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Growing role of fungi in the reproductive disorders of animals

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Abstract
Fungi are implicated in various clinical disorders of humans and animals. Reproductive problems caused by fungi are a significant concern for animals, especially domestic animals. Common reproductive disorders in domestic animals include endometritis, pyometra, retained placenta, abortion, anestrous, uterine and vaginal prolapse, and repeat breeding. Abortions in domestic ruminants cause significant economic losses for farmers. Various fungi species have been reported in numerous countries, such as Hungary, England, Germany, Israel, Poland, India, New Zealand, and the USA. *Aspergillus* species and *Candida* species are the most commonly associated fungi with abortion. Aspergillus fumigatus is responsible for over 70% of mycotic abortions recorded in cattle worldwide, although the exact mode of transmission of the infection is not known. Predisposing factors for mycotic abortion include moldy fodder or silage and the confinement of pregnant animals in humid, hot, old, and unhygienic houses. At present, there is no effective antifungal treatment against reproductive failure in bovines. Treatment of hay with a suitable fungicide during haymaking and avoiding moldy silage or hay feeding to pregnant animals is essential to prevent fungal reproductive problems. This review focuses on the emerging role of fungi in reproductive disorders in domestic animals.

Keywords: Abortion, animals, aspergillus species, candida species, endometritis, fungi, reproductive disorders

1. Introduction
For livestock production to be effective, reproduction is a prerequisite. Profitable animal production requires constant and effective reproduction since an animal's reproductive performance determines most of its productivity (Getahun *et al.*, 2021) [14]. Large animals are not worth maintaining if they don't often give birth to live calves. Reproductive efficiency is therefore, a valuable advantage for getting the most out of the animal. Reproductive disorders are the major cause of declining reproductive efficiency in cattle production, which is the primary determinant of overall productivity and profitability (Abdisa, 2018) [1]. Major reproductive disorders in domestic animals include endometritis, pyometra, retained placenta, abortion, anestrous, uterine, and vaginal prolapse, and repeat breading. These problems have a significant impact on the reproductive efficiency of the animal (Abdisa, 2018) [1]. Infectious agents are a major cause of reproductive disorders, and have received high priority in the bovine industry (Yoo, 2010) [42].
Among various infectious agents, fungi are known for their direct impact on the reproductive health of animals, and many fungal species are associated with common animal pathogens. *Aspergillus* species and *Candida* species are commonly linked to abortion in bovines. Fungi cause reproductive disorders in animals by either invading or colonizing the reproductive tract or being ingested; leading to the absorption of mycotoxins (Shokri and Yadollahi, 2017) [35]. The most significant effect of fungal infections of the reproductive tract is fungal miscarriage. However, fungi occasionally cause infection in other reproductive diseases such as vulvovaginitis, endometritis, and repeated intrauterine antibiotic therapy (Stout, 2008) [38].
Fungi are the most common cause of bovine abortions worldwide and are associated with 1-25% of all bovine abortions (Pal *et al.*, 1985) [24]. The exact route of infection is unknown. Although fungi are widespread in the soil, animal waste, and even the vegetative parts of plants and can enter the animal reproductive tract through these, the most common source of infection is skin or feces (Stout, 2008) [38]. To date, there is no effective antifungal treatment against mycotic abortion and endometritis in bovine. The management of fungal abortion depends on avoiding the feeding of moldy feed to pregnant animals and keeping them in hygienic, well-ventilated houses (Pal, 2007) [28].

Therefore, this paper delineates the growing role of fungi in reproductive disorders, particularly emphasizing on their etiology and prevention in domestic animals.

2. Literature review

2.1. Etiology

Fungi are relatively uncommon causes of disease in healthy and immune-competent humans and nonhuman vertebrates, even though hosts are constantly exposed to infectious propagules (Kohler *et al.*, 2015) [18]. Fungal reproductive problems are a serious concern among animals, especially bovines. Several species of fungi have been reported in many countries as causative agents of fungal reproductive disorders (Table-1). Mycotic abortion or mycotic placentitis is caused by different species of fungi, including molds and yeasts. The most common etiological agents involved in mycotic abortion are *Candida* species and *Aspergillus* species (Holler, 2012; Mee, 2020) [16, 19].

