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Research Paper

Subfertility in Buffaloes and the Association of Detected Milk Microbes

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ABSTRACT

Lactogenesis is a complex physiology and an outcome of a successful reproductive cycle in mammalians. The purpose itself is to nourish the newborn with the nutrients necessary for its protection and growth a continuation from the in utero survival strategy. Thus, mammary development and physiology are intriguingly linked to the uterine changes that happen in a normal reproductive individual. Milk is a highly nutritious food that also favours the growth of microorganisms that can enter through the bloodstream or the discharge system. Briefly, the mastitis-induced release of inflammation mediators such as cytokines, interleukins, and prostaglandin F2α are established to be associated with infertility. Before breeding, mastitis can disrupt hormonal patterns (depression of estradiol production, delayed surge of luteinizing hormone) and delay ovulation. The issue of clinical mastitis is obvious but when subclinical mastitis persists for long the problem gets compounded. Thus, microbes get easily established in the mammary glands of buffaloes whose udder and teat anatomy, love for dirt, and swamp to wallow makes them more vulnerable exposing them to various kinds of pathogenic and opportunistic microbes. Buffaloes, by nature, have issues with silent heat, seasonal anestrus, more sensitive to direct radiation heat stress when faced with a pathological attack of infectious microbes in the milk chamber compromises their fertility.

Keywords: Bacterial Load, Infertility, Silent Heat, Sub-clinical Mastitis

Buffalo holds a significant role in the world economy, as human prerequisites of proteins of high health benefit could be fulfilled by milk and meat accessibility coming from bison species, addressed in the world by more than 200 million heads (Borghese, 2017; Minervino *et al.* 2020). *Bubalus bubalis*, the domestic buffalo, also known as water buffalo or Asian buffalo includes two subspecies: the river buffalo (*B. bubalisbubalis*) and the swamp buffalo (*B. bubaliskerebau*) and is distributed in 77 countries in five continents (Minervino *et al.* 2020).

Buffalo milk is characterized by its high solids content and is a rich source of lipids, proteins, lactose, and minerals. Buffalo milk has long been valued for its important chemical composition because it determines the nutritional properties and applicability of traditional and industrial

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dairy products (Ahmad *et al.* 2013). Buffalo milk accounts for more than 12% of the world's annual milk production, second after cow milk (El Debaky *et al.* 2019).

Compared to cow milk, buffalo milk contains a higher content of nutrients, which gives it a rich taste and flavour, making it very suitable for the production of various dairy products, such as cheese, butter, ice cream, and yogurt (Nanda and Nakao, 2003). Like any raw milk, being a highly nutritious food, it favors rapid microorganism multiplication in it (Han *et al.* 2007). Microbiological quality of buffalo milk that can be induced mastitis are found to be *coliform* bacteria, *Escherichia coli*, lactic acid bacteria, *Listeria monocytogenes*, yeasts, moulds, *Staphylococcus spp*. and *Clostridium spp*. (Hussain and Akhtar, 2011). This article aims to focus on the role of detected milk microorganisms in affecting the fertility of Buffalo (Fig. 1).

Mastitis, affection of the mammary gland, is usually accompanied by physical, chemical, and bacteriological changes in milk and pathological changes in glandular tissue (Cressierand Bissonnette, 2011). Mastitis remains the most expensive disease in the world for dairy farmers causing a drop in milk production, increases in the cost of treatment, and culling process (Dhakal, 2002). In terms of economic losses, mastitis is considered to be the top three threats faced by farmers (Bhattarai *et al.* 2020). Although the symptoms of subclinical mastitis are not obvious, milk production can decrease by up to 20% per infection season (Blosser, 1979). Due to the affected quarters of animals, subclinical mastitis can reduce total milk production by two-thirds (Madutand Abdelgadir, 2011). Therefore, subclinical mastitis is a major problem for dairy animals raised in various rearing systems and deserves attention due to its potential impact.

Bacterial load in Milk and Mastitis

Buffalo raw milk, being a highly nutritious food, microorganisms can multiply rapidly in buffalo milk due to its high nutrient content. The microbiological quality of buffalo milk has revealed the presence of both Gram-positive and Gram-negative aerobes and anaerobes (Han *et al.* 2007). These microorganisms

