



Hormonal and Biochemical Profile of Follicular Cyst and its Responsiveness to Hormonal Therapy and Trans-gluteal Aspiration Technique in Crossbred Cattle

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ABSTRACT

Background: Artificial Insemination is being extensively used for genetic improvement and upgrading the genetic makeup of cattle in the Indian subcontinent and was mainly based on production parameters like milk production and feed conversion efficiency. Attempts to increase milk production over certain limits resulted in the reduction of reproductive efficiency and occurrence of some reproductive disorders (Arunmozhi *et al.*, 2023). The increased production has also been reported to result in ovulatory abnormalities, predominantly ovarian follicular cysts, which results in reduced fertility (Jeengar *et al.*, 2014). The present research work was carried out to evaluate the efficacy of the hormonal therapy for follicular cyst and trans-gluteal aspiration technique for correcting non-responsive ovarian follicular cyst in crossbred cows.

Methods: The crossbred cows presented with the history of more than six insemination and with nymphomaniac behavior for the past 5 months were included in the present study. Among the nymphomaniac cows, 25 animals which were observed having follicular cyst, based on rectal and ultrasonographic examination were selected. Incidence of follicular cysts were categorized on the basis of side of ovaries, size of cyst, follicular wall thickness, number of cystic follicles, breeds, lactation and response to the hormonal treatments. The blood samples were collected to estimate the level of estradiol and progesterone in the affected cows. Further, the serum and cystic fluid collected were analyzed for biochemical parameters. The conception rate was also recorded in the treated and trans-gluteal aspiration technique adopted cows.

Result: Cows (n=25) were treated with buserelin acetate (Day 0), prostaglandin F₂ alpha (Day 7) and again buserelin acetate (Day 9). It was found that 14 cows (56.0 %) responded and 11 cows (44.0%) were found to be non-responsive to hormonal therapy. Trans-gluteal aspiration of follicle was performed in these eleven cows along with modified Ovsynch protocol. Trans-gluteal approach was found to be effective with 72.73 per cent recovery without any consequences and only 27.27 per cent recurrence was documented. Hence, this technique can easily be adopted in field for correcting non-responsive follicular cyst in cows.

Key words: Crossbreed cow, Follicular cyst, Ovsynch, Trans-gluteal aspiration, Ultrasonography.

INTRODUCTION

Reproduction in dairy cow is considered to be economical when a calf is produced every year. In the recent past, Artificial Insemination was extensively used for genetic improvement; upgrading the genetic makeup of cattle on the Indian subcontinent was mainly based on production parameters like milk production and feed efficiency. An increase in milk production over certain limits results in the reduction of reproductive efficiency in dairy animals (Arunmozhi *et al.*, 2023). The increased production has also been reported resulting in ovulatory abnormalities, predominantly ovarian cysts (OCs), which result in reduced fertility (Jeengar *et al.*, 2014). Cystic ovarian disease was first observed in the early twentieth century and was recognized as one of the most important economical diseases of cattle (Jeengar *et al.*, 2014). Ovarian cysts were defined as a fluid filled or hard structures of 2.5 cm or more in diameter that persist on the ovarian surface for 10 or more days (Youngquist and Threlfall, 2007). This condition was synonymous with adrenal virilism, nymphomania, cystic ovarian degeneration, cystic ovaries and OCs

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(Garverick 1997). Silvia *et al.* (2002) defined a cystic ovary as having follicle-like structures with a minimum diameter of 17 mm that persist for more than 6 days in the absence of a corpus luteum and interfere with ovarian cyclicity. Recently, an ovarian cyst was defined as anovulatory follicles (< 2 cm) on one or both ovaries that fail to regress yet maintain growth and steroidogenesis, persist for more than 10 days and interfere with normal ovarian cyclicity in the absence of a corpus luteum (Vanholder *et al.*, 2006).

