RESEARCH ARTICLE

Prevalence, Risk Factors and Differential Diagnosis of Cystic Ovarian Degeneration in Crossbred Cows

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Abstract

The present study was conducted under field conditions on >90 days postpartum HF crossbred cows with cystic ovaries (n = 58). The diagnosis was confirmed by history and trans-rectal palpation and ultrasonographic examinations twice at weekly interval using 5.0–7.5 MHz frequency probe and was later authenticated with plasma progesterone assay and treatment response. The animals with follicular cysts were randomly treated with either conventional ovsynch or ovsynch + CIDR protocol with fixed time AI (n = 10 each), and those with luteal cysts with either double PG injections 11 days apart or modified Ovsynch protocol (n = 16 each). Among 58 cystic cows, the highest incidence (62.07 %) of the ovarian cyst was recorded in the age group of 5–7 years followed by above 7 years (36.21%) and 3–5 years (2.00%). The incidence was highest among cows of 3rd or more parity (70.69%) followed by 2nd parity (29.31%), and no case was seen in primiparous cows. Of the total 36.21% were follicular type cyst and 63.79% luteal type cysts. The right ovary had a high incidence of the cyst (51.72%) followed by the left ovary (36.21%), and bilateral (12.07%). Based on rectal palpation, the cystic ovary was classified to have follicular cyst in 36.21% (21/58) cases and luteal cysts in 63.79% (37/58). Ultrasound examination showed follicular and luteal cysts as 27.59% and 72.41%, whereas plasma P_4 (</> 1 ng/mL) analysis revealed this as 20.69% and 79.31%, respectively. The clinical diagnosis became more accurate with a combination of per rectal palpation and USG and was further improved by plasma progesterone assay. The mean diameters and a wall thickness of cysts varied highly significantly (p <0.01) between groups/protocols. The conception rates at induced estrus with FTAI in cows under ovsynch, ovsynch + CIDR, modified ovsynch and double PG protocols were 50.00, 40.00, 50.00 and 43.75 percent, respectively. It is thus concluded that in crossbred cows luteal cysts are more common than follicular cysts, particularly in prime aged animals of 3rd or 4th parity with more of left ovarian involvement. The differentiation of cyst type is best achieved with the combined use of USG and/or plasma progesterone assay with rectal palpation, and that ovsynch protocol appeared promising for the treatment of follicular cysts and Modified Ovsynch for luteal cysts.

Keywords: Crossbred cattle, Differential diagnosis, Ovarian cysts, Prevalence, Risk factors, Treatment response, Ultrasonography.

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Introduction

One of the most common ovarian dysfunctions during the postpartum period is the formation of ovarian cyst following ovulation failure (Opsomer *et al*., 1998). Cystic ovarian degeneration (COD) with the prevalence of 10–13% is a common and economically significant condition of dairy cattle affecting their fertility (Lopez-Diaz and Bosu, 1992; Johnson and Coates, 2004). The exotic and crossbred animals are highly susceptible to COD. Many predisposing factors are attributed to COD that include parity, high milk production, season, stress, and negative energy status (Lopez-Diaz and Bosu, 1992). Etiology of ovarian cysts is multifactorial, and it depends on the phenotypic, genetic, and environmental factors. The most widely accepted hypothesis describing the formation of a cyst is that the pre-ovulatory LH-surge is either absent, insufficient or occurs at a wrong time during dominant follicle maturation (Lopez-Diaz and Bosu, 1992; Hamilton *et al*., 1995). In determining the type of cyst, the accuracy of palpation per rectum can be increased by the simultaneous use of USG and/or plasma progesterone assay. Ultrasonography is an accurate tool to differentiate luteal and follicular cysts. Follicular cysts are more than 25 mm in diameter and have a smooth thin wall (<3 mm) and an anechogenic antrum, whereas luteal cysts have a thicker wall (3–5 mm) and grey echogenic patches within the antrum (Farin *et al*., 1992; Youngquist and Threlfall, 2007; Kahn, 2010). It is therefore imperative to use palpation per rectum with ultrasound or plasma progesterone assay to differentiate ovarian follicular cysts from luteal cysts. This study was therefore planned to know the prevalence, risk factors, and differential diagnosis of ovarian cysts in cattle.

