned to its initial position after being rolled 180 degrees. The birth canal should be inspected after each roll to see if the torsion has been rectified. The birth canal's stenosis and spiral folds will go away if the rolling technique is done correctly; if not, it should be done three or four times.

Schaffer explained a detorsion technique that involves securing the uterus with planks and rolling the dam in the same direction as the torsion (Yadav et al., 2023). However, because of their thick skin and protuberant abdomen, Indian buffaloes do not respond well to this technique. Accordingly, Sharma (Singh and Nanda, 1996) developed a modified version of the existing Schaffer's method by making specific alterations to enhance its effectiveness. The plank was resized to 11.9 feet in length, 9 inches in width, and 2 inches in thickness. During the rolling procedure, two or three medium-built assistants provide support: one remains at the lower end of the plank while another moves along its length, and a third helper applies pressure to the upper end of the plank to modulate force (Singh and Nanda, 1996). The modified Schaffer's method, has demonstrated a success rate that is 50% higher compared to the original Schaffer's technique (Prabhakar et al., 2007). It is recommended that failure be admitted and surgery be considered if the torsion is not alleviated after three rolls. Following correction of uterine torsion, buffaloes often experience failure of cervical dilation, which is considered a major obstacle to vaginal delivery, particularly when the fetus is dead (Prabhakar et al., 2007). The use of one litre of warm sodium carboxymethyl cellulose and three hourly massages (15 minutes each) have been proposed as one of the ways to produce adequate cervical dilatation for fetal delivery (Honparkhe et al., 2009). In cases where uterine torsion has been corrected but the fetus is dead and the cervix remains firm and lobulated, cesarean section is recommended (Honparkhe et al., 2009).

Prognostic Indicators

Uterine torsion is an extremely stressful condition that triggers alterations in both biochemical and endocrine parameters (Ghuman, 2010). In addition to hematobiochemical changes, it induces significant oxidative stress at the tissue level due to increased production of free radicals such as malondialdehyde, superoxide dismutase, and glutathione. These measurements provide a precise assessment of cellular and tissue damage, which aids in formulating an improved treatment plan and achieving a better prognosis (Bansal et al., 2011). Amer and Hashem (2008) documented alterations in liver-specific enzymes, glucose levels, and renal function tests that indicate the degree of damage sustained by the animal due to uterine torsion, as well as their prognosis. Ghuman (2010) observed that in acute uterine torsion cases, activation of the stress axis increases gluconeogenesis, resulting in hyperglycemia, whereas with the progression to a more chronic condition, hypoglycemia gradually develops. Elevated blood lactate levels can serve as a prognostic indicator of tissue damage in animals affected by uterine torsion (Murakami et al., 2017). Additionally, blood histamine concentrations provide valuable prognostic information, reflecting the overall extent of tissue injury due to the subsequent release of histamine. Other prognostic indicators include creatine kinase, which reflects muscle damage, and plasma haptoglobin, an acute phase protein produced during inflammatory responses, these markers can also be effectively utilized for prognosis (Amin et al., 2011).

Researchers are now very interested in trans-rectal Doppler sonography since it is a relatively less intrusive way to evaluate hemodynamic status. Several semi-quantitative indices, such as resistance index, pulsatility index, time-averaged maximum velocity, time-averaged mean velocity, and blood flow volume, have been established to evaluate organ perfusion (Ratta et al., 2016). These indices provide relative measurements of the resistance to blood flow and are utilized as prognostic indicators in cases of uterine torsion. Ratta et al. (2016) explained that the resistance index (RI), also known as Pourcelot’s ratio, reflects tissue perfusion during diastole, while the pulsatility index (PI), measured at a distal point, quantifies the resistance to blood flow caused by constriction in the vascular bed. However, there is a simultaneous decrease in blood flow volume, as well as in time-averaged maximum and mean velocities, accompanied by the presence of a pre-diastolic notch, indicating that end-diastolic flow is minimal or absent in cases of uterine torsion. Doppler indices such as the RI and PI were found to be elevated in bovines affected by uterine torsion, indicating a significant obstruction to blood flow (Hussein, 2013). The spectral waveforms of the middle uterine artery in bovines affected by uterine torsion exhibit distinct cardiac abnormalities, which correlate with a proportional decline in uterine blood flow as both the duration and severity of the torsion increase (Schonfelder et al., 2005). Trans-abdominal ultrasonography, alongside trans-rectal sonography, can serve as a valuable prognostic tool by assessing alterations in the uterine echotexture caused by inflammatory and necrotic changes in uterine tissue following uterine torsion (Devender et al., 2016). Doppler sonography of the middle uterine artery and fetal umbilical cord is highly useful for monitoring blood flow patterns during gestation in bovines, as well as assisting in the prognosis of uterine torsion cases.

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Mini Review

Alternative Approaches to Antimicrobial Therapy for Uterine Infections in Dairy Animals

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Abstract

Reproductive efficiency is a critical determinant of dairy herd productivity, with the goal of one calf per year in cattle and every 13-14 months in buffaloes. Postpartum uterine infections encompassing metritis, endometritis and pyometra pose a significant impediment to achieving this goal by delaying uterine involution, prolonging the calving-to-conception interval, and inflicting considerable economic losses. Although, conventional antimicrobial regimens remain the mainstay of treatment, their extensive use has raised concerns over milk residues and the alarming rise of antimicrobial resistance (AMR) in both livestock and humans. This approach has encouraged a global shift towards sustainable, non-antibiotic/alternative therapeutic strategies. Emerging alternatives including probiotics, chitosan micro-particles, mannose antagonists, bacteriophages, essential oils, recombinant cytokines, ozone therapy, metritis vaccines, and proteolytic enzymes, offer the potential to reduce pathogen burden, modulate uterine immune responses, promote tissue regeneration, and restore reproductive competence without fostering AMR. This review comprises current knowledge on these alternative therapies, evaluates their efficacy, and discusses future prospects for integrating them into reproductive management programs to improve dairy herd fertility and productivity.

Keywords: *Alternative therapeutics, Antimicrobial resistance, Ozone therapy, Postpartum uterine infection, Proteolytic enzymes*

The economic sustainability of dairy enterprises largely depends on the consistent production of calf crops with ideally one per year in dairy cattle and every 13-14 months in dairy buffaloes. Achieving optimal inter-calving intervals depends on normal calving, timely uterine involution, and rapid resumption of ovarian activity, all of which are influenced by postpartum uterine health. During parturition, vulvar relaxation and cervical dilation allow bacterial entry into the uterus (Azawi, 2008), resulting in contamination in 80–100% of animals. Although most cases resolve naturally during the puerperium, persistent infections remain common, with reported incidences of 25-40% for metritis, 18-20% for endometritis, and 25-40% for subclinical endometritis following calving. These conditions delay reproductive cyclicity and impair fertility (Sheldon, 2018).

Uterine infections are therefore a major postpartum concern, particularly in high-producing dairy animals where metabolic stress, retained fetal membranes, and unhygienic calving environments increase susceptibility (Gulia et al., 2023). If untreated, these infections prolong days open, elevate culling rates, and cause substantial economic losses (Gulia et al., 2022). Conventional management relies on antimicrobial therapy, but indiscriminate use has contributed to rising antimicrobial resistance (AMR), posing both animal-health and public-health challenges. This has intensified interest in non-antimicrobial alternatives for preventing and managing uterine infections. The present review outlines the major postpartum uterine infections in dairy animals and summarizes emerging alternative therapeutic approaches supported by current scientific evidence

Types of Uterine Infections

Uterine infections are typically classified based on clinical signs and severity, following theriogenological definitions (Sheldon, 2018). However, inconsistencies and lack of validation in these definitions limit precision and hinder reliable assessment of treatment strategies and their outcomes. Uterine infections are generally classified based on clinical presentation and severity into four categories: Puerperal (postpartum) metritis, Clinical endometritis, Subclinical endometritis, and Pyometra. These are collectively referred to as the *Metritis complex*.

- **Puerperal Metritis**: This condition typically occurings within 10 days postpartum is marked by systemic illness, uterine enlargement, foul-smelling purulent discharge, pyrexia, anorexia, reduced milk yield, and dehydration. It significantly extends the open period and inter-calving interval, leading to economic losses. It is also known as toxic or clinical metritis and the inflammation extends to all three layers of uterus (Lima, 2020).
- **Clinical Endometritis**: It is identified by the presence of purulent (over 50% pus) or mucopurulent (approximately equal proportions of pus and mucus) vaginal discharge after 21-26 days postpartum. The animal is not affected systemically with presence of almost normal appetite, water intake, body temperature with mild effects on total milk yield of the animals. Yet, presence of cervicovaginal discharge hinders the development of embryo and increases inseminations numbers required for conception with increased calving to conception interval (CCI) and prolonged inter-calving period. This is also known as leukorrhea or whites (Lima, 2020).
- **Subclinical (Cytological) Endometritis**: It involves inflammation of the endometrium without visible abnormal discharge and is diagnosed via uterine cytology. A polymorphonuclear cells (PMNs)/neutrophils count exceeding 18% (20-33 days postpartum), 10% (34-47 days postpartum), or 5% (>50 days

postpartum) indicates that animal is suffering from subclinical endometritis. As in this condition animal is having normal cervicovaginal mucus discharge without apparent clinical evidence of abnormality, therefore, it is termed as subclinical endometritis. The infection is evident with presence of abnormal cellularity in the uterus so it is also known as cytological endometritis (Sheldon, 2018).

- **Pyometra**: It is characterized by uterine distension resulting from the accumulation of purulent or mucopurulent exudate with closed cervix. The condition is typically associated with functional CL presence on the ovary, which sustains a progesterone-dominant environment conducive to its development. The animal remains in anestrus during pyometra (Sheldon, 2018). The clear classification and timely diagnosis of these conditions are crucial for effective therapeutic management and to minimize their impact on fertility.

Uterine Microbiome and Pathogenesis of Uterine infections

The term uterine microbiome is defined as the commensal non-pathogenic microbial community that maintains host homeostasis and is essential, as dysbiosis can disrupt physiological function and predispose the animal to disease. The microbial composition remains similar between animals that subsequently develop metritis and those that remain healthy until approximately two days after calving. Thereafter, animals that develop metritis exhibit a marked shift in their uterine microbiome, characterized by an increased relative abundance of Bacteroidetes and Fusobacteria and a decreased presence of Proteobacteria and Tenericutes. This dysbiosis is further distinguished by reduced microbial heterogeneity and diminished bacterial richness. Among uterine pathogens, *Escherichia coli* (*E. coli*) often acts as the pioneer organism, creating conditions that favour colonization by *Fusobacterium necrophorum* (*F. necrophorum*) and *Trueperella pyogenes* (*T. pyogenes*) later on. Pathogenic *E. coli* strains from endometritis cases demonstrate enhanced adhesion and invasiveness compared with isolates from healthy animal's uterus, with the *fimH* gene, encoding a D-mannose-specific adhesin, identified as a critical virulence factor. Notably, animal positive for *fimH* within 1-3 days postpartum had increased odds of harbouring *F. necrophorum lktA* at 8-10 days, which was linked to higher metritis risk, while *F. necrophorum* (*lktA)* positivity at 34-36 days post partum was strongly associated with *T. pyogenes fimA* and clinical endometritis at the same stage (Sheldon et al., 2010).

Alternative Therapies to Ameliorate the Adverse Impacts of Uterine Disease in Animals

The non-judicious use of antimicrobial agents has accelerated antimicrobial resistance (AMR) in human beings and animals, contributing to elevated morbidity, mortality, culling, and substantial economic losses in the dairy sector. Recognized as a global health threat, AMR has led to World Health Organization (WHO) guidelines on prudent antimicrobial use and spurred research into alternative therapies for uterine infections.

A range of alternative therapeutic strategies has been investigated for both the prevention and management of uterine diseases in animals. These interventions primarily aim either to suppress bacterial proliferation—using agents such as chitosan microparticles, dextrose, mannose essential oils, bacteriophages, and probiotics—or to enhance uterine immunomodulation through compounds such as pegbovigrastim, ozone, paraffin, recombinant bovine interleukin-8 (IL-8), and vaccines. Collectively, these strategies have been evaluated for the prevention of metritis (e.g., probiotics, chitosan microparticles, bacteriophages, pegbovigrastim, mannose, recombinant IL-8, vaccines, proteolytic enzymes), therapeutics of metritis (e.g., essential oils, ozone, chitosan microparticles), management of clinical endometritis (e.g., dextrose, paraffin, oyster glycogen), and prevention of clinical endometritis or subclinical (e.g., ozone, proteolytic enzymes, dextrose).

1. **Essential Oils:** Having carvacrol (4-isopropyl-2-methylphenol), which is a monoterpenic phenol, demonstrates potent antioxidant, anti-inflammatory, and antibacterial activities. Its proposed antimicrobial mechanism involves depletion of intracellular ATP, alteration of membrane potential, and increased cytoplasmic membrane permeability to protons and potassium ions, thereby inhibiting bacterial proliferation (Friedman, 2014).
2. **Mannose and Bacteriophage:** Mannose has been investigated as a competitive inhibitor of FimH, a key virulence factor of *E. coli*, thereby reducing the risk of metritis. Bacteriophages are obligatory intracellular parasites of bacteria. They lack their own metabolism, exhibit high host specificity, infecting only particular bacterial species or even specific strains of the pathogenic bacteria (Machado et al., 2012).
3. **Paraffin:** Intrauterine use of liquid paraffin facilitates the recruitment and migration of phagocytic cells specially macrophages and improves local uterine immune response (Ahmadi et al., 2019).
4. **Probiotics:** Prepartum intravaginal infusion of cocktail having lactic acid bacteria (*P. acidilactici* FUA3140, *Lactobacillus sakei* FUA3089 and *Pediococcus acidilactici* FUA3138) has been evaluated. These strains hydrolyse glycogen in the vaginal mucosa to produce lactic acid, thereby lowering vaginal pH to acidic levels (H_2O_2 production), which serves as a natural barrier against the ascending bacterial infection (Ametaj et al., 2014).
5. ***E. coli* Lipopolysaccharide (*E. coli* LPS Endotoxin):** Lipopolysaccharide (LPS), a structural component of the outer membrane of Gram-negative bacteria, plays a

pivotal role in pathogen recognition and the initiation of inflammatory responses. At low intrauterine doses (100-200 µg), it functions as an immunomodulator by engaging the Toll-like receptor 4 (TLR4)–myeloid differentiation factor 2 complex, thereby inducing cytokine production, activating both innate and adaptive immunity, and enhancing uterine defence through increased leukocyte counts, a higher proportion of polymorphonuclear neutrophils (PMN), and improved PMN viability (Moraes et al., 2017).

6. **Pegbovigrastim:** Pegbovigrastim is a recombinant, polyethylene glycol-conjugated form of bovine granulocyte colony-stimulating factor (G-CSF). As a hematopoietic growth factor, it promotes the proliferation, differentiation, and mobilization of neutrophil precursor cells into circulation (Zinicola et al., 2019).

7. **Metritis Vaccines:** The developed vaccine, formulations comprising of either major virulence factors (fimH, lktA, pyolysin Plo) and/or inactivated whole cells preparations (*Escherichia coli, Fusobacterium necrophorum, Trueperella pyogenes*), was administered intravaginally or subcutaneously. Formulations containing purified virulence factors demonstrated the greatest efficacy, providing superior protection against uterine infections (Meira et al., 2020).

8. **Recombinant IL-8:** Interleukin-8 is a pro-inflammatory chemokine produced by smooth muscle cells, epithelial cells, endothelial cells, and innate immune cells in response to pathogen-associated stimuli sensed primarily through Toll-like receptors. IL-8 binds to neutrophil surface receptors CXCR1 and CXCR2, promoting neutrophil activation, directed migration to the site of infection, and enhancement of phagocytic and bactericidal capacities. Administration of recombinant bovine IL-8 (rbIL-8) in dairy animals has been associated with reduced incidence and severity of puerperal metritis due to improved early postpartum neutrophil function and uterine immune responsiveness (Mitchell et al., 2003).

9. **Ozone:** Ozone (O_3) is a triatomic, highly reactive allotrope of oxygen characterized by its chemical instability. Ozone therapy exerts diverse biological effects primarily through controlled oxidative stress that activates the Nrf2–ARE pathway, enhancing antioxidant enzyme expression and redox balance. It demonstrates broad-spectrum antimicrobial activity by oxidatively disrupting bacterial membranes, fungal cell-wall components, and viral capsid proteins and nucleic acids. Ozone also modulates inflammation by reducing pro-inflammatory cytokines (IL-1β, IL-6, IL-12, TNF-α) and increasing IL-10. Its analgesic actions involve activation of antinociceptive pathways, endorphin release, nociceptor modulation, and improved tissue oxygenation and metabolism. Immunomodulatory effects include enhanced leukocyte activity, increased

secretion of regulatory cytokines and acute-phase proteins, and elevated immune cell and IgM levels. Additionally, ozone promotes wound healing through TGF-β upregulation, platelet activation, and accelerated epithelialization and collagen synthesis. Ozone-derived hydrogen peroxide further improves oxygen delivery by increasing erythrocyte ATP and 2, 3-DPG levels, thereby enhancing tissue perfusion and oxygenation (Escandón et al., 2020, Avila et al., 2022).

