Review Article

AN OVERVIEW OF OVARIAN FOLLICULAR CYST IN DAIRY CATTLE

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INTRODUCTION

Fertility of dairy cattle is dependent on five important factors viz., oestrus, ovulation, competent luteal function, healthy uterine environment and harmonious endocrine support. The former three are sequential physiological events which determine the crucial phase of fertility – the conception. Any disruption in any of these three events will lead to failure in conception. Ovulation is an important phenomenon that deals with the release of the female gamete from a mature follicle and makes it available for syngamy with the male counterpart aiding in conception. Ovulatory disturbances lead to various fertility disorders like anoestrum (anovulation), repeat breeding (delayed ovulation) and cystic degeneration (follicular and luteal cysts) (Arthur *et al*., 1989). Ovarian follicular cysts, if not diagnosed and treated promptly, may lead to sterility. With the advent of imaging and molecular diagnostics, the pathogenesis and sequelae are being updated regularly in the past two decades. Better understanding of aetiopathogenesis and clinical signs of ovarian follicular cysts will aid in making appropriate diagnostic and therapeutic approaches.

Classification of follicular cysts

Ovarian follicular cyst is a consequence of a mature follicle that fails to ovulate at the appointed time during the oestrous cycle. The condition was first described in the early 1900's and McNutt was probably the first physician to use the term "cystic" for these anovulatory persistent structures in the year 1927. Earlier, follicular cysts were defined as fluid filled structures of > 2.5 cm in diameter persisting for 10 or more days on the ovarian surface (Roberts, 1971). However, with the introduction of ultrasound scanning in veterinary practice, it was determined that follicles of Bos taurus cows and Bos indicus x Bos taurus crossbred cows typically ovulate at 13-17 mm and 10 – 14 mm in diameter respectively (Ginther *et al*., 1996; Satheshkumar *et al*., 2012). So, follicles that persist at that diameter or greater may be considered to be cystic. Silvia *et al*. (2002) defined them as follicle-like structures, with a minimum diameter of 17 mm and persisting for more than 6 days in the absence of a corpus luteum and clearly interfering with normal ovarian cyclicity. Over years, new definitions have been suggested based on the fate of these pathologic structures. According to Calder *et al.* (2001), follicular cysts undergo one of the following three fates.

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i. Cysts may remain dominant for a prolonged period without other follicular growth

ii. Cysts may lose dominance and be replaced by another cyst from a new follicular wave (cyst turnover), or

iii. Cysts may lose dominance and persist, however a new dominant follicle (DF) may develop and ovulate without affecting the cyclicity (Non-dominant follicular cysts - NDFCs).

Thus, understanding the etiology and pathogenesis of these pathologic structures is much needed to define the factors responsible for their development, persistence and final fate.

Recent concepts in aetiopathogenesis of follicular cysts

The primary cause and mechanism of follicular cyst development have been debated for decades, however, it is commonly believed that neuro-endocrine imbalance involving the hypothalamo–hypophyseal–ovarian axis is responsible for the condition. Normal follicular development requires gonadotropins and various intraovarian autocrine and paracrine factors. Follicular micro-environment comprising of follicular cells (theca and granulosa) and follicular fluid (FF) plays a major role in the autocrine and paracrine regulation. These follicular components provided the much needed physiological, biochemical and metabolic inputs for the maturation of the oocyte and the process of ovulation. The 'Two cell-Two gonadotropin' theory, the basis for steroidogenesis, is the major phenomenon in the cascade of endocrine events leading to ovulation. Any disturbance in this sequence of steroidogenesis is the prime cause for ovulatory failure. Anovulation may lead to regression of follicle resulting in anoestrus or its persistence and enlargement resulting in cyst formation (Roberts, 1971; Arthur *et al*., 1989).

The movement of water and ions through ion channels at the cell membrane is crucial for regulating ovarian folliculogenesis. The interplay between large molecules and ions, secreted by granulosa cells, precipitates an osmotic pressure gradient that draws water into the antrum (Rodgers and Irving-Rodgers, 2010). Follicles undergo expansion until osmolality equilibrium is achieved inside and outside the cells. This swelling, in turn, triggers the activation of K+ channels, which are crucial to the regulatory volume decrease (Kim *et al*., 2024). Thus it could be assumed that the size of the follicle is determined by the status of K+ channels. Disruptions in K+ ion channels can impact processes such as cell growth, apoptosis and volume regulation and thereby leading to excess fluid accumulation and increased volume of the structure.

