

## 'TRANS-GLUTEAL' FOLLICULAR CYST EVACUATION: A SUCCESSFUL TECHNIQUE TO CORRECT NON-RESPONSIVE FOLLICULAR CYSTS IN COWS

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### ABSTRACT

The research work was carried out to study the efficacy of 'trans-gluteal' follicular cyst aspiration technique in correcting the 'non-responsive' follicular cysts (NRFC). A total of 41 crossbred cows which were diagnosed as affected by follicular cystic degeneration, were utilized for the study. They were treated with human chorionic gonadotropin and prednisolone (Day 0), prostaglandin (Day 7) and GnRH (Day 9). It was found that six (14.6 %) animals with ovarian follicular cyst were found to be non-responsive for hormonal therapy. 'Trans-gluteal' approach of follicular cyst evacuation technique was employed in these six animals in combination with modified Cosynch protocol. It was found to be effective with 100 per cent recovery without any consequences and recurrence. Hence, 'trans-gluteal' approach of follicular cyst evacuation technique is a cost effective and user friendly method in correcting NRFCs in cattle.

**Keywords:** Cows, Follicular cyst, Non-responsive, Trans-gluteal evacuation

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Ovarian follicular cyst (OFC) is a consequence of a mature follicle that fails to ovulate at the appointed time during the oestrous cycle. With the advent of ultrasonographic imaging diagnostics, OFCs are defined as follicle like fluid filled structures, with a diameter of > 17 mm and persisting for more than six days on the ovarian surface in the absence of a corpus luteum and clearly interfering with normal ovarian cyclicality (Silvia *et al.*, 2002). However, Calder *et al.* (2001) opined a different category of OFCs which may lose their dominance and co-exist along with the normal follicular and luteal turnover without affecting the cyclicality. These cysts are found to be non-responsive to regular hormonal therapies and hence referred as 'non-responsive' follicular cysts (NRFC). In the recent years, we could observe an increase in the incidence of these NRFCs among the OFC conditions in crossbred cows (Gupta *et al.*, 2019). Since, these cysts are non-responsive to hormonal therapies, follicular evacuation is the only corrective measure. The present research work was carried out to study the efficacy of 'trans-gluteal' follicular cyst aspiration technique in correcting the NRFCs in comparison with the conventional hormonal therapy.

### MATERIALS AND METHODS

Crossbred cows which were brought to the Gynaecology Unit of Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu with the history of repeat breeding during the period from October 2020 to February 2021 (5 months) were subjected

for clinical examination and ultrasound screening. A total of 41 animals were diagnosed to be affected by cystic follicular degeneration, based on the clinical signs, biometry of the largest follicle (> 17 mm diameter) and persistence for more than 10 days in any one of the ovaries. All the animals were advised for mineral mixture supplementation throughout the therapeutic period. They were administered with human chorionic gonadotropin (Inj. hCG: 3000 IU; iv) and prednisolone acetate (100 mg; im) on Day 0 and prostaglandin (Inj. Cloprostenol; 500 µg; im) on Day 7. On Day 9, GnRH analogue (Inj. Buserelin acetate; 10 µg; i.m.) was administered and animals were inseminated twice at 24 h interval. Ultrasonographic examination of ovarian follicles was carried out on the day of hCG (0), PG (7) and GnRH (9) administrations and their diameters were recorded (Satheshkumar *et al.*, 2012). The follicular response to hormonal therapy, based on the nature of their luteinization, was documented. In six animals, ultrasonographic examination of ovaries on the day of GnRH revealed persistence of the larger diameter follicles which were observed on Day 0 indicative of non-responsiveness, while in the remaining animals, the cysts were regressed. All the 41 animals were advised to report in the subsequent cycle to assess the follicular status.

**Classification of NRFC:** A total of 23 animals reported in the subsequent cycle within 18-24 days. Ultrasonographic examination of ovaries revealed that eight animals developed a new cystic follicle on the ipsi- or contra-lateral ovary. While in the six animals, the non-responsive follicle was found to be still persisting with acutely increased

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diameter. These persistent follicular structures which were not responsive to hormonal therapy were considered to be NRFCs and were subjected to manual follicular evacuation technique.