10. **Proteolytic Enzymes:** Proteolytic enzymes combination such as chymotrypsin, trypsin, and papain has been utilized in intrauterine therapy to reduce the risk of uterine infections. Trypsin and chymotrypsin, both endopeptidases, are secreted as inactive zymogens (trypsinogen and chymotrypsinogen) and subsequently activated by enterokinase in the small intestine, with trypsin further facilitating the activation of additional proenzymes. In contrast, papain, a cysteine protease of plant origin, functions as both an endo- and exopeptidase and is typically derived from the latex and unripe fruits of papaya (*Carica papaya*) and mountain papaya (*Vasconcellea cundinamarcensis*). They degrade proteins and lipids, with trypsin and chymotrypsin acting optimally at alkaline pH and papain active across a broader range. These enzymes not only aid digestion but also modulate immune responses—papain, for example, influences CD14-mediated macrophage activity. Their potent proteolytic and fibrinolytic actions, especially in the alkaline environment of uterine infections, allow them to break down necrotic tissue, infectious debris, and lochia, thereby enhancing host defence, leukocyte migration, smooth muscle contraction, and uterine involution. They also exert direct antimicrobial effects by degrading structural proteins and lipids in bacterial, yeast, and protothecal cells, compromising membrane integrity. Collectively, these properties support their successful therapeutic use in postpartum uterine infections (Gulia et al., 2023).

Preventive and Therapeutics Alternatives Strategies for Retention of Fetal Membrane (RFM)

Djuricic et al. (2012) used two different intrauterine ozone preparations in Simmental cows with RFM (24–36 h postpartum) received either a 5-second ozone foam spray or six "ozone pearls" compared to cows without RFM and observed improved reproductive performance in terms of open days to first service and pregnancy, and overall pregnancy rates. Gulia et al. (2022) evaluated the intrauterine application of a proteolytic enzyme combination (trypsin, chymotrypsin and papain as 16 mg, 16 mg, and 8 mg, respectively) in dystocia-affected buffaloes. The treatment markedly reduced the mean expulsion time of fetal membranes (8.80 ± 1.50h vs. 27.40 ± 6.33h in controls) and lowered the incidence of RFM (26.67% vs. 73.33%).

Preventive and Therapeutics Alternatives Strategies for Metritis

Gulia et al. (2022) reported that intrauterine administration of a proteolytic enzyme combination in dystocia-affected buffaloes improved postpartum recovery, reflected by reduced neutrophil counts (55.05±1.63% vs. 64.92±1.46%), accelerated uterine involution (delayed uterine involution in 73.33% of controls), and a lower incidence of uterine infections (6.67% vs. 53.33%) at 45 days pp. Meira et al. (2020) demonstrated that vaccination with bacterial proteins and/or inactivated pathogens significantly reduced puerperal metritis (9.1% vs. 14.9% in controls), with the recombinant subunit vaccine (FimH, leukotoxin, pyolysin) being most effective (8.0% vs. 14.9%), while also lowering vaginal bacterial load and enhancing reproductive performance. This highlighted its potential as a preventive strategy against postpartum uterine diseases. Zinicola et al. (2019) investigated the impact of recombinant bovine interleukin-8 (rbIL-8) administration on postpartum uterine health and reported a substantial reduction in puerperal metritis incidence among cows. Specifically, the prevalence of metritis was 34.3% in the control group, compared with 8.11% and 6.35% in cows receiving low and high doses of rbIL-8, respectively.

Preventive and Therapeutics Alternatives Strategies for Clinical Endometritis

Avila et al. (2022) reported that direct intrauterine infusion of ozone (O_3) gas is effective in the treatment of endometritis in mares, leading to a measurable reduction in uterine inflammation (reduced neutrophils count from 10.67 ± 3.84 to 2.89 ± 3.59) and improvement in reproductive tract health. Biswal et al. (2013) observed that single intra uterine infusion of 500 mg Oyster glycogen (OG) in 50 ml of sterile phosphate buffer saline (PBS) on the day of estrus enhanced uterine neutrophilic influx, cleared bacterial infections and improved the overall conception rate. Zaben et al. (2024) concluded that intrauterine infusion of proteolytic enzymes can improve the physical properties of cervical mucus and enhance fertility outcomes in buffaloes with clinical endometritis. The proteolytic enzymes group animal's shows improve cervical mucus properties in terms of spinnbarkeit values from 7.24±0.79 to 15.79±0.64 and mean pH value from 8.22±0.08 to 7.10±0.03, respectively with highest first service conception rate as compared to control group (44.44% vs. 0%).

Preventive and Therapeutics Alternatives Strategies for Sub clinical Endometritis

Escandón et al. (2020) analysed the effect of intrauterine ozone therapy administered at 35 days after calving for prevention of subclinical endometritis in postpartum dairy cows and observed a substantial decline in the incidence of subclinical endometritis (5.0% vs. 50.0%), a reduction in the number of inseminations required per conception, and a shorter interval from calving to conception, along with an improvement in the first-service conception rate (50.0% vs. 16.2%). Singh et al. (2020) evaluated effects of intrauterine proteolytic enzymes administration (trypsin 8mg, chymotrypsin 8mg and papain 4mg in 20 ml normal saline) on endometrial inflammation and overall reproductive performance in the postpartum water buffalo cows diagnosed with subclinical endometritis. The treatment resulted in a marked modulation of uterine cytokine profiles, characterized by reduced interleukin (IL)-10 and elevated concentrations of tumour necrosis factor-α (TNF-α), IL-1β and IL-8. Additionally, buffaloes receiving proteolytic enzyme therapy exhibited a reduced open day's period, indicating improved reproductive efficiency.

Future Research Prospects on Bovine Uterine Infections

Reproductive efficiency is essential for dairy sustainability. With antibiotic efficacy declining, the need for effective non-antibiotic alternatives is increasingly urgent. Although several emerging options show promise, most evidence is limited to controlled or small-scale studies, with inadequate data on long-term effectiveness, field performance, dosing, and safety. Major research gaps remain in understanding uterine microbiome dynamics, pathogen virulence, host immune modulation, and the progression from normal postpartum inflammation to disease. Future work should emphasize large field trials, optimized delivery systems, and integration of precision diagnostics such as biomarkers and real-time monitoring. Improved insights into host–pathogen interactions and microbiome profiling may support tailored therapeutic approaches for managing uterine infections.

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Short Communication

Twisted Stomachs: Understanding Gastric Dilatation and Volvulus in Dogs

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Abstract

Gastric Dilatation Volvulus (GDV) Syndrome is a serious and acute life-threatening condition occurring more commonly in deep-chested, larger breeds of dogs. This brief review focuses on the clinical presentation, management, and survival outcome of this important condition of veterinary medicine. The syndrome combined with its relatively unknown etiology and high mortality rates demand a broader knowledge base and awareness amongst pet owners and veterinary professionals to recognize its severity and timely therapeutic intervention.

Key words: *Canine, Emergency surgery, Gastric dilatation volvulus, Stomach distension.*

Gastric Dilatation Volvulus (GDV) syndrome is an acute critical emergency condition that is caused by rapid gaseous filling of the stomach along with twisting on its mesenteric axis causing volvulus, which progresses to cardiopulmonary and hypovolemic shock and if left untreated, death (Sharp and Rozanski, 2014). The condition is of more prevalent in large breed dogs than smaller breed dog or cats. Epidemiological studies revealed a higher incidence of GDV in heavier-built, older, working military or farm dogs and sometimes in pure-bred male dogs (O'Neill et al., 2017). The condition due to its rapid progression and lower survivability rates, poses a significant necessity for the improvement of diagnostic approaches, its medicinal and surgical management.

Global Status of GDV – Incidence

A prospective cohort study on 1914 dogs reported a 5.7% cumulative incidence for all 11 breeds included in the study, with no significant difference between risks in large and giant breed dogs (Glickman et al., 2000). Another epidemiological study on 77,088 dogs in the United Kingdom established a 0.64% prevalence of GDV with Great danes and Akitas having the highest odd ratio of developing the condition (O'Neill et al., 2017). Besides, there are sporadic case reports on the GDV in dogs from various countries (Wangdi et al., 2025).

Occurrence of GDV in India

As per literature search, there is a lack of large-scale or systematic studies on GDV incidence or distribution from India. Scientific knowledge on this aspect is restricted to individual case reports and small series. There is an urgent need to bridge this gap by conducting planned epidemiological surveillance and reporting. Due to lack of structured case surveillance or multi-center veterinary research on GDV, true incidence, occurrence pattern, geographic distribution, and seasonal trends in India remain unknown.

An epidemiological study on esophageal and stomach disorders in Hyderabad reported a 4.72% prevalence of GDV amongst 42 affected canine patients (Suryawanshi, 2010). Another clinical study showed that out of 66 dogs presented with abdominal affections within a period of 14 months, 22 dogs suffered from gastro-intestinal conditions, two out of which were diagnosed with GDV, indicative of a 0.03% prevalence (Konwar, 2017).

Etiopathophysiology

The GDV syndrome is of multifactorial etiology which is not clearly identified; however, a combinatorial interplay of various risk factors has been identified through various retrospective and prospective studies. Larger, heavier, pure-bred, with familial predisposition, increased thoracic depth-to-width ratio, older age, working military dogs, history of less frequent feeding (once a day), rapid ingestion, gastric foreign body, and aerophagia are the most commonly identified risk factors for GDV (de Battisti et al., 2012). The temperament of the animal and stress also play a factor in the onset of the condition. Breeds at a significantly higher risk include Great Danes, German Shepherds, Irish setters, and Saint Bernards (Glickman et al., 2000).

The gastric mal-positioning due to volvulus concludes into a functional or mechanical outflow obstruction which prevents the eructation of air and gastric contents causing gastric distension and subsequently leads to a cascade of events resulting in Systemic Inflammatory Response Syndrome (SIRS), shock, disseminated intravascular coagulation and Multiple Organ Dysfunction Syndrome (MODS) (Sharp and Rozanski, 2014). The shift in the position of the fundus and pylorus from their normal positions at the left dorsal and right ventral abdominal positions respectively in a way that the pylorus goes cranial to the esophagus and the positions are interchanged (Fig. 1). The volvulus most commonly occurring in a clockwise direction and can vary with respect to the degree of the rotation ranging between 180 and 360 degrees (Fig. 2). The outflow obstruction causes local and systemic effects. The local effects include compromised gastric blood supply leading to reduced myocardial activity and gastric ischemia and necrosis. The gastrosplenic ligament causes splenic displacement and in turn may cause splenic infarction and torsion. The systemic effects are more severe occurring primarily due to the obstruction of blood flow through the caudal vena cava and hepatic portal vein which in turn, causes a reduced venous return, decreased cardiac output, hypotension, and shock. GDV also affects respiratory function leading to respiratory acidosis after the pressure on the diaphragm rises, acid-base abnormalities, and endotoxemia (Kumar, 2017; Fossum et al, 2019).

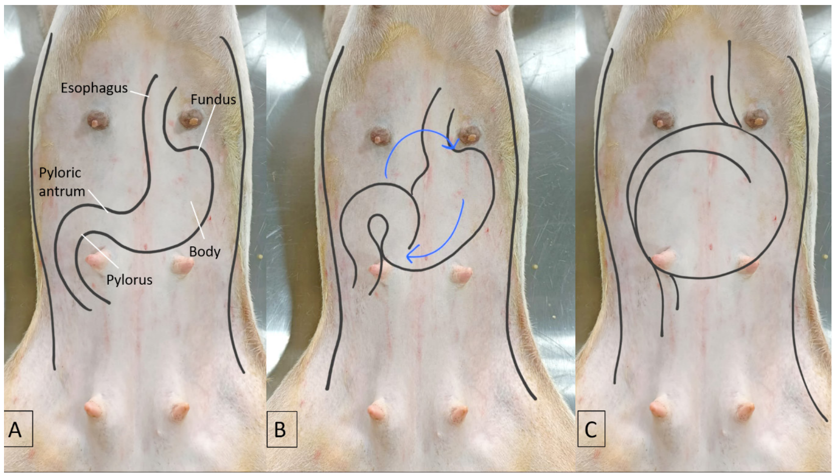

Fig. 1 Stomach rotation in gastric-dilatation volvulus, observed in dog in dorsal recumbency; (A) Normal positioning of stomach (pylorus and fundus) of normal dog; (B) the pylorus and pyloric antrum shift cranially relative to the body of the stomach and toward the midline; (C) Dilated stomach with pylorus and pyloric antrum ascended dorsally in the left cranial aspect of abdomen and fundus displaced ventrally to the right ventral abdomen (Monnet, 2003).

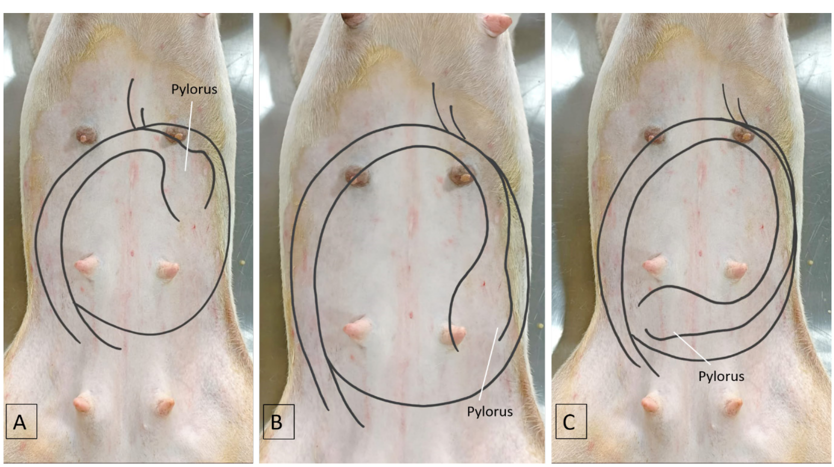

Fig. 2 Schematic representation of different degrees of rotation in Gastric-dilatation volvulus: (A) 180 degrees rotation with pylorus situated in left cranial quadrant; (B) 270 degrees rotation with pylorus visualised in left caudal quadrant; (C) 360 degrees rotation with pylorus twisting and returning to the initial position in the right caudal quadrant of abdomen.

Clinical Presentation

Affected dogs are usually presented with a tympanic, distended abdomen, with a history of non-productive retching, acute onset of restlessness, agitation, dyspnea, and signs of shock such as tachycardia, pale or cyanotic mucous membranes, and weak pulse. A few animals showed signs of increased pain, adopting a 'praying' posture (Broome and Walsh, 2003). The intensity and manifestation of the clinical signs are dependent upon the severity of distension and torsion and the duration of onset. A rapid examination is necessary to prevent further deterioration of the animal to a comatose state or death.

Diagnosis

An efficient and timely diagnosis is imperative which include analyzing history, signalment, and physical examination findings. Radiography is an important method for confirmation of diagnosis and ruling out other differentials such as food engorgement and Gastric dilatation, but it can be delayed until medicinal stabilization or may be done along with fluid administration. The right lateral and dorso-ventral radiographic views allow the filling of malpositioned pylorus with air for better visualization on radiographs. A right lateral radiograph reveals a characteristic "Double-Bubble" or "Popeye-arm" appearance due to the division of the gas-distended stomach into 2 compartments and on the dorsoventral view, the pylorus appears as a gas-filled structure to the left of midline (Fig. 2), while Gastric dilatation alone (without volvulus) presents a single distended compartment. Pneumoperitoneum, if visible on the radiograph is indicative of gastric perforation. The spleen owing to its proximal aspect's attachment to the study often follows the greater curvature of the stomach to the right, and can be difficult to visualize. A volvulus of 360 degrees would project the fundus and pylorus at their normal positions on radiography (Fossum et al., 2019). A right lateral radiograph showing gastric pneumatosis, malpositioned pylorus and spleen have been used to diagnose GDV radiographically (Wangdi et al., 2025). Hematobiochemical parameters generally indicate hypovolemic shock, disseminated intravascular coagulation (DIC) and metabolic acidosis depending upon the severity and duration. Early changes manifest themselves as hemoconcentration, leukocytosis, or leucopenia and thrombocytopenia in cases with DIC. Serum biochemistry parameters show elevated levels of lactate, liver enzymes, bilirubin, and creatinine, along with hypokalemia (Broome and Walsh, 2003; Verschoof et al., 2015).

