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**Original Research**

# Management of Follicular Cyst by Multiple Approaches in Crossbred Cows

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## Abstract

*A retrospective analysis was carried out in forty-nine numbers of Jersey crossbred cows, diagnosed with ovarian follicular cyst managed with four approaches. The first approach (n=49) to treat the follicular cyst was therapy with Inj. Buserelin acetate (20 µg; i.m.). 28 cases (57.14%) responded and ultrasonography revealed luteinisation of cyst, 42.85 per cent, 32 per cent and 25 per cent animals conceived after 1st, 2nd and 3rd AI respectively. In the second approach (n=21), the non-responded cases of first approach were administered with Inj. human chorionic gonadotropin (1500 I.U.; i.v.). Thirteen cases (61.9%) responded, 23.0, 53.8 and 23.0 per cent animals became pregnant after 1st, 2nd and 3rd AI, respectively. In third approach, eight non-responded cases (16.3%) were subjected to indigenous progesterone impregnated CIDR therapy and 75% (6/8) of cases responded successfully, out of which 83.3 and 16.6 per cent conceived with 1st and 3rd AI. Lastly two cases (4.0%) were diagnosed as non-responsive follicular cysts and were successfully treated by ultrasound guided trans-vaginal follicular cyst ablation technique. This study concludes the successful management of follicular cystic conditions with multiple approaches which can be easily adopted in the field conditions.*

**Keywords:** Crossbred Cattle, Follicular Cysts, Management, Multiple Approaches, Ultrasonography

## Introduction

Follicular cysts in cattle are defined as large follicle like structures present on one or both ovaries with a diameter greater than or equal to 2.5 cm in the absence of any luteal tissue and that persists for at least 10 days which clearly interfere with normal ovarian cyclicity (Vanholder *et al.,* 2006). This disorder is more common in high yielding dairy cattle characterized by ovulatory failure (Oltenacu *et al.,* 1990). The incidence ranges from 5 to 8 per cent in cattle (Vanholder *et al.,* 2006). Follicular cyst is more common in the early post-partum period (less than 60 days) during which period cows are under great metabolic stress. Many factors have been associated with its pathogenesis which include high milk production, negative energy balance, ketosis, twinning, peri-parturient problems, genetic predisposition, season and nutritional disorders (Roberts, 1986). Ovarian cyst results from the ovulation failure due to LH insufficiency. Follicular cyst can be diagnosed on the basis of history of nymphomania, per rectal examination, ultrasonography (USG) and plasma or milk progesterone assay. Ultrasonography is an important diagnostic tool to differentiate follicular and luteal cyst in the field conditions. On ultrasonographic examination, follicular cysts have a thin wall (≤3 mm) with uniformly anechoic follicular fluid (Jeffcoate and Ayliffe, 1995). Different therapeutic protocols, *viz.*, manual rupture, gonadotropin releasing hormone (GnRH), human chorionic gonadotropin (hCG), prostaglandin F2alpha (PGF2α) and progesterone have been used and evaluated with respect to their efficacy post-treatment (Peter, 2004). Prompt diagnosis and management is the key to reduce the economic loss to the farmers. Hence, this study presents the multiple sequential approaches to treat the follicular cyst in the field conditions and to improve reproductive performance post-treatment in crossbred cattle.

## Materials and Methods

A total of 49 crossbred cows, aged 2 to 8 years, which were diagnosed and treated for cystic degeneration of follicles at the Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli (Latitude: 8.741222; Longitude 77.694626; with the GPS coordinates of 8° 44' 28.3992" N and 77° 41' 40.6536" E) during August 2017 to March 2019 were analysed for this study.

