



# Reproductive performance in dairy cows with cystic ovarian disease after single treatment with buserelin acetate or dinoprost

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**ABSTRACT.** The treatment of cystic ovarian disease (COD) in dairy cows is still controversial, and some researchers recommend using gonadotropin-releasing hormone (GnRH) regardless of the type of cysts. The aim of this study was to comparatively evaluate the reproductive performance of cows diagnosed with follicular or luteal cystic structures, after treatment with either buserelin acetate (GnRH agonist) or dinoprost (prostaglandin F<sub>2</sub>-alpha or PGF<sub>2α</sub>). The diagnosis was established by ultrasonographic examinations performed twice a month starting 40–45 days after calving, until the cows were diagnosed pregnant after artificial insemination. Both types of cysts were treated either with 21 μg buserelin acetate or 25 mg dinoprost, resulting in four subject groups. After treatment, the estrus rate in cows with follicular cysts treated with dinoprost (55.1%) was significantly lower ( $P < 0.05$ ) than in cows treated with buserelin acetate (77.5%) or in cows diagnosed with luteal cysts (77.2% for dinoprost and 72.4% for buserelin acetate). The conception rate was higher ( $P < 0.05$ ) in cows with follicular cysts treated with buserelin acetate (67.7%) than in those treated with dinoprost (60%) or in those with luteal cysts (56.9% for dinoprost and 47.5% for buserelin acetate). These results show that it is preferable to differentiate between the two types of ovarian cysts before treatment. Luteal cysts can be treated with either PGF<sub>2α</sub> or GnRH analogues, with better results when PGF<sub>2α</sub> is used. Contrastingly, follicular cysts are better treated with GnRH analogues. When differentiation is not possible, GnRH analogues are recommended over PGF<sub>2α</sub>.

**KEY WORDS:** COD, follicular cyst, GnRH, luteal cyst, PGF<sub>2α</sub>

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Cystic ovarian disease (COD) is responsible for frequent reproductive failure in cattle, resulting in significant economic losses for the dairy industry. This disease is characterized by the failure of a mature follicle to ovulate at the appropriate time during the estrous cycle [21]. Anovulatory ovarian structures with a cavity greater than 20 mm in diameter, without a corpus luteum, are defined as ovarian cysts (follicular or luteal). The difference between follicular and luteal cysts (FC and LC) is that the wall is less than 3 mm in FC and greater than 3 mm in LC [4]. However, this differentiation is difficult in field conditions [9, 24] and is often omitted from the diagnosis.

COD incidence within dairy farms is variable, ranging between 5 and 25%, which is most likely due to different management strategies. The factors most commonly associated with disease prevalence are heredity, high milk production, age, lactation period, body condition score, seasonality, and phytoestrogens. However, it has been suggested that retained placenta, milk fever, metritis, and stress also contribute to the occurrence of COD [5].

As the cumulative evidence suggests, there are multiple causes of COD, which makes it difficult to both understand its origins and develop an effective treatment [21]. In the case of LC, Kahn [18] recommended luteolytic doses of prostaglandin F<sub>2</sub>-alpha (PGF<sub>2α</sub>) as the ideal treatment, which results in estrus within 3–5 days. However, it was also reported that the treatment results could be variable as it is difficult to accurately determine the mass of luteal tissue present [18]. Although a LC should have enough luteal tissue to respond to a single dose of PGF<sub>2α</sub>, Bartolome *et al.* [2] recommended using gonadotropin-releasing hormone

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(GnRH) to treat both LC and FC.

The objective of the present study was to test the use of a single hormonal treatment against COD by comparing the estrus, conception, and pregnancy rates, treatment to first estrus interval (TFEI), treatment to conception interval (TCI), and the calving to conception interval (CCI) in postpartum multiparous dairy cows. The animals were administered either 21  $\mu$ g buserelin acetate (GnRH agonist) or 25 mg dinoprost (PGF<sub>2 $\alpha$</sub> ), after ultrasonographic detection of ovarian cysts.

## MATERIALS AND METHODS

This study was conducted over 24 consecutive months, on 325 Holstein cows housed in a dairy farm located in northeastern Romania with an average milk production of  $6.6 \times 10^3$  kg per cow per 305 days. The cows were maintained in a free-stall housing system and were fed a diet as described in Table 1.