The exact mechanisms involved in the pathogenesis of OCs in dairy cattle are not fully understood, nevertheless, it may be due to the aberrant release of LH is neither due to reduced gonadotropin releasing hormone (GnRH) concentration nor due to reduced GnRH receptors but might be due to altered feedback mechanisms of estrogen on the HPG axis that lead to aberrant LH release (Gumen and Wiltbank 2002; Zaied *et al.*, 1980). Recently, kisspeptin has been reported to be mediator of GnRH release from the hypothalamus and kisspeptin plays a crucial role in controlling gonadotropin secretions and ovulation. Kisspeptin interacts with neurokinin B and dynorphin neuropeptides to control GnRH release and mostly the stress to an animal might inhibit kisspeptin and lead to altered GnRH or LH secretion, especially during lactation, which may cause anovulation (Yeo and Colledge, 2018).

Earlier ovarian cysts in cow were treated by using various hormonal and non-hormonal methods but with variable results (Jeengar *et al.*, 2014; Chauhan *et al.*, 2020) however, recently trans-gluteal aspiration of follicular cysts method is being used for the treatment of non-responsive OCs with a better success rate and being less expensive and non-invasive (Arunmozhi *et al.*, 2023). Therefore, the present experiment was designed to evaluate the efficacy

of the trans-gluteal aspiration technique for treating the non-responsive follicular cyst in crossbred cows.

MATERIALS AND METHODS

The Jersey and Holstein Friesian crossbred cows which were brought to the Gynaecology unit of Veterinary Clinical Complex, Veterinary College and Research Institute, Salem-636112 with the history of more than six repeated inseminations and nymphomaniac signs during the period from June 2023 to October 2024, were subjected to rectal and ultrasound examination for identification of ovarian functional structure. Trans-rectal ultrasonography was performed to record the location, size of follicle and size of corpus luteum measured as procedure described by Dodiya *et al.* (2022). A total of 25 cows were selected based on the clinical signs, presence of follicular cyst, biometry of the largest follicle (>17 mm diameter) and persistence on the same ovary, at the same site for more than 10 days (Fig 1). Incidence of follicular cyst was computed based on the side of ovaries (right, left or both), size of cyst (17-20 mm, 21-25 mm, 26-30 mm, 31 mm and above), follicular wall thickness (<1 mm, 1-2 mm, 2.1-3 mm and 3.1 and above), number of cystic follicles on the ovaries (one, two and three follicles), breeds (Jersey cross, Holstein Friesian cross and others), lactation (heifer, 1-2, 3-5, 6 and above lactation) and response to the hormonal treatments (responsive and non-responsive).

All the crossbred cows were advised for mineral mixture supplementation throughout the hormonal protocol period. They were administered buserelin acetate (Inj. Gynarich: 20 µg, im) on day 0 and cloprostenol sodium (Inj. Pragma 500 µg, im) on day 7 and buserelin acetate (Inj. Gynarich, 20 µg, im) again on day 9. Ultrasonographic

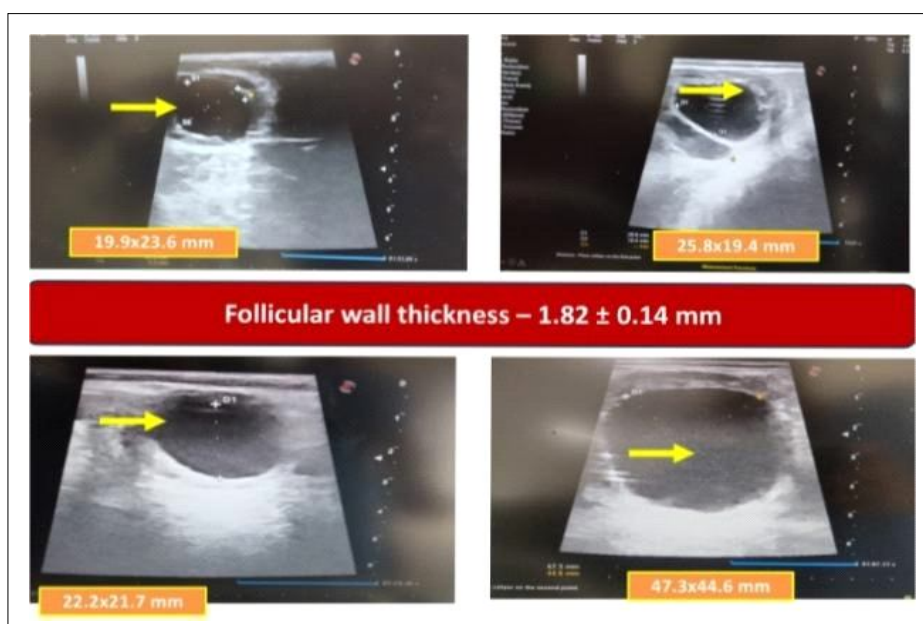


Fig 1: Follicular cyst in crossbred cows.