The cases were presented with the history of infertility associated with irregular estrous cycles, prolonged estrus period, nymphomania, repeat breeding were subjected for regular clinical observation, gynaeco-clinical examination and finally ultrasonographic investigation for confirmation. On the basis of anamnesis, rectal examination (large single or multiple fluid filled structures with 'pitting' on palpation) and ultrasonographic examination of ovaries (single or multiple uniformly anechoic follicular fluid filled structure > 25 mm and absence of corpus luteum), the cases were confirmed as ovarian follicular cysts. All the animals with follicular cysts (n=49) were subjected to Ovsynch protocol (Group I), wherein GnRH analogue (Buserelin acetate; Gynarich; Intas Pharma, Ahmedabad) was administered (20 µg; i.m.) on Day 0. Animals were examined ultrasonographically on Day 7 post-GnRH for the evidence of luteinisation of the cyst. A total of 28 animals had responded with luteinisation of cyst and were administered with synthetic prostaglandin (Cloprostenol; Pragma; Intas Pharma, Ahmedabad; 500 µg; i.m) on the same day, followed by GnRH on Day 9 (induced estrus) and two inseminations at 24 h interval on Day 10 and 11. The animals were followed for three consecutive cycles and inseminated if returned to estrus.

The 21 animals which had not responded to first GnRH with luteinisation were subjected to the modified Ovsynch protocol (Group II) by administering Inj. human chorionic gonadotropin (Chorulon; MSD Animal Health India; 1500 I.U.; i.v.) in place of first GnRH of above approach. In animals with luteinisation (n=13) on Day 7 post-hCG, the protocol was completed with PG and GnRH followed by inseminations as described previously. Eight animals which have not responded to hCG were further subjected to CoSynch + CIDR protocol (Group III). In this approach, buserelin acetate (10 µg; i.m.) was given on Day 0 and indigenously developed progesterone impregnated sponge (CIDR: 1.20 g natural progesterone 4-Pregnene-3, 20-dione, Sigma-Aldrich, in ethanolic solution) was inserted simultaneously intra-vaginally. After seven days, CIDR was removed and Inj. Cloprostenol Sodium (500 µg; i.m.) was administered. On day nine, the animals that were in estrus were inseminated twice at 24 h intervals along with GnRH during first insemination. The animals returned to estrus were followed for three more cycles and inseminated as described previously.

The animals which had not responded to any of the previous three protocols (n=2) were considered to be having 'non-responsive' follicular cysts. These animals were subjected to ultrasound guided follicular cyst ablation technique (Group IV). Under epidural anaesthesia, trans-rectal probe of the scanner was passed via rectum and positioned over the follicular cyst. A sterile 18G needle was guided through vagina within the folded fingers and

the follicular cyst was punctured by continuously viewing the live monitor image. The vaginal canal was flushed with 1% potassium permanganate solution. These animals were inseminated in the subsequent estrus. The pregnancy was diagnosed after 45 days of last AI by per rectal and ultrasonographical examination. The number and percentage of animals responded for the treatment in each group was recorded. The number and percentage of animals conceived in three consecutive cycles were also recorded. The percentage of animals responded and the conception rate were compared between all the treatment groups.

## Results and Discussion

Among the Group I of forty-nine animals which were subjected to first approach to treat the follicular cyst with Ovsynh protocol (Figures 1 to 3), 28 cases (57.14%) responded with luteinisation of cyst on Day 7. Out of 28 responded cases, 12 (42.85%) (Figure 7), 9 (32.14%) and 7 (25.00%) were conceived after 1st, 2nd and 3rd AI, respectively (Table 1). As per Leonardo and Collin (2004) natural or synthetic GnRH analogues are the most common treatment for follicular cyst which causes subsequent increase in LH secretion from anterior pituitary resulting in luteinisation of the cyst. These changes in the cyst switches steroidogenic synthesis pathway from the release of estradiol from the follicular cyst to progesterone from the luteinised cyst, which is responsive to prostaglandin-F2α (PGF2α) analogues. Garverick (1997) reported normal cyclic ovarian activity in 72% to 85% of the cows treated with GnRH and their pregnancy rates at first estrus ranged from 46% to 58%. In present study, 57.14 (28/49) % cows responded and pregnancy rate with 1st AI was 42.85%, which is in agreement with Leonardo and Collin (2004) and Garverick (1997).