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Materials and methods

The present study was carried out on animals selected from the field as well as from the organized private dairy farms under the Amul milk shed area in and around Anand, Gujarat, during September 2018 to May 2019. The study covered 58 HF crossbred cows with cystic ovaries during >90 days postpartum. Problem breeders were confirmed by history, transrectal palpation, and ultrasonographic examinations twice at weekly interval using linear array transducer with 5.0–7.5 MHz frequency. The diameters and wall thickness of ovarian cysts were recorded in millimeter. Ovarian cysts more than 22 mm in diameter, having a smooth, thin wall (<3 mm) and an anechogenic antrum were classified as Follicular cysts and those having a thicker wall (3–5 mm) and grey echogenic patches within the antrum as luteal cysts as per standard criteria (Youngquist and Threlfall, 2007). Blood samples were collected simultaneously and plasma was stored at –20°C to estimate progesterone levels using the RIA technique to confirm the findings retrospectively. The details of the age, parity, clinical signs, rectal palpation findings, sonographic findings, and breeding history were recorded to study the prevalence of COD. The animals with follicular cysts were then randomly treated with either conventional Ovsynch or Ovsynch + CIDR protocols with fixed time AI (n = 10 each), and those with luteal cysts with either Double PG injections 11 days apart or modified Ovsynch protocols (n = 16 each). A group of six cystic cows was also kept as untreated Control. The comparative efficacy and agreement between three diagnostic techniques adopted were then worked out including response and conception rate at fixed time AI following different protocols. The data were analyzed suitably using either descriptive statistics or ANOVA and DMRT (Snedecor and Cochran, 1994).

Results and discussion

Risk Factors and Prevalence of COD in Crossbred Cows

The prevalence of COD studied according to the age among the total of 58 crossbred cows revealed the highest incidence of ovarian cyst among cows with age 5–7 years (62.07%) followed in descending order by above 7 years (36.21%) and the least in younger 3–5 years (2.00 %) age group. Similar findings were recorded by Nelson *et al.* (2010). However, Noseir *et al.* (2013) found the highest incidence of ovarian cysts in 3–5 years age group of Holstein cows. This difference could be attributed to breed, production potential, nutritional management and agro-climatic conditions of the area. In a recent Wisconsin field study of dairy herds by Amer and Badr (2006), the incidence of ovarian cysts ranged from 16.2–47.4%. Mature cows had a higher incidence (39.0 %) of ovarian cysts than do first-calf heifers (11.0%).

The parity wise classification of affected crossbred cows revealed the highest incidence of ovarian cyst amongst cows of ≥3rd parity with 70.69% incidence followed by cows of 2nd parity (29.31%), while no case was found in 1st parity cows (Table 1). Cows in higher parity have an increased risk of being diagnosed with ovarian cysts (Fleischer *et al*., 2001). Increasing parity was considered a significant risk for the development of ovarian cysts in dairy cows (Nelson *et al*., 2010). The increasing incidence of COD found in crossbred cows with advancing age/parity was also in line with the earlier observations of Amer and Badr (2006).

Evaluation of type and location of ovarian cysts in crossbred cows studied revealed that 36.21% of cysts were follicular type and 63.79% were of luteal type. Among total cysts, the right ovary had the highest incidence of the cyst (51.72%) followed by the left ovary (36.21%) and bilateral cases (12.07%) (Table 2). Amongst total 21 cases of follicular cysts, the highest incidence of 27.59 % follicular cysts were found on the right ovary followed by bilateral (6.90%), and the lowest in the left ovary (1.72%). Amongst total 37 cases of luteal cysts, the highest incidence of 34.48% luteal cysts were found on the left ovary followed by the right ovary (24.14%) and the lowest in bilateral (5.17%) cases (Table 3).

Similar observations have been reported by Farin *et al*. (1990), who classified 67 ovarian cysts by serum progesterone concentrations as 47 luteal cysts and 20 follicular cysts. Leslie and Bosu (1983) clinically found 30 cases of follicular cysts and 32 cases of luteal cyst among 62

Table 1: Age and parity wise prevalence of COD in crossbred cows

Details	*Total cases*	*Age group*			*Parity/lactation*		
		3–5 years	*5–7 years*	*>7 years*	*1st*	*2nd*	*≥3rd*
Number	58	1	36	21	0	17	41
%	100	2.00	62.07	36.21	0.00	29.31	70.69

Table 2: Ovarian involvement and type of cysts in crossbred cows with COD

Details	*Total cases*	*Ovarian involvement*			*Type of cyst*	
		Right ovary	*Left ovary*	*Bilateral*	*Follicular cyst*	*Luteal cyst*
Number	58	30	21	7	21	37
%	100	51.72	36.21	12.07	36.21	63.79