Management

A multi-faceted approach is essential for a successful therapeutic intervention. The primary medicinal management involves rapid intravenous fluid therapy to counter hypovolemic shock and address metabolic derangements and gastric decompression. Shock doses of isotonic crystalloid solutions (up to 90 ml/kg boluses), or a combination

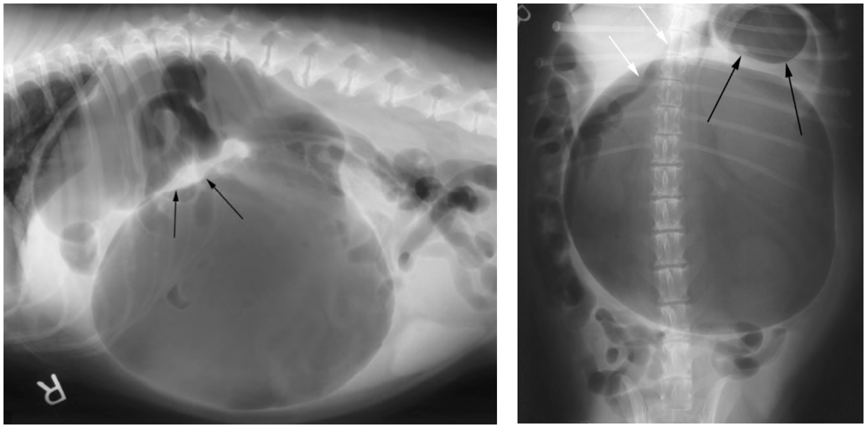

Fig. 2 Double bubble appearance on right lateral radiograph and ventro-dorsal radiograph of abdomen suggestive of 270 degrees of GDV (Fossum et al., 2019)

of isotonic crystalloids at a lower dose (20-40 ml/kg) along with 7% hypertonic saline (2-4 ml/kg), can be administered along-with regular cardiovascular assessment. Gastric decompression is of utmost importance to restore the normal venous return and gastric wall perfusion. The most commonly adopted techniques include passage of an oro-gastric tube under sedation or percutaneous gastric trocarization (Monnet, 2003).

Surgical Management

Surgery should be performed as soon as the animal's condition has been stabilized, even if the stomach has been decompressed. Surgical management is necessary for gastric detorsion and correction of splenic torsion. Gasterectomy may be required if the stomach wall is partially necrosed, after de-torsion, gastropexy need to be done to prevent re-occurrence of volvulus. Gastropexy can be done using Belt-loop, Incisional, tube, circumcostal (Fig. 4) techniques. Gastropexy in dogs with GDV aims to create permanent adhesions between the stomach and the body wall. There is currently no recommended 'gold standard' surgical procedure for gastropexy.

Surgeon expertise and comfort with the technique frequently affect the gastropexy approach. In dogs that have already undergone an exploratory celiotomy, the gastropexy is performed as part of an open approach to the abdomen; however, minimally invasive approaches might be explored for dogs receiving preventive gastropexy as an independent surgery. Regardless of the procedure used, gastropexy is performed on the right side, close to the last rib. The placement of the gastropexy site is crucial for preventing complications, since an anatomic "kink" produced inadvertently might cause partial pyloric outflow blockage (Allen and Paul, 2014). Minimally invasive options include the grid technique, endoscopically guided mini approach, and laparoscopic gastropexy.

Retrospective research by Benitez et al. (2013) indicated that incisional gastropexy is beneficial in reducing GDV in dogs, comparable to belt-loop gastropexy. An incision is made in the seromuscular layer of the gastric antrum and in the right ventrolateral abdominal wall. The edge of the abdominal incision is sutured to the gastric incision using a simple continuous pattern, making sure the muscularis layer of the stomach is in contact with the abdominal wall muscle. Gastric lumen is not opened as in tube gastropexy.

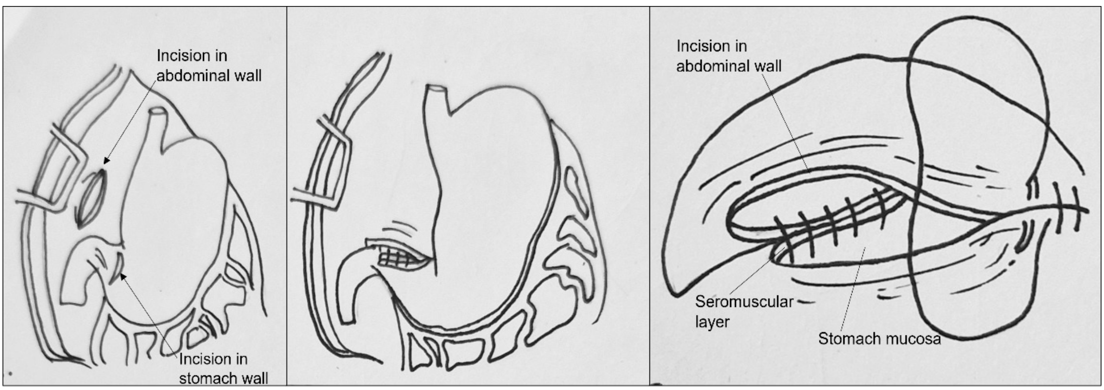

Fig. 4 Steps for incisional gastropexy (Fossum, 2019)

Postoperative Care

In the postoperative period, therapy focuses on maintaining tissue perfusion while also providing intensive monitoring for the prevention and early detection of ischemia-reperfusion injury and its associated complications, which include hypotension, cardiac arrhythmias, acute kidney injury), gastric ulceration, electrolyte imbalances, and pain. Intravenous (IV) fluid treatment is continued postoperatively, although it should be reduced depending on perfusion metrics in patients with uncomplicated GDV. Adequate post-operative analgesia using opioids or continuous infusions of ketamine or lidocaine should be employed. Antacid drugs are regularly given in the postoperative period and may comprise an H2 receptor antagonist, a proton pump inhibitor, or both. Sucralfate can be used as a gastroprotectant. Prokinetics are also recommended for dogs that have postoperative ileus or regurgitation (Bruchim and Kelmer, 2014). Postoperative cardiac arrhythmias, typically of ventricular origin, have been documented in 40%-70% of dogs with GDV, and their occurrence has been linked to a poor prognosis in several studies (MacPhail et al., 2006).

Electrolyte and venous blood-gas analysis should be conducted to assess potassium and magnesium levels, as well as acid-base status. If ventricular tachycardia (heart rate >180) persists despite adequate fluid resuscitation, treatment with lidocaine (2 mg/kg slow IV bolus followed by a constant rate infusion of 50 µg/kg/min) or procainamide

(2-4 mg/kg slow IV bolus followed by 10-40 µg/kg/min) is recommended (Bruchim and Kelmer, 2014). In a prospective study, lidocaine was administered @ 2 mg/kg given as an IV bolus, before any medical intervention, followed by a constant rate infusion of 0.05 mg/kg/min for 24 hours throughout the initial patient care period. Lidocaine therapy dramatically reduced AKI, cardiac arrhythmias, numerous coagulation problems, and hospitalization time as compared to 47 historical control dogs. The mortality rate in the therapy group was lower (10%) than in the control group (24%) (Bruchim et al., 2012) Serial abdominal focused assessment with sonography for trauma (aFAST) is advised in the postoperative phase to detect and sample abdominal effusions. Post-op care involves critical monitoring, analgesia and anti-microbial therapy.

Surgical Prognostic Factors

Timely surgery offers a fair prognosis, while delays or complications like gastric necrosis or perforation lead to poor outcomes. Preoperative plasma lactate levels can predict gastric necrosis and overall prognosis in dogs with GDV. Mortality rates can reach up to 45%, though rates as low as 10% are increasingly reported. Dogs with gastric necrosis face a tenfold higher risk of death than those without. Additionally, concurrent splenectomy is associated with higher mortality. Recurrence rates for GDV differ based on the surgical method, but most studies show rates below 10%. Tube gastropexy has the highest recurrence, ranging from 5% to 29%. If the stomach is repositioned without gastropexy, recurrence can be as high as 80%, while gastropexy can bring down the recurrence rate to less than 5% (Mackenzie et al, 2010; Allen and Paul, 2014).

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Clinical Article

Successful Surgical Resection of a Large Rectal Leiomyoma in a Buffalo: Long-Term Follow-Up

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Abstract

A 5-year-old, 550 kg, non-descript female buffalo was presented with the complaint of acute prolapse of a large rectal mass and the buffalo was straining to pass feces. The mass was lobulated, firm, and bleeding. Surgical resection was done under epidural anesthesia in the standing position. Two lobes of the mass, measuring 35 × 22 cm and 12 × 13 cm respectively, were excised separately from anal approach. The masses were present on the ventral surface of the rectal mucosa. Stay suture with silk no. 2 was applied at the proximal end of the rectal mass to prevent slippage during resection. The raw ends at the resection site were closed with polygalactin 910, 2-0 in an inverting suture pattern to achieve hemostasis and to repair the ulcerated region. Histopathology confirmed the mass as leiomyoma. The buffalo made an uneventful recovery with no signs of recurrence over a 10-month follow-up period. The present case recommends successful resection of rectal leiomyoma under epidural anesthesia and with an excellent prognosis.

Keywords: *Buffalo, Leiomyoma, Mass, Prolapse, Rectal, Straining*

Rectal tumors in bovines are rare, with limited case reports documented in literature (Prasad et al., 2017). Leiomyoma is the most frequently encountered rectal tumor, as confirmed histopathologically, while other reported neoplasms include leiomyosarcoma, fibroma, fibrosarcoma, myxoma, and carcinoid tumors (Kumar and Krishna, 2000; Prasad et al., 2017; Sangwan et al., 2019). Surgical excision is the preferred treatment when the mass is distinctly attached to the rectal mucosa and is surgically accessible. This report describes a case of a buffalo presenting with a large rectal leiomyoma, its surgical management and outcome.

Case History and Presentation

A 5-year-old, 550 kg, non-gravid female buffalo was presented to the veterinary teaching hospital with a complaint of a large prolapsed ulcerated rectal mass since 24 hrs. The owner did not report observed straining prior to the prolapse. No clear history of straining by the animal was provided by the owner. But the buffalo exhibited noticeable straining at the hospital during clinical examination.

On physical examination, two spherical ulcerated masses protruding out of anal opening were found to be attached to the rectal mucosa (Fig. 1a). Based on the clinical findings, surgical resection under epidural anesthesia was decided and explained to owner.

Surgical Treatment

The buffalo was restrained in a crate and 5ml of 2% lignocaine was aseptically administered in the epidural space. The protruded mass was washed with ample amount of water to clear all the debris and surrounding anal region and area under the tail base was scrubbed with chlorhexidine and cetrimide solution.

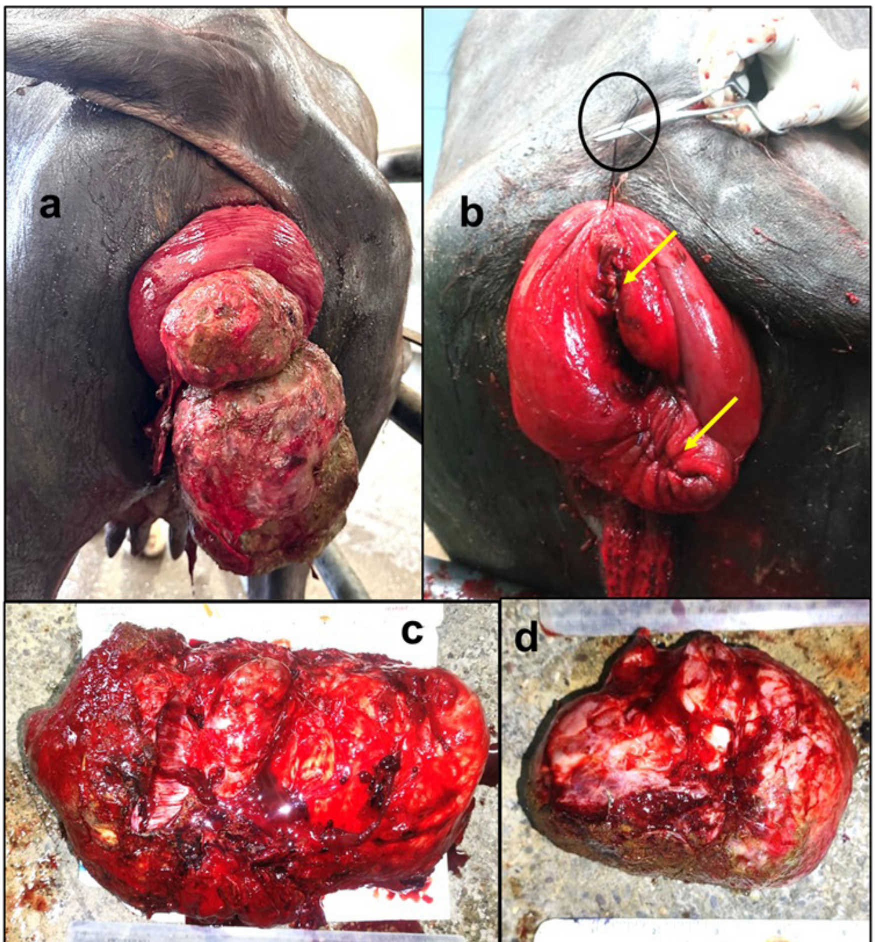

Fig. 1. Serial photographs showing the prolapsed rectal masses in a 5-year-old buffalo (a), inversion suturing of the rectal mucosa after removal of rectal mass (yellow arrow) (the black circle shows the stay suture applied to hold the rectum outside) (b), the resected large (c) and small (d) mass with scale.

A stay suture was applied on the healthy rectal wall cranial to the mass with silk no. 2 and was held with an artery forceps throughout surgery to avoid inward slipping of rectum when the masses were resected. Both the masses were manually dissected with fingers without use of scalpel blade or scissors. The raw edges were bleeding and were carefully sutured in inversion pattern using 2-0, polyglactin 910 (Fig. 1b). A mixture of ointment lignocaine and soframycin was applied on the suture lines. The stay suture was removed and the rectum was repositioned. The resected masses measured 35 × 22 cm and 12 × 13 cm (Fig. 1c and 1d). This approach facilitated mass removal without much hemorrhage and preserved rectal integrity. Histopathology, diagnosed the tumor as leiomyoma.

Post-Operative Care and Follow Up

Post operative care included systemic antibiotics amoxicillin-cloxacillin, 10mg/Kg body weight, IM for 5 days and gentamicin, 4mg/Kg IM, for 3 days, and analgesic meloxicam, 0. 2mg/Kg body weight for 3 days. Regular per-rectal application of soframycin and Lignocaine gel was advised. Dry fodder was not allowed for 15 days to help healing. The buffalo showed normal appetite and no straining in immediate post-operative period. At 10 month follow up, the buffalo was healthy and no re-occurrence was reported.

Discussion

Rectal tumors are seldom reported bovines. However, leiomyoma is the most common rectal tumour documented (Awadin and Mosbah, 2013; Prasad et al., 2017). Leiomyoma originates from smooth muscles of hollow, tubular organs like reproductive, gastro-intestinal or urinary tract (Tyagi et al., 1993; Sharma et al., 2012) and may be benign or malignant. It has smooth appearance and may reach a gigantic size before showing any clinical sign (Ducharme et al., 2004). Animal usually shows straining while defecating, if the origin is from the rectal wall. If the tumorous mass is big enough, it may protrude out of anal opening and hence can be ulcerated due to environmental trauma.

Surgical resection is the treatment of choice if the base of the tumour is approachable. Lignocaine is most preferred and cost-effective anaesthetic for epidural anesthesia. It can be combined with other agents like xylazine for better effects (Awadin and Mosbah, 2013). The application of lignocaine jelly and soframycin ointments in rectal and vaginal mucosa surgeries reduces pain and straining, supporting mucosal healing. Definitive diagnosis for the type of tumour can be made through histopathology.

In summary, Leiomyoma is the most common rectal tumor in bovines and can be successfully managed by surgical excision under epidural anesthesia, with an excellent prognosis.

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Mini Review

Targeted Dietary Interventions for Enhancing Gut Health in Dogs

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Abstract

Gut health is the fundamental aspect of canine health with implications for digestion, immunity and behavior. The gut microbiota of the dog, characterized by its high taxonomic diversity of microbes, is responsible for metabolite production, immune modulation and intestinal barrier function. Dysbiosis—imbalance of microbes—has been linked with chronic gastrointestinal and systemic disease. Nutritional interventions with modulation of the microbiota are effective through dietary supplementation. Prebiotics (e.g., fructo-oligosaccharides, inulin), probiotics (e.g., Lactobacillus, Bifidobacterium) and synbiotics have synergistic effects on microbial content, short-chain fatty acids production and gut barrier function. Postbiotics and fermented substrates enhance mucosal immunity and prevent inflammation by bioactive metabolomes. Dietary fibers like chicory root and beet pulp induce beneficial microbial fermentation, while omega-3 fatty acids from fish or algal oils exert anti-inflammatory effects. Phytonutrients, antioxidants and digestible plant proteins obtained from plants enhance gut health, oxidative status and nutrient absorption. Digestive enzyme supplementation also enhances digestive efficiency. Functional nutrition thus has potential as a non-pharmacological intervention to optimize canine gut health.

Keywords: *Canine gut health, Microbes, Prebiotics, Probiotics, Functional nutrition*

Dog gut health is a cornerstone of overall health, as it affects digestion, immunity, behavior and lifespan. The gastrointestinal tract harbors several communities of microbes, collectively known as the gut microbiome, whose activity is crucial for digestion, production of important metabolites and protection against pathogens. Dysbiosis, an imbalance of microbes, can lead to chronic diarrhea, inflammatory bowel disease, allergies and immune dysregulation (Rhimi et al., 2022). Targeted dietary intervention is an effective method for modulating the gut microbiome and promoting canine gut health. This is achieved through the incorporation of prebiotics, probiotics, postbiotics, synbiotics, dietary fibre and other functional nutrients in dog diets.

