

## Review Article

## Causes of Infertility in Bitch

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**Abstract** | The dog is considered as an important companion animal. The dogs perform a significant role in different departments also. For example police assistance, murder cases and identification of addicted medicines at airports. These functions can be performed only by healthy dogs. The infertility adversely affects the health of female dogs. The infertile bitches are unable to produce healthy and live puppies. The fertility of bitch requires the normal estrus cycle and ovulation within the reproductive tract followed by the union of sperm. The developed embryo must implant in the endometrium of uterus till the completion of pregnancy period and successful parturition. There are many causes responsible for the infertility in female dog. The common categories of infertility are structural, physiological, neoplasm and infectious agents. The objective of this review was to highlight the emerging causes of infertility.

**Keywords** | Bitch reproductive system, Infertility and estrus cycle

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## INTRODUCTION

Due to increased appreciation as a pet animals and increase in demand of high priced purebred dogs, now owners frequently consult their veterinarian regarding aspects of fertility. The causes of fertility in bitch are variable due to many factors. The normal fertility of bitch requires the fertilization of sperm within the fallopian tube of female reproductive tract and implantation in uterus till the delivery. The common causes of infertility in bitch are structural, physiological, neoplastic and infectious. The structural causes of infertility in bitch hamper in the fertilization or characterized by ambiguous genitalia in bitch. The second most important cause of infertility in bitch is physiological abnormalities. These abnormalities are manifested by unpredictable ovulation, split heat, silent heat and long anoestrus. The infectious causes of infertility which are transmitted at the time of estrus

or during coitus prevent the bitch from carrying the normal pregnancy. The brucellosis is a venereal disease that causes the infertility by abortion of fetus or loss of conception. Uterine infections can contribute to infertility, such as endometritis, pyometra/pyometritis and metritis (Kida et al., 2006). Despite good breeding management there is still many reasons that can result in infertility. In this review an effort is made to summarize various causes of infertility in bitch.

## STRUCTURAL AND PHYSIOLOGICAL CAUSES OF INFERTILITY

The structural abnormalities (congenital) including ovarian dysplasia associated with abnormal number of chromosomes (Jhonston et al., 1985) cause the infertility in bitch. The exogenous use of hormones during pregnancy causes the segmental aplasia of mullerian duct and absence of connection between mullerian ducts and urogenital sinus in fetus which

results in lifetime infertility (Christiansen, 1984). Complete aplasia of vagina causes the infertility (Wadsworth et al. 1978; Hawe and Loeb 1984) and the uterine fluid is accumulated like pyometra. The intersex animals have ambiguous genitalia and associated with abnormalities of chromosomes, gonads and phenotypic sex (Meyers-wallen, 1993). Aneuploidy which is chromosomal abnormality at meiosis and mitosis resulting into the product trisomy and monosomy is also an important cause of infertility in bitch. Many cases of aneuploidy intersex (XXY trisomy and X monosomy) are discovered in dogs (Mellink and Bosma, 1988; Meyers-Wallen, 1993; Goldschmidt et al., 2001; Goldschmidt et al., 2002), but the two cases of X trisomy have been described in the dogs (Johnston et al., 1985; Switonski et al., 2000). The bitch fails to exhibit the estrus at 24 months of age is called primary anestrus (Johnston et al., 1991). Primary anestrus can be caused by hypothyroidism, drug induced anestrus, debilitating diseases, chromosomal and ovarian abnormalities (Johnston et al., 2001). The level of progesterone in blood is greater than 2.0 ng/mL on monthly basis is an indication of functional ovary (Clark and Stainer, 1983). The exogenous use of drugs like progestin can be used for the inhibition of estrus cycle in bitch (Olson et al., 1992) which can be diagnosed by taking the history from honour. The ten percent bitches with hypothyroidism represent the anoestrus (Johnston et al., 2001, Johnson et al., 2002) and it is reversible with the use of L-thyroxin (Bell et al., 1971; Raymer et al., 1984). The ovarian abnormalities (agenesis and oophoritis) are responsible for anoestrus (Johnston et al., 2001). The exact diagnosis requires the histopathology of ovarian tissues (Bell et al., 1971, Roberts et al., 1986).

