Efficacy of three hormonal treatments for cystic ovarian disease and subsequent reproductive performance in dairy cows

Mohamed Elbehiry¹, Mostafa Mahboob¹, Mohamed Marey¹, Sayed Hattab², Rezk Ghallab³, and Ragab Dohreig⁴

¹Department of Theriogenology, Faculty of Veterinary Medicine, Damanhour University, Egypt  
²Department of Theriogenology, Faculty of Veterinary Medicine, Alexandria University, Egypt  
³Department of Theriogenology, Faculty of Veterinary Medicine Matrouh University, Egypt  
⁴Animal Reproduction Research Institute (ARRI), Agriculture Research Center (ARC), Egypt

ABSTRACT

Cystic ovarian disease (COD) is a common ovarian disorder that causes major reproductive failure in dairy cows leading to significant economic losses in the dairy industry. This study aimed to evaluate the efficacy and the response of cows with ovarian cysts to three different treatments regardless of the cyst type. At 50 days postpartum, 27 cows were assigned randomly into one of 3 treatment groups: GnRH group (n=12) treated with 100 μg gonadorelin as a single dose, GP group (n=12) treated with (100 μg gonadorelin on day 0 followed by 500 μg of cloprostenol sodium on day 7, CIDR group (n=12) treated with intravaginal progesterone-releasing insert CIDR on the day 0; and 100 μg gonadorelin then an injection of 500 μg cloprostenol sodium IM on day 7. The CIDR was removed on day 8, and a second GnRH injection was given on day 9. From day 9, animals were observed for estrus and inseminated with proven frozen-thawed semen. Control Group (n=15) represents cows that did not receive any treatment. Pregnancy was confirmed by ultrasound on day 28 post-insemination. Results showed that the GP group achieved the highest estrus induction rate (EIR). Thus, 44.4% of treated cows conceived in estrus less than 10 days and 100% by day 20. While GnRH group showed EIR with 25% in less than 10 days and 62.5% at the day 20. The CIDR group showed EIR (0%) in less than 10 days and 100% at the day 20. Total conception rate (3 cycles after treatment) GP recorded (88.9%) GnRH group (87.5%) and CIDR (85.7%), while pregnancy rates (PR) was highest (66.7%) in GP protocol compared to GnRH (58.3%), and CIDR (49.9%). In conclusion, our results showed that the (GP group) had the best effect on fertility. Thus, higher milk production is commonly associated with reduced fertility in dairy cows (Butler, 2003). Cystic ovarian disease is a common ovarian illness that causes major reproductive failure in dairy cows, resulting in substantial financial losses for the dairy industry. The incidence of cystic ovarian diseases is considered to be 10%, with studies ranging from 2.7 to 30% (Boë & Borç, 2020; Garverick, 1997). Follicular cysts in the ovaries are a common source of infertility and financial loss for dairy farms. The extend in calving intervals in cows with ovarian follicular cysts range from 22 days (Lee, Ferguson, & Galligan, 1988) to 64 days (Butler & Dobson, 1989); the usual interval is 40 to 50 days (Burlett et al., 1986). The average time between diagnosis and conception is 50 days (Bierschwal et al., 1975).

COD was previously defined as fluid-filled structures with a diameter of 2.5 cm or greater that remained on the ovarian surface for 10 days or longer without a corpus luteum (CL). (Garverick, 1997; Youngquist & Threlfall, 2007). COD is currently defined as follicle-like structures with a minimum diameter of 17 mm that last longer than 6 days or is larger than normal ovulatory follicles, and also the uterus is flaccid in absence of a corpus luteum (Bierschwal, 1966; Purohit, 2008). Ultrasound and plasma progesterone levels were recently used to make the most accurate diagnosis of the cyst and its type, either follicular or luteal (Kahn, Line, & Aiello, 2010; Rauch, Krueger, Miyamoto, & Bollwein, 2008).

The hormonal therapy for follicular and luteal cysts are different. Follicular cysts have been treated with hCG and GnRH analogues since the 1970s. In terms of treatment response and fertility, both appear to be equally successful (Peter, 2004), but the induced estrus would occur 5-21 days later (Kahn et al., 2010). Because of its luteolytic effect, prostaglandin F2 (PGF2α) has been used to treat luteinized cysts, and estrus signs can be seen within 2-3 days after treatment (Kesler & Garverick, 1982). As a result, they are the most effective therapeutic option for luteinized cysts (Kahn et al., 2010).