Table 3: Types of ovarian cysts and its site of predilection in crossbred cows with cystic ovarian degeneration

Details	Total cases	Follicular cyst			Luteal cyst		
		Right ovary	Left ovary	Bilateral	Right ovary	Left ovary	Bilateral
Number	58	16	1	4	14	20	3
%	100	27.59	1.72	6.90	24.14	34.48	5.17

COD cases in exotic cattle. However, in contrast to present findings, higher incidences of follicular type cysts and lower incidence of luteal type cysts have been documented by others (Douthwaite and Dobson, 2000; Chaudhary and Mehta, 2014) in different studies from India and abroad. The variations regarding occurrence of follicular and luteal cysts reported in different studies could be attributed to the breed, age/parity, endocrine and nutritional status, duration of existence of cyst, adrenal function, postpartum interval, lactation stage and production potential of animals, diagnostic modality, expertise of clinician, and preventive measures adopted as well.

Efficacy of Three Differential Diagnostic Methods of Ovarian Cysts

In the present study, identification and differentiation of the type of ovarian cysts were made on 58 cystic ovarian cows based on per rectal palpation, trans-rectal ultrasound examination, and plasma progesterone assay. Rectal palpations of cystic ovarian cases, could identify 36.21% cases as follicular cyst, having relatively large size soft fluctuating structure on either of the ovaries, and 63.79% as luteal cyst with thick wall and hard consistency, whereas plasma progesterone analysis revealed 20.69% cases of follicular cyst (<1 ng/mL plasma P_4) and 79.31% luteal cyst (>1 ng/mL plasma P_4). Ultrasound examination, on the contrary, showed some different percentages of cystic ovaries, wherein follicular cyst (<3 mm wall thickness) was confirmed in 27.59% and luteal cyst (>3 mm wall thickness) in 72.41% cases (Table 4). Thus, the accuracy of differential diagnosis of ovarian cysts varied among the three methods followed. The clinical diagnosis with per rectal palpation was inaccurate, but became more accurate with USG and was further improved by plasma progesterone.

Indeed, misdiagnosis of an ovarian cyst leads to incorrect therapeutic choice, which results in failure of response and delays the conception leading to economic loss to the farmer. The probability of a false diagnosis of ovarian cyst is larger with the structures diagnosed firstly as being a follicular cyst. Our observation was in agreement with Leslie and Bosu (1983), Farin *et al.* (1992), Ribadu *et al.* (1994), Hanzen *et al.* (2000) and Gundling *et al.* (2015) regarding prevalence of follicular and luteal cysts, and comparative accuracy/ reliability of three diagnostic approaches tested. These results reiterate the importance of ultrasound examination and hormonal diagnosis in the identification of the type of ovarian cyst before initiating costly hormonal treatment.

Table 4: Types of ovarian cysts according to the diagnostic methods used in crossbred cows

Method	No.	Type of cyst	
		Follicular cyst	Luteal cyst
Per rectal palpation	58	21 (36.21%)	37 (63.79%)
Transrectal USG	58	16 (27.59%)	42 (72.41%)
Plasma progesterone	58	12 (20.69%)	46 (79.31%)

Ultrasonographic Evaluation of Ovarian Cysts

The mean diameters of ovarian cysts and their wall thickness observed through ultrasonography in cystic cows put under ovsynch, ovsynch + CIDR, modified ovsynch and double PG protocols, and in control group varied highly significantly (p <0.01), the diameter being larger and wall thickness being smaller in groups treated for follicular cyst and vice versa for groups with luteal cysts (Table 5).

The mean diameters of ovarian cysts in all four groups were close to 25 mm (range 17–28 mm) defined for ovarian follicular and luteal cysts in the literature (Tebble *et al.,* 2001; Lopaz-Gatius and Lopez-Bejar, 2002; Enginler *et al.,* (2012). Further, the thickness of cyst wall in animals of Ovsynch group having the follicular type of cysts as revealed by manual palpation and plasma P_4 was also the lowest, whereas the same for animals of modified ovsynch and double PG groups having the luteal type of cysts was the highest and correlated with plasma progesterone profile. The intermediate mean wall thickness of cysts for animals of other groups particularly ovsynch + CIDR and untreated control group was due to the fact that these groups had few cows with cysts of either type. The results were also substantiated with conception rates at FTAI in different treatment groups.