**‘Trans-gluteal’ approach of follicular evacuation:**

‘Trans-gluteal’ approach of follicular cyst evacuation was the technique developed and applied to correct the NRFC in these six animals. Animal was restrained properly in the trevis. A small circumscribed area of mid-gluteal region through which the aspiration needle has to be inserted was shaved and thoroughly disinfected. Local infiltration of 2% lignocaine around the site of needle insertion was carried out. Lubricated, gloved left hand was passed trans-rectally by the first operator. The ovary which possessed the NRFC was held firmly closer to ipsi-lateral gluteal region and positioned in such a way that the cyst was exposed towards the gluteal region. Using the right hand, a sterile 18/20 G spinal needle (0.9 mm × 90 mm; BD, Spain) was passed through the gluteal region into the pelvic cavity (Fig. 1). The needle was guided to pierce the exposed cyst. Successful piercing of cyst was indicated by oozing out of cystic fluid through the spinal needle. A 20 ml sterile syringe was attached to the spinal needle by the second operator and cystic fluid was aspirated slowly. Nearly 3-7 ml of clear and dark yellow to orange coloured cystic fluid was aspirated from each NRFC. Collapse of the cyst due to fluid evacuation could be appreciated trans-rectally by the first operator. The evacuation was also confirmed ultrasonographically (Fig. 2 a,b). Anti-inflammatory, anti-histaminic and antibiotic coverage was followed for three days.

After follicular evacuation, modified Cosynch protocol was initiated on the same day with hCG (3000 IU; iv) followed by prostaglandin (Inj. Cloprostenol; 500 µg; im) seven days later and GnRH analogue (Inj. Buserelin acetate; 10 µg; im) 48 h post prostaglandin administration. All the animals were inseminated twice at 24 h interval in the synchronized oestrus and were followed to observe for the recurrence of the condition.

The efficacy of hormonal therapy and follicular evacuation techniques were assessed based on the percentage of recovery and recurrence of the cystic condition.

**Statistical analysis :** The mean diameter of the NRFCs on Day 0, 7 and 9 of hormonal protocol and on the day of subsequent oestrus (day of evacuation) were statistically analysed for significance using students ‘t’ test (Snedecor and Cochran, 1994).

**RESULTS AND DISCUSSION**

Sequential observations of the follicular entities

**Table 1**

**Sequential biometry (Mean ± SE) of responsive and non-responsive follicular cysts**

Cyst	Diameter (mm)			
	Day of first examination	Day 0 (hCG)	Day 7 (PG)	Day 9 (GnRH)
Responsive cyst (n=35)	20.7±0.3 (17.8-25.4)	21.0±0.3 (18.2-27.2)	-- (Luteinized)	--
NRFC (n=6)	20.6±0.7 (18.2-22.1)	20.9±0.7 (18.8-22.5)	26.1±0.5 (26.8-29.5)	22.9±0.5 (21.7-24.8)

Range within parenthesis

(Table) during the hormonal therapy revealed that 35 (85.4%) out of 41 animals with OFCs responded to hCG therapy with varying degrees of luteinization (> 3 mm follicular wall thickness) on Day 7 and regressed on Day 9 following prostaglandin. In the rest of the six animals (14.6 %), the cystic follicle found on Day 0 were observed with a mild degree of luteinization (< 2mm follicular wall thickness) on Day 7 and persisted without responding to PG, and hence considered to be NRFCs. The mean diameter of the NRFCs on Day 0, 7 and 9 were 20.9 ± 0.7, 26.1±0.5 and 22.9±0.5 mm, respectively. In the subsequent cycle, these NRFCs persisted and increased significantly (P < 0.01) in diameter (38.9 ± 0.5 mm). The uncontrollable acute increase in the diameter of non-responsive cysts was in accordance with the previous reports of Calder *et al.* (2001) and Satheshkumar *et al.* (2018). The incidence of animals with NRFCs (14.6 %) was higher than the previous report of 4.0 per cent recorded by Gupta *et al.* (2019).