Canine Gut Microbiome

The canine gut microbiome comprises numerous bacterial species, primarily from the phyla Firmicutes, Bacteroidetes, Proteobacteria, *and Actinobacteria* (Hernandez et al., 2022). These microorganisms' ferment dietary substrates into short-chain fatty acids (SCFAs), such as acetate, propionate and butyrate, which are utilized as energy by colonocytes and provide anti-inflammatory effects (Rhimi et al., 2022).

The integrity of the intestinal barrier and competitive exclusion of pathogenic organisms by an abundant and diverse microbiome are established in healthy adult dogs. Decreased microbial diversity and overgrowth of opportunistic bacteria (e.g., *Clostridium spp.*) are signs of dysbiosis, which is typically associated with gastrointestinal and systemic disease. Recent evidence suggests that dietary manipulations can modify the microbial composition towards more beneficial taxa, thus restoring homeostasis and a healthy gut condition in dogs (Baritugo et al., 2023). Hence there is an urgent need to summarize the feed additives/ supplements in the canine diet which can enhance gut health. So, an effort has been made to discuss this topic under following sub-headings:

High-Quality Plant Proteins

Plant protein sources offer palatably digestible alternatives to animal sources, with reduced allergenicity and maintaining gut integrity. Hydrolyzed soy protein has been found to effectively manage inflammatory bowel diseases by enhancing mucosal barrier integrity and increasing the number of beneficial bacteria like *Lactobacillus, Bifidobacterium*, and *Faecalibacterium*. Varied plant protein-rich diets (e.g., chickpeas, lentils) provide required amino acids without overburdening digestion, promoting optimal nutrient absorption (Di Cerbo et al., 2017).

Digestible Lipids and Marine Oils

Omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are derived from fish oils and algae oils have anti-inflammatory actions that reach into the gut. These lipids promote mucosal homeostasis and suppress gut inflammation through reduced production of pro-inflammatory eicosanoids (Baritugo et al., 2023; Di Cerbo et al., 2017). A clinical study showed that a dog diet using fish oil demonstrated increased consistency of stool and decreased markers of intestinal inflammation (Baritugo et al., 2023). Oils derived from algae are a sustainable option, are high in DHA and have been added to extrude adult dog diets with beneficial gut health effects (Di Cerbo et al., 2017).

Fermentable Fibers

Fermentable fibers are substrates for gut health-promoting bacteria, resulting in the production of short-chain fatty acids (butyrate, acetate and propionate) which are an important source of energy for colonocytes and improve local immunity (Calvert, 2025). Chicory root has the active ingredient inulin is a fructan prebiotic that is resistant to small intestinal digestion and selectively stimulates the growth of *Bifidobacterium* and *Lactobacilli* (Di Cerbo et al., 2017; Calvert, 2025). Sugar beet pulp has soluble as well as insoluble fibers, inducing fecal bulk which maintains SCFA levels in the gut (Di Cerbo et al., 2017). Pumpkin and sweet potatoes are natural prebiotic fibers that promote SCFA production, which strengthens the gut barrier and reduces inflammation (Calvert, 2025).

Prebiotics

Prebiotics are indigestible food components that are added to the basal diet which specifically promote the growth and function of beneficial gut flora. In canine nutrition, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), inulin, resistant starch and psyllium husk are the most commonly used prebiotics (Atuahene et al., 2024). Prebiotic supplementation at 1–3% of the daily diet has been associated with improved stool consistency, increased SCFA concentration and improved immune markers in healthy as well as gut-compromised dogs (Atuahene et al., 2024; Di Cerbo et al., 2017). Fructo-oligosaccharides (FOS) fiber molecules are fermented by *lactobacilli* and *Bifidobacterium* and result in enhanced SCFA production, improved stool quality and reduced colon pH, thus inhibiting pathogen growth (Atuahene et al., 2024). Psyllium husk is a bulk-forming fiber that increases faecal bulk and water content. Psyllium is a fermentable substrate and growth stimulant for health-promoting bacteria (Calvert, 2025; Di Cerbo et al., 2017).

Probiotics

Probiotics are live non-pathogenic microbes that induce health benefits to the host. In dogs, the most studied species are from genera such as Lactobacillus, Bifidobacterium, Enterococcus and Bacillus. Probiotic doses are generally 10^9 to 10^{11} CFU daily. Strain, dose and viability are characteristics that have impacts on the effectiveness of the probiotics (Atuahene et al., 2024). *Lactobacillus acidophilus* & *Enterococcus faecium* strains reduce the duration and severity of acute diarrhoea, normalize transit time and decrease the level of pro-inflammatory cytokines in faeces (Montserrat-Malagarriga et al., 2024). Probiotics, administered in adequate amounts, confer health benefits by inhibiting pathogenic adhesion, enhancing barrier function, and modulating the immune system (Dhuria and Kaur, 2024).

Synbiotics

Synbiotics combine probiotics (live beneficial microorganisms) and prebiotics (their fermentable food) in a single product, achieving a synergistic effect that enhances gut health more than when given alone. Normal doses of synbiotics are 10^8 to 10^{11} CFU probiotics with 1–3 g prebiotics daily (Montserrat-Malagarriga et al., 2024). The concept is that prebiotics encourage survival and colonization of the probiotic strain co-administered and both combined more effectively control microbial composition and metabolic activity. When a specific probiotic strain, such as *Lactobacillus rhamnosus* or *Bifidobacterium longum* is paired with a specific prebiotic substrate such as fructo-oligosaccharides or inulin, the prebiotic acts as a selective growth stimulator and aids in the survival, colonization and metabolic function of the probiotic upon reaching the colon (Di Cerbo et al., 2017; Atuahene et al., 2024).

Postbiotics and Fermented Foods

Postbiotics are microbial non-viable products or metabolic end-products like SCFAs (butyrate, acetate and propionate), bacterial cell wall fragments and peptides which have health-promoting properties (Salminen et al., 2021). Feeding postbiotic-rich fermented substrates (e.g., kefir, fermented soybean meal) to dogs yields a high concentration of postbiotics, e.g., lactic acid and bioactive peptides, without risk of live microbes (Cheng et al., 2024). Postbiotics in the diet can inhibit pathogenic bacteria through organic acids and bacteriocins, increase antioxidant capability, modulate immune responses, support mucosal repair, decrease colonic inflammation and promote brain health through the gut-brain axis (Salminen et al., 2021).

Phytonutrients

Phytonutrients, also known as phytochemicals, are plant compounds that have a role in general health including the gut health of dogs. These compounds include carotenoids, polyphenols and phytosterols, which aid in regulating gut function and microbial balance. Carotenoids like beta-carotene and lutein are responsible for reducing oxidative stress in intestinal tissue, thereby preserving cellular integrity and gut barrier function (Baritugo et al., 2023). Many of these phytonutrients are available in commercial nutraceutical formulations, including carotenoid supplements, polyphenol-rich herbal extracts and phytosterol-enriched plant oils that can be incorporated into canine diets.

Polyphenols, which are common in fruits, seeds and herbs, can modulate gut microbiota by positively influencing the growth of beneficial bacteria and inhibiting pathogens. Phytosterols in plant oils and nuts benefit gut health by lowering inflammation in the intestines, supporting immune responses, and supporting the formation of short-chain fatty acids (SCFAs) through microbial fermentation, which supports gut integrity and energy metabolism (Di Cerbo et al., 2017).

Antioxidants

Antioxidants play a crucial role in maintaining and promoting the gut health of dogs through the protection of gastrointestinal cells from oxidative damage and assisting with overall digestive function. Some of the most significant antioxidants in dogs include vitamin E, vitamin C, beta-carotene, selenium and polyphenols. All these compounds can neutralize free radicals and hence inhibit cellular damage and maintain a healthy gastrointestinal tract (Baritugo et al., 2023; Cheng et al., 2024). Supplementation with antioxidant blends, such as those containing vitamin E, vitamin C, and beta-carotene, has been shown to enhance immune status, reduce DNA injury and improve antioxidant status in dogs (Baritugo et al., 2023). Recent research also demonstrates the benefits of novel antioxidant compounds such as chito-oligosaccharides, which boost antioxidant enzyme activity and modulate the intestinal microbiota, promoting a balanced and healthy gastrointestinal flora (Cheng et al., 2024).

Digestive Enzymes

Digestive enzymes maintain gut health of the dogs since they break down proteins (protease), fats (lipase) and carbohydrates (amylase) into absorbable nutrients such as amino acids, fatty acids and sugars respectively. Commercially, digestive enzyme supplements are widely available as powders, tablets or as additives in premium dog foods, often formulated with multi-enzyme blends to maximize digestive efficiency. Additive enzymes are especially beneficial in dogs suffering from gastrointestinal disease, old animals or animals fed with processed food by promoting proper digestion, as they maximize nutrient absorption, firm up stool and aid in a healthy gut microbiome (Di Cerbo et al., 2017).

Future Research Prospects

Despite notable advancements, there remain substantial gaps in knowledge regarding dietary modulation of gut health in dogs. Future studies should include assessment of efficacy, dosage and duration of exposure to a strain-specific probiotic in dogs. More studies are required on postbiotics, particularly for their potential role in systemic immunity and the gut-brain axis signaling. The collaborative interactions among phytonutrients and antioxidants with microbiota also need exploration to provide evidence-based feeding recommendations. Beyond this, metabolomic and genomic data analysis may be utilized to discover microbial analysis for predicting of responsiveness to the diet. Such research shall offer a firmer scientific basis for the development of precision nutrition practices in canine gut health.

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Mini Review

The Critical Balance: Optimizing Omega-6 to Omega-3 Ratios for Canine Health

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Abstract

Maintaining a proper balance between omega-6 and omega-3 fatty acids is essential for canine health, influencing immune response, skin and coat condition, joint health, brain development, and digestive well-being. Diets high in omega-6 fatty acids can promote inflammation, while supplementing with omega-3s supports resilience and longevity. Ensuring a well-balanced ratio of these fatty acids through proper formulation and supplementation helps prevent chronic diseases and promotes vitality across all life stages in dogs. Practical strategies focusing on the selection of dietary fat sources, inclusion of antioxidant fortifiers such as vitamin E, and regional adaptation of feeding guidelines can enhance long-term health and nutritional resilience in canines. This article reviews the biological significance, ideal dietary ratios, health implications, and practical approaches to maintaining an optimal omega-6 to omega-3 balance in canine nutrition.

Keywords: *Dog nutrition, Joint support, Omega-6:3 balance, Skin and coat health, Vitality*

Fatty acids are essential components of a dog's diet, playing critical roles in maintaining cell membrane integrity, supporting immune function, modulating inflammation, promoting brain health, and maintaining a healthy coat. Among these, omega-6 and omega-3 fatty acids are of particular importance, as dogs cannot synthesize them endogenously and must obtain them through their diet (Burron et al., 2024; Lenox, 2016). The balance between these two families of fatty acids—commonly expressed as the omega-6 to omega-3 ratio—is especially significant for canine health. An imbalance, particularly an excessive intake of omega-6 relative to omega-3, has been associated with chronic inflammation, impaired immune function, and an increased risk of conditions such as arthritis, cancer, and cardiovascular disease (Wander et al., 1997).

The increasing prevalence of chronic inflammatory disorders, dermatological issues, and lifestyle-related diseases in dogs highlights the growing need to optimize dietary fatty acid composition. Modern commercial pet foods are often rich in omega-6 fatty acids but deficient in omega-3s, leading to nutritional imbalances that can compromise immune and metabolic health. This article is important because it integrates current research with practical feeding recommendations to help veterinarians, nutritionists, and pet owners achieve a healthier omega-6 to omega-3 ratio in canine diets, ultimately improving longevity and overall well-being.

The Biology of Omega-6 and Omega-3 Fatty Acids

Omega-6 and omega-3 fatty acids (FAs) are polyunsaturated fats that serve as precursors for bioactive lipid mediators such as eicosanoids, prostaglandins, and leukotrienes, which play key roles in regulating inflammation and immune responses (Burron et al., 2024).

- **Omega-6 Fatty Acids:** Linoleic acid (LA), the primary omega-6 fatty acid, is commonly found in vegetable oils such as corn, sunflower, and safflower oils. Arachidonic acid (AA), another important omega-6 fatty acid, is primarily derived from animal fats. These fatty acids play essential roles in promoting inflammation, which is necessary for wound healing and the body's response to infection. However, when consumed in excess, omega-6 fatty acids can contribute to chronic inflammation and negatively impact overall health (Lenox, 2016).
- **Omega-3 Fatty Acids:** Plant-based alpha-linolenic acid (ALA), found in flaxseed, camelina oil, chia seeds, and walnuts, along with marine-derived eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from sources like fish oil, exhibit anti-inflammatory properties. These fatty acids compete with omega-6 metabolites and inhibit the synthesis of pro-inflammatory eicosanoids (Bauer, 2007).

Metabolism of Omega-6(n-6) and Omega-3 (n-3) Fatty Acids in Dogs

In dogs, both omega-6 and omega-3 fatty acids undergo similar enzymatic processes in the body to form long-chain polyunsaturated fatty acids (PUFAs) that are biologically active. These steps involve desaturation (adding double bonds) and elongation (adding carbon atoms).

- **Omega-6 Pathway**
  - Linoleic acid (LA; 18:2 n-6) → γ-linolenic acid (GLA; 18:3 n-6) via Δ6-desaturase
  - GLA → Dihomo-γ-linolenic acid (DGLA; 20:3 n-6) via elongase
  - DGLA → Arachidonic acid (AA; 20:4 n-6) via Δ5-desaturase
  - Arachidonic acid serves as the precursor for pro-inflammatory eicosanoids (prostaglandins and leukotrienes).
- **Omega-3 Pathway**
  - Alpha-linolenic acid (ALA; 18:3 n-3) → Stearidonic acid (SDA; 18:4 n-3) via Δ6-desaturase

  - SDA → Eicosatetraenoic acid (ETA; 20:4 n-3) → Eicosapentaenoic acid (EPA; 20:5 n-3) via elongase and Δ5-desaturase
  - EPA → Docosahexaenoic acid (DHA; 22:6 n-3) through further elongation and desaturation steps.
- **Species Limitation**
  - Dogs have low Δ6-desaturase activity, which limits conversion of ALA (plant-based omega-3) to EPA and DHA.
  - Therefore, direct dietary sources of EPA and DHA (such as fish oil or algal oil) are necessary to achieve optimal physiological levels.
- **Functional Impact**
  - The balance between AA (n-6) and EPA/DHA (n-3) in cell membranes influences inflammatory response, immune modulation, and overall metabolic health in dogs.

The Ideal Omega-6:3 Ratio for Canines

The optimal dietary ratio of omega-6 to omega-3 fatty acids in dogs has been the subject of extensive research. The National Research Council (NRC) recommends a broad range from 2.6:1 to 26:1 (NRC, 2006), while the Association of American Feed Control Officials (AAFCO) sets a maximum allowable ratio of 30:1 (AAFCO, 2021). However, emerging evidence suggests that lower ratios—particularly those below 5:1—are more effective in reducing inflammation and supporting overall canine health (Wander et al., 1997).

- **High Ratios (10:1 and above):** Diets high in corn, soybean, or sunflower oils can exacerbate inflammation, impair immune function, and increase oxidative stress. Wander et al. (1997) showed a 31:1 ratio suppressed cell-mediated immunity and raised lipid peroxidation compared to 5.4:1 and 1.4:1 in aged Beagles. The seminal investigation by Wander et al. (1997) provided a critical framework for understanding the immunomodulatory effects associated with elevated omega-6: omega-3 ratios. Nevertheless, accumulating evidence from recent studies (Bobeck, 2020) suggests that breed-specific metabolic differences, baseline dietary composition, and the oxidative stability and source of dietary oils can substantially modulate these immunological responses. Although the original findings remain pivotal, their extrapolation to contemporary canine diets—characterized by extensive processing, variable ingredient quality, and prolonged storage—necessitates careful re-evaluation. Accordingly, the translation of controlled experimental ratios into practical feeding strategies demands validation across diverse breeds, diet formulations, and management conditions to ensure biological and clinical relevance.

- **Low Ratios (5:1 or lower):** Diets with balanced omega-3 inclusion have shown improvements in skin, joint comfort, and neurodevelopment (Roush et al., 2010; Lenox, 2016). Carlisle et al. (2024) reported that 70 mg/kg combined EPA+DHA improved the Omega-3 Index and reduced pain scores in small-to-medium breeds.
- **Role of Vitamin E Supplementation:** Diets with disproportionately high omega-6:omega-3 ratios tend to enhance lipid peroxidation and oxidative stress due to the higher content of polyunsaturated fatty acids susceptible to oxidation. Supplementation with vitamin E (α-tocopherol) has been shown to protect cell membranes by scavenging free radicals and stabilizing unsaturated fatty acids, thereby reducing the negative effects associated with excess omega-6 intake (Wander et al., 1997; Traber and Atkinson, 2007). Adequate inclusion of vitamin E—either through fortified diets or as an additive alongside omega-3 supplementation—can mitigate oxidative damage, improve immune response, and enhance the stability of dietary lipids during storage. In practical feeding systems, particularly under tropical conditions, combining vitamin E with omega-3 supplementation could be a cost-effective strategy to counteract oxidative stress and maintain fatty acid integrity.