The secondary anoestrus or elongation of interestrus interval is the failure of start of estrus cycle by 10 to 18 months of the preceding estrus cycle (Rosychuk et al., 1983, Peterson et al., 1997). The secondary anoestrus can be caused by hypothyroidism, stress and endocrine abnormalities. Persistent estrus comprises of proestrus and estrus with more than six weeks (Johnston et al., 1994) or mating for 21 to 28 consecutive days in one estrus cycle (Feldman and Nelson. 2004). The persistent estrus can also be defined as the presence of cornified cells of vaginal epithelium for 21 to 28 days due to production of endogenous estrogen or use of exogenous estrogen (Perkins and Thomas., 1993). The causes of persistent estrus are follicular cysts, tumour of granulose cells and exogenous use of estrogen

(Reimers et al., 1983) and idiopathic lymphocytic oophoritis (Jeffcoate, 1991). The level of estrogen in serum is not frequently elevated in persistent estrus (Freshman et al., 1991) and level of progesterone in serum stays in the preovulatory range (<2.0 ng/mL) (Freshman et al., 1991, Perkins and Thomas, 1993). The normal ineteretrous interval in bitch is 130-150 days (Reimers et al., 1983). The bitch becomes subfertile when this interval is less than 120 days (Peterson et al., 1997; Reimers et al., 1983). Breed has significant influence on duration of estrus interval (Reimers et al., 1983; Olson et al., 1989; Meyers-Wallen et al., 1992). Diseases like (cystic endometrial hyperplasia) also cause the short interval of estrus (Reimers et al., 1983; Nickel et al., 1991; Meyers-Wallen et al., 2007). The bitch shows estrus behaviour without ovulation in proestrus followed by short anestrus of few days and then normal estrus cycle in split heat (Jhonston et al., 2001).

The insufficient release of gonadotropin may be the cause of split heat (Sokolowski et al., 1977). Split heats confuse with short estrus interval but the level of progesterone does not exceed from 4-6ng/ml in split heat and no ovulation takes place (Feldman and Nelson, 2004). Anovulation is failure of ovulation in consequence the concentration of progesterone fails to exceed from 4-8 ng/ml during the estrus cycle (Freshman, 1991). The anovulation is also connected to split heat and clinical findings of anovulation are only one percent (Rogers et al., 1970; Olson et al., 1988). The insufficient release of LH from anterior pituitary gland is the suggested cause of anovulation (Freshman, 1991). The reported therapies for anovulation are GnRH or HCG (Allen et al., 1982) but the treatment can fail due to heredity. Hypothyroidism is also associated with infertility and is characterized by anestrus, irregularities in estrus cycle and longer proestral bleeding (Johnson, 1980; Nesbitt et al., 1980). The reproductive abnormalities associated with hypothyroidism are due to elevated level of prolactin in serum (Chastin and Ganjam, 1986). Diagnosis of canine hypothyroidism can be difficult, because the clinical signs are varied and not characteristic in many instances (Li et al., 1986). Combination of the variables free T4 and fasting serum cholesterol levels might have helped to confirm hypothyroidism in this case (Larsson et al., 1988). The pseudo-pregnancy a syndrome is commonly present in non-pregnant bitches after 6-12 weeks of estrus and characterized by the signs of vulvas swelling, lactation and mammary gland enlargement (Jones et al., 1988).

The pharmacological approaches including estrogens, progesterone and testosterone are used and more recently dopamine agonists (Gobello et al., 2001).

### NEOPLASMS (TUMOURS)

Neoplasias of vagina are the most widespread type of tumour of reproductive system of bitch (Manothaiudom et al., 1991). The important neoplasias of vagina are transitional cell carcinoma (TCC) of the urethra, transmissible venereal tumour (TVT), squamous cell carcinoma (SCC), leiomyoma, and fibro papilloma. These Vaginal tumors are commonly present in bitch of old age except the TVT which is usually present in young dogs. The age for the vaginal tumours in bitch is more than ten years of age. The bitch with vaginal neoplasia is clinically characterized by protrusion of mass, dysuria and continuous discharge from vagina (Manothaiudom et al., 1991, Olson 1984).