Characteristically, cows with follicular cysts have higher mean LH concentration, LH pulse amplitude and LH pulse frequency than cows with normal oestrous cycles. Frequent GnRH or LH pulses lead to prolonged growth of follicles and increased follicular oestradiol (E2) synthesis (Marques *et al*., 2024). Concentrations of E2 and progesterone (P4) in follicular fluid are higher in follicular cysts than in normal DF (Roth *et al*., 2012). Intrafollicular synthesis of E2 is directly proportional to the number of granulosa cells. As granulosa and theca cell mass increase with increase in follicular diameter, the total steroid hormone content in increased follicular cysts. Thus, follicular cysts are steroidogenically

active structures in their early stages, resulting in frequent and exaggerated oestrus like signs. However, in chronic cases the follicular cell characteristics alter leading to defective steroidogenesis resulting in anoestrum (Roberts, 1971).

NDFCs are non-steroidogenic cysts which are hormonally inactive and 'inert' space occupying structures. They are thin-walled fluid filled structures uniquely characterized by their uncontrollable acute or chronic increase in diameter and extremely non-responsive to regular hormonal therapies. No nymphomaniac or anoestrus symptoms could be observed in the affected animals, instead the cows are cyclic with normal follicular turnover but remains infertile (cyclic non-breeders or repeat breeders). They do not influence the normal oestrous cycle because of their inertness, so they can occur together with a corpus luteum unlike the follicular cysts (Calder *et al*., 2001). They have higher intrafollicular concentrations of P4, lower E2 concentrations and lower expression of steroidogenic enzyme, gonadotropin receptor, and oestrogen receptor-β mRNAs than follicular cysts and DFs (Xu *et al*., 2023). NDFCs fit the description of chronic cysts with degenerated granulosa cell layers, no basement membrane and theca layers containing hyalinized collagen and luteinized zones. Low intrafollicular E2 concentrations, absence of aromatase and 17α-hydroxylase activity and lack of P450arom mRNA expression in NDFCs were likely related to degeneration of the granulosa cell layer (Grado-Ahuir *et al*., 2011; Ortega *et al.*, 2015). Reduced LH and FSH binding receptors were also reported in these chronic oestrogen-inactive cysts, which might be the reason for non-responsiveness to exogenous hormones. Thus, loss of dominance in cysts is associated with atresia of granulosa cells and decreased expression of steroidogenic enzyme and gonadotropin receptor mRNAs.

Physico-biochemical characterization of follicular cysts

The study on physical characteristics revealed that FF of normal developing follicles are straw yellow to yellow in colour and those from follicular cysts are dark yellow to orange in colour. Maniwa *et al*. (2005) and Khan *et al*. (2011) suggested that the darker colouration of cystic fluid could be due to the interfusion of blood into the cysts.

Cystic follicular fluid was slightly viscous when compared to FF from normal follicles and frequently coagulated in petri plates, if left unprocessed which might be attributed to the presence of coagulating proteins like prothrombin and thrombin (Satheshkumar *et al*., 2021). Active thrombin initiates the polymerization which converts soluble fibrinogen into insoluble fibrin during the cascade of blood coagulation events (Chapin and Hajjar, 2015). In-vivo ultrasonographic examination of cystic follicles in cattle and buffaloes too revealed sparse intra-follicular fibrous strands (Fig.1). Based on our observation, the coagulating properties of cystic FF might be attributed to the presence of these coagulating proteins.

The glucose concentration in normal developing follicles increased significantly as the follicle size increased (Satheshkumar *et al*., 2021). The normal DFs have the ability to filter and reserve the high concentrations of glucose from blood for utilization in their development to the mature Graafian follicle (Nandi *et al*., 2008). On the contrary, the glucose and

triglyceride concentrations in cystic fluid are significantly higher when compared to normal DFs. As indicated by Gosden *et al*. (1988), it could be assumed that relatively smaller number of granulosa cells in cysts might be consuming these metabolites from a relatively larger amount of fluid leading to the accumulation of metabolite. Based on the biochemical study, it could be concluded that impaired metabolism and improper utilization of the resources by the follicular cells might be the major reason for high concentrations of glucose and triglycerides in FF of cysts when compared to that of normal DF.