Health Benefits of an Optimum Ratio of Omega-6 to Omega-3 Fatty Acids

1. Inflammation and Immune Regulation

Omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), exert potent immunomodulatory effects by integrating into cell membranes and modifying eicosanoid synthesis pathways. This incorporation shifts metabolic activity toward the production of less pro-inflammatory mediators, thereby attenuating chronic inflammation and promoting immune homeostasis (Lenox, 2016).

Clinical evidence demonstrates that dietary supplementation with fish oil, a rich source of EPA and DHA, can significantly reduce inflammatory biomarkers in the synovial fluid of osteoarthritic dogs, corresponding with reduced joint inflammation, pain relief, and improved mobility (Bauer, 2007). Similarly, a veterinary therapeutic diet enriched with fish-derived omega-3 fatty acids has been shown to enhance locomotor function and daily activity performance in dogs with naturally occurring osteoarthritis (Moreau et al., 2014).

2. Skin and Coat Health

Omega-6 fatty acids, particularly linoleic acid, are essential for preserving the integrity and function of the skin's lipid barrier, thereby supporting normal epidermal structure and hydration. In contrast, omega-3 fatty acids play a pivotal role in mitigating allergy-induced itching, inflammation, and scaling. Achieving an optimal dietary balance between these fatty acids has been shown to enhance coat glossiness and alleviate clinical signs of dermatitis, ultimately improving overall dermatologic health in canines (Lenox, 2016). However, the predominance of high omega-6 formulations in many commercial pet foods—driven largely by reliance on plant-based oils—may inadvertently contribute to chronic dermatological sensitivities and subclinical inflammation. Incorporating balanced levels of omega-3 fatty acids can help modulate inflammatory cytokine activity within the skin, restoring immune equilibrium. Future diet formulations should therefore emphasize precisely tailored omega-6:omega-3 blends, optimized for dermatological resilience—especially in breeds predisposed to allergic conditions or those living in polluted, humid, or high-temperature environments, where oxidative and inflammatory stressors are elevated.

3. Prevention and Control of Cancer

Excessive intake of omega-6 fatty acids has been associated with tumor promotion, whereas omega-3 fatty acids exhibit well-documented anti-inflammatory and anti-tumor properties. In canine oncology, lower circulating omega-3 concentrations have been reported in dogs with mammary tumors, while dietary omega-3 supplementation has been linked to prolonged survival times in cases of lymphoma (Tuzlu et al., 2021), suggesting a potential protective and adjunctive role for omega-3s in neoplastic disease management.

Evidence from both veterinary and human oncology indicates that omega-3 fatty acids inhibit tumor progression through modulation of COX and eicosanoid pathways, alterations in membrane signaling, and reduction of oxidative stress. However, variations in tumor type, dosage, and dietary composition limit the establishment of universal clinical protocols, positioning omega-3s as supportive adjuncts rather than standalone therapeutic agents.

In a pivotal study, Olgivie et al. (2000) demonstrated that dogs with lymphoma fed a diet enriched with menhaden fish oil and arginine showed longer survival and disease-free intervals compared to those receiving a soybean oil–based control diet.

4. Neurological Development

Docosahexaenoic acid (DHA) plays a pivotal role in the development and functional maturation of the brain and retina in puppies. Supplementation during pregnancy, lactation, and early postnatal life has been shown to significantly enhance learning ability, memory, and visual acuity in offspring (Araujo et al., 2022; Rodrigues et al., 2023). These findings underscore DHA's indispensable contribution to neurological and sensory development in canines. A diet enriched with omega-3 fatty acids has been shown to alleviate convulsive symptoms in dogs with epilepsy (Schlanger et al., 2002), while supplementation in drug-resistant cases resulted in an approximately 85% reduction in seizure frequency (Scorza et al., 2009).

The critical importance of DHA during gestation and early growth highlights the need for its adequate inclusion in the diets of breeding females and developing puppies. In many Indian feeding systems, however, both commercial and home-prepared diets are typically deficient in marine-derived ingredients, leading to suboptimal DHA intake during critical developmental periods. Recent studies (Rodrigues et al., 2023) reinforce DHA's benefits while emphasizing the influence of co-nutrients such as choline and vitamin A, as well as the necessity of protecting DHA from oxidative degradation during processing and storage.

To address these challenges, DHA-fortified feeds or algal-based DHA supplements, stabilized with natural antioxidants, should be prioritized during late gestation, lactation, and weaning. Such strategies can enhance neurodevelopmental, visual, and behavioral outcomes in offspring (Mota et al., 2024). The use of stabilized algal oil–derived DHA also provides a sustainable and oxidation-resistant alternative to fish oil, addressing both nutritional efficacy and environmental sustainability concerns (Mota et al., 2024).

Future studies should focus on evaluating the effects of maternal and neonatal DHA supplementation on neurobehavioral development, stress adaptation, and trainability in indigenous and crossbred dogs under Indian climatic conditions. Such research would provide valuable insights into species-specific fatty acid metabolism and support the development of regionally optimized feeding strategies to enhance neurological development and cognitive performance in canines

5. Cardiovascular and Renal Health

Omega-3 fatty acids play a critical role in maintaining cardiovascular and renal health in dogs. They have been shown to reduce the incidence of arrhythmias, preserve myocardial function in cases of congestive heart failure, and lower systemic blood pressure while decreasing proteinuria in dogs with renal disease (Bauer, 2007). These effects are largely attributed to the anti-inflammatory, anti-thrombotic, and membrane-stabilizing properties of EPA and DHA, which collectively support improved cardiac output and renal filtration efficiency.

To establish standardized omega-3 dosage protocols for cardiovascular and renal disease management, large-scale multicentric clinical trials under Indian conditions are warranted. Such studies would help determine the optimal EPA:DHA ratios, dosage ranges, and feeding durations required for consistent therapeutic efficacy across diverse breeds, dietary systems, and management environments.

6. Gut Health and Microbiome

A balanced omega-6:omega-3 ratio is increasingly recognized as a key factor in maintaining intestinal health and microbial equilibrium. Such balance supports a diverse and stable gut microbiota, mitigates intestinal permeability (“leaky gut”), and reduces the abundance of pro-inflammatory bacterial populations. Furthermore, omega-3 fatty acids may enhance short-chain fatty acid (SCFA) production, which in turn strengthens the gut epithelial barrier and promotes immune homeostasis (Fu et al., 2021).

Although mounting evidence supports a role for omega-3 fatty acids in modulating the gut microbiome, results are inconsistent. Some studies report increased SCFA production and greater microbial diversity after omega-3 supplementation, while others show variable or diet-dependent shifts. These discrepant outcomes likely reflect differences in baseline dietary fiber, host genetics, and omega-3 dose or duration of administration. Future nutritional approaches may benefit from combining omega-3 fatty acids with prebiotics or probiotics, offering synergistic benefits through concurrent modulation of microbial ecology and mucosal immune function.

Commercial and Practical Adoption

Despite extensive experimental validation of omega-6: omega-3 balance, commercial implementation remains limited. Most pet foods depend on low-cost plant oils such as corn, soybean, and sunflower oil, resulting in omega-6 dominance. In recent years, premium and therapeutic diets—especially those for dermatological, joint, or cardiac support—have incorporated fish, algal, or flaxseed oils (typically 4:1–10:1 ratios). This marks a gradual shift toward evidence-based formulation.

However, cost constraints, oxidation risk, and ingredient sourcing challenges continue to hinder widespread use. Under tropical conditions, temperature and humidity accelerate lipid degradation, making antioxidant protection and storage stability essential. Utilizing cold-pressed flaxseed, camelina, or mustard oils, fortified with natural antioxidants (vitamin E, rosemary extract), offers sustainable and affordable alternatives.

In regions such as India, where diets are mainly cereal- and vegetable oil–based, high omega-6 content predisposes dogs to chronic inflammation. Reformulating diets using marine or algal-derived omega-3 sources, validated through local field studies, can restore physiological balance. Technologies such as microencapsulation, algal-DHA incorporation, and blended lipid systems (ratios $<5:1$) improve both bioavailability and oxidative resistance.

Bridging the research-to-practice gap requires collaboration between veterinary nutritionists and pet food manufacturers. Together, they can develop regionally adapted, cost-effective fortified diets enriched with EPA and DHA—particularly for aging or large-breed dogs prone to osteoarthritis and inflammation—ensuring that scientific advances translate into tangible clinical outcomes.

Practical Food Tips to Obtain the Best Omega-6:3 Ratio

Achieving a desirable omega-6 to omega-3 ratio in canine diets requires informed ingredient selection and balanced supplementation. To assist in choosing appropriate fat sources, Table 1 summarizes the typical omega-6 : omega-3 ratios of common vegetable and marine oils used in dog diets.

Table 1. Approximate Omega-6 : Omega-3 Fatty Acid Ratios in Common Oils

Oil / Fat Source	Approximate n-6 : n-3 Ratio	Remarks / Nutritional Notes
Corn oil	46 – 60 : 1	Very high in omega-6; promotes inflammation if not balanced with omega-3.
Soybean oil	7 – 10 : 1	Moderate omega-3 content but still omega-6 dominant.
Sunflower oil	40 – 70 : 1	Extremely high in omega-6; should be minimized in canine diets.
Safflower oil	70 – 100 : 1	One of the richest omega-6 sources; lacks omega-3s.
Canola oil	2 – 3 : 1	Balanced profile; a good neutral oil option.
Flaxseed (linseed) oil	1 : 3 – 1 : 4	Rich in ALA (omega-3); limited conversion to EPA/DHA in dogs.
Camelina oil	2 – 3 : 1	Stable under warm conditions; emerging alternative to fish oil.
Mustard oil	5 – 6 : 1	Moderate omega-3 content; regionally available in India.
Olive oil	10 – 12 : 1	Low PUFA content; mainly monounsaturated.
Fish oil (salmon, sardine, anchovy)	1 : 10 – 1 : 20	Excellent omega-3 source (EPA + DHA); strongly anti-inflammatory.
Algal oil	1 : 10 – 1 : 15	Sustainable DHA source; suitable for vegetarian formulations.

Values are approximate ranges derived from compositional analyses of edible oils (NRC, 2006; Bauer, 2007; Lenox, 2016; Burron et al., 2024). Ratios may vary depending on source, processing method, and storage conditions.

Practical Recommendations

- **Limit** use of oils with very high n-6 (corn, sunflower, safflower).
- **Increase** EPA/DHA via fish or algal oil supplements where feasible (Lindqvist et al., 2023).
- **Select** commercial feeds containing fish oil, salmon meal, or flaxseed; avoid those relying primarily on corn/soy.
- **Supplementation**: 50–75 mg combined EPA+DHA per kg BW/day has been used to correct ratios in omega-6 dominant diets (Lenox, 2016).
- **Plant vs. Marine Sources**: While flaxseed supplies ALA, conversion to EPA/DHA is limited—marine or algal sources are preferable for therapeutic EPA/DHA (Burron et al., 2024).
- **Stability**: Use antioxidants (vitamin E, tocopherols) and good storage practices to reduce oxidation—critical in hot climates.

Author's Note: In India, blending flax/camelina/mustard oils with small amounts of fish or algal oil, plus antioxidant fortification, is a cost-effective strategy. Establishing guidelines for safe supplementation levels and stability standards for omega-3-rich oils under tropical storage conditions could enhance the reliability of practical feeding recommendations.

Future Scope of Research

Despite extensive evidence from controlled studies in Western countries, there is a pressing need to generate region-specific data under Indian and other developing-country conditions. Future research should focus on:

- Defining optimal omega-6:3 ratios for indigenous and crossbred dogs under varying climatic and dietary conditions.
- Assessing the stability and bioavailability of omega-3 supplements in tropical environments, where oxidation and storage issues are common.
- Exploring locally available, sustainable sources of omega-3 fatty acids—such as algal oil, camelina, or linseed—as cost-effective alternatives to imported fish oil.
- Investigating the long-term effects of omega-3 supplementation on reproductive performance, immune competence, and gut microbiota in canines.
- Extending comparative studies to livestock and companion animals to establish unified dietary guidelines tailored to Indian feeding practices.
- Examining antioxidant strategies (vitamin E, natural extracts) for improving shelf life and efficacy.

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Short Communication

Leveraging Indigenous Breeds and Smart Technologies for Sustainable Livestock Production in India

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Abstract

India's livestock sector is at a crossroads: it must meet rising demand for animal-sourced foods while reducing environmental impacts and improving rural livelihoods. Indigenous breeds of cattle, buffalo, sheep, and goats possess unique adaptive traits—thermotolerance, disease resilience, feed efficiency on low-quality forages—that are invaluable under climate change. Simultaneously, smart technologies (precision livestock farming, genomic selection tools, low-cost sensors, decision-support systems, and supply-chain traceability) can accelerate productivity gains while protecting biodiversity and welfare. This paper reviews the potential for synergizing indigenous breed strengths with appropriate smart technologies to create resilient, inclusive, and low-carbon livestock systems in India. We synthesize evidence on indigenous breed characteristics, outline smart technology applications feasible for smallholders, present integrative farm- and landscape-level models, discuss socio-economic and policy levers, and identify critical research and implementation gaps. Finally, we propose a scalable roadmap prioritizing community-led breeding, sensor-enabled management, data governance, and gender-equitable extension to mainstream these integrated solutions across agro-ecological zones.

Keywords: *Genomics, Indigenous breeds, Precision Livestock Farming, Resilience, Smallholders, Sustainability*

India's livestock sector contributes substantially to rural incomes, nutrition, and employment. With over 500 million large and small ruminants, the sector supports millions of smallholder households, particularly women (Government of India, 2019). However, productivity gaps, feed constraints, disease pressures, heat stress, and greenhouse gas emissions present major challenges (Rojas-Downing et al., 2017). Indigenous breeds, developed over centuries under local conditions, show traits such as thermotolerance, parasite resistance, and ability to utilize low-quality feed that confer resilience but are often undervalued in productivity-centric policies (Singh et al., 2022). Conversely, smart technologies offer tools for precision management, early disease detection, efficient breeding, and better market linkage (Halachmi and Guarino, 2016). Aligning indigenous breed strengths with targeted smart technologies creates an opportunity to enhance productivity without compromising adaptability, animal welfare, or biodiversity.

1. Indigenous Breeds of India: Assets and Challenges

1.1. Overview of adaptive traits

Indigenous breeds of cattle (e.g., Gir, Sahiwal, Red Sindhi), buffalo (e.g., Murrah, Jaffarabadi), sheep (e.g., Deccani, Nali), goats (e.g., Jamunapari, Osmanabadi), and poultry (Desi breeds) possess traits shaped by local selection: heat tolerance, tick and parasite resistance, efficient utilization of fibrous diets, disease resilience, and fertility under low-input systems (FAO, 2015).

1.2. Genetic diversity and conservation status

India's livestock genetic diversity is rich but fragmented. Many indigenous breeds face dilution from indiscriminate crossbreeding, loss of traditional management knowledge, and economic pressures favouring high-yielding exotic genetics (ICAR, 2013). Conservation of adaptive alleles requires both in situ (on-farm) and ex situ (gene banks) strategies, and incentives that make maintaining indigenous breeds economically viable.

1.3. Constraints to realizing productivity from indigenous breeds

Key constraints include limited organized breeding programs, poor recording and pedigree systems, inadequate nutrition and healthcare, market biases, and lack of value-added supply chains for indigenous-breed products (Thorpe et al., 2018).

2. Smart Technologies Relevant to Indian Smallholders

Smart technologies can be classified across domains: sensing & monitoring, genomics & breeding, decision support & data analytics, supply-chain traceability, and market & finance linkages. The emphasis here is on low-cost, resilient, and locally adaptable technologies (Neethirajan, 2020).

2.1. Sensing & Monitoring

Wearables and low-cost sensors (e.g., pedometers, accelerometers in collars or ear tags, temperature sensors) stream data on movement, rumination, feeding behaviour and body temperature. These feed into IoT networks (edge gateways, cloud) and analytics/AI modules which detect abnormalities such as estrus, illness, and reduced feeding earlier than manual methods (Darvesh et al., 2023).

In the Indian smallholder context (often indigenous breeds, small herd sizes), these allow continuous monitoring even when labour is constrained, enabling timely intervention and better resource use. Importantly, using AI/IoT means thresholds can be adapted to local breed baselines (rather than imported exotic-breed norms), and alerts can be delivered via mobile phones in rural settings with intermittent connectivity.

Weight proxies and photogrammetry use smartphone-based heart-girth estimation or computer-vision image analysis to infer body condition score or live weight when scales are unavailable. This is useful for indigenous breeds under variable feed regimes, enabling smallholders to monitor condition and adjust feeding/reproductive management without high cost.

Environmental sensors (temperature/humidity/THI loggers, soil-moisture probes, water-quality sensors) monitor micro-climate and fodder/water conditions. When integrated with animal sensor data (e.g., collars), the system can trigger alerts for heat stress, poor water quality or forage shortage very relevant for Indian smallholder systems reliant on local paddocks and indigenous breeds. The review by "Review on tech enabled precision dairy farming" highlights that IoT along with analytics can improve productivity, animal health and resource management in India.

