



A systematic review of predictive, diagnostic, and prognostic biomarkers for detecting reproductive diseases in cattle using traditional and omics approaches

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ABSTRACT

Reproductive diseases and illnesses pose significant challenges in cattle farming, affecting fertility, milk production, and overall herd health. In recent years, the integration of various omics approaches, including transcriptomics, proteomics, metagenomics, miRNAomics, and metabolomics, has revolutionized the study of these conditions. This systematic review summarised the findings from studies that investigated reproductive disease biomarkers in both male and female cattle. After extracting 6137 studies according to exclusion and inclusion criteria, a total of 60 studies were included in this review. All studies identified were associated with female cattle and none were related to reproductive diseases in bulls. The analysis highlights specific biomarkers, metabolic pathways, and microbial compositions associated with bovine reproductive disease conditions, providing valuable insights into the underlying molecular mechanisms of disease. Pro-inflammatory cytokines such as IL-1 β , IL-8, IL-4, IL-6, TNF α and acute-phase response proteins such as SAA and HP have been identified as promising biomarkers for bovine reproductive diseases. However, further research is needed to validate these markers clinically and to explore potential strategies for improving cow reproductive health. The role of bulls as carriers of venereal diseases has been underestimated in the current literature and therefore needs more attention to understand their impact on infectious reproductive diseases of female cattle.

1. Introduction

Reproductive diseases in cattle are of global concern, affecting both developed and developing countries. Reproductive diseases and disorders in cattle are responsible for significant economic losses, estimated \$1 billion per year, which represents a substantial financial burden on the livestock industry worldwide (USDA, 2010, Deka et al., 2021). Reproductive disorders such as endometritis, metritis, vaginitis, and cervicitis may be caused by a variety of infectious agents and factors, and pose significant challenges for cattle industries, impacting fertility, productivity, and overall herd health (Sheldon et al., 2006). Unravelling the complex molecular mechanisms underlying these diseases is crucial for developing effective diagnostic tools, therapeutic interventions, and

management strategies. In recent years, multi-omics approaches have emerged as a novel tool (Wang et al., 2024) to define the molecular landscape of complex diseases in cattle with potential to improve disease diagnosis, treatment, and prevention. The omics approaches such as transcriptomics (Wang et al., 2009), proteomics (Ma et al., 2022), metabolomics (Ashokan et al., 2022), and metagenomics (Chen et al., 2022b) have superseded traditional methods (i.e. qPCR, RT-PCR, microarrays, ELISA, Western blots, Flow cytometry) in disease biomarker discovery. The traditional methods of biomarker identification are target-specific and require intensive time and labour resources (Mccoll et al., 2019). In contrast, omics approaches provide un-targeted and comprehensive data sets describing complex biological responses at greater sensitivity and accuracy (Horgan and Kenny, 2011, Acunzo

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et al., 2015, Mccoll et al., 2019, Dai and Shen, 2022). Although several reviews have been published on postpartum reproductive diseases of cattle (Amin and Hussein, 2022, Leblanc, 2023), this systematic review focused on biomarkers for all reproductive diseases in cattle using both traditional and omics approaches. To our knowledge such a review has not previously been undertaken.

Transcriptomics examines the entire set of RNA transcripts by RNA/cDNA sequencing and can include mRNA, differential splice variants, small RNAs and microRNAs (miRNAs) (Wang et al., 2009). It provides valuable information on the regulation of gene expression and functional pathways in different tissues in response to disease pathologies. Microarrays and reverse transcriptase polymerase chain reaction (RT-PCR) are the traditional techniques of gene expression that allow the expression of predetermined genes to be assessed using specific probes/primers. This can introduce bias to results compared to transcriptomic techniques (RNA-seq), which can identify all sequences present in a cell, tissue, organ or whole organism (Wang et al., 2009, Lowe et al., 2017, Hong et al., 2020). Sequencing of MicroRNA/miRNAs (microRNAomics) have emerged as a promising omics approach for biomarker identification due to the vital roles of these molecules in biological pathways by posttranscriptional regulation of gene expression including immune responses (Corbeil et al., 1974, Lim et al., 2003, Bartel, 2004, Lawless et al., 2014, Acunzo et al., 2015, Dong et al., 2017, Han et al., 2017). MicroRNAs have been identified as potential candidate biomarkers for infectious diseases in cattle such as paratuberculosis, tuberculosis and mastitis (Wang et al., 2016, Luoreng et al., 2018, Ma et al., 2019, Srikok et al., 2020). Multi-omics studies are integrations of different approaches such as transcriptomics, RNA-Seq and proteomics. Multi-omics approaches have the potential to identify biomarkers for specific diseases such as: mastitis using mRNA, miRNA and DNA methylation omics data (Wang et al., 2024); and human endometriosis using proteomics and transcriptomics (Li et al., 2023); drug resistant tuberculosis using DNA methylation, transcriptome and proteomic analyses (Wu et al., 2024); and bovine viral diarrhoea virus infections using host transcriptomics and proteomics (Zhang et al., 2023).

Proteomics facilitates an in-depth study of cellular, tissues and organismal proteomes including protein expression, structure, function, interactions and modifications related to stage of disease (Domon and Aebersold, 2006). With the advancement of high-throughput chromatography and Mass Spectrometry (MS), proteomics has become more accessible and provides potential tools for diagnostic interventions and disease-specific biomarkers such as clinical proteomics can differentiate human endometriosis and healthy patients with 96 % sensitivity and 100 % specificity while elevated expression of CXCL5 and IL1RN identified as potential biomarker for endometriosis (Zhao et al., 2015, Ji et al., 2023). Proteomics has shown increasing potential in developing diagnostic, prognostic, and therapeutic biomarkers for livestock diseases, including mastitis, tuberculosis, and infectious reproductive illnesses (Zhang et al., 2018a, Gao et al., 2019, Bathla et al., 2020, Almeida et al., 2021).

Analysis of the microbiome enables the elucidation of an entire microbial community from a specific environment and allows correlation with health and disease (Ehrlich, 2016). The analysis of the microbiome can be conducted through amplicon sequencing, targeting the 16 S rRNA gene for bacteria and the 18 S rRNA gene for eukaryotes, as well as through a metagenomics approach. Metagenomics is distinct from amplicon sequencing, as it does not require PCR amplification, but very commonly this term is used by researchers to describe 16 S amplicon sequencing in microbiome studies (Alves et al., 2018). Amplicon sequencing uses targeted specific sequencing (usually 16 S and/or 18 S variable fragments) providing the identity of specific taxonomic groups, however results can be biased due to variations in binding efficiencies of different PCR primer pairs and also variations in rDNA gene copy numbers among different species (Větrovský and Baldrian, 2013, Tremblay et al., 2015). In contrast, metagenomics offers a more unbiased approach (PCR-free) analysing mixed communities of

microorganisms, including all prokaryotic and eukaryotic organisms such as bacteria, virus, fungi, parasites, and other microbes in a given environment community (Alves et al., 2018, Zhang et al., 2021b). A recent study comparing microbiome sequencing methods of the bovine reproductive tract demonstrated the superior accuracy of metagenomics using long read sequencing over 16 S amplicon sequencing (Ong et al., 2022).