Agreement between Different Diagnostic Methods followed

The agreement among three different methods of diagnosis of follicular and luteal cysts used in crossbred cows and thereby their accuracy is shown in Table 6. The data reveal that agreement of rectal palpation (follicular cyst: FC-21, luteal cyst: LC-37) with USG and plasma progesterone assay for follicular cyst was 71.43 and 57.14% and for luteal cyst 97.30 and 100.00%, respectively, whereas the agreement of rectal palpation with both USG and plasma progesterone assay for follicular cyst was 57.14% and for luteal cyst it was 97.30 %.

The agreement of USG finding (FC-16, LC-42) with that of rectal palpation and plasma P_4 assay for follicular cyst

Table 5: Mean (±SE) initial diameters of ovarian cysts and their wall thickness assessed by transrectal ultrasonography and fertility response in cows put under different treatment protocols

Criteria	*Groups/treatment protocols*				
	Ovsynch protocol	*Ovsynch + CIDR*	*Modified ovsynch*	*Double PG injection*	*Untreated control*
Diameter of cyst (mm)	24.16 ± 0.79[a]	24.00 ± 0.58[a]	21.25 ± 0.56[b]	22.17 ± 0.31[b]	23.53 ± 0.78[a]
Thickness of cyst wall (mm)	1.85 ± 0.09[c]	2.70 ± 0.49[b]	3.37 ± 0.11[a]	3.70 ± 0.21[a]	2.59 ± 0.42[b]
Conception rate at FATI (%)	50.00 (5/10)	40.00 (4/10)	50.00 (8/16)	43.75 (7/16)	0.00 (0/06)

Means bearing uncommon superscripts within the row ([abc]) differ significantly (p <0.05)

Table 6: Agreement among three different methods of diagnosis of ovarian cyst in crossbred cows

Agreement of	*Follicular cyst*	*Luteal cyst*
Per rectal palpation with...	21	37
USG	15 (71.43%)	36 (97.30%)
Progesterone P4	12 (57.14%)	37 (100.0%)
USG and Progesterone	12 (57.14%)	36 (97.30%)
USG with...	16	42
Per rectal palpation	15 (93.75%)	36 (85.71%)
Progesterone P4	12 (75.00%)	40 (95.24%)
Per rectal and progesterone	12 (75.00%)	36 (85.71%)
Plasma progesterone with...	12	46
Per rectal palpation	12 (100%)	37 (80.43%)
USG	12 (100%)	42 (91.30%)
Per rectal and USG	12 (100%)	36 (78.26%)

was 93.75 and 75.00% and for luteal cyst 85.71 and 95.24%, respectively. The agreement of USG with both rectal palpation and plasma progesterone assay for follicular cyst was 75.00% and for luteal cyst 85.71% (Table 6). Further, the agreement of plasma progesterone assay (FC-12, LC-46) with the findings of per rectal palpation and USG for follicular cyst was 100 % each, while for luteal cyst per rectal palpation and USG had agreement of 80.43 and 91.30 %, respectively, whereas the agreement of plasma progesterone profile with both rectal palpation and USG for follicular and luteal cyst was 100 and 78.26%.

One of the aims of the present investigation was to assess the accuracy with which different common methods of diagnosis were able to differentiate between follicular and luteal cysts. Ultrasound was more accurate in aiding the diagnosis of follicular cysts than luteal cysts and combined with progesterone measurements it gave the most accurate assessment of the type of cysts. These observations were in agreement with the findings of Douthwaite and Dobson (2000). As in the present study, Farin *et al.* (1992) also found it more difficult to differentiate luteal cysts by rectal palpation alone, while in an earlier study, the same authors found ultrasound to be more accurate and repeatable in differentiating luteal from follicular cysts because it was easier to diagnose luteal structures (Farin *et al.,* 1990). This is probably because the luteal tissue can be visualized by ultrasound, but cannot necessarily be palpated (Ribadu *et al.,* 1994). In the present study, the mean plasma progesterone concentrations were significantly higher in the cows with luteal cysts, the walls of luteal cysts were thicker, and progesterone concentrations were positively correlated with wall thickness for both the types of cyst, as observed in several other studies (Farin *et al.,* 1990; Douthwaite and Dobson, 2000).

From the study, it is concluded that in crossbred cows luteal cysts are more common than follicular cysts, particularly in prime aged animals of 3rd or 4th parity with more of left ovarian involvement. The differentiation of cyst type is best achieved with the combined use of USG and/or plasma progesterone assay with rectal palpation, and that Ovsynch protocol appeared promising for the treatment of follicular cysts and Modified Ovsynch for luteal cysts.

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