2.2 Genomics and Breeding Tools

Genotype-based selection uses single-nucleotide polymorphism (SNP) panels and genomic estimated breeding values (GEBVs) to select for traits such as disease resistance, heat tolerance and feed-efficiency while retaining adaptive alleles (Hayes et al., 2013). Low-cost genotyping-by-sequencing (GBS) enables community or cooperative nucleus herds of smallholders to identify superior sires/dams from their own indigenous breed base, reducing dependence on exotic genetics and helping maintain adaptation to local conditions.

In India, studies on indigenous breeds have used ddRAD/GBS to identify large SNP numbers in breeds such as Sahiwal, and cross-bred evaluations in South India have used genomic evaluation models (Khan et al., 2025). For smallholders, this means they can progressively build locally-relevant genetic improvement programs, suited for indigenous breeds under the small-scale dairy farming context.

2.3 Decision Support Systems & Analytics

2.31 Offline-first mobile apps: These apps work on low-cost Android phones, require minimal connectivity, support ration formulation, disease checklists, reproductive calendars and welfare scoring in local languages. They help smallholders (including users of indigenous breeds) to access decision support even with literacy or connectivity constraints.

2.32. Machine learning (ML) models / analytics: ML algorithms (e.g., random forest, SVM, XGBoost, neural networks) have been used globally for tasks such as predicting mastitis risk, forecasting growth trajectories, feed conversion ratios (Tian et al., 2024). While specific models for Indian indigenous breeds are less numerous, analogous frameworks apply. For example:

- Random Forest and Decision Trees used in Indian collar + sensor pilot achieved ~91-92% accuracy for health status detection (Darvesh et al., 2023).
- A time-series based XGBoost model (though in China) reached AUC ~0.75 in mastitis risk prediction, indicating the applicability of ensemble methods in dairy health analytics (Guo et al., 2025).

For indigenous breeds and smallholder farms the key is to re-train models with local data (distinct behaviour, lower yields) and integrate the output into simple, actionable interfaces for farmers or cooperative extension agents.

2.4 Traceability & Market Linkages

QR-enabled traceability works as follows: each product (milk, ghee, etc.) carries a unique QR code (or GS1 standard QR/2D-barcode) linking to metadata such as farm/animal origin, collection date/time, processing batch, transport conditions and quality tests. When scanned by a consumer or stakeholder, the QR reveals the product's journey, enabling verification of authenticity and premium provenance (GS1 India) (Khanna et al., 2022).

In the Indian dairy smallholder / indigenous-breed context this has key benefits:

- Enables premium branding (e.g., "indigenous Sahiwal A2 milk from village cooperative") by providing verifiable origin and quality history.
- Improves transparency in the value-chain: smallholders can get recognition for quality/trace features, potentially securing better prices.

Enhances consumer trust and differentiation, important in competitive markets where adulteration/quality concerns are high.

Thus, QR-based traceability provides a tangible market linkage mechanism from smallholder farms (including indigenous breed producers) to premium product market segments.

3. Synergies: Combining Indigenous Genetics with Smart Technologies

Integrating the strengths of indigenous breeds in India with smart technologies requires careful design so that local farmer knowledge is augmented (not displaced), and technology is adapted to low-input, small-holder realities.

3.1 Community-led breeding enhanced with genomics

Community breeding programmes can maintain nucleus herds of superior indigenous animals, plus use low-cost genotyping to identify high-performing sires/dams, monitor inbreeding and feed performance data via mobile apps into genomic estimated breeding values (GEBVs).

In India, for example, BAIF's "Indigenous Breed Conservation and Genetic Improvement" strategy emphasises selection of superior animals from indigenous breeds and uses genomic/biotechnological tools (Bhagat, 2024). Also, National Dairy Research Institute (NDRI) has launched a genomic-selection programme for the indigenous Sahiwal breed aimed at smallholders and multi-breed herds in India (Arora, 2025).

3.2 Precision feeding for low-quality diets

Using sensor-driven bodyweight and activity data, combined with mobile decision-support systems (DSS), farmers can tailor supplementation strategies even when feed quality is low or variable. Smartphone-based DSS can suggest locally-available supplement mixes (crop residues, by-products) maximise nutrient density at minimal cost. This supports indigenous breeds (which are often reared under sparse-resource conditions) by maintaining body-condition and feed conversion efficiency.

Indian studies show major deficits in fodder quantity/quality and stress the need for feeding interventions: for example, a Haryana study found large deficits in concentrate feed and green fodder for dairy animals under Indian conditions (Verma et al., 2023).

3.3 Early disease detection and preventive health

Wearables and computer-vision-based monitoring tools can flag at-risk animals early e.g., collars with accelerometers/temperature sensors, video monitoring of behaviour changes. For indigenous breeds, early detection helps preserve their resilience advantage by preventing small illnesses from escalating into major losses. Moreover, community animal-health hubs equipped with rapid diagnostics and tele-veterinary services can integrate with sensor/AI alerts to enable timely intervention.

Indian work includes an IoT-based "smart neck collar" study conducted in seven districts of Punjab, which collected temperature, pulse and activity data from 150 dairy cows and used ML classifiers to detect anomalies. Another project at Indian Institute of Information Technology Allahabad (IIIT-A) is developing video-based monitoring for early detection of cattle diseases in India.

3.4 Market differentiation and value capture

Traceability systems (QR codes, blockchain) allow dairy products derived from indigenous breeds (e.g., A2 milk, grass-fed goat milk, heritage-breed ghee) to be credibly branded and marketed for premium price. Certification, storytelling (heritage breeds, climate-smart production) reinforce consumer willingness to pay more. For smallholder networks of indigenous-breed dairy farmers this offers a path to value-capture beyond mere volume.

To summarize, India's indigenous livestock breeds are resilient, climate-tolerant, and well suited to local conditions but remain underutilized. Smart technologies such as sensors, genomic tools, mobile apps, and traceability systems can unlock their full potential. Blending traditional knowledge with these modern tools can improve productivity, animal welfare, and income while conserving biodiversity. Community-led breeding, data-driven management, and digital market linkages can make indigenous livestock systems more efficient, inclusive, and sustainable for the future.

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Short Communication

From Microscopy to Molecular Tools: New Frontiers in Avian Coccidiosis Control

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Abstract

Coccidiosis is one of the most predominating devastating economical diseases of the poultry caused by the seven species of genus Eimeria belongs to phylum Apicomplexa. Among these Eimeria tenella, E. brunetti, and E. necatrix are the most pathogenic species responsible for clinical outbreaks. The routine diagnosis of the disease depends upon the presence of oocysts in the faecal samples and demonstration of the typical lesions on necropsy. These traditional methods lack ability to differentiate various Eimeria species. Molecular tools are widely used for the species confirmation and thus recommending specific control measures. Multidrug drug resistance against the anticoccidials is a global concern. Integrated approaches comprising of use of anticoccidials through shuttle and rotation programme, and alternatives to resistant drugs including application of phytochemicals, prebiotics, probiotics, vaccination and adoption of strict hygienic managemental practices can help to combat this issue.

Keywords: *Coccidiosis, Control measures, Diagnosis, Eimeria, Poultry*

Poultry farming is one of the fastest growing and most dynamic animal production sectors in India. It plays a key role in providing affordable animal protein, generating employment, and improving rural livelihoods. India is the second largest producer of eggs and ranks fifth in poultry meat production in the world, with total production of 142.77 billion eggs and 10.25 million tonnes of meat (BAHS, 2024). Of the many infectious diseases that pose a threat to poultry industry, coccidiosis is one of the most common gastrointestinal infections costing the world's economy ~ £104 billion per annum including India (Blake et al., 2020).

Previously documented report identified nine potential *Eimeria* species affecting the poultry (Chapman 2003), which later briefed into seven species namely *Eimeria tenella*, *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti, E. mitis*, and *E. praecox* that accounted for avian coccidiosis. Of these species, *E. tenella, E. brunetti,* and *E. necatrix* are highly pathogenic causing high morbidity and mortality at poultry farms. However, the other remaining species are mild to moderately pathogenic. The prevalence of avian coccidiosis in different Indian states ranged from 12.27- 81.03 percent (Bora et al., 2024).

Avian coccidiosis is influenced by several environmental, mangemental, and host-related risk factors (Singh et al., 2021). Warm, humid conditions and poor litter management promote the survival and spread of *Eimeria* oocysts. Young birds are more susceptible due to lack of immunity and nutritional deficiencies or stress can worsen disease severity. The continuous rearing of the birds in contaminated sheds and the development of resistance to anticoccidials have been documented for periodic outbreaks (Singh et al., 2021)

Biology: The transmission of *Eimeria* to the susceptible birds occurs via ingestion of the fully sporulated oocysts (infective stage), which develops from the unsporulated (non infective) oocysts shed in the faeces by the infected birds. This phase is called sporogony (exogenous) that occurs in 2-4 days depending on the environmental conditions (oxygen, temperature and relative humidity). Fully sporulated oocysts (four sporocysts each with two sporozoites) after ingestion first releases sporocysts by the mechanical action of the gizzard and then release sporozoites by the action of bile and enzymatic protein degradation in the intestine. After the ingestion, *Eimeria* undergoes complex seven days life cycle including both asexual (2- 3 generation of schizogony) and sexual stages (gametogony) within the epithelial cells of the intestine (Mehlhorn, 2015).

Pathogenesis and Clinical Signs: The severity of the disease depends on the *Eimeria* species involved, the level of infection, and the immune status of the host. Highly pathogenic species are *E. tenella* and *E. necatrix* as schizogonic phase occurs in the crypts of Lieberkühn and lamina propria of caeca and small intestine, respectively. This course of action results into extensive haemorrhages, bloody diarrhoea, anemia and mortality particularly in young chicks. However, the other species cause damage to the mucosal lining of the intestinal tract responsible for leakage of proteins and fluids that results into watery diarrhoea, dehydration, and stunted growth.

Diagnosis: The coccidiosis is routinely identified based on the presence of the oocysts in microscopic analysis of faecal droppings and gross lesion score monitoring (LSM) in case of death of the affected birds. The detailed criteria of different *Eimeria* species identification based on part of the gut infected, pathognomic lesions, shape and size index of the infective sporulated oocysts and pre-patent period (PPP) is given in (Table 1) (Quiroz-Castañeda and Dantán-González, 2015). These traditional methods are laborious, time consuming and prone to error in identification of different *Eimeria* species. This limitation is overcome by a computational software tool known as COCCIMORPH that aids in confirmation of *Eimeria* species based on uploaded pictures of the sporulated oocysts (Castanon et al. 2007). This computational tool was found insensitive due to its inability to differentiate *E. tenella, E brunetti,* and *E. praecox* oocysts (Kumar et al., 2014).

Table 1: Identification characteristics of different *Eimeria* species

Species	**Predilection Site**	**Pathogenicity & Gross Lesion**	**Shape & Size Index of Oocysts**	**Pre-patent Period (Days)**
E. tenella	Caecum	Highly pathogenic. Massive haemorrhages, ballooning of caeca due to bloody contents accumulation	14-31 µm by 9-25 µm	6
E. necatrix	Jejunum, ileum & caecum	Highly pathogenic. Ballooning of anterior and middle part of small intestine, gut lumen filled blood, mucosal lining of the intestine shows black and white foci giving salt and pepper appearance	Ovoid, 15-25 µm by 14-20 µm	6-7
E. brunetti	Caecum, rectum	Highly Pathogenic. Mucosal lining of the gut tissue shows pin pointed and erosion of the epithelial lining	Ovoid,14-34 µm by 12-26 µm	5
E. maxima	Jejunum, ileum	Moderate to highly pathogenic. Marked haemorrhages on the gut wall. Dropping initially mucoid watery later becomes bloody	Ovoid, 27-34 µm by 16-28 µm	5-6
E. acervulina	Duodenum, ileum	Mild pathogenic. Anterior part of the intestine shows red and white foci	Ovoid,12-23 µm by 9-17 µm	4
E. mitis	Ileum	Mild pathogenic. Malabsorption of the nutrients and vey mild enteritis	Sub spherical, 10-21 µm by 9-18 µm	4-5
E. praecox	Duodenum, jejunum	Very mildly pathogenic, Mucoid intestinal watery contents	20-25 µm by 16-20 µm	3-4

Over the decades, several molecular diagnostic approaches like polymerase chain reaction (PCR) with high sensitivity and specificity have been developed that enable to differentiate the mixed *Eimeria* species (Kumar et al., 2014). The conventional and nested PCR require post PCR gel electrophoresis. Real time PCR owes the ability to quantifying the parasite loads beside *Eimeria* species differentiation (Kundu et al., 2020). Next-Generation Sequencing (NGS) the new emerging tool has the tendency to detect the genetic mutation that is associated with anticoccidial resistance in addition to the diagnosis (Franzo et al., 2025).

Control Strategies

Effective control measures for coccidiosis are based on the integration of the chemotherapy, immune-prophylaxis and management approaches.

Chemoprophylaxis: The anticoccidials drugs belongs to two categories; coccidiostat (inhibit the multiplication and growth) and coccidiocidal (destroy the developmental stages). Broadly the anticoccidial drugs are classified as ionophorous compounds (ionophores) and synthetic drugs (chemicals). The ionophores contains lasalocid, monensin, narasin, maduramicin, nicarbazine, robenidine and synthetic drugs includes amprolium, clopidol, diclazuril, ethopabate, halofuginone and sulphonamides. Among these ionophores are widely used as prophylactic drugs. The emergence of a multidrug resistance is a major concern in poultry industry across the globe. To combat this issue, shuttle and bio-shuttle programme have been adopted. The shuttle programme is a strategy that uses different anticoccidial drugs in the starter, grower, and finisher feeds to prevent the development of resistance e.g. use a chemical anticoccidial in the starter feed and an ionophore in the finisher feed. A bio-shuttle program that combines a coccidiosis vaccine with the anticoccidial drugs in the feed for controlling coccidiosis.

Vaccination: Immunization against coccidiosis is a best alternative to medications to resolve the multidrug drug resistance concern in poultry. Since the launch of the first commercial live vaccine against the coccidiosis in America in 1952, several vaccines are marketed globally (Zaheer et al., 2022). Currently, three types of vaccines; attenuated, non-attenuated and recombinant are widely used under field conditions in other countries except India. Attenuated vaccines are those where oocysts have been attenuated (weakened) through selection for early life cycles or passage through hosts. Non attenuated are wild type vaccines that contain virulent *Eimeria* strains at low doses. Both attenuated and non-attenuated vaccines are not economical, reversion to the infection and their availability is limited. This demerit is ruled out by the production of the recombinant antigen based vaccines that are safer and highly immunogenic and induce the specific immunity. Many recombinant antigens of *Eimeria* species have been recognized as vaccine candidate, but no any antigen yet commercialized (William, 2002). The only commercial subunit vaccine prepared from the purified protein isolated from the gametocyte of *E. maxima* is available as CoxAbic®. Immunization induces the immunity within 3-4 weeks depending upon the host genetics, time and frequency of the infection and parasitic load. Vaccination practice is not very common in India because of administration method and there is no surety that all birds in the flock are immunized with the same dose of the coccidia. To overcome this limitation "*in ovo*" immunization approach that permits to inoculate the pathogen into 18-day embryonated chicken eggs. By this technique specific and consistent administration of the vaccine to the amniotic cavity of the chick embryo is being induced (Lee et al., 2022). But now-a-days coccidiosis vaccines are gaining momentum in India. The commercially available vaccines in India are Stallen's Livacox Q by (Hester Biosciences Ltd), IMMUCOX®3 / IMMUCOX®5 (Ceva poultry pharma Ltd.) and Evant® (HIPRA India Pvt Ltd.). These vaccines contain 3-5 *Eimeria* species for use in broilers, breeder and layers birds.

Mangemental Strategies

a. **Biosecurity & Hygiene:** Clean and disinfect the sheds by adopting all-in/all-out management system. Remove litter completely and thoroughly clean floors, walls, and equipment with detergents and disinfectants like sodium hypochlorite solution, formaldehyde and ammonium hydroxide which are effective against oocysts. Avoid introducing contaminated equipment or footwear into the shed to prevent mechanical transmission of the oocysts. Prevent access of wild birds, rodents, and insects to prevent the mechanical transmission of the oocysts.

b. **Litter Management:** Keep litter dry as wet litter promotes sporulation of oocysts. Ensure that ventilation systems operate efficiently to sustain optimal air flow and to keep relative humidity below 30%, thereby reducing the likelihood of pathogen proliferation. Prevent overcrowding by adhering to recommended stocking densities (for broilers, approximately ~0.07–0.09 m^2/bird) which helps limit litter moisture accumulation and stress-related susceptibility to disease. Implement routine removal and replacement of litter to further minimize environmental contamination and interrupt the coccidian life cycle.

c. **Nutritional & Supportive Management:** Provide balanced feed with adequate vitamin A, E, K, selenium, and methionine as it improves the gut health and immune response. Add probiotics, prebiotics, and phytogenic additives (e.g., oregano, garlic, turmeric) to help modulate gut flora. Provide electrolytes and vitamins in water during outbreaks to reduce stress (Ahmad et al., 2024).

d. **Use of Phytochemicals:** The bioactive compounds derived from plants such as saponins, tannins, flavonoids, alkaloids, and essential oils proved their efficacy as an alternative to synthetic anticoccidial drugs, which face challenges like drug resistance and residue concerns. The effect of various phytochemical/herbal medicine, their extracts, and bioactive molecules showed anticoccidial properties on different *Eimeria* species of poultry (El-Shell et al., 2022). Commonly studied herbs are neem (*Azadirachta indica*), turmeric (*Curcuma longa*), garlic (*Allium sativum*), oregano (*Origanum vulgare*), wormwood (*Artemisia annua*), green tea (*Camellia sinensis*), and black cumin (*Nigella sativa*). Their extracts work by inhibiting oocyst sporulation and parasite development, stimulating the immune system, reducing oxidative stress, and protecting intestinal tissues.