Metabolomics used to identify and quantify hundreds of known and unknown metabolites (untargeted) with a very small volume of sample and is an ideal tool for understanding the pathobiology of disease, characterisation, screening, and prediction of disease and drug discovery (Adamski and Suhre, 2013, Rinschen et al., 2019, Zwierchowski et al., 2020, Zhang et al., 2022). Metabolomics use MS and Nuclear Magnetic Resonance (NMR) to measure cellular biochemical activities, allowing association with phenotypes compared to the gene. The obtained data can be correlated with other omics approaches described above such as transcriptomics and proteomics to understand the host-pathogen interactions of disease and biomarker (Rinschen et al., 2019, Tounta et al., 2021).

Although omics for the identification of biomarkers in cattle and other species of livestock have been increasing during the last few decades, their application for early diagnosis and prediction of reproductive diseases still requires more attention. This study aims to systematically review and summarise the biomarkers for reproductive disease in cattle identified to date, using both traditional and novel omics approaches.

2. Methodology

2.1. Key words and search strategies

The study utilized a comprehensive search strategy that involved searching seven databases, namely PubMed, Embase, Scopus, Google Scholars, Web of Science, CAB Directory and Cochrane library, for relevant articles published between 2010 and 1st July 2023. The search terms included a combination of relevant medical subject headings (MeSH) and keywords related to reproductive disorders and diseases of cattle. The search terms used for biomarkers were: 'Biomarker' or 'Biomarkers' or 'MicroRNA' or 'miRNA' or 'Proteomics' or 'Proteome' or 'Metabolome' or 'Metabolomics' or 'Transcriptomics' or 'Transcriptome' or 'Glycomics' or 'Glycome' or 'Microbiome' or 'Microbiota' or 'Metagenomics', for species: 'Cattle' or 'Bovine' or 'Heifers' or 'Cows' or 'Dairy' or 'Beef' or '*Bos indicus*' or '*Bos taurus*' or 'Bull' and for reproductive diseases: 'Reproductive Disease' or 'Reproductive Disorder' or 'Reproductive Tract Infection' or 'Infectious Reproductive' or 'Bacterial Reproductive' or 'Genital Disease' or 'Sexually Transmitted Disease' or 'Venereal Disease' or 'Animal Reproduction' or 'Endometritis' or 'Metritis' or 'Vaginitis' or 'Cervicitis' or 'Oophoritis' or 'Placentitis' or 'Balanoposthitis' or 'Vesiculitis' or 'Balanitis' or 'Posthitis'.

The extracted articles were imported into EndNote 20 (The Endnote Team, 2013) and then transferred into Covidence software (www.covidence.org) for the screening process as an microsoftword.xml file. Duplicate articles (n=5635) from different databases were automatically removed by Covidence software and 10 duplicates were removed manually. Remaining articles (n=6137) were selected for title and abstract screening. For the first comprehensive screening process two reviewers screened the title and abstract of the articles based on keywords, inclusion and exclusion criteria (Supplementary File Table 1) and conflicts were resolved by a third reviewer. After completion of the screening, a total of 132 articles were selected for full-text review and reviewed by two authors independently. Out of these, 72 articles were excluded based on the further downstream of exclusion and inclusion criteria (listed in Fig. 1) and a total of 60 articles were included for systematic review. The steps adopted in this systematic review were based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Fig. 1)

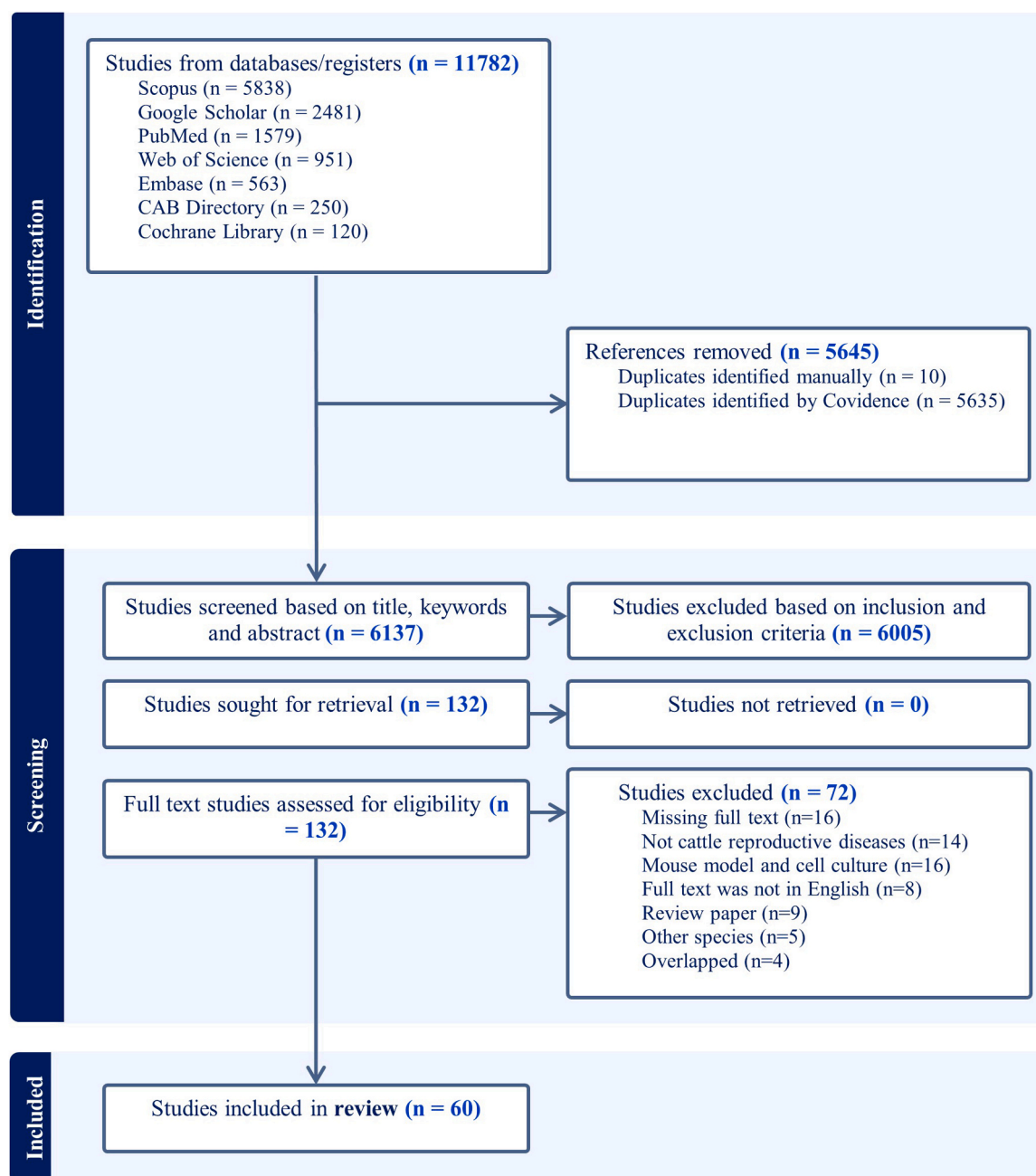


Fig. 1. The steps from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) utilised for this systematic review.