Table 1: Some of etiological agent of mycotic abortion and endometritis in animals

Genus	Species	References
Moulds		
Aspergillus	*A. fumigatus, A. Terreus, A. flavus, A. niger, A. wentii, A. nidulans, A. versicolnr*	(Ali and Khan, 2006; Pal *et al.*, 1985; Pal, 2007; Pal, 2015; Ozavci and Kirkiran, 2014) [5, 24, 28, 27, 21]
Absidia	*A. corymbifera, A. ramose*	
Mortierella	*M. wolfii, M. asterodies, M. zychae*	
Rhizopus	*R. pusillus, R. arrhizus, R. boyinus*	
Mucor	*M. psillus*	
Rhizomucor	*R. pusillus*	
Curvularia	*Curvularia geniculata*	
Emericella	*Emericella nidulans, E. rugulosa,*	
Exophila	*Exophila jeanselmei*	
Fusarium	*Fusarium* spp.	
Penicillium	*Penicillium* spp.	
Paecilomyces	*Paecilomyces* spp.	
Pseudoallescheria	*Pseudoallescheria boydii*	
Wangiella	*Wangiella dermatitidis*	
Lecythosphora	*Lecythosphora hoffmannii*	
	Yeasts	
Candida	*C. albicans, C. etchesii, C.krusei, C. rugosa, C. guilliermondii, C. glabrata, C. zeylanoides, C. norvegica, C. viswanathii, C. tropicalis, C. catenulata, C. kefyr, C. maltosa, C. parapsilosis, C. pseudotropicalis, C. lusitaniae*	(Blanchard and Filkins, 1992; Hassan *et al.*, 2013; Pal, 2007; Villarroe and Maggiulli, 2012) [7, 15, 28, 40]
Cryptococcus	*C. neoformans, C. laurentii, C. gatii*	
Geotrichum Torulopsis	*Geotrichum candidum, Torulopsis glabrata*	

2.2. Species affected

Reproductive problems caused by fungal pathogens have been diagnosed in many animal species, such as cattle, buffaloes, goats, sheep, horses, and pigs from different countries around the world (Akange *et al.*, 2013; Al Humam, 2014; Pal, 1988) [3, 4, 26]. The infection is most commonly seen in dairy cattle (Pal, 2007) [28].

2.3. Major fungal reproductive disorders in animals

Reproductive disorders cause enormous economic losses in dairy cattle production worldwide due to various reasons such as loss of replacement animals for the herd, longer inter-calving periods, decreased milk production, increased open days, additional costs for veterinary inputs, and unnecessary culling (Yoo, 2010) [42]. Reproductive disorders in domestic animals include early embryonic death, abortion, stillbirth, anestrous, dystocia, endometritis, pyometra, retained placenta, uterine and vaginal prolapse, repeat breeding, and increased calving interval. Mycotic abortion is among the most common disorders in the dairy industry in many parts of the world (Pal, 2007) [28], and endometritis has occasionally been discovered in reproductive illnesses (Pal, 1990; Shokri and Yadollahi, 2017) [23, 35].

2.3.1. Mycotic abortions

A fungal abortion results in significant financial losses for both the individual farmer and the cattle-breeding industry at large. One of the most frequent causes of sporadic abortion in cows is a mycotoxin infection of the placenta (Ali and Khan, 2006) [5]. The global prevalence of fungal abortion in cows varies between 2-20%. Several species of yeasts and fungi can induce mycotic abortion. *Aspergillus* spp. and *Candida* spp. are the fungi most frequently linked to abortion (Holler, 2012; Mee, 2020) [16, 19]. In this context, Pal (1998) [26] is credited to elucidate the etiologic role of *A. niger* in the abortion of buffalo for the first time in the world.

2.3.2. Mycotic endometritis

Endometritis is defined as an inflammation of the uterine mucous membrane characterized by a mucoid, muco-purulent, or purulent discharge from the vulva that develops three weeks or later after parturition (Sheldom *et al.*, 2008) [34]. As an opportunistic pathogen, fungus is more likely to induce uterine infection when specific risk factors are present, including immunosuppression, pneumovagina, extended intrauterine antibiotic treatment, and chronic endometritis. Several authors have observed varying incidences of fungal endometritis in repeat breeder cows, ranging from 10.5% to 33.3% (Sharma and Singh, 2012; Ramsingh *et al.*, 2013) [33, 31]. In another study, the incidence of pathogenic fungal infections was 17.98% in repeat breeding buffaloes and cattle (Ahmed and Bhattacharyya, 2015) [2]. *Aspergillus terreus* was identified as the cause of endometritis in cattle for the first time in Gujarat, India (Pal *et al.*, 1990) [23]. The first record of equine

endometritis due to *Candida albicans* in Gujarat, India was documented by Pal and co-workers, 2002) [25].