Probiotics: Probiotics are live beneficial microorganisms (*Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Enterococcus* spp.) that improve the gut health and enhance the bird's immune defense against *Eimeria* infections. These probiotics compete with other pathogens for nutrients and binding sites, produce the substances that possess antimicrobial activity, stimulate gut immunity, enhance the intestinal barrier and feed conversion efficiency of the birds. Studies have shown that probiotic supplementation can reduce oocyst shedding, decrease intestinal lesion scores, and improve growth performance and feed efficiency in infected chickens (Ahmad et al., 2024).

Prebiotics: Prebiotics like mannan-oligosaccharides, fructo-oligosaccharides, inulin, β-glucans stimulate gut friendly microbiota such as *Lactobacillus* and *Bifidobacterium* which reduce the growth of *Eimeria*. Prebiotics can reduce lesion severity, lower oocyst shedding, and improve performance (growth and feed efficiency), especially when used alongside vaccines or probiotics (El-Shall et al., 2024).

Future Research Perspectives on Avian Coccidiosis

Genomic tools and molecular diagnostics will support early detection, strain monitoring, and selection of genetically resistant poultry lines. Natural alternatives such as probiotics, phytochemicals, essential oils, and immune modulators are expected to replace conventional anticoccidials. Precision farming technologies, AI-assisted monitoring, improved litter management, and strong biosecurity will also reduce environmental oocyst load. Overall, the future emphasizes integrated, data-driven approaches combining vaccination, nutrition, genetics, and management for long-term and sustainable coccidiosis control.

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Short Communication

Cryptosporidium and *Giardia* infections: Zoonotic implications for Dairy Farm Management

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Abstract

Cryptosporidium and Giardia are two major zoonotic enteric protozoans that affect domestic animals in the tropics and subtropics parts of the globe and lead to massive economic losses to the livestock owners. These parasitic infections are transmitted through contaminated food and water to animals and humans. In dairy cattle farms, they act as major reservoirs for waterborne outbreaks in the livestock and increase the societal healthcare costs, potential litigation and reputational damage to the livestock and agricultural sector. Microscopic examination of faecal smears specifically stained with Ziehl-Neelsenand Lugol's Iodine stain as a routine diagnostic approach for screening of Cryptosporidium oocyst and Giardia cyst, while Competitive ELISA offers a more sensitive option. For zoonotic subtypes and assemblages, amplification of SSU rRNA and gp60 gene for Cryptosporidium species and β-giardin gene for Giardia species distinguishes animal infective subtypes from humans. Control of zoonotic parasitic diseases is a multidisciplinary approach involving the integration of chemotherapy, grazing, adapting effective management strategies, targeting therapeutic interventions and proper biological control measures which is important to mitigate these pervasive economic and public health risks.

Keywords: *Cattle, Cryptosporidium, Economic loss, Giardia, Parasite, Zoonosis*

In India, there are about 193.46 million of cattle and ranks first in the world in terms of cattle population with significant contribution in the overall economic development of our nation. Over 65% population of Indian resid sector, it is the main sources of livelihood especially in rural areas, where agriculture is the primary source of livelihood, the cattle sector plays crucial role in sustaining rural economies (Gautam et al., 2007).

Cryptosporidium and *Giardia* are common intestinal protozoan parasites impacting the health and productivity of dairy calves worldwide, including India. Among a variety of infectious agents that cause diarrhoea in calves, *Cryptosporidium parvum* and *Giardia duodenal is* are particularly prevalent in India causing acute symptoms affecting growth and production with severe economical losses to the livestock sector and dairy cattle farmers. Both parasites are recognised as important disease of zoonotic disease transmission which is a significant public health concern and impairs the development and socio-economic improvements, and they have a common link with poverty (Santin et al*.,* 2020)

Pathogenesis and Clinical Signs

The calves of age 5 to 20 days are more susceptible as compared to older animals for cryptosporidial infection. Whereas, calves aged 1to 5 months are commonly infected with giardiasis. Transmission of both parasites is quite challenging as they readily contaminate the environment with 10^{10} total oocysts/cyst output per infected calf can over a week. The common route of transmission in the dairy cattle calves is through direct contact with the feces of infected animals (oocyst and cyst) or indirectly from contaminated feed, water and soil especially among calves kept under intensive management system. The newly born calves can also be infected by transmission of oocysts/cysts around the time of calving (periparturient period) with the species of *Cryptosporidium parvum* (IIa subtype) and *Giardia duodenalis* (assemblage A) which are of zoonotic importance (Alves et al., 2001; Robertson et al, 2014). A comparative summary on the various aspects of the pathogenesis on *Cryptosporidum and Giadia* have been discussed in Table 1.

Diagnosis

Microscopy coupled with differential staining techniques is considered as a conventional method for detection of oocysts of *Cryptosporidium* by modified Ziehl-Neelsen staining technique (mZN) (Henricksen and Pohlenz, 1981) and simple iodine wet mount method for *Giardia* identification (Soulsby, 1982). Apart from simple microscopic examination, other sensitive diagnostic technique included direct immunofluorescent antibody test (IFAT) and molecular tools like PCR assays and PCR-RFLP have the potential of addressing many of the limitations of traditional methods and are frequently introduced in many of previous studies for genus or species specific detection of *Cryptosporidium* and *Giardia* species at large scale sample analysis. Polymerase chain reaction (PCR) is more sensitive and in particular allow species identification and subtyping of *C. parvum* and identification of assemblages of *Giardia duodenalis* will help to differentiate the zoonotic subtype (Louro et al., 2025). Recently, quantitative PCR have been established for quantification of many genetic targets as compared with classical PCR assays. Whereas, droplet digital PCR (ddPCR) gives a higher precision in quantitation of *Cryptosporidium* DNA based on two loci (18S rRNA and actin) in the faecal samples of cattle, goat and humans. By use of different primers and probe sets, we can identify the infections through multiplex real time PCR protocols. In order to differentiate six distinct regions of target genes, loop mediated isothermal amplification (LAMP) which is highly sensitive, simple and cost effective method for detection of *Cryptosporidium* and *Giardia* infection in calves.

Table 1 Comparative summary on pathogenesis of *Cryptosporidum and Giadia*

Features	***Cryptosporidum***	***Giardia***
Age	Calves (1-4 weeks old)	Calves (1-6 months old)
Pathogenesis	Invades epithelial cells, villous atrophy with shortening of intestinal microvilli and inflammation which leads to malabsorption.	Cyst attaches to intestinal mucosa via a sucking disc, causing enterocyte apoptosis, barrier dysfunction and reduced enzyme activity causing maldigestion and malabsorption.
Site of attachment	Intracellular but extracytoplasmic (within the epithelial cells of the intestine, tips of microvilli)	Extracellular (attaches to the surface of host enterocytes using a ventral adhesive disk)
Oocyst/Cyst	Oocysts (thin walled and tick walled) are of2-4µm size containing 4 naked sporozoites. Thick walled oocysts excreted in faeces while thin walled rupture within the host cause auto-infection	Cysts (7-10 µm) having an axostyle at middle and 4 nuclei which are shed in faeces. No auto-infection.
Clinical Signs	Profuse watery diarrhoea, dehydration, weight loss, lethargy, inappetence, abdominal pain and potential death in severe cases.	Asymptomatic, intermittent steatorrhea diarrhoea, abdominal pain, poor growth and reduced production.
Faecal Consistency	Pasty to watery, mucoid, sticky and foul-smelling diarrhoea.	Watery and less mucoid
Duration	Self-limiting in healthy calves, lasting a few days to two weeks.	Chronic and infection last for several months.

Genotypes of Zoonotic Implications

There are more than 44 species and 120 genotypes of *Cryptosporidium* recognized globally, *C. hominis* and *C. parvum* are the main species affecting humans (Ryan et al., 2021). The *C. parvum* species is of primary concern, as it is a leading cause of human cryptosporidiosis globally and cattle are considered its main reservoir. The zoonotic Apicomplexan *C. parvum* is considered the most common enteropathogen of neonatal calves. The most common subtyping gene are 18 *SSU rRNA*andgp-60 subtype, which are used for the identification and differentiation of geographic and temporal differences in the transmission of *Cryptosporidium* species which is important for public health significance. Among the subtypes of *C. parvum* (IIb, IIc, IIe – Iii), IIa and IId are found in both humans and ruminants which are responsible for causing zoonotic cryptosporidiosis. Among all subtype families of *C. parvum*, IIa (especially IIaA15G2R1) is considered the most prevalent in cattle (Imre et al., 2011).

Giardia duodenalis is a complex of seven distinct genotypic assemblages (A to G) that can only be distinguished by PCR amplification and sequencing of appropriate genes. Assemblages A and B are diagnosed in humans, dogs, cats, livestock and also wild mammals (Feng and Xiao, 2011). However, some human-associated assemblages (A and B) have been detected in cattle, suggesting the potential for some cross-species transmission, but it is less frequent than *C. parvum*. Thus, molecular techniques have now been used as a sensitive tool particularly to allow the identification of species, subtypes and assemblages of these zoonotic pathogens.

Treatment and Control

The effective control, treatment and management of *Cryptosporidium* and *Giardia* infections emphasis on antiparasitic therapy, supportive care and prevention strategies.

Cryptosporidiosis: Many chemotherapeutic agents have been tested against cryptosporidiosis but few drugs have shown the promising results. The anticoccidials agents (Sulphonamides, amprolium, toltrazuril) have been used against cryptosporidiosis in dairy farms. The poor response to many chemotherapeutic agents has been explained by the parasite's unique intracellular location, which may serve as "escape mechanism" to protect the parasite from anticryptosporidial drugs. A limited number of chemotherapeutic agents (azithromycin, nitazoxanide, paromomycin, macrolide, spiramycin, metranidazole and refaxmin) have demonstrated efficacy in animal models and under clinical trials. The current optimal therapy for cryptosporidiosis is azithromycin and is the most common drug for treating cryptosporidiosis in humans and animals. Therapeutic efficacy of azithromycin in naturally infected cattle calves from Jammu region showed the maximum reduction in number of oocysts per gram of faeces and increase in body weight gain (Ganai et al., 2017). A report from Ludhiana, Punjab described the successful use of a combination therapy of metronidazole and furazolidone (along with supportive care) to treat a cryptosporidial outbreak in calves, leading to clinical and parasitological recovery (Randhawa et al., 2012). Although, scientific interest on natural products with anti cryptosporidial properties is increasing in recent years; traditional system of medicine having anti-inflammatory, antiproliferative and antioxidant properties have shown promising anti cryptosporidial activity in *in vivo* and *in vitro* studies such as turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), garlic (*Allium sativum)*, mint (*Mentha spicata*), chamomile (*Chamaemelum nobile*) and pomegramnate (*Punica granatum*) peel extracts. Immunodominant *Cryptosporidium* antigens identified from natural infection, some of the promising vaccine have been developed and tested in experimental conditions against cryptosporidiosis from MSD Animal Health (Bovilis Cryptium®) which was recently approved in Great Britain, a widely available and fully effective commercial vaccine but has not yet marketed in India (Obiad et al., 2012).

Giardiasis: The use of chemotherapeutic agents against giardiasis in calves enhances the performance, reduce clinical signs and prevent environmental contamination. One of the effective and beneficial drug is metronidazole which helps in eliminating *Giardia* infection in calves by improving the mucosal microvillus structure and function within seven days of initiating treatment. Various herbal extracts have shown the significant anti giardial activity in experimental animals and in vitro studies. The various extracts showing reduced drug resistance and side effects includes: Garlic (*Allium sativum*), ginger (*Zingiber officinale*), cinnamon (*Cinnamomum zeylanicum*), Wormwood (*Artemisia annua*) and Pippali Rasayana (Ayurvedic formulation). Giardia Vax® is a commercially available vaccine licensed for use in dogs and cats in USA and it is commercialized to significantly reduce the incidence, severity, and duration of cyst shedding. The vaccine consists of a crude preparation of disrupted, axenically cultured *G. intestinalis* trophozoites (sheep isolate). The fully effective vaccine against giardiasis in bovines has not been yet published and marketed in India.

This article emphasised latest diagnostic techniques which help in detection of zoonotic *Cryptosporidium* and *Giardia* subtypes/assemblages in the infected host. In Indian cattle dairy farms, the diagnosis of the infections which are the common cause of diarrhoea in calves relies on conventional techniques rather than genotyping confirmation of zoonotic strains via molecular characterization of the infection. Therefore, by adopting latest diagnostic tools, broad molecular surveillance, and biosecurity measures and involvement of modern drug delivery system may help in reducing environmental contamination and zoonotic transmission of parasitic infections in dairy cattle farms.

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Short Communication

Argas persicus: A Menace to the Indian Poultry Industry

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Abstract

Argas persicus, commonly called the fowl tick or poultry soft tick, is a significant ectoparasite of domestic and wild birds, especially in tropical and subtropical areas. Its nocturnal, intermittent feeding habits and ability to survive lengthy periods without a blood meal make it highly resilient in poultry environments. A. persicus is of notable veterinary concern because of its role in transmitting important pathogens, including Borrelia anserina (the cause of avian spirochetosis), and its capacity to cause high morbidity and mortality in affected poultry. Heavy infestations can lead to considerable economic losses, particularly in backyard and smallholder poultry operations. Management of A. persicus requires a combination of acaricidal treatments, habitat adjustments, improved hygiene in poultry houses, and the elimination of crevices that shelter off-host stages. The emergence of acaricide resistance in many tick species and the limited availability of effective alternatives emphasise the importance of integrated, sustainable tick management strategies. Improved epidemiological monitoring and region-specific control plans are essential for reducing the impact of A. persicus on poultry health and productivity.

Keywords: *Argas persicus, Ectoparasite, Indian scenario, Menace, Poultry Industry.*

Argas persicus, commonly known as the fowl tick or poultry tick, is one of the most widely distributed soft ticks affecting domestic and wild birds across tropical and subtropical regions. As a member of the family Argasidae, *A. persicus* differs biologically and behaviourally from hard ticks, exhibiting nocturnal feeding, multi-host parasitism, and rapid engorgement (Zahid et al., 2021). Backyard poultry rearing is a deeply embedded part of rural livelihoods in India, especially among small and marginal farming households. India's poultry sector is rapidly expanding due to rising demand for eggs and broiler meat, driven by commercialization and improved genetics. It remains a major contributor to the livestock economy while also supporting rural livelihoods through backyard poultry. The poultry tick thus represents a significant ectoparasitic challenge with direct implications for animal health, productivity, and economic sustainability. Its ability to transmit important avian pathogens increases its veterinary importance.

The prevalence *A. persicus* in India varies greatly, with sporadic reports from various regions, including Punjab (Kour et al., 2024; Sumbria et al., 2024). In some areas like Telangana, prevalence has been reported to reach up to 35%. This review provides an expanded examination of the biology, pathogenicity, epidemiology, diagnosis, and control of *A. persicus,* highlighting its growing relevance in the Indian context.

Biology and Life Cycle

The biology of *A. persicus* is closely connected to its life cycle and behaviour. The tick has a multi-stage life cycle that includes egg, larva, several nymphal stages, and adult. The ticks are characterized by their compact, flattened, oval bodies and a distinctive bluish-grey to blue-black coloration. The dorsal surface of adult ticks appears leathery with a finely granular texture. They have pulvilli and claws on their legs. Females exhibit a clearly horizontal genital aperture, while males display a semi-circular one. The larval stage is marked by three pairs of legs, well-developed palpi, and a hypostome. *A. persicus* typically attaches to soft, concealed, and less-feathered regions of poultry, including the axilla, neck and breast folds, vent area, head, comb, wattles, and other skin folds. Unlike hard ticks, which stay attached to the host for long feeding periods, *A. persicus* feeds briefly—often 15 to 60 minutes—mainly at night and is therefore categorised as an intermittent feeder. After feeding, the ticks hide in dark, protected cracks in poultry sheds. This behaviour makes detection difficult and helps maintain infestations. Larvae are the only stage that stays attached to the host for several days, while nymphs and adults finish feeding quickly owing to their intermittent feeding habits. But they repeatedly feed on their hosts. The adult females lay their eggs in cracks, crevices, or litter, which continually contaminates the environment. High fecundity and the ability of off-host stages to survive for long periods—sometimes several months—allow *A. persicus* populations to persist even when feeding opportunities are infrequent (Zahid et al., 2021). Climatic factors such as warmth, moderate humidity, and the availability of shelters strongly influence tick proliferation. India's tropical climate, combined with the prevalence of traditional poultry housing structures, creates ideal conditions for the life cycle progression of *A. persicus*, making it a recurring problem in rural settings.