2.2. Description of the terminology

The term “traditional” approaches for biomarker detection refers to studies that employed expression and amplification of targeted genes, proteins and regions for microbial genome sequencing. Targeted gene expression methods included RT-PCR (Reverse Transcriptase- Polymerase Chain Reactions), quantitative RT-PCR (qRT-PCR), and microarray, while targeted protein expression included Enzyme-linked-immunosorbent Assays (ELISA), Western blots, and Flow cytometry. Targeted amplicon sequencing used to define microbiome analysis using 16 S rRNA, and 16 S rDNA gene. In contrast, the term “novel” was used for studies that adopted high-through-put modern omics approaches such as proteomics, transcriptomics, microRNAomics, metagenomics, and metabolomics for biomarker discovery. The term “type of sample” was used to describe sample collection method used by researchers in their article.

The term “reproductive disease” was used to describe the common reproductive tract infection and illness used by researchers in their article. “Endometritis” is the inflammation of endometrium classified as clinical and subclinical endometritis. Presence of purulent or mucopurulent discharge detectable in the vagina after 21 days of postpartum is defined as “clinical endometritis” while absence of detectable vaginal discharge and presence of >18 % or >10 % neutrophil in vaginal cytological samples collected after 21–33 or 34–47 days of postpartum is called “subclinical endometritis”. “Metritis” is inflammation of uterus characterised by an enlarged uterus and the presence of foetid odour pus with or without fever within 21 days of postpartum (Sheldon et al., 2020). Purulent vaginal discharge (PVD) is a symptom of the inflammatory process and is characterised by the presence of mucopurulent discharge in the vagina. It most often confused with clinical endometritis, but researchers found that PVD may occur independently without the incidence of endometritis (Dubuc et al., 2010, Williams, 2013).

Endometritis and PVD are related but not synonymous conditions (de Boer et al., 2014). “Cervicitis”, “Vaginitis” and “Salpingitis” are used to describe inflammation of the cervix, vagina and fallopian tubes respectively. “Repeat breeding” is a state in which a cow, without apparent clinical abnormalities, is unable to achieve conception after being bred or inseminated three or more times with a fertile bull or using fertile bull semen (Amiridis et al., 2009, Funeshima et al., 2021). “Retained placenta” is used to describe a cow who failed to expel the foetal membrane after 24 hours of parturition (Yazlik et al., 2019). However, in this review, the authors categorised the reproductive diseases and syndromes according to the terms used by the authors of the cited research articles.

2.3. Features of data extraction and analysis

Data extraction was performed on the 60 studies, using a pre-defined data extraction excel sheet. The sheet included the following variables: country or region of study, year of study, breed of cattle, age, sex, type of approaches (traditional vs novel), type of sample, name of reproductive disease, number of samples, technique used for biomarker and number of animals. In the case of female cattle, number of parity and days of postpartum (DPP) were also included. The geographical origin included in the studies were classified according to World Health Organization (WHO) group (<https://www.who.int/countries/>). Studied related to both *in vitro* and *ex vivo* sampling from infected cattle were included. Descriptive statistics were used to summarize the data, and the results were presented using tables and graphs. Data were analysed using GraphPad Prism v10.0.2.

3. Results

The key findings extracted from individual articles based on reproductive diseases, type of samples, type of approaches, techniques used for omics and summary of the results are presented in a tabular form and attached as a supplementary file (Supplementary File Table 2).

3.1. Advancement of biomarker studies for reproductive disease in cattle

A total of 60 articles for bovine reproductive disease biomarkers published between 2010 and 1st July 2023 were included in this review. A peak in the number of articles published was seen for 2018, this number may be matched by the full calendar year 2023 (only 6 months charted here), Fig. 2.

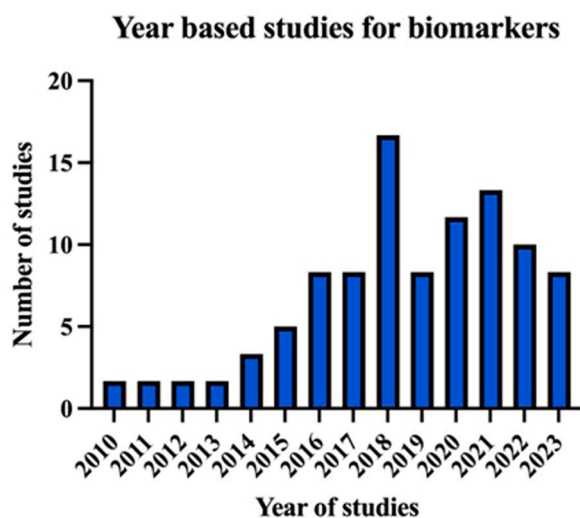


Fig. 2. Bar graph outlining the number of biomarker studies between 2010 and 2023 for reproductive disease in cattle.

3.2. Geographical distribution of studies related to biomarker for reproductive disease in cattle

For the geographical distributions, studies were categorised according to WHO regions including five regions- Americas, Europe, Western Pacific, South-east Asia and Eastern Mediterranean. The highest number of studies were found in the Americas region (43.0 %) followed by Europe (27.0 %) and Western Pacific (18.0 %). In the Americas region, studies were from USA (20.0 %), Canada (15.0 %), Brazil (5.0 %) and Argentina (1.7 %). In Europe, studies were found from Ireland (8.3 %), Austria (3.3 %), Germany (3.3 %), Ireland (3.3 %), Italy (3.3 %), Israel (3.3 %), Turkey (3.3 %), Russia (1.7 %), Poland (1.7 %) and Portugal (1.7 %). In the Western Pacific region, studies were from China (15.0 %), New Zealand (1.7 %) and Japan (3.3 %). For the South-east Asia region, studies were from India (3.3 %) and from the Eastern Mediterranean region, Iran (1.7 %) (Fig. 3).

3.3. Approaches used for biomarker study for reproductive disease in cattle

Of the 60 studies analysed (Supplementary File Table 2), 63 % used traditional approaches of protein and gene expression and 37 % used modern high throughput omics approaches (Fig. 4). Five omics technologies were found in modern approaches: metabolomics (59 %), proteomics (27 %), microRNAomics (5 %), transcriptomics (5 %) and metagenomics (5 %) for the identification and characterisation of biomarkers for reproductive diseases in cattle (Fig. 4).

For traditional methods, the most common approach were microbiome studies (45 %) using 16 S rDNA amplicon sequencing, followed by targeted gene expression using qRT-PCR (24 %) expression of specific miRNAs and mRNAs (8 %), Microarrays (3 %), and targeted protein expression using ELISA (11 %), Flow cytometry (5 %), and Western blots (5 %). (Fig. 4).

3.4. Type of reproductive diseases and disorders studied for biomarker(s) identification in cattle

Reproductive diseases found in this review categorised into 9 groups based on the terms used by researchers in their studies named endometritis, metritis, salpingitis, necrotic vulvo-vaginitis, cervicitis, retained placenta, purulent vaginal discharge (PVD), repeat breeding and Bovine Genital Campylobacteriosis (BGC) (Fig. 5a). Endometritis recorded as clinical endometritis (CE), subclinical endometritis (SCE), uterine infection and combined with other uterine infections such as metritis and retained placenta (Fig. 5b).