Protein profile of follicular fluid and follicular cells in follicular cysts

Protein profile analysis of FF and granulosa cells in normal developing and cystic follicles revealed presence of a 70kDa protein resembling Heat shock protein 70 (Hsp70), a 30 kDa protein resembling Insulin like growth factor binding protein (IGFBP) and a 180 kDa protein resembling vascular endothelial growth factor (VEGF) (Satheshkumar *et al*., 2019 and 2021). Hsp70 is usually upregulated in response to cell stress, which indicated the role of stress factors ranging from negative energy balance to high ambient temperature in the development of ovarian cystic degeneration in cattle. Increased expression of Hsp70 was documented in FF and GCs of bovine follicular cysts when compared to normal follicles by Maniwa *et al*. (2005). Stress due to negative energy balance and high ambient temperature seems to be some aetiologic factors (Zhu *et al*., 2024). As referred by Braw-Tal *et al*. (2009) and Mazerbourg and Monget (2018), persistence and high activity of IGFBPs would have reduced the levels of IGF bioavailability and associated with anovulation and cyst formation. The implication of VEGF in the aetiology of polycystic ovary syndrome was well documented in humans. Elevated levels of VEGF, an important regulator of ovarian angiogenesis might be contributing to the continuous growth and persistence of follicles (Greenaway *et al*., 2004).

Clinical signs in cattle with follicular cysts

Clinical signs include behavioral changes which are characteristic of frequent oestrus like activities referred to as nymphomania (i.e., excessive mounting, standing, and bawling with noticeably deeper tone). In chronic cases, anoestrum with loss of uterine tone, relaxation or stretching of the sacrosciatic and sacroiliac ligaments giving the raised tail head appearance, commonly referred to as 'Sterility hump' (Roberts, 1971) are appreciated. Development of masculine physical characters is common in prolonged untreated cases due, probably, to faulty steroidogenesis i.e., failure in conversion of androgens to oestrogen. On contrary, no nymphomaniac or anoestrus symptoms could be observed in NDFC cases, instead the cows are cyclic with normal follicular turnover but remains infertile (cyclic non-breeders or repeat breeders). The NDFCs are thin-walled fluid filled structures uniquely characterized by their uncontrollable acute or chronic increase in diameter and will be co-existing along with a corpus luteum unlike the follicular cysts. Hence, differential diagnosis of cystic category is of prime importance before attempting the therapy.

Diagnosis of follicular cysts

Diagnostic approaches for bovine follicular cysts include history and clinical signs, transrectal palpation, ultrasonography and plasma or milk progesterone assay.

By routine gynaeco-clinical transrectal palpation, ovarian cysts are identified as typically larger than normal ovulatory follicles with an increased overall ovarian diameter along with a flaccid uterus in the absence of a corpus luteum. Although transrectal palpation has long been the diagnostic approach, it is difficult to distinguish between follicular and luteinized cysts solely by palpation. The accuracy of diagnosing ovarian cysts can be increased by combining transrectal palpation with ultrasonography. A persistent fluid filled structure of > 17 mm in diameter with < 3mm follicular wall thickness in the absence of corpus luteum in either of the ovary can be diagnosed as a follicular cyst. Similar structure with > 3mm follicular wall thickness is considered as a luteal cyst. Thus, ultrasonography is effective in detecting follicular and luteal cysts with high accuracy.

The NDFCs could be differentiated from follicular cysts by their relatively larger diameter, co-existence with corpus luteum and normal cyclicity of the animals (Satheshkumar *et al*., 2020).

Therapeutic approaches to treat follicular cysts

Hormonal approach to alleviate follicular cysts should be with the aim to induce luteinization of the cystic structure followed by lysing the structure and breeding in the subsequent oestrus.

GnRH analogues are effective in a proportion of cases with aovulatory follicular cysts. GnRH stimulates the release of LH (with a maximum plasma LH concentration being reached 90 to 150 minutes post administration), which initiates the luteinization process, as indicated by increased serum P4 levels 7-10 days post treatment (Jeengar *et al*., 2014). After the GnRH treatment and subsequent luteinization, the cyst becomes responsive to the luteolysin, prostaglandin-F2α (PGF2α) and timed breeding could be attempted (Arthur *et al*., 1989).

However, a large proportion (25-39%) of cows with ovarian cysts treated are not responding to GnRH therapy (Tebble *et al*., 2001). The treatment of follicular cysts with hCG is found to be more effective than GnRH (Ragul *et al*., 2024). In the recent years, we could observe an increase in the incidence of NDFCs among the crossbred cows (Gupta *et al*., 2020; Satheshkumar *et al*., 2022). The NDFCs are extremely non-responsive to regular hormonal therapies. Deficient LH receptors in NDFCs could explain the non-responsiveness of cows to exogenous GnRH or hCG that are often used to treat cysts. Hence mechanically collapsing the inert cyst is the only option to get rid of the ailment. Trans-rectal manual rupture of follicular cysts is not preferred due to the well-known potential risk of adhesion formation. Transvaginal ultrasound-guided follicular aspiration is a safe and good alternative approach to treat the NDFCs. The conventional trans-vaginal approach of follicular aspiration requires too many ultrasonographic probe accessories and skilled physicians. Above all, the cost involvement for these investments also hinders the procedure. 'Trans-gluteal follicular cyst evacuation' (Fig.2) is recently developed and found to be effective in correcting the NDFCs (Satheshkumar *et al*., 2022). Even this approach is now utilized in regular treatment of follicular cysts (Ragul *et al*., 2024).