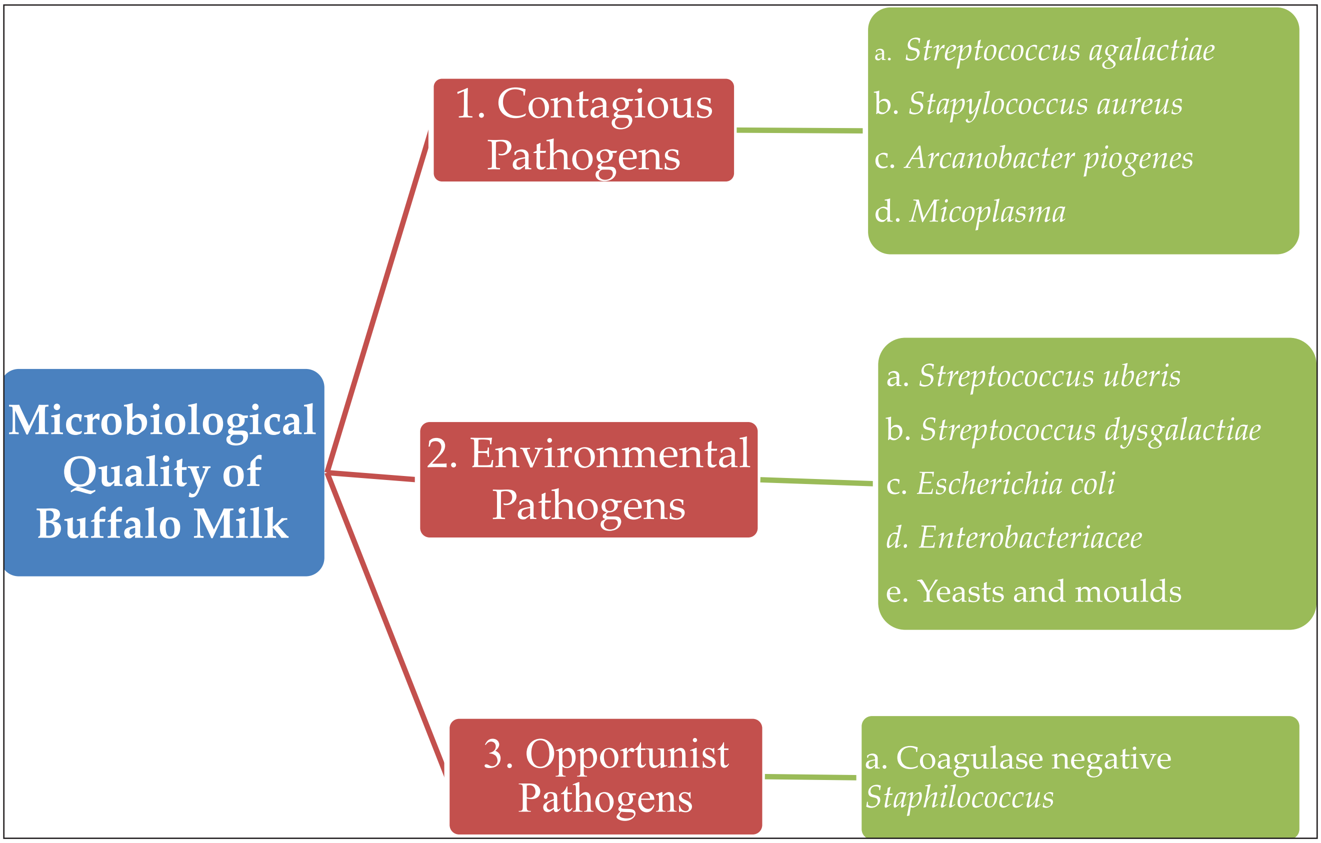

Fig. 1: Microbiological quality of Buffalo Milk (Fagiolo and Lai, 2007; Salvador *et al.* 2012)

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multiply and may enter through the bloodstream or get discharged into the system. Both clinical mastitis and subclinical mastitis are encountered in buffalo (Bhattarai *et al.* 2020). Clinical mastitis is characterized by sudden onset, swelling, and redness of udder, pain, and reduced and altered milk secretion of affected quarters. Milk may have clots, flakes, or water in consistency and is accompanied by fever, depression, and anorexia (Khan and Khan, 2006). While subclinical mastitis is characterized by no obvious signs in the udder and milk, but milk yield can drop as much as 20% per infected quarter, and somatic cell count increases, having a greater impact in older lactating animals than in the first lactation one (Radostits *et al*. 2007).

Subclinical mastitis can be diagnosed by Somatic Cell Counts (SCC), California Mastitis Test, Whiteside test (WST), or Surf field mastitis test (SMT). Among them, SCC is a measure that is widely used to assess mammary health (Ali *et al*. 2011). Milk SCC includes all types of cells: polymorphonuclear leukocytes (PMN), macrophages, and lymphocytes. The SCC increase is mainly due to the PMN increase. The main function of PMNs is to ingest and destroy invading microorganisms and to secrete inflammation regulators (Tripaldi *et al.* 2010). The number of SCCs found in subclinical mastitis ranges from 200,000 to 1.2 million, while the number of SCCs found in clinical mastitis exceeds 1.2 million (Hagnestam-Nielsen *et al.* 2009). Clinically detectable and treatable diseases have less risk to future fertility performance than undetected subclinical diseases of longer duration (Azawi, 2010). Subclinical mastitis in early lactation prolongs the interval to the first insemination, while clinical mastitis has no significant effect on reproductive performance. Therefore, subclinical mastitis should be diagnosed on the farm to improve the loss from disease (Klaas *et al.* 2004).