The highest number of studies investigated bovine endometritis (43 %) including clinical and subclinical forms, followed by metritis (30 %), uterine infections (7 %), combined studies on metritis-endometritis (5 %) and retained placenta (5 %), respectively. Studies were recorded (1.7 %) for each category of salpingitis, vaginitis,

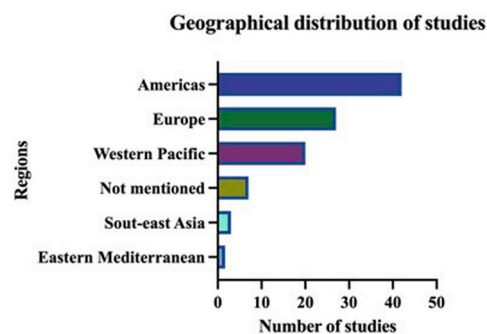


Fig. 3. Geographical distribution of biomarkers studies related to the study on bovine reproductive disease identified in cattle.

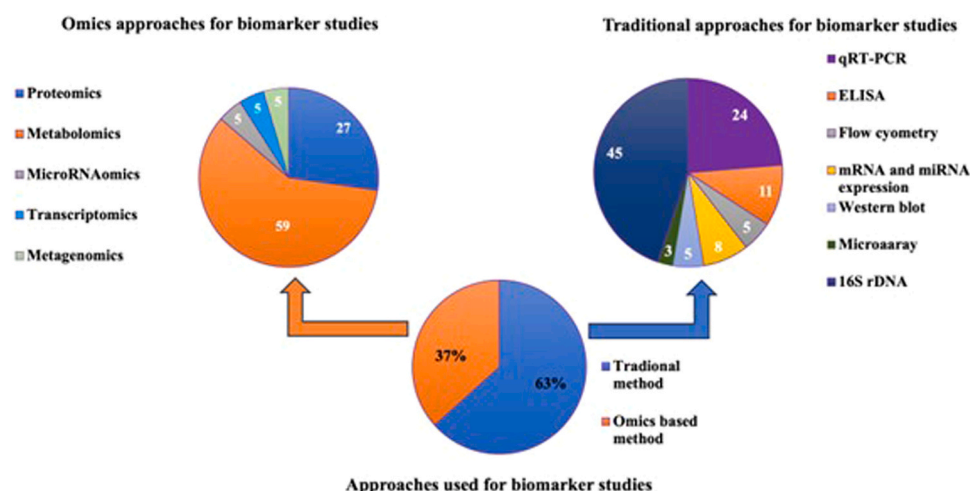


Fig. 4. Pie charts illustrating study approaches used for the identification of biomarkers for bovine reproductive diseases.

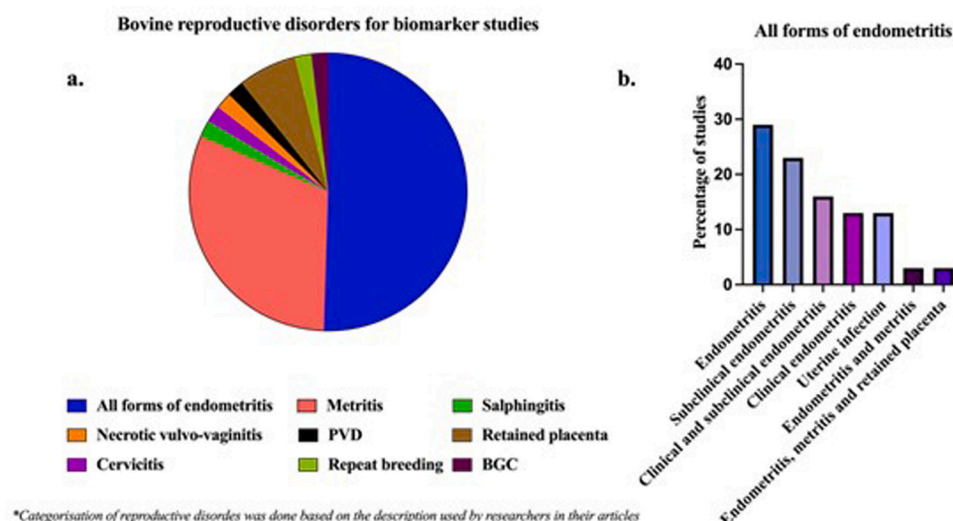


Fig. 5. a) Number and categorisation of reproductive disorders found in cattle recorded from 2010 to 1st July 2023, for biomarker study, b) Bar graph describing the distribution of all forms of endometritis recorded alone or with other reproductive disorders. The terminology used here follows the terms used to describe the diseases or disorders in the original publications.

cervicitis, repeat breeding, PVD and BGC (Fig. 5a).

3.5. Sex, age, breed, and parity of female cattle

All 60 studies recorded in this review were conducted on female cattle and surprisingly no studies determined if the disease was related to mated bulls with similar serum or reproductive tract biomarkers. Holstein Friesian breeds were used in 75 % of studies while 10 % of studies included other dairy breeds, and the remaining 15 % did not mention the breed studied (Fig. 6). In most of the studies, age, parity and days of postpartum of cows were not mentioned in detail. The 'parity' of experimental cattle used by the researchers ranged from 1 to 5 (32 %) while 12 % of studies used mixed parity and 32 % did not mention parity.

'Days of postpartum (DPP)' ranged between 0 and 60, and highest number of studies included 0–28 DPP for biomarker studies (Fig. 7). The age of the cattle used in the studies ranged from 1 to 10 years old. Up to 73 % of the studies did not mention the age, and 17 % used up to 6 years age old of cattle.

3.6. Population, size and type of sample

In 73 % of the studies, there was a variation between the population size of cattle and the number of samples collected. Conversely, in 27 % of the studies, the number of samples collected for biomarker analysis was consistent with the population size. The cattle population sizes across the studies varied from 8 to 1000, while the number of samples collected for biomarker analysis ranged from 6 to 100.

Samples were predominantly collected from the uterus, cervix and vagina depending on the type of disease and techniques used. Depending on the type of biomarker and approach used, blood samples were collected. In 38 % of the studies, various forms of blood samples were collected (serum, plasma, white blood cell, peripheral blood nuclear cells) with and without other reproductive tract samples (Fig. 8). The highest number of studies used blood samples for metabolomics (52 %) followed by the targeted expression (30 %) and proteomics (9 %) approaches.