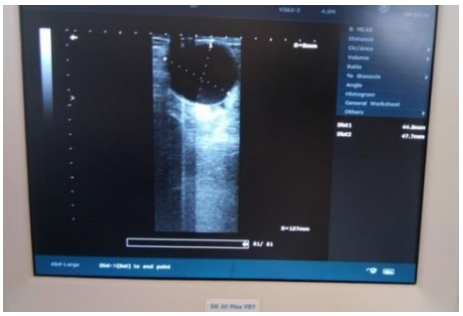

**Figure 1:** Day 0- Anechoic follicular fluid filled cavity > 52 mm with wall thickness less than 3 mm.

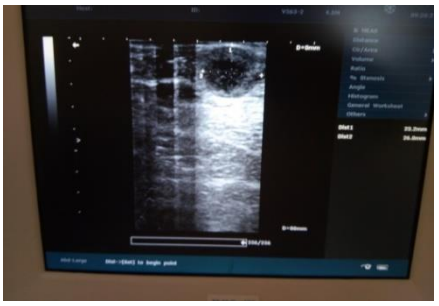

**Figure 2:** Day 7- Hypoechoic luteinised cyst treated with Inj. Cloprostenol sodium 500 µg i/m

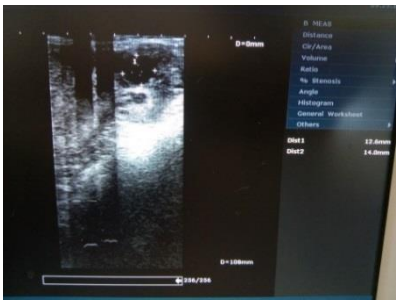

**Figure 3:** Day 9- Luteolysis of luteinised cyst, Development of anechoic dominant follicle >12 mm treated with Inj. buserelin acetate 10 µg, i/m

**Table 1:** Assessment of multiple approaches in management of follicular cyst

| Group | Treatment approach | Animals responded to treatment | Conception rate (%) | | |
|---|---|---|---|---|---|
| | | | 1st AI | 2nd AI | 3rd AI |
| I (n=49) | Buserelin acetate + Cloprostenol + Buserelin acetate | 28(57.14) | 12 (42.85) | 9 (32.14) | 7 (25.00) |
| II (n=21) | hCG + Cloprostenol + Buserelin acetate | 13 (61.90) | 3 (23.07) | 7 (53.85) | 3(23.07) |
| III (n=8) | Indigenous CIDR with Cloprostenol sodium + Buserelin acetate | 6(75.00) | 5 (83.33) | 0 | 1(16.67) |
| IV (n=2) | Ultrasound guided trans-vaginal follicular cyst ablation technique | 2 (100.00) | 2(100.00) | 0 | 0 |

*(% in parenthesis)*

In group II, non-responded 21 cases (42.85%) to GnRH were subjected to second approach, *i.e.*, Modified Ovsynch using hCG on Day 0 thirteen cases (61.9%) underwent luteinisation of cyst on seventh day post-treatment with hCG and fixed timed AI was performed on day 10 and 11. Out of 13 turned up cases 3 (23%), 7 (53.8%) and 3 (23%) animals conceived with 1st, 2nd and 3rd AI, respectively (Table 1). In previous comparative studies, buserelin acetate and human chorionic gonadotropin (hCG) produced similar effects (Garverick *et al*., 1976). However, in our study the success rate of animals responded to hCG treatment was 61.9% which had not responded to previous GnRH analogue. So, the observation of present study was in contradiction to the findings of Garverick *et al.* (1976).