Bacterial Intervention and Mechanisms of infertility

Fever was associated with reduced feed intake and deterioration in the physical condition of the animal (Kadzere *et al.* 2002). Buffaloes with clinical mastitis may have a high fever, which may make their condition worse, and their negative energy may be more pronounced which could delay the resumption of ovarian cyclicity (Japheth *et al.* 2021). Mastitic buffalo with bacterial loads causes inflammation of the mammary gland, leading to the release of inflammatory mediators such as cytokines (especially TNF-α), cortisol, prostaglandin F2-α, reactive oxygen species (ROS), and classic inflammatory mediators (H. Verma *et al.* 2018). Fertility disorders in those mastitic buffaloes are mostly connected with the activity of these inflammatory mediators (Malinowski and Gajewski, 2010). Maturation of bovine oocytes in the presence of tumor necrosis factor (TNF- α) reduced the proportion of fertilized oocytes that develop into the blastocyst stage (Soto *et al.* 2003). Increased secretion of prostaglandins leads to premature luteal dissolution, which results in embryonic/fetal death (Gasparrini, 2019). Embryos developed in the presence of TNF-α, or prostaglandin F2-α, had either increased numbers of apoptotic cells (dead cells) or impaired development of blastocysts (Bhardwaj *et al*. 2016; Verma *et al,* 2018). Exposure of mastitic buffalo to endotoxins secreted by gram-negative bacteria can lead to increased blood levels of cortisol, a hormone that prevents the release, and Luteinizing Hormone (LH) peak (Azawi, 2010). Even after the etiological agents are fixed, there's a long-term effect on pregnancy loss. The fertility index of buffalo with persistent bacterial load in the mammary gland is lower as the time from parturition to the first insemination was longer as well as the service period and gestation period of calving (Ghani *et al.* 2019).

Role of Bacterial Load of Milk in Infertility of Buffalo

Before conception, mastitis can also alter hormonal patterns, such as inhibiting estradiol production, thereby delaying the rise in luteinizing hormone, which in turn delays ovulation, thus affecting fertility (Fig. 2) (Al-jabri *et al.* 2019; Mansour *et al.* 2016).

The bacterial load reduces the appearance of natural estrus and the ability to conceive after reproduction (Mansour *et al*. 2017). It also increases the tendency to miscarriage. The bacterial load has a time-dependent and destructive effect on conception rates 10 days before artificial insemination and 30 days after artificial insemination (Mansour *et al.* 2016; Shrestha *et al.* 2021; Verma *et al*. 2018). Water buffalo are seasonal breeders and exhibit silent