Following blood, other important reproductive tract samples were collected including endometrial cytobrush (35 %), ex vivo uterus sample collection (12 %), endometrial biopsy (8 %) and uterine swab (8 %), respectively. Other samples included endometrial scrapes, uterine flush,

Breeds used for biomarker studies

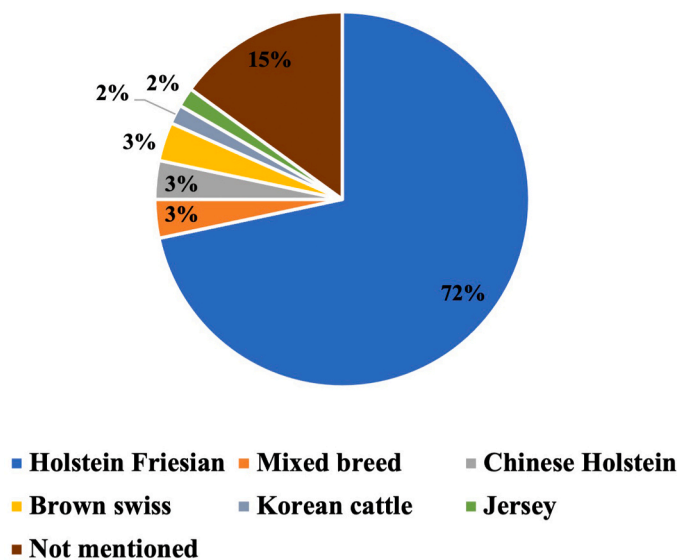


Fig. 6. Breeds of cattle used for biomarker studies for reproductive diseases in cattle.

Day of post-partum used for biomarker studies

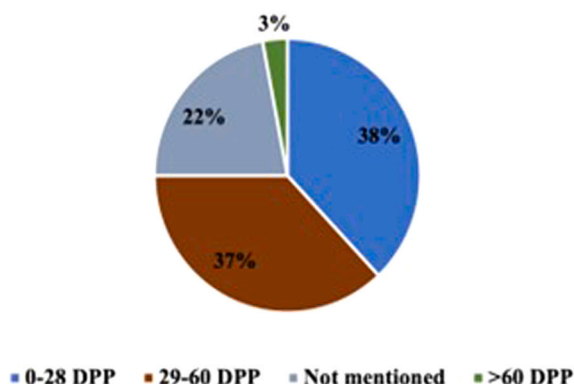


Fig. 7. Number of days post-partum (DPP) considered for biomarker studies in reproductive diseases of cattle.

uterine lavage, uterine secretion, vaginal swab, vaginal biopsy, vaginal scrapes, vaginal discharge, and cervical swabs collected from the upper or lower reproductive tracts.

3.7. Predictive, diagnostic, prognostic and immune biomarkers for bovine reproductive diseases

3.7.1. Pro-inflammatory cytokine, chemokine and adipokine biomarkers

A number of studies (~18 %) identified the development of bovine reproductive disease associated with complex signalling processes involving with the detection of bacterial components by innate immune cells via Toll-like receptors, production of tumour necrosis factor- α (TNF- α) and other pro-inflammatory cytokines as well as chemokines (Loyi et al., 2015, Brewer et al., 2020, Horlock et al., 2020, Kasimanickam et al., 2020, Vallejo-Timarán et al., 2020, Islam et al., 2021, Li et al., 2021, Kneidl et al., 2022, Sadeghi et al., 2022). Cytokines act as important mediators to initiate the inflammatory cascade and help to

clear out the uterine infection by influx of immune cells. Cytokines associated with disease includes several members of proteins including interleukins (IL, IL-1, IL-6, IL-8, IL-1 β , IL-8), chemokines, interferons, mesenchymal growth factors, adipokines and TNF α , among these interleukins play a crucial role in various pathological, immunological and physiological function in the reproductive tract of cattle. A list of potential cytokines, chemokines and adipokines described in this review as biomarkers for bovine reproductive diseases is summarised in Table 1.

Depending on the severity of endometritis and the oestrous stage, transcripts of pro-inflammatory cytokines vary in cows (Loyi et al., 2015, Raliou et al., 2019). Cows that fail to restore homeostasis by activation of pro-inflammatory cytokines after 21 days of postpartum (DPP), developed endometritis (Foley et al., 2015, Raliou et al., 2019). A reduction in expression of IL-1 in endometritic cows (Islam et al., 2021) and an increased expression of IL-4, IL-1 β and TNF α levels was seen in cows with clinical endometritis, suggesting the potential of these inflammatory markers for early detection and monitoring of clinical endometritis (Kim et al., 2014, Adnane et al., 2017, Adnane et al., 2018, Brewer et al., 2020, Islam et al., 2021). Similar to endometritis and metritis, expression of cytokines was also associated with salpingitis, cervicitis and retained placenta. Severity of salpingitis was proportionally associated with increased expression of CD14, IL1 β and IL-8 (Sadeghi et al., 2022) while increased level of IL-8, IL-1, and α 1-acid glycoprotein (AGP) was found to be associated with cervicitis (Vallejo-Timarán et al., 2020). Significantly decrease in expression of IL-4, IL-2 and increased expression of IL-10, appears to consistently indicate early diagnosis of retained placenta (Li et al., 2021). The identification of biomarkers associated with these conditions provides valuable information on the pathogenesis of disease and therapeutic targets for future use.

Adipokines, also called as adipocytokines, are produced by adipose tissue, and like adiponectin, chemerin, and visfatin, play an important role in reproductive function and immunity and were identified as potential biomarkers to distinguish healthy and diseased cows (Reverchon et al., 2014, Pereira et al., 2020). Increased rate of adiponectin in both plasma and uterine fluid precisely differentiates healthy cows with persistent endometritis depending on days after parturition; 87 % at 21 DPP, 98 % at 45 DPP while 100 % in uterine fluid of endometritis affected cows at 45 DPP (Pereira et al., 2020).

3.7.2. Acute phase response protein biomarkers

Acute-phase response proteins (APPs) are the outcome of the acute phase response due to inflammation, tissue injury, infections and immunological disorders and are stimulated by pro-inflammatory cytokines (Petersen et al., 2004, Jain et al., 2011, Cray, 2012). Serum Amyloid A (SAA) and Haptoglobin (HP) are two important AAPs that indicate reproductive health and inflammation (Humblet et al., 2006, Bazzano et al., 2022). Although an increased level of HP and SAA were found in endometritis, metritis, PVD and retained placenta, SAA was highlighted as an emerging biomarker for endometritis due to its significant increased expression in the endometrium, compared to HP in reflecting the severity of endometritis (Foley et al., 2015, Kaya et al., 2016, Zhang et al., 2018b, Miller et al., 2019). HP and SAA had lower sensitivity (<50 %) but higher specificity (>90 %) as a biomarker in determining reproductive disease status in cattle (Humblet et al., 2006, Kaya et al., 2016). In addition, other proteins, such as desmin, α -actin-2, heat shock protein (HSP)27, MHC class I heavy chain (MHC-Ih), transferrin, KCTD11 and ceruplasmin have shown promise as diagnostic markers for bovine endometritis, and serum fibrinogen as a predictive biomarker for bovine metritis. (Choe et al., 2010, Paiano et al., 2021).