Administration of progesterone may disturb the endocrine state necessary for the maintenance of follicular cysts, causing them to regress (Hatler, Hayes, Anderson, & Silvia, 2006). It seems to have substantial negative feedback on LH pulse frequency (Kinder, Kojima, Bergfeld, Wehrman, & Fike, 1996), suppressing LH in cows with cysts and permitting normal follicles growth (Calder, Salfen, Bao, Youngquist, & Garverick, 1999). Cows with persistent follicles can be effectively synchronized and time inseminated with progesterone, GnRH, and PGF2, but GnRH plus PGF2 only had a limited response (López-Gatius, Santoloria, Yániz, Rutlant, & López-Béjar, 2001). In beef donor cows with cysts that had been present for a long time, treatment with CIDR was helpful in recovering ovulation and reestablishing normal cyclicity (Douthwaite & Dobson, 2000). In cows with follicular cysts, a CIDR and GnRH injection can cause synchronous follicular wave emergence, similar to that seen in cows with regular estrus cycles (Kim, Kim, & Kang, 2006). A single dose of GnRH or hCG followed by PG at 7 days later also is a common strategy to treat cysts. Since the standard Ovsynch timed-insemination protocol utilizes GnRH and PG, this program has been used routinely to treat cystic ovarian conditions (Jeengar et al., 2018). The topic of which treatment is the most cost-effective is frequently asked. Understanding the etiology of this disorder is improving, and treatment

Keywords: Cystic ovarian disease; GnRH; CIDR; Estrus

*Corresponding author: Mohamed Elbehiry  
E-mail address: Mohamed.Elbehiry2@vetmed.dmu.edu.eg
options have improved as well. As a result, the goal of this study was to assess the response of cows with ovarian cysts to three therapies and compare the efficacy of the best treatment regardless of the cyst type.

2. Materials and Methods

2.1. Animals and grouping

This study used 51 lactating multiparous Holstein cows from a commercial dairy farm on the Cairo Alexandria desert road. Individual pens were almost identical in form, size, and quantity of housed cows. Cows were fed two distinct TMR (total mixed ration) diets based on their lactation stage, with an immediate postpartum diet fed between 1 and 21 days in milk (DIM) and a lactating diet fed for the rest of lactation. Maize silage, alfalfa hay, soybean meal, steam-rolled corn, whole cottonseed, calcium salts of palm oil, and a mineral, vitamin, and protein supplement were included in the cows’ diets. The two diets were created with NRC in mind (NRC, 2001). Lactating Holstein cows weighing 500 Kg and producing 35 Kg of milk per day with a 3.5 percent fat content. Water was available to cows at all times. As part of a normal reproductive herd health program, all cows were checked for ovarian cysts twice at 40 to 50 DIM using an ultrasound (9 MHz) linear array B-mode veterinary ultrasound transducer (Sonoscape ASV, Shenzhen, China).

![Ultrasound image of ovary showing anechoic follicular cyst with a diameter (42X45.7 mm).](image)

Follicular cysts were identified in 51 cows as follicular structures larger than 17 mm in diameter (Figure 1) that remained for the two examinations without the presence of a corpus luteum (CL), and the cows were then assigned into four treatment groups at random:

- **Group 1**: CIDR in combination with GnRH and PGF2α
- **Group 2**: GnRH
- **Group 3**: GnRH and prostaglandin
- **Group 4** (Control group)

Twelve (n=12) multiparous puerperium cows were diagnosed having cystic ovaries with ultrasound. Each insert is impregnated with 1.38 g of progesterone and is designed for intravaginal insertion, and 1 mL GnRH (100 µg gonadorelin acetate 100 µg/mL; Ovurelin®, Bayer, NSW, Aust) then an injection of 500 µg cloprostenol sodium IM (2 mL of Ovuprost®, Bayer, Aust) on day 7. CIDR was removed on day 8 and a second GnRH injection was given on day 9 (Figure 2).

![CIDR, GnRH and Prostaglandins for treatment of ovarian cyst](image)

Twelve (n=12) multiparous puerperium cows were diagnosed having cystic ovaries with ultrasound, and were kept as a separate experimental unit. For each treatment, animals were observed for estrus twice per day, 30 minutes each time and checked on SCR. In addition, observed standing estrus during routine handling was recorded. Each animal in estrus was inseminated with proven thawed semen. Resident veterinarian made all inseminations. Trans-rectal ultrasonography on ovaries of all cows was done. Cows that did not return to estrus were examined by ultrasound on day 28 post insemination for pregnancy.