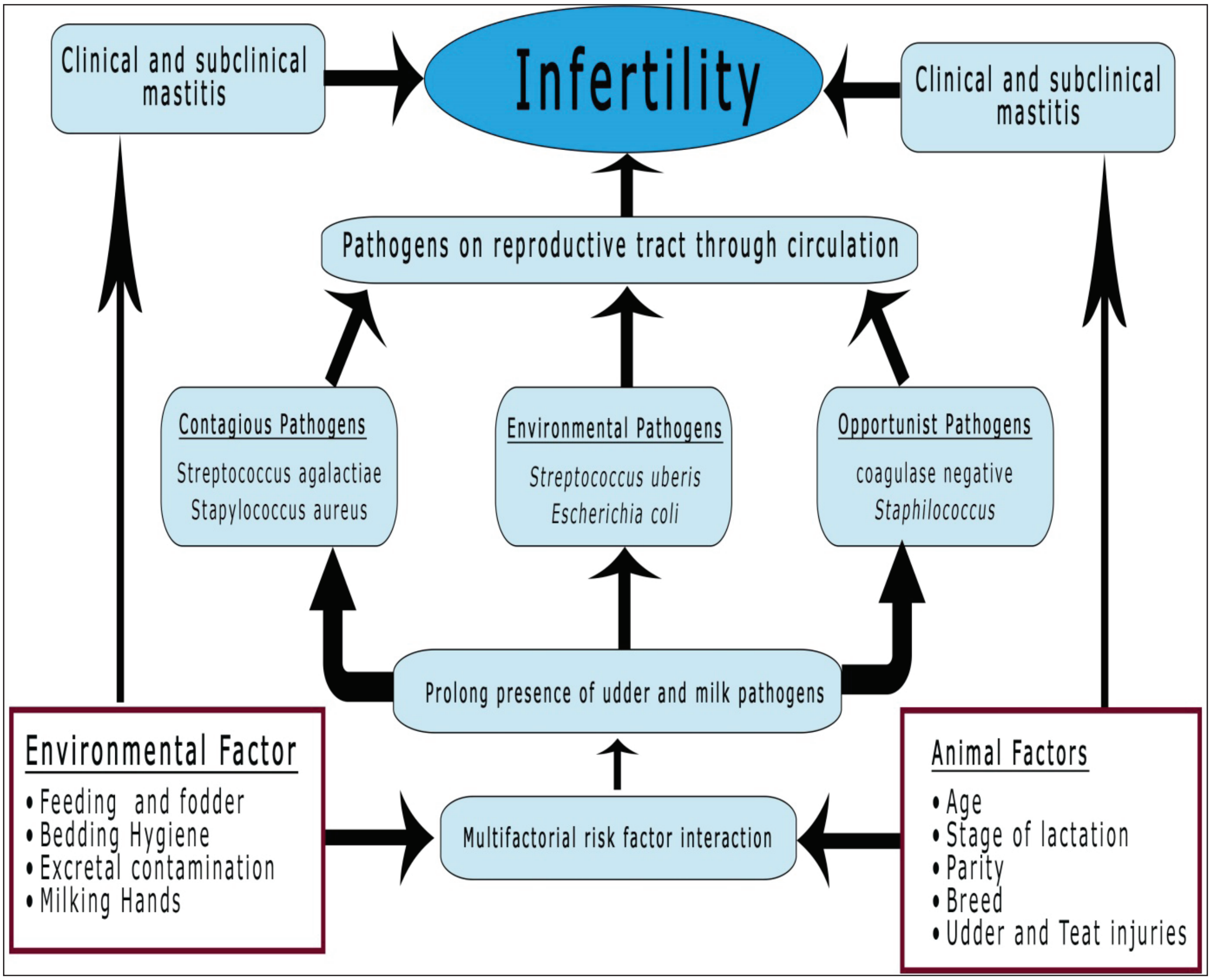

Fig. 2: Relation between infertility and bacterial load on milk

heat that is more sensitive to direct radiant heat stress (Phogat *et al*. 2016). Environmental factors along with pathological attacks by infectious microorganisms in the milk chamber compromise fertility (Ali *et al*. 2009).

Effect on Corpus Luteum

Luteal size and progesterone (P4) secretion are important indicators for buffalo's functional corpus luteum (CL) (Rahaman *et al.* 2012). CL development and function differ between pregnant and non-pregnant buffalo (Vecchio *et al*. 2012). A high premature luteolysis can lead to a short luteal phase induced by Gram-negative bacteria (such as *E. coli*), which alters inter-estrus interval (Bantawa *et al.* 2019). While, Gram-positive bacteria (such as *Staphylococcus aureus*) can reduce progesterone levels, which ultimately reduce CL function (Mansour *et al*. 2016). Increased prostaglandins and tumor necrosis factors lead to early regression of the CL after artificial insemination (AI), results in pregnancy termination (Wakayo *et al*. 2014).

Effect on Ovulation

Before mating or AI, the bacterial load significantly reduces the secretion of pulsatile luteinizing hormone secretion that induces low secretion of estradiol production close to estrus, delays luteinizing hormone surge and ovulation (Dahiya *et al*. 2018). Inhibition to the estradiol production results in the slow growth of ovarian-dominant follicles and reduces the number of ovulations which leads to reduces productivity and increases economic loss (Srinivasan *et al*. 2021). In nearly one-third of bacterial infections, there is a low or delayed LH surge before ovulation due to low secretion of estradiol (Kumar *et al.* 2018). Summer heat effect on fertility of buffaloes and seasonal anestrus is new area of immense interest (Kaphle 2021).

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Effect on Oocyte Competence

Prostaglandin as well as tumor necrosis factor produced during bacterial infection reduces the percentage of blastocyte formation (Soto *et al*. 2003). Once the oocyte is fertilized, if the bacterial load increases, the tumor necrosis factor will increase the number of apoptotic blastomeres (Malinowski and Gajewski, 2010). The follicular fluid of gram-negative and gram-positive bacterial toxins reduces the rate of cleavage and blastocyte formation of an embryo (Azawi, 2010).

CONCLUSION

Milk is a noninvasive biological specimen that can mirror physiology, pathology and clinical status of an individual. Assay of milk for endocrine profile can come close to what is circulating in the body. Milk which is a very nutritious food source is prone to invasion by various types of microbes. These microbes also reflect clinical and subclinical disease status like this and they are associated with fertility status in an individual. Thus, detection of the microbes, their distribution and load can speak volumes of the reproductive capacity. Buffalo which in itself is a trouble maker when it comes to regular reproductive cycle due to silent heat and seasonal anestrus stands to benefit from regular milk microbe detection protocols.

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