3.7.3. Exosomal biomarkers

Exosomes are small nanovesicles derived from endosomes and found almost in all body fluids, provide a more precise reflection of cellular processes such as immunity, signal transmission, antigen presentation and inflammation (Gurung et al., 2021, Buzas, 2023). The identification

Blood sample used for biomarker studies

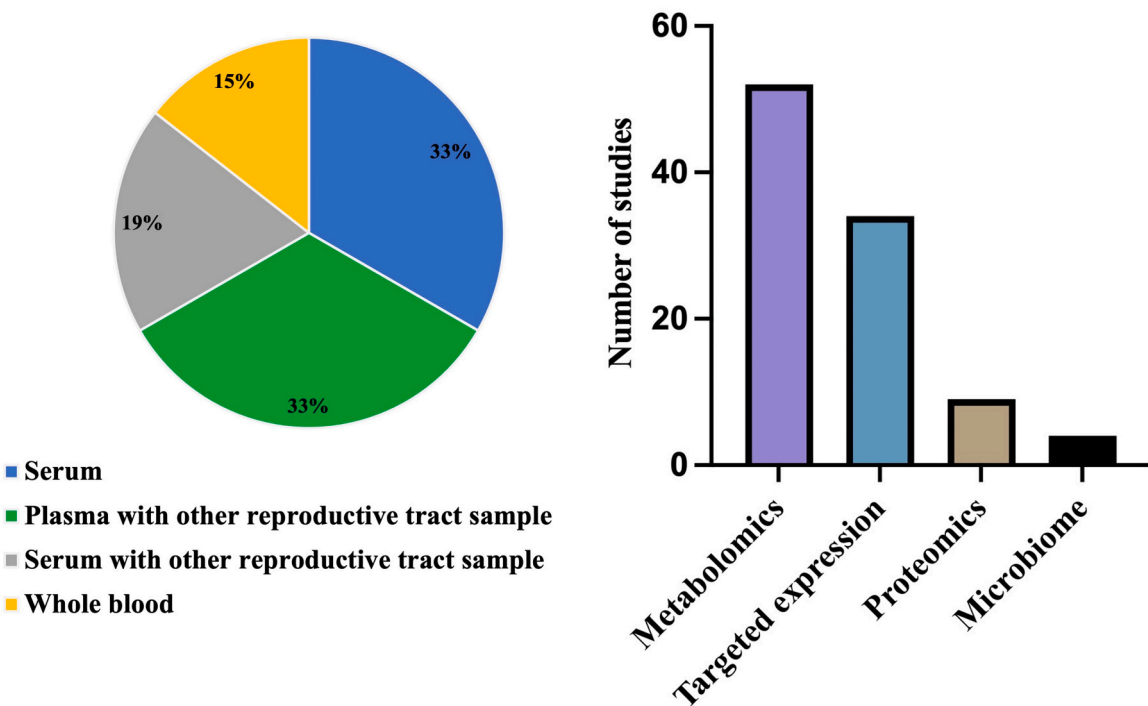


Fig. 8. Blood samples used for traditional and novel approaches of biomarker studies in cattle for reproductive diseases.

Table 1

List of cytokines, chemokines, and acute-phase response proteins as biomarkers for bovine reproductive diseases including disease state and type of biomarkers.

Biomarkers	State	Name of Diseases	Type of biomarker	Reference
Cytokines, chemokines and adipokines				
IL-1	Decreased	Endometritis	Diagnostic	(Islam et al., 2021)
IL-1 β IL-4 and TNF α	Increased	Endometritis	Diagnostic	(Islam et al., 2021)
IL-1A, IL-1 β , TNF α and SERPINB4	Increased	Endometritis	Diagnostic	(Brewer et al., 2020)
IL-1 β	Increased	CE	Predictive	(Adnane et al., 2017)
IL-1 β and MUC1	Increased	RB	Predictive	(Kasimanickam et al., 2014)
IL-1 β , IL-6, IL-8 and TNF α	Increased	CE and SCE	Diagnostic	(Loyi et al., 2015)
IL1RA/IL1B ratio	Decreased	SCE	Prognostic	(Kneidl et al., 2022)
IL-2, IL-8 and IL-10	Increased	RFM	Diagnostic	(Li et al., 2021)
IL-1 β IL-8, CD14 and CASP3	Increased	Salpingitis	Prognostic	(Sadeghi et al., 2022)
IL-1 and IL-8	Increased	Cervicitis	Diagnostic	(Vallejo-Timarán et al., 2020)
Adipokines	Increased	SCE	Diagnostic	(Pereira et al., 2020)
Acute-phase response proteins (APPs)				
HP and SAA	Increased	Endometritis	Prognostic	(Zhang et al., 2018b)
HP	Increased	PVD and SCE	Diagnostic	(Bogado Pascottini and Leblanc, 2020)
HP	Increased	RP and Metritis	Diagnostic	(Pohl et al., 2015)
SAA, HP with Ceruplasmin	Increased	Endometritis	Diagnostic	(Kaya et al., 2016)
SAA and HP	Increased	CE	Diagnostic	(Foley et al., 2015)
HP and Frutosamine free fatty acid (FAA)	Increased	Metritis	Diagnostic	(Menta et al., 2023)
SAA and HP-20 homolog with immunoglobulin M-like proteins	Increased	Endometritis	Diagnostic	(Miller et al., 2019)
Desmin, α -actin-2, HSP27, MHC-Ih, transferrin, and KCTD11	Increased	Endometritis	Diagnostic	(Choe et al., 2010)
Serum fibrinogen	Increased	Metritis	Predictive	(Paiano et al., 2021)

SCE: Subclinical Endometritis; CE: Clinical Endometritis; PVD: Purulent Vaginal Discharge; IL: Interleukin; HP: Haptoglobin; SAA: Serum Amyloid A; HSP: Heat Shock Protein; TNF α : Tumour Necrosis Factor alpha; MHC: Major Histocompatibility Complex; KCTD11: Potassium Channel Tetramerisation Domain-Containing 11; RP: Retained Placenta; RB: Repeat breeding

of altered exosomal proteins which were consistent with immune response in the presence of uterine infection highlighted the potential role of exosomes in early detection of bovine reproductive disease and opened up new avenues for exploring non-invasive diagnostic approaches for uterine infections (Almughlliq et al., 2018). Bovine uterus-derived exosomes and transcriptome have been shown to affect pregnancy and development of the embryo under endometritis

conditions and may cause infertility in cows (Wang et al., 2019). Comparing exosomal samples from endometritis and healthy cows by deep sequencing method, 20 differentially expressed miRNAs were found in which the 10 most abundant miRNAs were selected for further validation. Three upregulated (AC_000161_317, bta-miR-708, bta-miR-91b) and six down-regulated miRNAs (bta-let-7d, bta-miR-1, bta-miR-122, bta-miR-146a, bta-miR-3600, bta-miR-451) were consistent

with deep sequencing results, providing an insight of their importance in pathogenesis of endometritis (Wang et al., 2019). Further, exosomal miR-218 has been found to regulate development of bovine endometritis by targeting pathway of transforming growth factor-beta (TGIF2) (Chen et al., 2022a). Future investigations into the downstream targets of identified miRNAs and their regulatory effects on inflammatory pathways could provide valuable insights into the bovine reproductive disease mechanisms and discovery of new potential biomarker(s) for diagnosis and therapeutics.

3.7.4. Metabolomic biomarkers

Metabolic inflammation and maladaptation to postpartum metabolic requirements is potentially linked to bacterial proliferation and immune activation which may lead to postpartum reproductive diseases in cattle (Zhang et al., 2017b, Hailemariam et al., 2018, Bogado Pascottini and Leblanc, 2020, Bogado Pascottini et al., 2023, Figueiredo et al., 2023). Increased concentrations of serum non-esterified fatty acid (NEFA), β -hydroxybutyric acid (BHB) at 2 weeks of postpartum, were early indicators of a systematic inflammation in cows with SCE, PVD and metritis while increase in antepartum superoxide dismutase activity (SOD) act as a predictive biomarker for retained placenta (Yazlik et al., 2019, Bogado Pascottini and Leblanc, 2020, Menta et al., 2023).