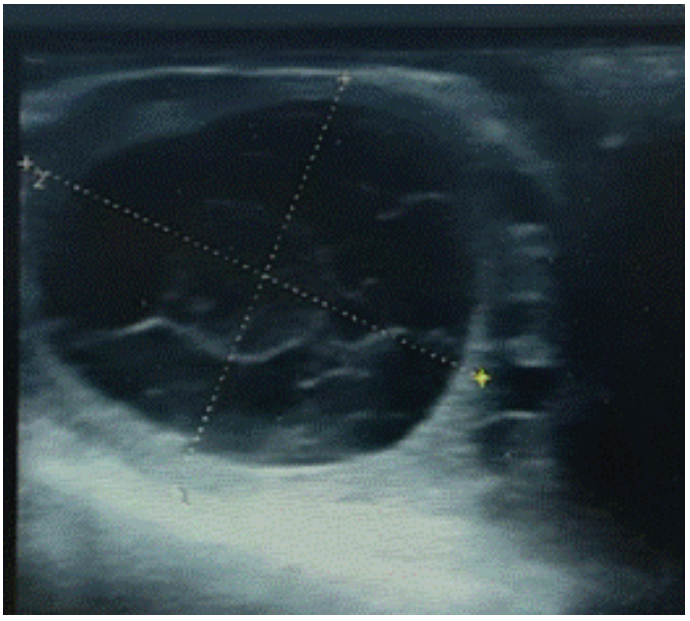

Fig.1. Ultrasonographic image of follicular cyst with intra-follicular fibrous strands

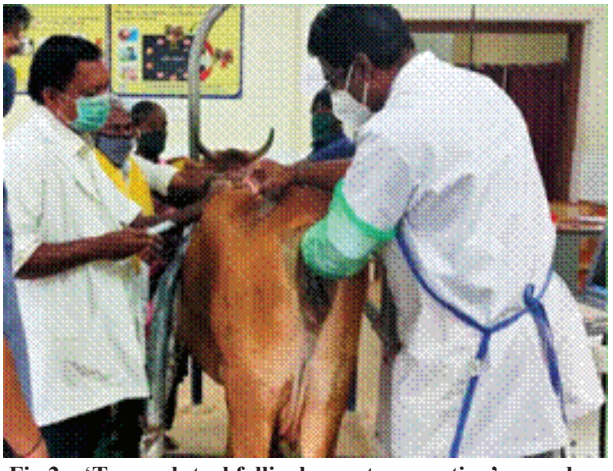

Fig.2a. 'Trans-gluteal follicular cyst evacuation' procedure

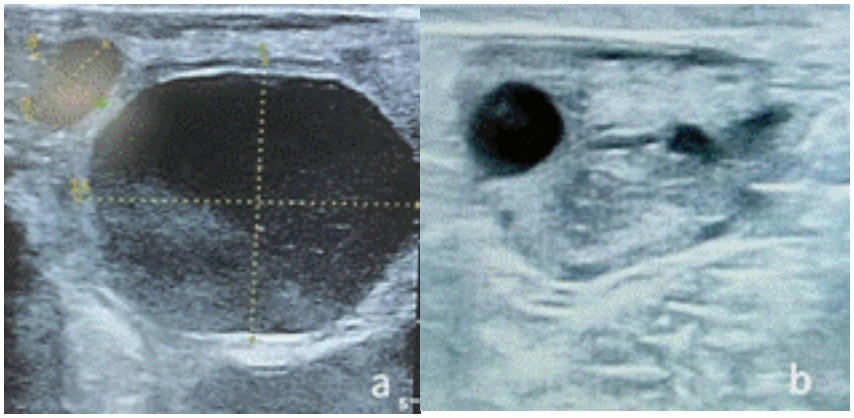

Fig.2b. NDFC before (a) and after (b) evacuation

CONCLUSION

As per recent developments, follicle like fluid filled structures with minimum of 17 mm diameter and persisting for more than 6 days in the absence of corpus luteum are considered to be follicular cyst. Disturbances in ovarian steroidogenesis disrupts ovulatory process leading to cystic conditions. In recent years, non-steroidogenic non-responsive inert follicular cysts are reported. Hence proper diagnosis by advocating advanced imaging techniques is warranted in deciding the appropriate therapeutic measures. Further research should be concentrated on proper understanding of aetiopathogenesis in order to prevent the occurrence of follicular cysts.

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