The usual diagnostic methods for these tumours are physical examination, complete blood history, palpation (digital examination), vaginoscopy, vaginal cytology and surgical biopsy. Vaginal cytology is most helpful in diagnosing TVT, TCC, and SCC. Transmissible venereal tumour is transmitted during coitus, contact of mucous membranes and licking the wounds of infected dog. The feature of metastasis is rare in these TVT but may involve lymph nodes and skin. The TVT are characterized by excessive bleeding from the genital organs along the cauliflower like growth on penis or vagina (Santos-do-Amaral et al., 2007). The TVT cells have unique circular nucleus with vacuolated cytoplasm and may regress without any sign (Manothaiudom et al., 1991). There is effective treatment of TVT with vincristine or irradiation (Rogers, 1997). Awan et al. (2014) treated the thirty dogs of TVT with surgical excision, vincristine sulphate and combination of both. The only vincristine sulphate was used with the dose of 0.025 mg/kg body weight for 6 weeks at alternative weeks whereas after the one week of surgical excision vincristine sulphate was used for three weeks with the interval of one week. The dogs with surgical excision showed 30% recurrence of TVT while dogs with only chemotherapy prolong the treatment for six weeks. The shortest duration of 4 weeks of healing was observed in the surgical excision along the chemotherapy. The chemotherapy treatments express different results according to their environmental conditions (Scarpelli et al., 2010) but the vincristine sulphate has good results with least recurrence and no side effects (Varughese et al., 2012). The common site for Transitional cell carcinomas and SCC is the ter-

iminal part of urethra of the bitch and can occupy the vagina or vestibule or both. Occasionally, fine-needle aspirates may provide a definitive diagnosis of TCC or SCC. Cells in TCC exhibit anisocytosis, anisokaryosis, and have an increased nucleus to cytoplasm ratio (Manothaiudom et al., 1991). The bitch has specific characteristic of epithelial neoplasms and about 40-50 % of these growths originate from the epithelial surface of ovary. The growth of these epithelium is most commonly papillary (adenoma or adenocarcinoma) or cystic (adenoma or adenocarcinoma) (Greenlee and Patnaik, 1985; MacLachlan et al., 2002).

One or both ovaries could be affected by papillary adenocarcinomas. The unique feature of these tumours is involvement of ovarian structures and metastasis in kidneys, lungs and mesenteric lymph nodes. Cystadenomas develop from the rete ovarii, commonly affecting one or both ovaries and composed of multiple thin walled cysts and filled with fluid (Greene et al., 1979, Herron., 1983). These tumours are responsible for various abnormalities in reproductive cycle such as anestrus, nymphomania, excessive growth of muscles and alopecia or they might be symptomless (Theilen and Madewell, 1979; Yamini et al., 1997). Yotov et al. (2005) reported cyst adenocarcinomas in an 11-year-old bitch which has clinical signs of fast exhaustion, swelling of abdomen and discharge from vulva. Although, the previous studies had not reported obvious clinical abnormalities (Greene et al., 1979,). The ultrasonographic findings were suggestive of cyst with intermittent vulvar discharges and increased number of white blood cells (Yotov et al., 2005). Ovariohysterectomy is the most effective treatment for such kind of tumour. Leiomyosarcomas are malignant tumours and grow slowly in muscles of liver, spleen, intestines, urinary bladder and uterus of domestic animals (Kapatkin et al., 1992). These tumours are not dependent on any breed for growth but old dogs are more susceptible for these tumours (Patnaik et al., 1977; Kapatkin et al., 1992). The uterine tumours are rare in canine and only 0.4% tumours are reported of all the tumours of dog. Leiomyosarcomas shares only 10 percent of tumours in older dogs and rarely associated with clinical signs (Kapatkin et al., 1992; Klein, 2001). The clinical signs manifested by uterine tumours are dependent on size of tumour and metastasis of disease. The tumours are linked with presence of hard mass along with vomiting, off feed, ascities at belly and weight loss (Morrow, 1986; Madewell and Theilen, 1987; Murphy et al., 1994). Uterine leiomyosarcomas are reported in bitch with abdominal disten-

sion, dyspnea and dullness due to its extended volume but without general symptoms in fourteen years old bitch (Serin et al., 2010). The exact diagnosis of leiomyosarcomas requires histopathology, ultrasonography and radiography examination (Murphy et al., 1994; Harvey, 1998) but a successful removal was obtained with surgery in short time (Serin et al., 2010).