2.2. Ultrasound scanning

A real-time B-mode scanner (Sonoscape A5) with a 5L vet rectal linear array auto-adapted frequency transducer range of 6 to 9 MHz was used for ultrasonography. To avoid air interface, ultrasonic gel was employed to obtain high-quality images with minimal image artefacts. Before transrectal scanning, animals were adequately restrained in headlocks, and the scanning unit was carried by an assistant on the side opposite the operator’s arm inserted rectally. The scanner was placed at the level of the operator’s eyes, and ambient light was reduced for good observation. The animal rectum was evacuated of all feces before introduction of the handheld transducer. The transducer scan head was covered with coupling ultrasonic gel and was inserted through the rectum with the scan head pressed firmly against the rectal mucosa to prevent air interface. For orientation, the transducer was first moved along the dorsal surface of the reproductive tract to evaluate the uterine horns and body, then laterally to examine the ovaries. The image was saved and kept on the screen. Ultrasonography was advised for diagnosing cystic animals with one or more follicle-like structures that were at least 17 mm in diameter, lasted more than 6 days in the absence of a corpus luteum, and interfered with normal ovarian cyclicity (Silvia et al., 2002). After treatment, animals were observed for estrus, then ultrasonographically evaluated for ovulating follicles measuring 13-17 mm (Ginther, Knopf, & Kastelic, 1989), then inseminated. On day 28 after insemination, each animal that did not return to estrus was checked by ultrasonography for pregnancy.

2.3. Statistical analysis

The results are reported as the mean ± the standard deviation of the experiments. Each animal was treated as a separate experimental unit. For statistical analysis, GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA) was utilized. Student's t-test (for two groups) or one-way ANOVA followed by Tukey post hoc tests were used to determine statistical significance between groups (for more than two groups). At p 0.05, the results were considered statistically significant. Furthermore, the Chi-square test was employed to determine the degree of heterogeneity in response between the various groups.

3. RESULTS

Table 1 shows the data on estrus exhibition and estrus induction rate: The GP protocol had the highest estrus induction rate (EIR) of 75 %, followed by GnRH with 66.7 % and the CIDR group with the lowest EIR (58.3%). Total conception rate (3 cycles after treatment) was 88.9% in the GP protocol, 87.5 % in the GnRH group, and 85.7 % in the CIDR protocol, while pregnancy rates (PR) were highest (66.7 %) in the GP protocol, GnRH (58.3 %) and CIDR (49.9 %). Table 2 shows reproductive performance represented by interval to estrus exhibition from end of treatment in days. There was a statistically significant difference compared to control in the interval recorded in all groups with mean ± SD of (11.3 ± 4.2) in GP Group, (15.5 ± 5.9) in GnRH Group and (10 ± 0.0) in CIDR Group compared to control group (26.7 ± 1.5). Table 3 There was a statistically significant difference between groups was determined by one-way ANOVA (F (3, 24) =16, X2 = 0.00). A Tukey post hoc test confirmed the previous statistically significant difference between each group and the control group as mentioned in Table 2. Table 4 shows reproductive performance represented by interval to conception from end of treatment in.
GnRH, 993). The positive treatment results of the (GP) for the use of 
ular growth and eventually . Exogenous 
t GnRH 
triggers the release of LH to assist follic
Lammoglia, Lewis, Neuendorff, & Guthrie, 1996).
Gatius and Lopez and uterine pathology that follows the long sta
fertility often requires longer time due to the disturbance in endocrinology 
1997
Statistically significant difference between groups, as shown in 
GP groups compared with the control group.
There was a statistically significant difference in the results of GnRH and 
CIDR and (29.4 ± 22.6) with GNRH while that of control was (67.5 ± 7.8).
Days with a mean and SD of 
(Table 5
Table 4
Table 3
Table 1
Table 2
Table 3
days with a mean and SD of (20.5 ± 16.7) with GP, (29.3 ± 30.9) with 
and (29.4 ± 22.6) with GNRH while that of control was (67.5 ± 7.8).

4. DISCUSSION
Ovarian cysts can be classified into follicular and luteal cysts (Garverick, 1997). Both of them decrease the reproductive efficiency leading to prolonged calving interval and subsequent less production. Regaining fertility often requires longer time due to the disturbance in endocrinology and uterine pathology that follows the long standing cases (Purohit, 2008), so we started our diagnosis early (40-50 days post-partum). Protocols of treatments for ovarian cysts are numerous and variable, and have changed considerably over the years (Peter, 2004; Purohit; 2008; Woolums & Peter, 1994). In this study we tried three protocols for treatment and evaluated the response to treatment in form of EIR, conception rate, interval to estrus, interval to insemination and pregnancy rate.