examination of ovarian follicles was carried out on the day 0, 7 and 9 and their diameters were recorded. In 14 cows, ultrasonographic examination of ovaries on the day 7 revealed luteinization of follicle or absence of cystic follicle as observed on day 0, while in 11 cows larger follicles which were observed on day 0 were also observed on day 7 indicating non-responsiveness to the hormonal treatment. These follicular cysts were considered as non-responsive follicular cyst (Table 1). Subsequently these non-responsive follicular cysts were subjected to manual rupture.

Procedure for trans-gluteal follicular aspiration technique

To perform trans-gluteal follicular aspiration, the ipsilateral gluteal muscle region was aseptically prepared and 3 ml of 2% lignocaine administered at sacro-coccygeal space and 2 ml of lignocaine was administered in various sites of gluteal region to induce local anesthesia for the trans-gluteal aspiration of follicle (Fig 2). The affected ovary was held between two fingers per-rectal and it was brought to ipsilateral gluteal muscle region. The ovary was positioned in such a way that the cyst is exposed towards gluteal muscle. By using right hand, 18G spinal needle was passed through gluteal muscle region and the follicular cyst was pierced with needle. The piercing of the cyst was confirmed

by presence of follicular fluid through needle. Now, a sterile 20 ml syringe was fixed with needle and follicular fluid was aspirated (Fig 3). Around 10-15 ml of follicular fluid was aspirated from the affected ovary. After follicular aspiration, the ultrasonographic examination was performed to confirm the complete aspiration of follicle. The collapsing of follicle was observed through ultrasonography (Fig 4). Immediately were also observed on day-7 after follicular aspiration, they were administered with buserelin acetate (20 µg, im) on day 0 and cloprostenol sodium (500 µg, im) on day 10 and second dose of buserelin acetate (20 µg, im) on day 12 and 24 hrs after GnRH administration Fixed Time Artificial Insemination was carried out based on dominant follicle on the ovary (Fig 5). Recorded the consequences of follicular cyst after evacuation and pregnancy diagnosis was performed day 45 after artificial insemination.

Blood sampling and hormonal estimation

Blood collection was carried out on day 0, 7 and 9 of treatment period. Blood samples were collected from jugular venipuncture and the serum was separated by centrifugation at 3000 rpm for 15 min and stored at -20°C until analysis. The stored serum samples were utilized for the estimation of estradiol and progesterone concentration by Enzyme Linked Immuno Sorbent Assay (ELISA) using