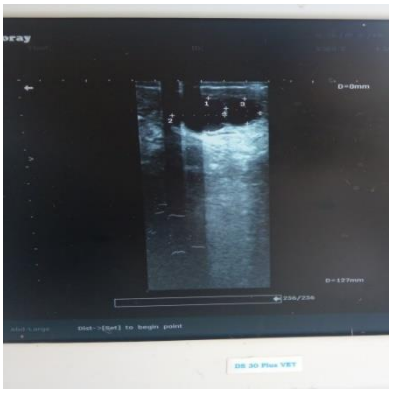

**Figure 4:** Day 0-Two anechoic follicular fluid cavity size 24 mm and 23.5 mm Insertion of indigenous progesterone sponge Inj. buserelin acetate 20 µg, i/m

**Figure 5:** Day 9- Non-responsive follicular cysts 30 mm in diameter Ablation of cyst, Arrow1: Hyperechoic needle tip in anechoic cystic fluid, Arrow 2: Anechoic follicle

**Figure 6:** Anechoic preovulatory follicle after ablation of cyst

Thirdly, the non-responded eight cases (16.3%) (Group III) to GnRH and hCG were further subjected to CoSynh + CIDR protocol Figure 4) and in responded cases fixed time AI was performed on day 9 and 10. Six (75%) animals responded and after 1st and 3rd AI, 5 (83.3 %) and 1 (16.7 %) animals, respectively, were conceived (Table 1). As per Stock and Fortune (1993) the increased plasma progesterone derived from the progesterone passerines reduces the frequency of the LH pulses which in turn removes the gonadotrophin support for the follicular cyst and emerging the new follicular wave and produces a follicle which undergoes final maturation and when the source of progesterone is removed it results in increased frequency of the LH pulses which causes the luteinisation of the cyst and subsequent treatment with prostaglandin causes the lysis of the luteinised cyst and ovulation of dominant follicle. Estrus rates ranging from 82% to 100% and conception rates at first estrus ranging from 18% to 28% have been reported after progesterone treatment (Douthwaite and Dobson, 2000). Present study revealed 75% estrus rate with 83.3 % conception rate with first AI (Table 1) which is in agreement with Zulu *et al.* (2003).

Fourthly, two cases (4.0%) (Group IV) were diagnosed as non-responsive follicular cysts to previous three approaches (Figure 5) and were treated by ultrasound guided trans-vaginal follicular cyst ablation technique on day 9 of CoSynch + CIDR therapy. Both these cases were having a dominant follicle >18 mm (Figure 6) at subsequent estrus and were inseminated along with inj. Buserelin acetate (10 µg; i.m.). Both the cases responded and became pregnant with 1st AI. As per Singh *et al.* (2017), transvaginal ultrasound guided cyst ablation can be effectively used as an alternative non-hormonal method for treating cystic ovarian follicles in dairy cattle with 80% animals shown spontaneous estrus with 33.3% pregnancy rate with 1st AI. In our study 100% animals showed the spontaneous estrus and conception (Table 1), although the number of cases subjected to this approach was less. So, this approach can be considered as an option for the management of non-responsive follicular cyst in the field conditions, but the further detailed study is required in future to determine the advantages and disadvantages of this approach.

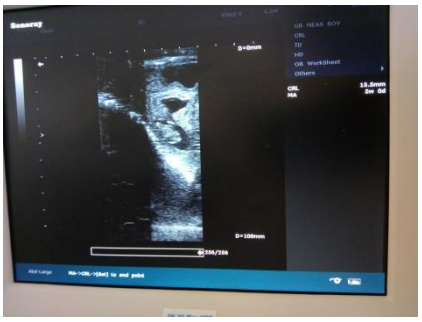

**Figure 7:** Early pregnancy approximate 35 daysCRL 15.5mm

## Conclusion

This study concluded that GnRH analogues and hCG therapy are the non-invasive easiest approaches to treat the cases of follicular cyst in the field conditions. Progesterone impregnated indigenous device (Progesterone sponge, CIDR) is the most effective approach in terms of reproductive performance in the field conditions. Ultrasound guided trans-vaginal follicular cyst ablation technique can be considered as an option for the non-responsive follicular cyst with some more studies in future. Ultrasonography is a reliable tool for timely diagnosis of the cyst and assessment of the responsiveness of the follicular cyst with multiple approaches.

## Acknowledgement

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## Conflict of Interests

There is no conflict of interest.

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