Follicular cysts were identified more frequently (84.6 %) than luteal cysts (15.4 %), regardless of the type of the cyst (GnRH, PGF2α, and CIDR), assuming synergistic effects on the luteinization of follicular cysts and its subsequent regression (PGF2α) according to Carroll, Pierson, Hauser, Grummer, and Combs (1990), Douthwaite and Dobson (2000), and Lopez- 
Gatius and Lopez-Bejar (2002). In previous study, exogenous injection of GnRH increased LH production from the pituitary gland (Hanzen, 1984), GnRH-induced LH secretion in postpartum cows is enhanced (Randel, Lammoglia, Lewis, Neuendorff, & Guthrie, 1996). This could have a positive effect on cyst luteinization and/or ovulation of another follicle via endogenous and/or exogenous GnRH. Furthermore, removing the Eazi- 
Breed CIDR causes a rapid drop in plasma progesterone levels, which triggers the release of LH to assist follicular growth and eventually 
by promoting the mRNA expression of galanin, a neuropeptide involved in the release of gonadotropins during the preovulatory surge (Brann, Chorich, & Mahesh, 1993). The positive treatment results of the (GP) protocol and CIDR were independent of the kind of cyst, despite the fact that the luteolytic action of the PGF2α application would have predicted higher results for luteal cysts.
Dinsmore, White, and English (1990) found that giving GnRH and PGF2α at the same time had no negative effect on PGF2α's luteolytic effect in luteal cysts. Furthermore, cows given GnRH alone or GnRH plus CIDR. Because of the 

Using 
PGF2α regimen, 34.9% EIR was 

Table 4
Table 5

Table 1. Estrus induction, total conception and pregnancy rate following treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of cows within the group</th>
<th>Cows detected in estrus No.</th>
<th>% (a)</th>
<th>Conception at 1º AI No.</th>
<th>% (a)</th>
<th>Conception at 2º AI No.</th>
<th>% (a)</th>
<th>Conception at 3º AI No.</th>
<th>% (a)</th>
<th>Total conception results No.</th>
<th>% (a)</th>
<th>Pregnancy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>4</td>
<td>26.7%</td>
<td>---</td>
<td>---</td>
<td>1</td>
<td>25%</td>
<td>1</td>
<td>25%</td>
<td>2</td>
<td>50%</td>
<td>13.3%</td>
</tr>
<tr>
<td>GnRH</td>
<td>12</td>
<td>8</td>
<td>66.7%</td>
<td>4</td>
<td>50%</td>
<td>2</td>
<td>12.5%</td>
<td>7</td>
<td>87.5%</td>
<td>58.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIDR</td>
<td>12</td>
<td>7</td>
<td>58.3%</td>
<td>4</td>
<td>57.1%</td>
<td>1</td>
<td>14.2%</td>
<td>6</td>
<td>85.7%</td>
<td>49.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td>12</td>
<td>9</td>
<td>75%</td>
<td>6</td>
<td>66.7%</td>
<td>1</td>
<td>11.1%</td>
<td>8</td>
<td>88.9%</td>
<td>66.7%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a) = percent calculated from total detected in estrous within each group

Table 2. Interval to estrus exhibition from end of treatment in days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Frequency of estrus exhibition from end of treatment in days</th>
<th>Mean +/- SD</th>
<th>Test of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;10 days</td>
<td>10-&lt;20</td>
<td>20-&lt;30</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>GnRH</td>
<td>2 (25%)</td>
<td>5 (62.5%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>CIDR</td>
<td>-</td>
<td>7 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>GP</td>
<td>4 (44.4%)</td>
<td>4 (44.4%)</td>
<td>1 (11.2%)</td>
</tr>
</tbody>
</table>

Table 3. One-way ANOVA

<table>
<thead>
<tr>
<th>Interval to estrous exhibition from end of treatment in days</th>
<th>F</th>
<th>df1</th>
<th>df2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.8</td>
<td>3</td>
<td>24</td>
<td>0.00*</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Interval to conception from end of treatment in days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Interval to conception from end of treatment in days</th>
<th>Mean +/- SD</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>67.5 ± 7.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GnRH</td>
<td>29.4 ± 22.6</td>
<td>T=2.25*</td>
<td>P= 0.05</td>
<td></td>
</tr>
<tr>
<td>CIDR</td>
<td>29.3 ± 30.9</td>
<td>T=1.6</td>
<td>P= 0.15</td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td>20.5 ± 16.7</td>
<td>T=3.7*</td>
<td>P= 0.00</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Interval to conception from end of treatment in days