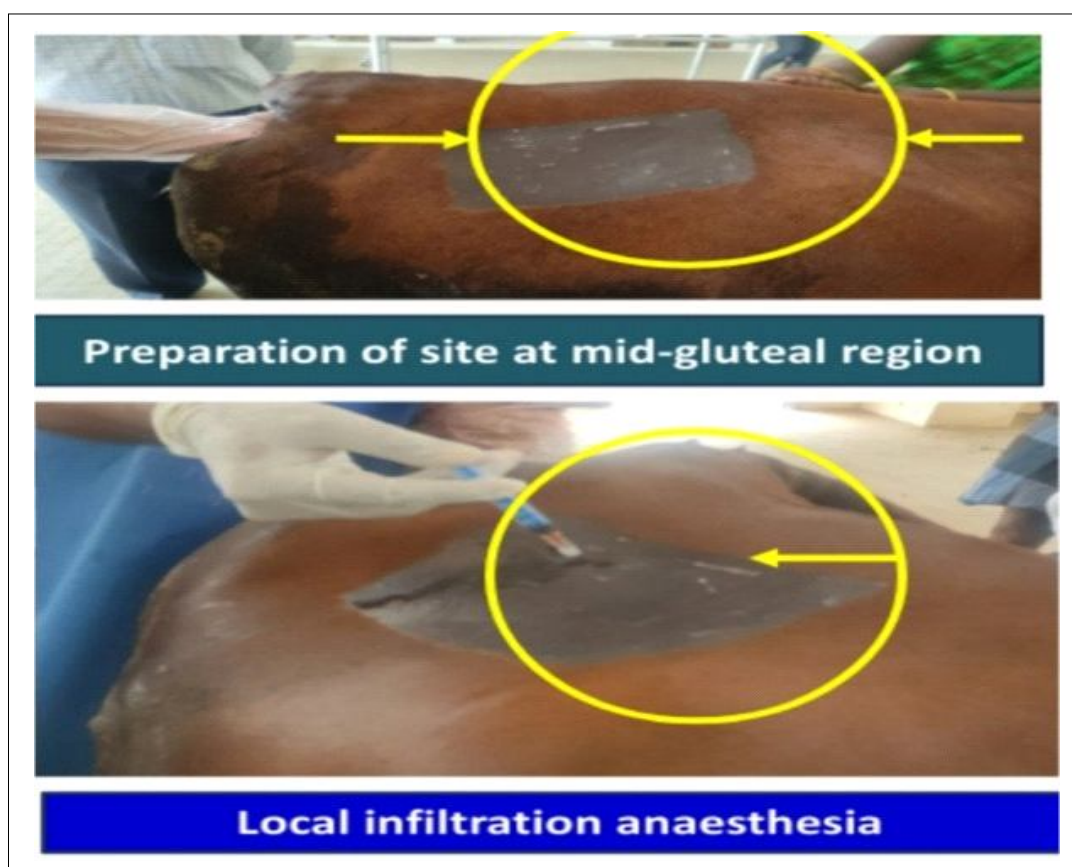


Fig 2: Preparation of site for Trans-gluteal aspiration of cyst.

the Calbiotech, Inc (CBI) kit in the centralized clinical laboratory, Madras Veterinary College, Chennai.

Biochemical studies

The serum and cystic fluid collected by follicular aspiration from chronic follicular cystic cows were analyzed in the Veterinary Diagnostic Laboratory, Veterinary Clinical Complex, VCRI, Salem for biochemical parameters like

BUN, Glucose, Creatinine, Total protein, Albumin, Globulin, ALT, AST, Calcium and Phosphorus.

Conception rate

All the crossbred cows were subjected to Fixed Time Artificial Insemination by using Jersey and HF crossbred frozen semen straws and the AI was followed the repeater cows who have returned to estrus upto the third consecutive



Fig 3: Trans-gluteal aspiration of follicular cystic fluid from follicular cyst in the ovary.



Fig 4: Collapsing of follicle after aspiration of follicular fluid.

cycle. The conception rate was calculated as per the standard protocol.

Statistical analysis

Statistical analysis was performed with the two-tailed independent sample t-test and p values < 0.05 were considered statistically significant. All statistical analysis was done using SPSS Statistical software.

RESULT AND DISCUSSION

In the present study, 25 crossbred cows diagnosed and confirmed as suffering with follicular cyst during the period from June 2023-October 2024 were included. It was observed that the incidence of follicular cyst in right ovary (72.0%) was more as compared to that of left ovary (16.0%), which perhaps due to more ovulation from right ovary in bovine. The size of the follicular cyst was recorded as 17 mm to 48.2 mm. However, the diameter of follicular cyst was recorded as 17.0-20.0 mm, 21.0-25.0 mm, 26.0-30.0 mm and 31.0 mm in 48.0, 24.0, 8.0 and 20.0 per cent of cows, respectively. The follicular wall thickness of 1.1-2.0 mm diameter was recorded in more number of cows (36.0 per cent) with a preponderance of single follicular cyst in 84.0 percent affected cows. It was further observed that

Jersey crossbred cows notably had higher numbers of follicular cyst as compared to HF crossbred cows (56.0 Vs 36.0 per cent) and more numbers of follicular cyst recorded in the animals of 1-2 lactation followed by 3-5 lactation period (Table 2).