Plasma metabolic biomarkers can predict periparturient diseases four weeks before its development with 87 % sensitivity and 85 % specificity (Hailemariam et al., 2014b). Three metabolites were found to differentiate cows with metritis and healthy cows and may be used as a predictive biomarker for periparturient diseases; these included carnitine-C0, propionyl carnitine-C3 and phospholipid-(lysophosphatidylcholine acyl C14:0). Similarly, five amino acids (aspartic acid, glutamic acid, serine, threonine and tyrosine), a sugar (myoinositol), and urea were shown to increase 8 and 4 weeks before parturition, and at the time when retained placenta was diagnosed (Zhang et al., 2021a). Metabolites were significantly altered in cows with endometritis, metritis, retained placenta and repeat breeding, suggesting the importance of those metabolites as predictive, diagnostic, prognostic, and therapeutic markers for the diseases.

3.7.5. Microbiome biomarkers

In this review 30 % of studies were detailing the reproductive tract microbiome, 29 % of the studies utilised 16 S rDNA amplicon sequencing, targeting the variable region ranging from V1-V6, and only one study used metagenomics using MiSeq Illumina sequencing (Yildirir et al., 2022). In addition to 16 S and metagenomics, researchers recently used MALDI-TOF MS to understand the dynamics and diversity of reproductive tract microbiota in reproductive diseases of cattle (Ballas et al., 2022, Paiano et al., 2022). The highest number of studies on the microbiome was for metritis (15 %) and endometritis (10 %) followed by PVD, bovine necrotic vulvovaginitis (BNVV), and BGC. Depending on the target region of bacteria, V4 (65 %) was the most used variable region amplifying either alone or with the V3, V5, and V6 regions (Brodzki et al., 2014, Jeon et al., 2015, Moreno et al., 2022). Endometrial cytobrush was used in 33 % of the microbiome studies followed by uterine swabs (22 %), uterine secretions (17 %) and vaginal swabs (11 %).

The bovine vagina is composed of diversified microorganisms with a mixture of aerobic, facultative anaerobic and anaerobic bacteria (Santos and Bicalho, 2012, Moreno et al., 2022) and harbours the same dominant bacterial phyla as the uterus, but in different proportions including, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Fusobacteria* (Peng et al., 2013, Jeon et al., 2015, Rodrigues et al., 2015). The presence or absence of certain bacterial phyla might serve as a biomarker for both optimal and suboptimal reproduction status and as an indicator for reproductive health and therapeutic interventions of certain reproductive diseases (Baker et al., 2018, Deng et al., 2019). Alterations in microbial compositions and abundances were associated with various reproductive illnesses, including endometritis (Brodzki et al., 2014, Bicalho et al.,

2017b, Becker et al., 2023), metritis (Santos et al., 2011, Cunha et al., 2018, Sicsic et al., 2018, Wang et al., 2018b, Chen et al., 2020), PVD (Bicalho et al., 2017a); and vaginitis (Shpigel et al., 2017). Endometritis in the cow was associated with increased abundance of *Fusobacteria* and *Bacteroidetes* with decrease of *Proteobacteria*, while metritis was notably associated with increased levels of *Fusobacteria*, *Bacteroidetes*, and *Firmicutes*, along with decreased presence of *Proteobacteria* (Santos and Bicalho, 2012, Brodzki et al., 2014, Jeon et al., 2016, Cunha et al., 2018, Sicsic et al., 2018, Wang et al., 2018a, Chen et al., 2020, Kudo et al., 2021, Moreno et al., 2022, Tasara et al., 2023). *Bacteroidetes pyogenes* was found as a significant bacteria involved in metritis (Jeon et al., 2016, Cunha et al., 2018, Chen et al., 2020) and described as an emerging pathogen for metritis with *Porphyromonas levii* and *Holococcus ovis* (Cunha et al., 2018)). *Trueperella* was recognised as a unique genera for endometritis (Wang et al., 2018a, Kudo et al., 2021) while *Histophilus* and *Mogibacteriaceae* were found to be associated with endometritis after calving (Kudo et al., 2021). *Porphyromonas* and unidentified genera from *Pasturellaceae* were shown to be associated with cow classified as repeat breeders (Moreno et al., 2022).

Higher abundance of *Bacteroidetes* and *Fusobacteria* in cows with PVD, along with of *Trueperella* species exclusively associated with pathogenesis of PVD in cows, highlighting their potential as diagnostic markers (Bicalho et al., 2017a, Shpigel et al., 2017, Ballas et al., 2022). While BNVD, a foetal post-parturient disorder in cows with necrotic vulvovaginal lesion identified as a polymicrobial disease with *Fusobacteria*, *Firmicutes*, and *Bacteroidetes* as the dominant phyla. The presence of specific bacteria, including *Parvimonas*, *Porphyromonas*, *Veillonellaceae*, and *Mycoplasma*, indicated the occurrence of BNVD (Shpigel et al., 2017). De Carli et al. (2023) found that presence of *Campylobacter fetus* subsp. *venerealis* did not significantly change the cervicovaginal microbiota of heifers and suggested that infected cows with *C. fetus* subsp. *venerealis* infection returned to oestrus without any apparent cooperation with other microorganisms (De Carli et al., 2023).

Overall, the reviewed studies emphasised the importance of understanding the compositions and abundances of the uterine and vaginal microbiota in maintaining cow reproductive health. Dysbiosis in these microbial communities may lead to various reproductive illnesses, and specific bacterial genera were associated with different conditions. The identified microbial markers have the potential to serve as valuable diagnostic tools for early detection and intervention in reproductive diseases in dairy cows.

4. Discussion

The present systematic review aimed to identify and integrate the existing literature on the use of different approaches including modern omics, to summarise and provide an overview on predictive, diagnostic, prognostic, and immune biomarkers for reproductive diseases in cattle. We searched to include all the studies related to biomarkers for all reproductive diseases in cattle including male and female, however a total of 60 studies were selected after a careful review from 2010 to 1st of July 2023 with the highest number of studies were in 2018 (17 %).

4.1. Approaches used for reproductive disease biomarker studies in cattle

Although 63 % of the studies used traditional methods and 37 % omics, omics applications have been increasing in the last few years. Of the traditional approaches, 45 % of studies used targeted amplicon sequencing to analyse the reproductive tract microbiota and 24 % of studies used qRT-PCR for targeted gene expressions. After revealing unique and diversified microbiota of the bovine vagina using culture-independent 16 S amplicon sequencing by Swartz et al. (2014) and Laguardia-Nascimento et al. (2015), microbiome studies have gained attention and uncovered the association of reproductive tract microbiota with dysbiosis.

Interestingly, metabolomics (53 %) and proteomics (32 %) were

found as the highest used omics in novel approaches (32 %) and in traditional approaches (68 %), microbiome using 16 S sequencing was highest (42 %). With improved access and availability of advanced high throughput liquid chromatography (LC) and Mass Spectrometry (MS), use of metabolomics and proteomics for biomarker identification in bovine reproductive diseases increased significantly over the last 5 years (Bilić et al., 2018, Perera et al., 2022).