### Bacterial Causes of Infertility

The diseases of uterus in bitch are directly linked with estrus cycle are categorized as major causes of infertility in bitch. Uterine infections can contribute to infertility, such as endometritis, pyometra/pyometritis and metritis (Kida et al., 2006). Despite good breeding management there is still many reasons that can result in infertility. Pyometra is also one of them and occurs at 4 to 16 weeks after estrus. The pyometra which is also called cystic endometrial-hyperplasia is the worst disease of uterus in bitch (Kida et al., 2006). Usual time for the development of pyometra is 20-70-days after the end of heat (Bigliardi et al, 2004). It is disorder of diestrus and characterized by the excessive proliferation of endometrium under progesterone with inflammatory cells in the layers of uterus (Zdunczyk et al., 2006). The uterine immunity is decreased under the influence of increase concentration of progesterone (Sugiura et al., 2004). The cystic ovaries, long estrus and neoplastic ovarian disorder are also the major causes of pyometra (Kida et al., 2006). The changes in the hormones of ovaries in blood and their receptors in uterus help in the adhesion of *E. coli* within the uterus (de Bosschere et al, 2002). The use of progesterone to stop the estrus stimulates the inflammatory response within the uterus (Noakes et al., 2001). De Cock et al, (2002) proposed that Insulin like Growth Factor 1 has higher concentrations in the epithelial cells of endometrium and helps in the formation of cystic endometrial hyperplasia. Hormonal imbalance and bacterial infections are the potential causes of cystic endometrial hyperplasia (Arora et al., 2006). In recent studies, relating with *E. coli* induced pyometra, the most susceptible time for pyometra is 11-21 days after LH peak (Tsumagari et al., 2005). The signs of pyometra are vaginal discharge due to degeneration of endometrium is mucoid and like tomato soup (Switonski et al., 2000). The other signs of pyometra are off feed, increase in body temperature and polyuria (Bedrica and Sacar, 2004; Fransson et al., 1997). On ultrasonography, the uterus looks fluid-filled (Bigliardi et al., 2004). The pyometra is commonly of two types; one is close and other open. The closed pyometra is characterized by the increase of body

temperature due to intoxication. The concentration of leukocytes is also increased from 15 000–60 000/mm<sup>3</sup> in the closed pyometra (Bigliardi et al, 2004). The histology changes are large cystic endometrial glands, bacterial colonies and degenerative changes in morphology of nucleus (Groppetti et al., 2010). The ovariohysterectomy is treatment of choice for the old bitches (MacIntire et al., 2004). The Young bitches with open pyometra, are treated with prostaglandin (PGF<sub>2</sub>α) on the complaint of owner for the continuity of reproductive activity (Gilbert et al., 1989). PGF<sub>2</sub>α is injected at the dose of 250 ug/ kg (very high dose need clarification) subcutaneously after every 12 h for 3-5 days (Meyers-Wallen et al., 1986) until the uterus regains its normal structure. Vaginitis is the inflammation of vagina in bitches regardless of age and breeds and characterized by purulent discharge from the vagina (Johnson, 1991). The vaginitis is sequel to some causes of inflammation which originate from the skin and perineal regions (Freshman et al., 1991; Jarvinen, 1981). The mucoid to purulent discharge is present in 80-90% cases of vaginitis (Parker, 1998; Johnson, 1991). The vulvar licking and urinary incontinence are also the common causes during the vaginitis (Cowell et al., 1999). The primary causes of adult vaginitis are; 26-60% urinary tract infections, 20-36% congenital and 15% are urinary tract infections (Parker, 1998, Johnson, 1991). Infectious causes result into typically vaginitis (Olson, 1984). The uniform growth of bacteria in vagina is common suggestion for pathogenic (Freshman, 1991). But the abundant number of neutrophil due to phagocytosis of bacteria in diestrus must be distinguished (Cowell et al., 1999).

*Brucella canis* is also the main infectious agent responsible for creation of reproductive disturbances in dogs. The brucellosis can be transmitted through contact with aborted fetus or placenta and vaginal tissues but the most important transmission is through venereal (Moore and Gupta, 1970). The bacteria appear in blood after two to three weeks of infections and the incubation period for the manifestations of clinical signs of reproductive system vary (Shin et al., 1999). The most important clinical sign of brucellosis in bitch is abortion at 30-57 days of gestation and more common at 45-55 days of gestation and these abortions are characterized by serosanguineous for 4-6 weeks after parturition (Shin et al., 1999; Hollett et al., 2006). The estrus and breeding pattern of bitch is not changed in brucellosis (Hollett et al., 2006). The infected bitch produces the consecutive abortion and aborted pups can die within few hours after parturi-

tion. Later on, the birth of normal pup that can cause the disease can occur (Shin et al., 1999; Wanke, 2004). Infected kennel suffer from abortion, weak pups and poor conception rates (Brennan et al., 2008). The death of embryo after 2-3 weeks of breeding can take place (Shin et al., 1999, Hollett et al., 2006). The infected bitches may suffer with abortion or other congenital abnormalities like siezuring and leucocytosis in their pups (Hollett et al., 2006).