Pathogenicity and Veterinary Importance

The pathogenic potential of *A. persicus* mainly arises from its repeated blood-feeding behaviour and the resulting cumulative blood loss in birds. Chickens, turkeys, ducks, and other domestic fowl show different levels of susceptibility, with young chicks being especially vulnerable. Chronic infestations can cause anaemia, weight loss, decreased egg production, reduced fertility, and weakened immune responses (Zahid et al., 2021). . Birds may exhibit pale combs, lethargy, and restlessness, particularly at night when ticks are actively feeding.

A. persicus is also associated with tick paralysis in poultry, and it ends fatally if neglected or left untreated for an extended period of time. Beyond direct blood loss, *A.*

persicus also serves as an effective vector for several bird pathogens. The most notable is *Borrelia anserina*, which causes avian spirochaetosis, a disease marked by fever, depression, greenish diarrhoea, and high mortality rates. Additionally, the tick transmits *Aegyptianella pullorum* and various Rickettsia-like organisms. Differential diagnosis of *A. persicus* includes other poultry-infesting ticks, as well as mites such as *Dermanyssus gallinae* and *Ornithonyssus sylviarum*. Lice infestations and allergic dermatitis may also produce similar clinical signs. The combined negative effects of tick infestation and related haemoparasitic diseases can lead to significant production losses, especially in smallholder poultry systems.

Epidemiology and Importance in the Indian Context

In India, *A. persicus* is common in areas where rural poultry farming is widespread. States such as Andhra Pradesh, Odisha, West Bengal, Tamil Nadu, Uttar Pradesh, Punjab, and several northeastern states often report the presence of the fowl tick, especially in villages with traditional poultry shelters (Kour et al., 2024; Sumbria et al., 2024). Mud-walled or thatched structures with many cracks, wooden perches, and poor hygiene provide ideal microhabitats for the tick. Backyard poultry in India is expanding through government programs like the National Livestock Mission (NLM), Rashtriya Krishi Vikas Yojana (RKVY), and various state-level livelihood initiatives. While these efforts greatly improve household income and nutritional security, increases the susceptibility of hosts and the risk of ectoparasite transmission. Limited awareness among poultry farmers about tick control, lack of routine cleaning, and limited access to veterinary services further increases the challenge.

Studies in India have frequently associated outbreaks of avian spirochaetosis with the presence of *A. persicus*, especially after the monsoon season when humidity favours tick survival. The economic impact is substantial, as many rural families rely on poultry for eggs, meat, and additional income. Severe *A. persicus* infestation leads to significant production losses in poultry due to anemia, reduced egg output, poor growth, tick paralysis and increased mortality. It also imposes an economic burden through treatment costs, reduced flock performance, and potential disease transmission. Therefore, *A. persicus is* not just an ectoparasite but also a barrier to rural development and livelihood resilience.

Diagnosis

Diagnosis of *A. persicus* infestation primarily relies on inspection of poultry housing structures. During the day, ticks hide in crevices, so it is crucial to carefully examine walls, perches, roosting materials, and nearby shelters. Birds may show signs of anaemia, decreased egg production, or overall poor health, warranting further investigation. Microscopic identification of the ticks found confirms the species. *A. persicus* are small, flat, oval-shaped, with a distinctive bluish-grey to blue-black colouration, giving rise to the name "blue tick." Unfed larvae possess three pairs of legs with well-developed palpi and a hypostome, while adults exhibit a leathery, granular dorsal surface along with pulvilli and claws. Females and males display characteristic horizontal and semi-circular genital apertures, respectively, and adults are broader posteriorly, with females showing notable maternal behaviour toward their larvae (Kour et al., 2024).When avian spirochaetosis is suspected; Giemsa-stained blood smears can detect *Borrelia anserina* spirochetes, which appear as slender, spiral-shaped organisms (usually 8–20 µm long) with deep purple to blue coloration. They often exhibit wavy, helical curves and may be seen extracellularly among red blood cells. and Other diagnostic tools, such as dark-field microscopy, PCR, serological assays like ELISA/IFA, and, less commonly, culture in specialised media have improved the detection of associated pathogens.

Control and Management

Control of *A. persicus* requires a multifaceted approach targeting both the host and the environment. Sealing cracks, replacing wooden perches with metal or PVC alternatives, and maintaining clean floors significantly reduce tick hiding places. Regular sweeping, litter removal, cleaning cracks and crevices, promoting a cage system over deep litter, and whitewashing walls with lime are measures widely practised in rural India. Chemical control measures involve using acaricides like pyrethroids or organophosphates around the premises. These chemicals are sprayed carefully to target crevices where ticks hide. Topical treatment of birds is less effective because ticks feed for a short time, but it can offer limited relief in severe cases. Phytochemicals such as neem oil are popular because they are safe, affordable, and locally available. Neem-based emulsions or sprays applied to shelters can significantly lower tick populations. There are reports of using Neem and Babul extracts against *A. persicus* with good results. Alongside tick control, vaccination against avian spirochaetosis can be implemented in high-risk areas. However, in India, access to these vaccines is limited, and tick control remains the main approach. Farmer awareness programs and community-based control efforts are essential for reducing infestation rates at the village level.

Future Research Prospects

Despite its increasing recognition as an emerging ectoparasite of poultry, significant research gaps about *A. persicus* remain in India. Current prevalence data are patchy and mostly region-specific, emphasizing the need for nationwide epidemiological surveys to determine its true distribution. Studies on genetic diversity and transmission dynamics are scarce, and systematic monitoring of acaricide susceptibility is lacking, despite growing concerns about resistance. Additionally, the vector potential of *A. persicus* for avian pathogens like *B. anserina* has not been thoroughly studied under Indian conditions. Addressing these gaps through integrated research will help develop effective and sustainable control strategies for both backyard and commercial poultry systems.

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Mini Review

Recent Advances in the Chemotherapeutic Strategies for Canine Lymphoma

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Abstract

Canine lymphoma remains the most common hematopoietic malignancy in dogs. Although multi-agent such as Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone (CHOP) based protocols continue to deliver complete remission rates exceeding 80%, long-term survival has improved only marginally over the past twenty years, with T-cell phenotypes consistently a laggard when compared to B-cell disease. This review summarizes current prognostic stratification, recent refinements in CHOP protocol delivery, the growing role of lomustine-based strategies for high-risk and T-cell lymphoma, Anatomic variants with distinct behaviour, and drug repurposing and novel therapeutics in the pipeline, concluding with practical recommendations for contemporary clinical practice.

Keywords*: Canine lymphoma, Chemotherapy Drug repurposing, Lomustine, T-cell lymphoma, Veterinary oncology,*

Lymphoma represents 7–24% of all canine tumors and approximately a vast majority of all hematopoietic malignancies (Bennett et al., 2023). The disease is remarkably heterogeneous, manifesting as multicentric, mediastinal, alimentary, or extranodal forms, and, more importantly, as B-cell or T-cell immunophenotypes with markedly different biological behaviour (Bennett et al., 2023; Lai et al., 2024). Since the widespread adoption of cyclophosphamide–doxorubicin–vincristine–prednisone (CHOP) protocols in the 1990s, initial response rates were excellent and very consistent across institutions (Lai et al., 2024). In spite of that, the median overall survival in unselected populations has remained between 10 and 14 months, highlighting the urgent need for more effective strategies, particularly for dogs with T-cell lymphoma (Einhorn et al., 2024; Lai et al., 2024).

Biological Behavious and Classification of Lymphoma:

Lymphoma classification mainly divides cancers into Hodgkin Lymphoma (HL) and Non-Hodgkin Lymphoma (NHL), with NHL and further divided by cell type (B-cell, T-cell) and growth rate (indolent, aggressive, very aggressive). Key subtypes include aggressive DLBCL, slow-growing Follicular Lymphoma (FL), and aggressive Burkitt

Lymphoma, all classified based on cell origin (B/T), appearance (Reed-Sternberg cells for HL), and molecular features, guiding treatment (Jamil & Mukkamalla, 2025).

Prognostic Factors

Immunophenotype, reliably determined by flow cytometry or PCR for antigen receptor rearrangements (PARR) at diagnosis, shows the most powerful prognostic influence (Limmer et al., 2022; Einhorn et al., 2024). Dogs with B-cell lymphoma routinely achieve median survival times of 12–18 months with standard therapy, whereas those with T-cell lymphoma typically survive only 6–8 months (Limmer et al., 2022; Bennett et al., 2023; Einhorn et al., 2024). Additional well-validated prognostic markers with poorer outcomes include WHO substage b (systemic signs), anemia, thrombocytopenia, hypercalcemia, cranial mediastinal involvement, and advanced clinical stage (Bennett et al., 2023). Incorporation of these variables into pretreatment counseling allows for more accurate prognosis and tailored therapeutic planning (Bennett et al., 2023).

Refinement rather than Revolution for CHOP-Based Protocols

Multi-agent CHOP protocols, whether delivered over the traditional 19–25 weeks or in more recent abbreviated 12–15-week schedules, continue to produce complete remission in 80–92% of cases (Limmer et al., 2022; Bennett et al., 2023). Many prospective and majority retrospective studies have now confirmed that shortened protocols maintain equivalent progression-free and overall survival while substantially reducing treatment duration, cumulative myelosuppression, client cost, and hospital visits (Limmer et al., 2022; Bennett et al., 2023). In contemporary series using modern supportive care, dogs with B-cell lymphoma commonly achieve median progression-free survival of 230–280 days whereas one-year survival rates of 45–65%, with gastrointestinal toxicity and neutropenia remaining predictable and generally manageable (Bennett et al., 2023).

Lomustine-Centric Approaches for T-Cell and Refractory Disease

The relative doxorubicin resistance of many T-cell lymphomas has driven the rising adoption of lomustine, an oral nitroso-urea with favorable pharmacokinetics and activity against non-dividing cells (Einhorn et al., 2024). Primary treatment with cyclophosphamide–lomustine–vincristine–prednisone (CLOP) or sequential strategies employing 6–8 weeks of CHOP induction followed by lomustine consolidation/ maintenance have dramatically improved outcomes in T-cell cohorts (Limmer et al., 2022; Einhorn et al., 2024;). Recent retrospective analyses report median overall survival of 291–374 days in T-cell lymphoma treated with lomustine-inclusive protocols—figures that approach those historically seen only in B-cell disease and represent one of the best step forward to manage T-cell diseases (Limmer et al., 2022).

Anatomic Variants with Distinct Behavior

Certain anatomic presentations continue to pose unique challenges. Alimentary lymphoma exhibits highly variable chemosensitivity; some dogs experience prolonged remission with CHOP alone, whereas others progress rapidly despite aggressive therapy (Lai et al., 2024). Hepatosplenic γδ T-cell lymphoma remains among the most aggressive entity, with median survival times frequently less than 60 days even with intensive multi-agent chemotherapy, demonstrating the need for novel approaches in these rare but destructive forms (Lai et al., 2024.

Drug Repurposing and Novel Therapeutics

High-throughput screening of FDA-approved human medications has identified several unexpectedly potent agents with strong anti-lymphoma activity in canine cell lines and xenograft models, including artesunate, niclosamide, itraconazole, and dronedarone (Nishida et al., 2025). Their established safety profiles and oral bioavailability make them extremely attractive for rapid clinical translation (Nishida et al., 2025). Concurrently, targeted small molecules such as toceranib, verdinexor (Laverdia), rabacfosadine (Tanovea), and isoform-selective PI3K inhibitors are undergoing prospective evaluation (Vlodaver et al., 2024). Although anecdotal complete remissions are encouraging, large randomized trials are still needed to define their ultimate role.

Response Evaluation and Rescue Therapy

Objective response is assessed using Veterinary Cooperative Oncology Group RECIST v1.1 criteria, (which is the globally accepted standard for measuring how solid tumors respond to cancer treatment in clinical trials like CT/MRI for checking shrinkage of tumors) with regular physical examination, lymph node measurements, and ancillary diagnostics as indicated (Einhorn et al., 2024; Lai et al., 2024). At relapse, rescue protocol selection is individualized based on prior treatment, duration of first remission, current immunophenotype, organ tolerance, and owner priorities (Bennett et al., 2023). Commonly employed options include single-agent lomustine, Mechlorethamine, Vincristine, Procarbazine, Prednisone (MOPP) or protocols, L-asparaginase-containing combinations, and CHOP re-induction when the initial remission exceeded 6–8 months (Limmer et al., 2022; Bennett et al., 2023).

Persistent Challenges

Even after several years of research efforts the overall survival curves for canine lymphoma have shifted only moderately since the introduction of doxorubicin (Bennett et al., 2023). Progress continues to be hampered by the predominance of small, single-institution retrospective studies, limited regular molecular profiling, under-representation of T-cell and slow progressing subtypes in clinical trials, and financial constraints that restrict many owners to less intensive protocols (Bennett et al., 2023).

Current Clinical Recommendations

For dogs with indolent or B-cell lymphoma and favorable prognostic features, a 12–15-week CHOP-based protocol remains the first-line standard of care (Limmer et al., 2022). In cases of confirmed T-cell lymphoma or high-risk B-cell disease (substage b, anemia, hypercalcemia, etc.), a lomustine-containing regimen—CLOP from the outset or short CHOP followed by lomustine switch—should be strongly considered (Bennett et al., 2023; Einhorn et al., 2024). Early and open discussion of expectations, quality-of-life goals, and financial considerations is key to helping owners make informed decisions (Bennett et al., 2023).

Future Research Prospects

Canine lymphoma is highly responsive to chemotherapy; however, it is still rarely curable with current treatment options.[2] The progress has been gradual but clinical relevant and can be achieved through shorter protocols, strategic use of lomustine, and accelerated evaluation of repurposed and targeted therapies. The next major improvements will require prospective multi-center trials, routine incorporation of immunophenotyping and next-generation sequencing, and broader access to novel therapeutics[2] which will help in better life expectancies and more feasible treatment options for the owners.

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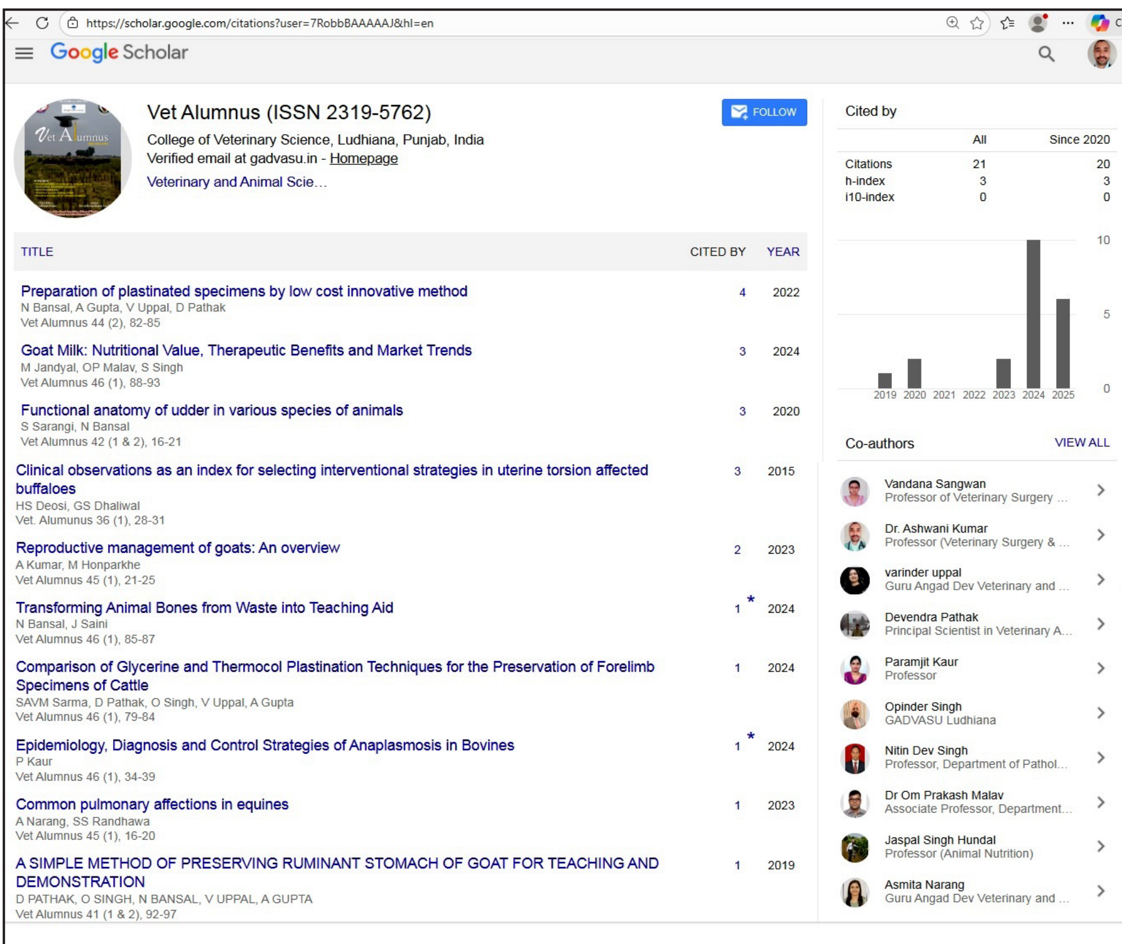

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