4.2. Omics: as emerging horizon for biomarker studies in reproductive diseases of cattle

Ongoing progress in omics has broadened our knowledge to detect and confirm biomarkers for infectious and non-infectious diseases. Over the years omics are emerging for biomarker study in reproductive disease of cattle for its wider range of molecular information with sensitivity as well as availability of modern high-throughput technologies.

This review identifies metabolomics and proteomics as important emerging sciences in livestock which provide valuable information on the biological pathways and metabolic changes associated with reproductive diseases. For example, SCE, PVD and retained placenta associated with metabolic inflammation and maladaptation to postpartum metabolic requirements revealed in metabolomics study (Dervishi et al., 2018b, Bogado Pascottini and Leblanc, 2020). Shifts in the uterine metabolome associated with disturbances in amino acid, lipid, and carbohydrate metabolism, potentially linked to bacterial proliferation and immune activation (Figueiredo et al., 2023). Proteomics has been used, not only to identify immune biomarkers for reproductive diseases such as cytokines and acute phase response proteins, but also to characterise reproductive tract microbiome and their association with clinical and subclinical endometritis (Ballas et al., 2022, Paiano et al., 2022). Use of MS based technologies such as UPLC-QTOF-MS, MALDI-TOF-MS, gas chromatography (GC-MS), high pressure liquid chromatography (HPLC) and Nuclear Magnetic Resonance Spectroscopy (NMRs) advanced the investigation of metabolic association with reproductive diseases over the years (Zhang et al., 2017a, Almughlliq et al., 2018, Dervishi et al., 2018a, Li et al., 2021, Wang et al., 2021). Researchers identified potential role of miRNAs as biomarker for endometritis and metritis, while exploration of miRNAomics in understanding bovine reproductive disease is still in its early stage (Hailemariam et al., 2014a, Kasimanickam and Kastelic, 2016, Salilew-Wondim et al., 2016).

Metagenomic shotgun sequencing is also an important emerging technology to understand the in-depth taxonomic classification of microbial community and their association with disease by pathway analysis. Miseq illumina sequencing was used in one study to identify the association of endometrial microbes with endometritis (Yildirim et al., 2022). Amplicon sequencing widely used to describe reproductive tract microbiome over metagenomics for cost effectiveness (Weinroth et al., 2022) however, it is important to consider that metagenomic data not only provides insights into the taxonomic makeup of the studied ecosystem but also reveals information about the functional genes present in the sample. This valuable information, which is not attainable through 16 S rRNA gene sequencing, sets metagenomics apart (Laudadio et al., 2018, Durazzi et al., 2021).

4.3. Biomarkers: an indicator of bovine reproductive diseases, knowledge gaps and future directions

The integration of biomarkers using traditional and omics approaches have significantly contributed to our understanding of reproductive diseases in cattle, however use of omics in reproductive disease of cattle is still low. Studies have led to new insights into the mechanisms underlying of reproductive diseases and have identified potential biomarkers that can aid in early diagnosis and targeted treatment strategies. As diagnostic and predictive biomarkers, increased of IL-1, IL-6, IL-1 β , TNF α for endometritis, IL-10, IL-2, IL-6 for retained placenta, IL-8, IL-1 β , CD14 for salpingitis and IL-1, IL-8 for cervicitis found in this

review (Adnane et al., 2017, Miller et al., 2019, Vallejo-Timarán et al., 2020, Li et al., 2021, Sadeghi et al., 2022). Increased acute phase response proteins such as SAA and HP found in almost all reproductive diseases except in repeat breeder. Dysbiosis of reproductive tract microbiome and their association with reproductive diseases identified through metagenomics and 16 S amplicon sequencing. An increased abundance of *Fusobacteria*, *Bacteroidetes* and *Firmicutes* with decreased abundance of *Proteobacteria* associated with endometritis, metritis, purulent vaginal discharge and vaginitis (Shpigel et al., 2017, Cunha et al., 2018, Kudo et al., 2021, Ballas et al., 2022, Moreno et al., 2022). Bacterial infection also altered the uterine tissue transcriptome, micro-RNome and transcriptome of pregnancy regulated genes leading to infertility in cows (Salilew-Wondim et al., 2016, Genis et al., 2018, Horlock et al., 2020, Dickson et al., 2022). However, to fully establish the clinical utility of these biomarkers, wider use of emerging omics-based approaches such as metabolomics, proteomics, metagenomics, transcriptomics and micrRNAomics could be implemented for further validation in larger populations including longitudinal studies of biomarkers in reproductive diseases of cattle.

Although studies in bulls are mostly related to bull fertility and semen quality (Chacón et al., 2023, Solanki et al., 2023), one surprising finding from the current study was the lack of studies on infections in bulls and the role of bulls in natural mating systems as carriers of venereal or infectious diseases. Not one single study was identified which focussed on bull samples and their potential link to reproductive diseases including uterine infection in female cattle. As bulls play an important role in venereal disease transmission and are the asymptomatic carriers that can cause reproductive diseases in cows (Givens, 2018, Carli et al., 2022, Polo et al., 2023), future research could focus on the identification of biomarkers for carrier bulls as an additional measure to control disease transmission.

Lack of biomarker studies on specific venereal diseases such as Bovine Genital Campylobacteriosis (BGC), trichomoniasis etc in cattle also observed. These are economically important infectious diseases which lead to significant losses in cattle industries causing infertility, abortion and reproductive losses (Bondurant, 2005, Michi et al., 2016, Carli et al., 2022, Polo et al., 2023). Only one study addressed BGC in cows to identify biomarkers using novel methodologies (De Carli et al., 2023). To address these issues and gain a more comprehensive understanding of infectious reproductive diseases, it is essential to focus on venereal diseases and infections in bulls who may act as an asymptomatic carrier of disease in natural mating system. Studying biomarkers can help researchers uncover the associations between the bulls' immune response, pathophysiology, and metabolic pathways, shedding light on the role of bulls in the transmission of infectious reproductive diseases.

5. Conclusions

Reproductive diseases cause significant losses in cattle. Biomarker studies using omics approaches will lead to improved diagnostic tools and better management and targeted treatment of reproductive diseases in cattle. The increasing use of integrated data or multi-omics from transcriptomics, proteomics, metabolomics and metagenomics are helping researchers to understand the molecular landscape of reproductive diseases to identify significant biomarkers in cattle without limiting specific targets which may be overlook when using traditional approaches. However, traditional approaches are needed for the validation of identified biomarkers in larger populations including longitudinal studies of reproductive diseases of cattle. Future research could focus on linking biomarkers for infectious reproductive pathogens present in bull carriers which may influence the development of reproductive diseases in cows.

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Hannah Siddle: Writing – review & editing, Validation, Supervision. **Chian Teng Ong:** Writing – review & editing, Methodology. **Mehrnush Forutan:** Writing – review & editing, Supervision. **Ali Raza:** Writing – review & editing, Supervision. **Gry Boe-Hansen:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Sogra Banu Juli:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Ala Tabor:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Appendix A. Supporting information

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