The greatest prevalence of brucellosis occurs in dogs of commercial kennels (CFSPH, 1984) and significant reproductive losses can occur. The infertility due to brucellosis was first time reported in USA (Monroe et al., 1975). The canine brucellosis is raising disease in some countries due to movement of dogs across the countries (Hollett et al., 2006; Brower et al., 2007). Canine brucellosis is common in central and South America and South part of USA (Lucero et al., 2008; Lovejoy et al., 1976) and in Canada (Bosu and Prescott, 1980; Brennan et al., 2008). In the continent of Asia, the infection of brucellosis has been reported in Japan (Saegusa et al., 1978; Hayashi and Isayama, 1977), India (Srinivasan et al., 1992), Korea (Park et al., 2001), China (Jiang, 1989) and in Africa from Nigeria (Adesiyun et al., 1986). The dogs in kennels houses with canine brucellosis are often euthanized. The antibiotics with long term therapy have no beneficial effects (Wanke et al., 2006). The different treatments are evaluated with combination of different antibiotics like tetracycline, streptomycin and sulphonamides for two weeks (Flores-Castro and Carmichael, 1981; Zoha and Walsh, 1982) or use of enrofloxacin for 4 weeks (Wanke et al., 2006). There are no successful results of culturing, and for culturing there are many biohazard for the laboratory workers (Wooley et al., 1978). Serologic test is commonly used with polymerase chain reaction (PCR) tests (Keid et al., 2007) for the detection of bacteria.

Streptococci are gram-positive cocci are the normal micro flora of genital tract (Johnston et al., 2001). These bacteria are associated with abortion but the isolated bacteria have no ability of causing the reproductive problems. Campylobacter is commonly present in faeces of normal dogs (Torre et al., 1993), it causes the abortions in bitch (Bulgin et al., 1984). In the suspected cases of campylobacter, the sample should handle carefully due to zoonotic problems (Johnston et al., 2001). Salmonella is transmitted to animals through the contamination of food and water and is also the source of abortion and weak puppies

in bitch (Greene, 2006). E. coli is normally isolated in metritis and pyometra of bitch (Johnston et al., 2001) and partial abortion (Linde, 1983). Mycoplasma and Ureaplasma are isolated from the normal and infertile reproductive tract of bitch (Doig et al., 1981) and these organisms cause the infertility, fatal death, emaciated puppies and stillbirth (Johnston et al., 2001). Canine herpes virus can cause the infertility via abortion and early embryonic (Megid et al., 1999). The common route for the transfer of virus is respiratory or reproductive tract (Anvik, 1991; Greene, 2006) and latent infection can be reactivated due to pregnancy stress and transplacental infections lead to weak or mummified fetus. The canine distemper is caused by morbillivirus (Greene, 2006) causes the abortion and congenital infections in bitch (Krakowka et al., 1977; Greene, 2006). The systemic infections as well as transplacental infections are also occur due to this virus (Johnston et al., 2001). Transplacentally infected puppies can develop the neurologic signs in six weeks after birth (Greene, 2006). Placentitis is caused due to Toxoplasmosis in bitch (Dubey and Lappin, 2006).

Experimental infections have proved that *N. caninum* can be transmitted through transplacental routes (Dubey and Lindsay, 1989). And death of fetus, mummification and weak puppies born (Dubey and Lappin, 2006).

## CONCLUSION

In the above review, all the reproductive problems were discussed which are responsible for causing the infertility in bitch. The most important of them is uterine infections (pyometra). Due to unique estrus cycle and long phase of diestrus, this problem causes the subsequent loss of fertility and health of bitch. It is suggested that there is need of unique management of canine estrus cycle during the diestrus phase. No report of brucellosis is available in Pakistan, so there is necessitate to make the epidemiological survey of brucellosis in this country.

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