It has been observed that insufficiency of the pre-ovulatory LH surge was the major factor in the predisposition of formation of follicular cyst (Ortega *et al.*, 2015). Similar results as that of present study, Pesantez *et al.* (2016) also documented that multiparous cow had a higher incidence of OFC (27.5%) than primiparous cows (15.5%). However, the mechanism explaining the association of parity or age with OFC remains unknown. This difference may be related to increased milk production in multiparous versus primiparous cows, as cows with higher milk production have an increased incidence of OFC (Lopez-Gatius *et al.*, 2002, Fitzgerald *et al.*, 2014).

Routine observations of the follicular status during the hormonal intervention revealed that 14 (56.0%) out of 25 cows with follicular cyst responded to buserelin injection with varying degrees of luteinization (>3mm follicular wall thickness) on day 7 and regressed on day 9 following cloprostenol sodium injection. In the rest of the 11 cows (44.0%), the cystic follicle found on day 0 were observed

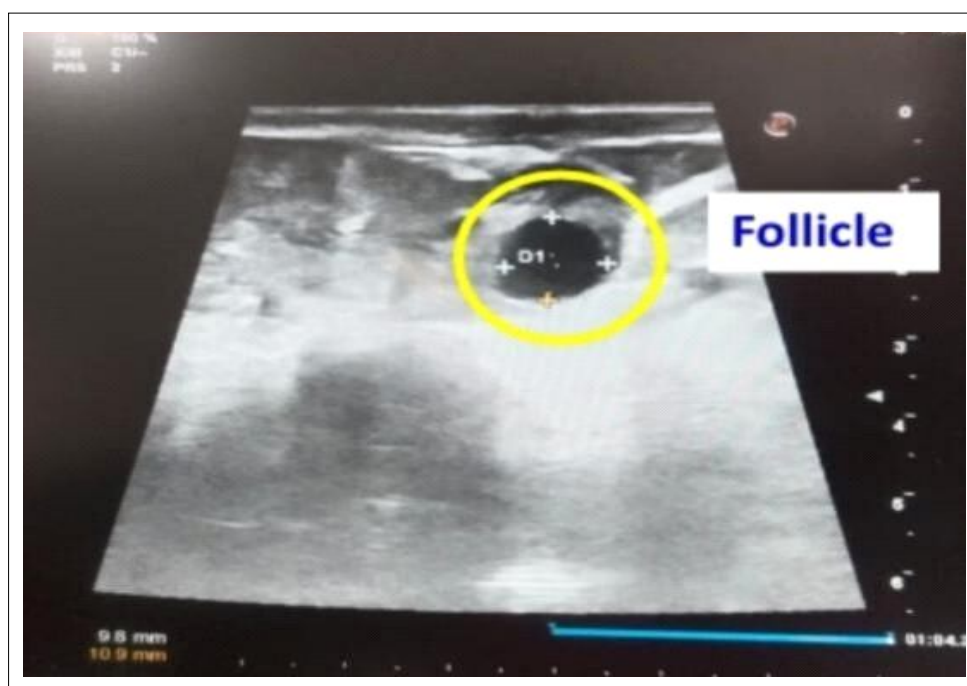


Fig 5: Dominant follicle after treatment.

Table 1: Cystic follicle diameter during different treatment period in crossbred cows.

Categories based on response to hormone	Follicular Diameter (mm)		
	Day 0	Day 7	Day 9
Responsive cyst (n=14)	19.6±0.71	Luteinized (>3mm)	-
Non-responsive cyst (n=11)	30.4±2.45	31.3±2.21	31.1±2.10

with a mild degree of luteinization (< 2mm follicular wall thickness) on day 7 and persisted without responding to hormones and hence considered to be non-responsive follicular cyst (Table 2). The occurrence of cows with non-responsiveness to the hormones (44.0 %) was higher than the previous report of 14.6 and 4.0 per cent recorded by Sathees kumar *et al.* (2022) and Gupta *et al.* (2019), respectively.