Each multiparous cow had a voluntary waiting period of 60 days postpartum. Estrus detection was carried out by observations twice daily and was based on the behavioral signs of cows (stands to be ridden, bellows frequently, is nervous and excitable, sometimes rides other cows) along with a clinical examination of the genital tract (mucus discharge and swollen vulva). The cows suspected to be in estrus were clinically examined and only those with physiologically normal, clear, and viscous mucus were inseminated to maximize the chances of pregnancy. Animals with cloudy, purulent, sanguineous or watery vaginal discharge were not inseminated, as uterine infection was suspected, which is a condition associated with COD [22]. An artificial insemination (AI) technician used semen from a single bull to serve the selected cows approximately 12 hr after the onset of estrus (one straw per cow, one insemination per estrus). Pregnancy was diagnosed 40–45 days after AI by ultrasonographic detection of the embryonic heartbeat.

Transrectal ultrasonographic examinations were performed twice a month for all cows starting 40–45 days after calving, until they were diagnosed pregnant. For this examination, a scanner (WED 3000 V, Well.D Medical Electronics Co., Ltd., Shenzhen, China) with a linear-array transducer of 5–7.5 MHz was used. All ultrasound examinations were performed by the same veterinarian, with experience in ultrasonography of the genital tract in cows. Based on literature, cows with ovarian structures with a diameter greater than 2 cm [2], along with a flaccid uterus in the absence of a luteal structure were considered to have ovarian cysts [15, 35]. Cysts with a wall thickness greater than 3 mm and gray echogenic appearance were categorized as luteal, whereas cysts with a wall thickness greater than or equal to 3 mm and a uniformly anechogenic antrum were categorized as follicular [16, 35]. Furthermore, cows with a history of clinical diseases such as endometritis, mastitis, lameness, digestive disorders, and cows diagnosed with clinical endometritis by transrectal palpation were not used in this study. After the voluntary waiting period of 60 days, multiparous dairy cows diagnosed with different ovarian cysts and no apparent uterine disease received an intramuscular dose of either 21  $\mu$ g buserelin acetate (Receptal, MSD Animal Health, Boxmeer, the Netherlands) or 25 mg dinoprost (Dinolytic, Pfizer, Ixelles, Belgium). This experimental design resulted in four subject groups: cows diagnosed with FC and treated with dinoprost; cows diagnosed with FC and treated with buserelin acetate; cows diagnosed with LC and treated with dinoprost; and cows diagnosed with LC and treated with buserelin acetate. The Tukey-Kramer multiple comparison test was used to compare the rates of estrus, the conception rates per total number of inseminated cows, the pregnancy rates per total number of treated cows, and the TFEI, TCI, and CCI among the groups.

## RESULTS

There was no significant difference between the estrus rates in cows diagnosed with either follicular or luteal cysts that were treated with buserelin acetate (77.5% for FC and 72.4% for LC,  $P > 0.05$ ). In case of dinoprost treatment, the estrus rate was lower ( $P < 0.05$ ) for cows diagnosed with FC (55.1%) compared with cows diagnosed with LC (77.2%), which presented an estrus rate similar to those treated with buserelin acetate for both ovarian structures (Table 2). Additionally, there was a significant difference ( $P < 0.05$ ) between the conception rates of inseminated cows diagnosed with the two different ovarian structures; the rate was significantly lower in cows with LC treated with buserelin acetate (47.5%) compared with those treated with dinoprost (56.9%), as well as compared with cows with FC treated with either drug (67.7% for buserelin acetate and 60% for dinoprost). The highest percentage of successful pregnancies after treatment was also recorded in the group with FC treated with buserelin acetate (52.5%), while poor results were obtained after treating FC with dinoprost (15.4%). The TFEI was lower ( $P < 0.05$ ) in cows with LC treated with dinoprost (5 days) compared with cows with FC that received the same treatment (17.6 days) as well as cows treated with buserelin acetate (17.8 days for LC and 17.9 days for FC). The cows treated with buserelin acetate presented the same TFEI regardless of the type of ovarian cystic structure, and similar to those diagnosed with FC treated with dinoprost ( $P > 0.05$ , Table 2). In contrast, the TCI values were similar in cows with LC treated with dinoprost (40.3 days) and with FC treated with buserelin acetate (40 days), and these results were superior ( $P < 0.05$ ) to those obtained by using dinoprost for FC (69.7 days) and by using buserelin acetate for LC (49 days). As indicated by the CCI, the treatment of cows presenting LC was equally effective with either drug (120.7 days for buserelin acetate and 119.8 days for dinoprost), and the values were not significantly different from those