In the six non-responsive animals, it was interesting to observe coexistence of a normal DF in any one of the ovaries along with the larger cystic follicle (Fig. 2a). These DFs responded to hCG by ovulation, transformed into a CL (Day 7) and regressed on Day 9, but the cystic follicle found on Day 0 persisted in the ovary. Cattle are usually mono-ovular species, but multiple ovulations do occur occasionally. During the follicular turnover, only one follicle will deviate and attain the dominance while the rest of the recruited follicles will undergo atresia. Co-dominance might occur when more than one follicle could deviate at the same time (Macmillan *et al.*, 2018). In the present clinical cases, such phenomena of co-dominance would have occurred, but one of the follicles persisted as cyst instead of ovulation. Based on the characteristics of NRFCs like non-responsiveness to the routine hormonal therapy, acute increase in diameter and coexistence with a responsive DF, it could be hypothesized that the condition is more restricted to the individual defective follicle rather than the generalized deficient endocrine status of the



Fig. 1. 'Trans-gluteal' approach of follicular cyst evacuation technique



Fig. 2. Ultrasonographic document of follicular evacuation- (a) Pre evacuation - NRFC with mild luteinization. DF also noticed, (b) Post evacuation - Completely evacuated cyst. DF noticed, (c) Seven days post evacuation - CL noticed

animal. These NRFCs would have lost its dominance due to lack of development of LH receptors in the granulosa cells. With the probable presence of FSH receptors, these follicles might be increasing in size acutely in response to FSH. Further molecular studies are warranted to confirm the hypothesis.

The animals with NRFCs were normally cyclic with regular follicular and luteal turnover, but they were found to be repeaters posing the problem on their fertility. Further, non-responsiveness and acute increase in cystic volume warranted follicular evacuation to avoid further consequences by these inert space occupying structures. Previously, manual and ultrasound guided methods of 'trans-vaginal' follicular cyst evacuation were reported by Satheshkumar *et al.* (2018) and Pugazharasi *et al.* (2020), respectively. The former technique was found to be laborious and time consuming, while the ultrasound guided follicular evacuation requires sophisticated instruments along with skilled personnel. On the other hand, 'trans-gluteal' approach of follicular cyst evacuation is found to be of easy application with minimal intervention, less time consuming, cost effective and without the need of sophisticated equipment. The technique is also highly efficient with 100 per cent recovery without any consequences.

Recurrence of OFC condition was observed in eight (19.5 %) out of 41 animals subjected for hormonal therapy, but there was no recurrence of cystic condition in the animals subjected to follicular evacuation technique. In the present technique, follicular evacuation was combined with modified Cosynch protocol, which ensured complete

luteinization of the evacuated cyst (Fig. 2c) and its regression following prostaglandin. Pugazharasi *et al.* (2020) reported recurrence of follicular cyst following ultrasound guided follicular cyst aspiration, which might be due to not combining the hormone therapy after evacuation. Amiridis (2009) also concluded that persisting cases of OFCs could be treated by combining aspiration with a hormonal regime.

All the six animals (100.0 %) with NRFCs responded to hormone therapy post evacuation by exhibiting oestrus with normal DF and regressed CL. Following insemination, three (50.0 %) animals were confirmed of pregnancy by 45-60 days, while the remaining three animals returned to next cycle within 20-22 days. The ovaries were normal without any adhesions or any other consequences. Thus 'trans-gluteal' approach of follicular cyst evacuation technique in combination with hormone therapy is proved to be effective in correcting the NRFCs in crossbred cattle.

## CONCLUSION

The incidence of NRFCs among the cystic conditions is found to be increasing in crossbred cattle. It could be attributed to the defective follicular micro-environment with deficient endocrine receptors in the granulosa cells. Elaborate molecular studies are required to confirm the aetio-pathogenesis of the NRFCs. 'Trans-gluteal' approach of follicular cyst evacuation technique is found to be cost effective and user friendly in correcting the NRFCs and can be recommended for routine application in field conditions.

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