2.4. Fungal pathogens important for reproductive disorders in animals

2.4.1. *Aspergillus* species

Aspergillus fumigatus is a common cause of reproductive disorders that can lead to devastating consequences, such as abortions, and only rarely by other species. *Aspergillus fumigatus* is the most commonly diagnosed causal organism (62%), followed by zygomyectes, which account for 21% of the cases (Pal, 2015) [27]. Pal *et al.* (1990) [23] also reported that *Aspergillus terreus* is the cause of endometritis in cows. This fungus proliferates in decomposing hay, poorly preserved silage, and soil and produces a non-airborne, pathogenic spore. Abortion occurs when fungal spores enter a pregnant cow's bloodstream, settle at the junction of the maternal and fetal placentas, and grow and attack the placental tissues (Walker, 2007) [41].

The mycotic abortions were confirmed by the isolation of *Aspergillus fumigatus* fungi from moldy hay as well as from fetal Abomasal contents (Parthiban *et al.*, 2015) [29]. Pregnancy in a cow with metabolic derangements from stress may predispose the pregnant cow to fungal infection. The incidence of the condition is high in late summer or early autumn due to the presence of a large number of fungal spores in pastures during this period (Ali and Khan, 2006) [5].

2.4.1.1. Clinical signs and diagnosis

The clinical manifestations of aspergillosis depend on the infective dose, spore distribution, pre-existing diseases, and the host immune response (Dahlhausen *et al.*, 2004) [10]. Any condition that reduces the cow's resistance to infection increases the chances of mycotic abortion. The spores localize to the uterine caruncle and induce inflammation-induced abortion after 2-5 weeks of proliferation. On gross examination, the placenta often has swollen, necrotic cotyledons, and the intercotyledonary membrane appears diffusely thickened, wrinkled, and resembling leather (Figure-1). Occasionally, aborted fetuses have characteristic fungal plaques, 1-10 cm in diameter, involving the skin around the eyelids, neck, dorsum, and thorax (Cordes and Shortridge, 1968) [9]. This is due to fungal proliferation in the amnion, penetration of the epidermis, the fetal inflammatory reaction as well as hyperkeratosis. *Aspergillus* abortion can be diagnosed by the identification of fungal colonies that grow on culture media and histologically by placental changes with visible fungal hyphae confirming infection (Garcia *et al.*, 2008) [13].

Source: (Prameela, 2019; Otter, 2020) [20, 30]

Fig 1: Mycotic abortion and placentitis. (a) Mycotic abortion and placenta in cattle caused by *Aspergillus* spp. (b) Portion of placenta showing thickened cotyledons infected with *Aspergillus* spp. in a cow.

2.4.2. *Mortierella wolfii*

Frequent reports of *Mortierella wolfii* have been made on the North Island of New Zealand and occasionally in Australian cattle (Gabor, 2003) [12]. Pregnant cows that have ingested silage that has not been adequately dried before use have been linked to the appearance of *M. wolfii* infection. If contaminated silage is inhaled, the spores can enter the pulmonary vascular bed and the arterial circulation. In almost 20% of cases, pneumonia that proves deadly occurs 4-5 days after the abortion (Cordes *et al.*, 1972) [8]. Similar in pathogenesis to *A. fumigatus*, *M. wolfii* grows in the uterine caruncle, causing extensive tissue necrosis and inflammation leading to placentitis and abortion following hematogenous spread. This fungus is responsible for about 6.8% of bovine abortions in North America and 46% in New Zealand (Knudtson and Kirkbride, 1992; Pachauri, 2013) [17, 22]. In a study, Krudtson and Kirkbride (1992) [17] also mentioned that mycotic endometritis suggested that cows should be aborted before encephalitis developed.

2.4.2.1. Clinical signs and diagnosis

Mycotic abortion usually occurs in the third trimester of pregnancy, and clinical signs in the dam are frequently observed apart from the retention of the placenta. Grossly, the placenta appears thickened, edematous, and necrotic. It is important to monitor for any clinical signs of *M. wolfii* infection in cows, especially after abortion. While other clinical signs may not be present, infected cows can develop post-abortion pneumonia and may even die within 72 hours after abortion. Experimentally, after intravenous injection of *M. wolfii* spores, mycotic placentitis was seen in cows in the early stages before abortion. Red infarcts were present in the caruncle and after abortion; most caruncles were uniformly dark red and full of hyphae (Ozavci and Kirkiran, 2014) [21].

The diagnosis of mycotic abortion is associated with the presence of fungi, which include placental lesions that are diffusely thickened, necrotic, hemorrhagic, and dermatitis. A small portion of fetal skin, placental tissue, fetal internal organs, or abomasal contents are examined by using

potassium hydroxide (Roland and Triber, 2003) [32]. For the identification of *M. wolfii*, the samples are subcultured on saline and dextrose agar and incubated at room temperature for 3 days. After staining with lactophenol cotton blue dye and sticky tape preparation, mycelium can be seen under a light microscope (Ozavci and Kirkiran, 2014) [21].