**Table 1.** Composition of diets fed to cows used in the study

Ingredient (kg/day)	Summer <sup>a)</sup>	Rest of the year <sup>b)</sup>
Alfalfa hay	2.5	3
Green-cut alfalfa and hairy vetch	35–40	0
Alfalfa silage	0	6.5–7
Corn silage	15	25
Concentrate supplement	5–6	5–6
Calcium carbonate	0.15	0.15
Mineral supplement	0.1	0.15

a) June–August; b) September–May.

**Table 2.** Comparison of responses in cows with luteal or follicular cysts to a single treatment with buserelin acetate (21 µg, gonadotropin-releasing hormone or GnRH agonist) or dinoprost (25 mg, prostaglandin F<sub>2</sub>-alpha or PGF<sub>2α</sub>)

Parameter	Treatment and ovarian structure			
	Buserelin acetate		Dinoprost	
	Luteal cyst	Follicular cyst	Luteal cyst	Follicular cyst
Estrus rate <sup>d</sup> (%)	72.4 <sup>a</sup> (50/69)	77.5 <sup>a</sup> (31/40)	77.2 <sup>a</sup> (78/101)	55.1 <sup>b</sup> (43/78)
Conception rate <sup>e</sup> (%)	47.5 <sup>c</sup> (19/40)	67.7 <sup>a</sup> (21/31)	56.9 <sup>b</sup> (33/58)	60.0 <sup>b</sup> (12/20)
Pregnancy rate <sup>f</sup> (%)	27.5 <sup>b</sup> (19/69)	52.5 <sup>a</sup> (21/40)	32.7 <sup>b</sup> (33/101)	15.4 <sup>c</sup> (12/78)
TFEI <sup>g</sup> (days) (mean ± SE)	17.8 ± 1.1 <sup>b</sup>	17.9 ± 0.9 <sup>b</sup>	5 ± 0.4 <sup>a</sup>	17.58 ± 1.2 <sup>b</sup>
TCI <sup>h</sup> (days) (mean ± SE)	49 ± 1.2 <sup>b</sup>	40 ± 1.9 <sup>a</sup>	40.3 ± 1.3 <sup>a</sup>	69.7 ± 0.7 <sup>c</sup>
CCI <sup>i</sup> (days) (mean ± SE)	120.7 ± 2.1 <sup>a</sup>	125.2 ± 2.1 <sup>a</sup>	119.8 ± 3.2 <sup>a</sup>	140.7 ± 1.9 <sup>b</sup>

a), b) and c) Values indicated by the same superscript alphabet significantly differ among rows ( $P < 0.05$ ). d) Percentage of cows that underwent estrus from the total number of cows included in the group. e) Percentage of pregnant cows from the total number of inseminated cows. f) Percentage of pregnant cows from the total number of cows included in the group; g) Treatment to first estrus interval. h) Treatment to conception interval. i) Calving to conception interval.

observed in cows with FC treated with buserelin acetate (125.2 days,  $P > 0.05$ ). The group with FC treated with dinoprost showed higher CCI (140.7 days,  $P < 0.05$ ) compared with the other three groups (Table 2).

## DISCUSSION

The accurate diagnosis of ovarian cysts currently requires a combination of transrectal palpation, transrectal ultrasonography, and the plasma progesterone assay. However, on-farm progesterone kits are not widely available in many countries [34], and variable threshold progesterone values have been reported in literature [35]. For this reason, farm veterinarians usually rely only on ultrasonography as a complementary method to transrectal palpation and symptomatology for assessing ovarian cysts. Since this study was intended particularly for helping farmers and farm veterinarians to determine the most effective and practical approach for COD in cows, the differentiation of ovarian cysts was based only on transrectal palpation and ultrasonography, which are widely available in the field. Although ultrasonography is not completely accurate, it represents nevertheless a robust method for differentiating ovarian cysts in cows, with accuracy ranging 75–95%, as reported by various authors [4, 10, 12, 27].