<table>
<thead>
<tr>
<th>Interval to conception from end of treatment in days</th>
<th>F</th>
<th>df1</th>
<th>df2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.27</td>
<td>3</td>
<td>19</td>
<td>0.11</td>
<td></td>
</tr>
</tbody>
</table>

8
of Probo et al., who reported 71.3 % EIR (Probo et al., 2011), Waheeb & Hatab (70.3 %) (Waheeb & Hatab, 2017), and Bierschwal et al., who reported 82 % EIR following GnRH injection at varying doses (Bierschwal et al., 1975). Within 30 days of treatment, Mollo et al reported 64 % (Mollo, Stradaiaioli, Gloria, & Cairoli, 2012) and 48 % (Saad, 2013). Within 21 days after therapy, it was 37.1 % (Kim et al., 2006) and 28 % (Dinsmore et al., 1990). GnRH was found to be useful in treating both follicular and luteal cysts in the ovaries. Its mechanism of action can be described in two ways: GnRH either causes the cyst to luteinize or stimulates ovulation. And formation of new corpora lutea. GnRH injection, on the other hand, was demonstrated to cause ovulation in follicles other than the cystic one present at the time of therapy (Ambrose, Schmitt, Lopes, Mattos, & Thatcher, 2004).

Pregnancy rate (PR) was substantially greater in the GP group (66.7 %) than in the other treatment protocols (58.3 percent, 49.9 % in GnRH, and 49.9 % in CIDR, respectively). Takizak and colleagues obtained a higher PR of 76.6 % using the same GP technique (Takizak, Kafi, Mokhtari, & Heidari, 2015), which is similar to Waheeb and Hatab's PR of 65.21 % (Waheeb & Hatab, 2017). Other investigations found lower percentages of around 18 % (Bartolome et al., 2000), 13 % (López-Gatius & López-Bejar, 2002), and 5.9 % (López-Gatius et al., 2001). GnRH alone was able to obtain PR (58.3 %), which is within the previously reported ranges (46.42 % - 71.42 %) of (Bierschwal et al., 1975). Dinsmore et al. attained a higher percentage of 71.8 % (1990). Using a single injection of GnRH, a PR of roughly 20 % was recorded in cystic Frisian cows (Mollo et al., 2012), while a PR of 63 % was reported in cystic Frisian cows (Mollo et al., 2012). (Waheeb & Hatab, 2017). In our study, the CIDR group reported EIR (58.3 %). Gatus and coworker reported (83.3 %) EIR after 9 days of using the progesterone-releasing intravaginal device “PRID” (López-Gatius et al., 2001), and 100 % EIR after 12 days of PRID (Douthwaite & Dobson, 2000), but Waheeb reported (37.36 %) EIR after 12 days of PRID (Waheeb & Hatab, 2017).

Gatus and coworker reported (83.3 %) EIR after 9 days of using the progesterone-releasing intravaginal device “PRID” (López-Gatius et al., 2001), and 100 % EIR after 12 days of PRID (Douthwaite & Dobson, 2000), but Waheeb reported (37.36 %) EIR after 12 days of PRID (Waheeb & Hatab, 2017). Follicular cyst has been linked to high levels of LH and an increase in its pulse frequency, which enhance the development and persistence of large cysts (Calder et al., 1999). The PR rate for the CIDR group was 49.9 % (6 pregnant out of 12 total treated). Using the same CIDR technique, other investigations showed PR of 41 % (Ambrose et al., 2004) and 57.1 percent (Amer & Badr, 2008). Waheeb & Hatab recorded a PR rate of 47.36 % (Waheeb & Hatab, 2017). It was 50 % after 12 days of PRID (Douthwaite & Dobson, 2000), 27.8 % after 9 days of PRID (López-Gatius et al., 2001), and 20 % after 10 days of PRID (Mollo et al., 2012). Overall, the results of the current study’s therapeutic protocols demonstrated that COD may be effectively treated even in the lack of cyst classification diagnostic tools. When ultrasonography and the P4 assay were employed to determine cyst type in other research, the results were identical (Probo et al., 2011; Takizak et al., 2015).

5. Conclusion

Treatment of cystic cows with GnRH followed 7days later with PGF2α (GP protocol) recorded highest EIR and pregnancy rate (75 % and 66.7 % respectively); GnRH (66.7 % & 58.3 %) CIDR (58.3 % & 49.45 %). The GnRH and PGF2α protocol are an effective therapy in dairy cows diagnosed with COD.

Author contributions

All authors contributed to the conception and realization of the work. All the authors have contributed to the paper redaction and given their approval to the final version of the manuscript.

Conflict of interests

There are no conflicts of interest stated by the authors.

References