Among the crossbred cows with follicular cyst those responded to the hormonal therapy and inseminated on subsequent estrous cycle, 8 cows were recorded pregnant

and 6 cows returned to the estrus and 4 cows were conceived in second and 2 cows were in third service (Table 2). Those cows with follicular cyst non-responsive to the hormonal therapy enrolled for trans-gluteal aspiration of cystic fluid and in combined with modified Ovsynch protocol and found that the cyst regressed in eight cows (72.73%) and in three cows (27.27%) the cyst reoccurred. Among the responded cows which were inseminated on subsequent cycle, five cows were found pregnant (62.50%) and three cows (37.50%) returned to estrus (Table 2).

Table 2: Incidence of follicular cyst in crossbred cows (n=25).

	Observations	No. of cows	Percentage
I	Ovary involved		
1	Right ovary	18	72.00
2	Left ovary	4	16.00
3	Both	3	12.00
II	Size of the follicular cyst		
1	17.0 - 20.0 mm	12	48.00
2	21.0 - 25.0 mm	6	24.00
3	26.0 - 30.0 mm	2	8.00
4	31.0 mm and above	5	20.00
III	Follicular wall thickness		
1	0.1 - 1.0	6	24.00
2	1.1 - 2.0	9	36.00
3	2.1 - 3.0	8	32.00
4	3.1 and above (partially luteinized)	2	8.00
IV	Crossbred affected		
1	Jersey cross	14	56.00
2	Holstein Friesian cross	9	36.00
3	Others	2	8.00
V	Lactation		
1	Heifer	5	20.00
2	1-2 lactation	11	44.00
3	3-5 lactation	7	28.00
4	6 and above lactation	2	8.00
VI	Response to the hormonal treatment/protocol		
1	Responsive follicular cyst	14	56.00
2	Non-responsive follicular cyst	11	44.00
VII	No. of cystic follicle on each ovary	One follicle	Two follicles
1	Right	17 (68.0%)	4 (16.0%)
2	Left	4 (16.0%)	-

Table 3: Serum Estrogen and Progesterone profile during different days of hormonal treatment in the follicular cystic condition of cross bred cows.

Hormone profile	Category of follicle	On the day of selection	Day 0 (GnRH)	Day 7 (PGF2 alpha)	Day 9 (GnRH)
Estrogen (pg/ml)	RC	34.56±1.54	37.82±1.26	17.32±0.43 ^a	39.81±1.13
	NRFC	40.16±1.73	41.83±2.26	41.12±1.03 ^b	41.24±1.17
Progesterone(ng/ml)	RC	0.69±0.02	0.66±0.08	2.42±0.30 ^A	0.41±0.09
	NRFC	0.38±0.04	0.38±0.03	0.58±0.09 ^B	0.43±0.06

Values with superscript a within same column differ significantly (P<0.05) from values with superscript b.

Values with superscript A within same column differ significantly (P<0.05) from values with superscript B.

Table 4: Mean concentrations (Mean \pm SE) of various biochemical parameters in serum and follicular fluid of cystic cows.

Parameter	Serum	Follicular fluid
Glucose (mg/dl)	72.0 \pm 2.73	61.37 \pm 8.71
Total protein (g/dl)	8.03 \pm 0.30	6.07 \pm 0.20
Albumin (g/dl)	3.69 \pm 0.11	3.24 \pm 0.09
Globulin	4.45 \pm 0.35	2.83 \pm 0.20
BUN (mg/dl)	12.64 \pm 4.27	12.30 \pm 6.07
Creatinine (mg/dl)	0.90 \pm 0.13	0.98 \pm 0.08
ALT (IU/L)	23.56 \pm 1.73	15.42 \pm 5.71
AST (IU/L)	70.02 \pm 10.03	58.58 \pm 7.38
Calcium (mg/dl)	13.98 \pm 1.11	12.92 \pm 0.94
Phosphorus (mg/dl)	6.36 \pm 1.00	7.59 \pm 0.51