Current treatment regimens for COD involve GnRH, human chorionic gonadotropin, progesterone-releasing devices, and the Ovsynch protocol [9], which combines the use of GnRH with PGF<sub>2α</sub>. Even though the Ovsynch protocol appears to be the most logical approach, the pregnancy rates with timed inseminations following treatment are low, as with other hormonal treatments [15]. In addition, according to literature, there are risks associated with use of hormonal products in livestock, and animal health and fertility should be improved by selection and good management rather than by extensive use of hormones [26]. Furthermore, in our opinion, the extensive use of such products should not be encouraged but rather limited to situations where other treatment options are not available or prove to be inefficient. Thus, for health concerns and practical reasons, our study aimed at testing the use of a single hormonal treatment after diagnosis performed with easily accessible tools.

GnRH analogues such as buserelin acetate are the main ovulation inducing agents in dairy cows [20], which are being widely used for the treatment of ovarian cysts [23] and reportedly also prevent FC [19]. Our study tested the dogma that cows with COD, particularly those with FC, should be treated with GnRH [4]. The treatment protocols for COD are numerous and variable [22, 25, 28, 37], and selection of a suitable protocol is influenced by many variables like the costs and expected treatment benefits, cost of animal replacement, and the breeding value of the animal [3, 7, 8, 17, 30, 33]. Bartolome *et al.* [2] established that the differentiation of the type of cysts is not always necessary because treatments for both conditions are similar. However, it was demonstrated that the interval from treatment to resumption of ovarian activity is affected by the characteristics of ovarian cysts, with faster recovery reported for the luteal type [24]. In addition, in our study, the estrus rate in cows with FC treated with dinoprost (PGF<sub>2α</sub>) was significantly lower than those of the other three groups (cows diagnosed with LC treated with dinoprost or buserelin acetate and cows diagnosed with FC treated with buserelin acetate). Lower estrus rates in cows with FC treated with PGF<sub>2α</sub> were also reported by other authors [14]. Additionally, superior conception rates were observed in cows diagnosed with FC treated with buserelin acetate as compared with cows diagnosed with LC that received the same treatment. In contrast, cows suffering from LC had higher conception rates when treated with dinoprost than with buserelin acetate. Moreover, differentiating the type of cysts and choosing the appropriate treatment markedly influenced the number of successful pregnancies as well as the TFEI, TCI, and CCI.

Our findings suggest that although difficult, it is necessary to clearly define and differentiate ovarian cystic structures when diagnosing COD. Peter [22] defined COD as the presence of a mature follicle that fails to ovulate during the estrous cycle. Mature follicles typically ovulate when they grow to 13–17 mm in diameter [11]; therefore, follicles that persist at this size or grow larger may be considered to be FC [13]. Silvia *et al.* [32] defined FC as ovarian structures with a minimum diameter of 17 mm that persist for more than 6 days without a corpus luteum and clearly interfere with normal ovarian cyclicality. LC develop when ovulation fails to occur and the theca undergoes luteinization [29]. In most cases (62–85%), cows with LC remain in anoestrus [6, 36] due

to progesterone production. Ball and Peters [1] found that LC are more likely to persist over long periods of time and can lead to nymphomania in some animals, particularly in the early stages. Newly formed ovarian cysts seem to be FC, which may persist and later develop into LC, or regress following the ovulation of another follicle, which is considered one type of spontaneous recovery [9]. Therefore, sometimes differentiating between the two types of ovarian cysts is difficult. For these cases, treatment with a GnRH agonist is more suitable, offering comparable recovery rates for both types of cysts, as reported in our study as well as previous literature [24].

During this study, we noted that treating FC with dinoprost or buserelin acetate resulted in similar TFEI, but significant differences were recorded for TCI, with better values for buserelin acetate treatment. Thus, although both treatments seem equally suitable for inducing ovulation, dinoprost might be less effective in supporting fertilization and/or early embryonic development. It was previously demonstrated that COD in dairy cows also has negative effects on oviductal functional integrity [31], which suggests that a therapeutic approach should not only consider ovulation but also the conditions for the early embryo.

In conclusion, this study suggests that differentiation between the types of ovarian cysts in dairy cows is desirable before determining and administering treatment. Combining transrectal palpation with ultrasonography is particularly helpful in this regard and practical in farm conditions. For cases when differentiation between FC and LC is not possible, GnRH agonists provide effective treatment against COD. However, when the diagnosis reveals the presence of LC, PGF<sub>2α</sub> treatment should be preferred, whereas its use against FC is less effective.

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