2.4.3. *Candida* species

Candida species are opportunistic fungi that are commonly found in the mouth, vagina, and digestive tracts of humans and many domestic animals. Tissue damage and extensive or protracted antimicrobial treatment have been linked to fungal invasion-induced illness (Arné and Lee, 2020) [6]. Compared to reproductively normal cows and buffaloes, there is a higher prevalence of *Candida* spp. isolation from cervical mucous cultures of cows with fertility issues (Sinha *et al.*, 1980) [36]. *Candida*-induced endometritis has been documented in equines and has been linked to early embryonic death and infertility. Mycotic abortions are a common but occasional occurrence in a wide range of animals, primarily due to *Candida albicans*; however, other *Candida* spp. and yeasts have also been seen in animal cases. There have been reports of fungal abortions caused by *Candida* spp. in various countries including Hungary, England, Germany, Israel, Poland, India, New Zealand, and the USA (Foley and Schlafe, 1987) [11]. Some of the species of *Candida* mentioned in later studies include *Candida albicans*, *Candida krusei*, *Candida tropicalis*, and *Candida parapsilosis*.

2.4.3.1. Clinical signs and diagnosis

Candida species normally inhabit the mucosa, skin, nails, eye, joint, bone, digestive tract, respiratory tract, genital mucosa, and mammary glands of animals (Pal, 2002) [25]. Under normal conditions, these organisms may transform into pathogens, causing different infections, and the clinical signs observed in animals due to fungal infections are non-specific and may result in abortions. The diagnosis of fungal infection is made by identifying the fungus through culture of the placental or fetal tissues, examining these tissues under a microscope, or directly examining cotyledons after clearing with potassium hydroxide solution. Grossly, the placenta appeared thickened and covered by a dense exudate with necrosis (Figure- 2) (Stefanetti *et al.*, 2014) [37]. To isolate *C. albicans*, the sample should be cultured on Sabourand's dextrose agar with chloramphenicol. After incubating for 3 days at 37°C, colonies of the fungus appear whitish, shiny, and convex, with a diameter of 4 to 5 mm (Prameela, 2019) [30].

Source: (Stefanetti *et al.*, 2014) [37]

Fig 2: The chorionic surface of the placenta is covered by thick brown exudates and yellowish necrotic areas due to *Candida* abortion

2.5. Control and prevention

At present, there is no effective antifungal treatment against mycotic abortion and endometritis in animals; rather, supportive therapy is the mainstay of care. Management practices to reduce fungal reproductive disorder include: Avoiding mold silage or hay feeding to pregnant animals is essential. Fungal spores can pose a serious risk to pregnant cows, leading to potential health issues such as mycotic abortion. The dust has been shown to consist chiefly of fungal spores of various types, but more especially the spores of mycotic abortion. It is crucial to provide clean and dry feed to ensure the well-being of the animals (Prameela, 2019) [30]. Regularly inspecting and maintaining the quality of silage and hay can help prevent exposure to harmful spores and protect the health of the herd. To prevent further mold growth, hay should be treated with an appropriate fungicide throughout the haymaking process. Additionally, it is best to avoid housing animals in very small places since there is evidence that the air in overcrowded cowsheds has a high concentration of fungal spores, which can lead to miscarriages. When pregnant animals are kept in an open barn, they may be fed loose hay. Mycological testing of the artificially inseminated semen is essential to determining whether fungi are present. The frequency of mycotic abortions in domestic animals might decrease with the use of these steps (Pal, 2007) [28].

3. Conclusion and Recommendations

Fungi play a crucial role in animal reproductive health, with fungal abortion posing significant concerns for both farmers and the cattle-breeding industry. These abortions result in substantial economic losses. They are a leading cause of bovine abortions worldwide, accounting for 1-24.9% of cases. Fungal endometritis affects 10.5% to 33.3% of repeat breeder cows. *Aspergillus* spp. and *Candida* spp. are commonly associated with abortion. As the non-specific

clinical signs of fungal abortions indicate, laboratory diagnosis is crucial for confirmation. Prevention strategies such as proper ventilation, regular cleaning of animal housing, and avoiding mold silage or hay feeding to pregnant animals can help reduce the risk of fungal reproductive problems. Based on the above conclusion, the following recommendation will be forwarded: Effective anti-fungal medicines for reproductive disorders in domestic animals should be developed. Antibiotic infusions should not be used repeatedly, as this might increase the risk of fungal endometritis. It is emphasized to conduct further research on the epidemiology and economic impacts of fungi in various reproductive disorders of domestic animals.

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