Interestingly, Satheeskumar *et al.* (2022) has documented that 100 per cent regression of follicular cyst after evacuation of cyst by trans-gluteal approach and they recorded 50.0 per cent of conception rate in crossbred cows. Recurrence of cyst even after aspiration, might result more secretory nature of follicular wall and this may also leads to continuous stimulation of endometrium there by resulting in thickening of endometrium and thickened endometrium may not be able to support the development of early embryo (Ashitha *et al.*, 2020). However, ultrasound guided transvaginal follicular aspiration technique or trans-gluteal aspiration method has been considered as treatment of choice for the non-responsive cystic follicle which has not responded for the hormonal treatment (Pugazharasi *et al.*, 2020; Satheeskumar *et al.*, 2022) and it may be advocated for using in such animals for restoring the fertility in the affected Jersey crossbred animals. Furthermore, the nutritional supplementation during the postpartum period will reduces the incidence of infertility due to silent estrus, endometritis and cystic ovarian degeneration in crossbred cows (Dhami *et al.*, 2017).

The mean serum estradiol and progesterone concentration during different days of hormonal treatment in cows affected with follicular cyst are presented in Table 3. The serum estradiol concentration was significantly higher in cows affected with non-responsive cyst than responsive cyst on day 7. The cows destined to form cysts have higher concentrations of LH than cows that will ovulate normally, particularly during the last several days of follicular maturation. This high level of gonadotropic support may be essential for maintenance of cysts. The estrogen concentration in the present study was similar to Hamilton *et al.* (1995) who have recorded that the follicles destined to form cysts secrete higher concentrations of estradiol than follicles that will ovulate normally, possibly due to their exposure to higher levels of LH. These follicles continue to secrete a high level of estradiol for at least 4 days after they reach preovulatory size. Despite the excessive secretion of estradiol, cystic cows do not release a preovulatory surge of LH (Zaied *et al.*, 1980) and this is because of failure of estradiol to stimulate release of a surge of GnRH from hypothalamic centers responsible for inducing the preovulatory surge in LH from anterior pituitary (Silva *et al.* 2002).

The serum progesterone concentration was significantly higher in cows affected with responsive cyst than non-responsive cyst on day 7. Further, follicular cysts contain relatively high concentrations of progesterone in follicular fluid (Cook *et al.*, 2001), much higher than in normal preovulatory or atretic follicles (Calder *et al.*, 2001). The capacity for progesterone biosynthesis is much greater in follicular cysts than in normal follicles. The extent to which ovarian follicular cysts secrete progesterone is not clear. The progesterone concentration in the present study was similar to Silvia *et al.* (2002) observed that some cows with ovarian follicular cysts had peripheral concentrations of progesterone that ranged from 0.1 to 1 ng/mL and sixty-six percent of those cows had progesterone that fell in the intermediate range, 0.1-1.0 ng/mL.

The mean concentrations of various biochemical parameters in serum and follicular fluid of and cystic cows are presented in Table 4. The mean serum and follicular fluid biochemical parameters like BUN, glucose, creatinine, total protein, albumin, globulin, ALT, AST, calcium and phosphorus were within normal reference values which were in correspondence to reports of Ragul *et al.* (2024) who have also observed the similar serum biochemical parameters in cystic cows. However, some data showed that the cystic follicle was characterized by the lower concentrations of glucose, cholesterol, total protein and higher urea levels than those of the normal follicle (Mimoune *et al.*, 2018). The follicular fluid composition varies greatly depending on the stage of follicular development and changes in the composition of the follicular fluid greatly affects the ovulation and may leads to development of follicular cyst in dairy animals (Kalmath and Ravindra, 2007).

CONCLUSION

The incidence of non-responsive follicular cyst is nowadays increasing among the dairy cattle which lead to culling of valuable high milk yielding animals. It can be concluded from the present study that the follicular cyst can be initially treated with hormones followed by second dose of the GnRH. It was also observed that if the follicular cyst ranges from 17-25 mm then the hormonal treatment may be effective and cystic follicle may not be responsive, if the size is more than 25 mm. Therefore, Trans-gluteal aspiration is the choice of treatment for non-responsive follicular cyst as well as larger size cystic follicle and the technique is easier, less invasive and to treat all these animals, follicular aspiration technique will be highly useful, since transvaginal ultrasound guided follicular aspiration technique was highly costlier. This novel trans-gluteal follicular aspiration technique will be highly useful and successful to treat these animals at field level.

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Conflict of interest

The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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