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Original article

Effect of progesterone-releasing intravaginal device on reproductive performance of cows with cystic ovarian follicles after puerperium

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Abstract

The aim of the study was to investigate the influence of cystic ovarian follicles (COFs) occurring after puerperium on fertility and the effect of their treatment with progesterone releasing device on reproductive performance in dairy cows. The study was carried out in 3 herds of Polish Holstein-Friesian cows under herd health program. COFs were diagnosed by ultrasound above 60 days p.p. They were defined as follicular structures with a diameter > 2.5 cm in the absence of a corpus luteum at two repeated examinations at the 14-days interval. On the day of COF diagnosis blood samples were collected to measure progesterone (P_4) concentration. On the basis of the wall thickness measurement and progesterone concentration at the first examination, the COFs were differentiated into follicular and luteal cysts. The experimental group consisted of 23 animals with COF. The cows were treated with PRID Delta (Ceva Animal Health, Poland), containing 1.55 g of progesterone, for 7 days. The cows without oestrus signs within 14 days after treatment were re-examined. If COF persisted, the cows were treated again with PRID Delta. The treatment was repeated maximum three times. Fifteen cows with ovarian cysts were left untreated and served as a control group. The cows with COF were matched to healthy cows without COF. The cows with COF had worse fertility performances compared to cows without COF. There were significant ($p < 0.05$) differences in conception rates, number of services per conception and days open between these groups. Compared with non-treated cows with COF, in cows with COF treated with PRID Delta conceptions rates and number of services per conception were similar, but the days open were significantly ($p < 0.05$) lower in cows treated than in non-treated (183.3 days vs. 277.6 days). There were no differences in reproductive performances between the follicular and luteal cysts in treated and non-treated cows. In conclusion, the results of our study confirm the negative impact of COF after puerperium on fertility in dairy cows. Furthermore the results indicate the usefulness of PRID Delta for the treatment of COF occurring after the puerperium regardless of cysts type.

Key words: cows, cystic ovarian follicles, progesterone insert, fertility

Introduction

Ovarian cysts are a common and economically significant condition of dairy cattle affecting fertility (Lopez-Diaz and Bosu 1992, Gröhn et al. 1997, Fourichon et al. 2000, Peter 2004).

In the past they were defined as follicle-like structures, present on one or both ovaries, with a diameter of at least 2.5 cm lasting for a minimum of 10 days in the absence of luteal tissue (Day 1991, Peter 2004). Currently, ovarian cysts are referred to as cystic ovarian follicles (COF) and defined as follicular structures with the diameter of at least 17 mm persisting on the ovary for 7 days or more in the absence of a corpus luteum and interfering normal ovarian cyclicity (Garverick 1997, Jeengar et al. 2014). The incidence of ovarian cysts in dairy cows ranges from 6% to 30% (Opsomer et al. 1998, Vanholder et al. 2006, Probo et al. 2011, Cattaneo et al. 2014).

COF can be divided into follicular and luteal cysts, depending on their structural and functional characteristics. Follicular cysts have a thin wall (< 3 mm) and secrete no progesterone, whereas luteal cysts have a thicker wall (> 3 mm) and secrete progesterone (Douthwaite and Dobson 2000, Vanholder et al. 2006). COFs are dynamic structures. Newly formed follicular cysts may persist, develop into luteal cysts, or regress following the ovulation of another follicle (Lopez-Gatius and Lopez-Bejar 2002, Lopez-Gatius et al. 2002, Peter 2004, Vanholder et al. 2006).

The pathogenesis of COF remains unclear, but a dysfunction of the hypothalamus-pituitary-ovarian axis results in insufficient preovulatory LH-surge and anovulation is likely. This dysfunction has a multifactorial etiology, in which genetic, phenotypic and environmental factors are involved (Peter 2004, Vanholder et al. 2006).

There are numerous treatment methods of COF that involve GnRH, hCG, progesterone, or Ovsynch protocols (Kesler et al. 1980, Lopez-Gatius and Lopez-Bejar 2002, Bartolome et al. 2005, Gundling et al. 2015, Rudowska et al. 2015). However, some authors question the necessity for treatment of ovarian cysts due to the high spontaneous recovery rate (Grunert 1999, Kruse et al. 2016).

Treatment with progesterone reduces LH secretion, restores the responsiveness of hypothalamus to oestradiol, induces regression of cysts and leads to development of normal ovulatory follicles (Calder et al. 1999, Todoroki et al. 2001, Brito and Palmer 2004, Bartolome et al. 2005).

Many COFs that develop during the early postpartum period (p.p.) regress spontaneously (Carroll et al. 1990, Peter 2004), which questions the necessity

for treatment during this period (Kesler and Garverick 1982, Lopez-Gatius et al. 2002). However, some studies suggest that treatment in puerperium can improve reproductive performance (De Vries et al. 2006, Prasse 2007).

Various progesterone releasing intravaginal devices, such as the progesterone-releasing intravaginal device (PRID) and the controlled internal drug-releasing insert (CIDR) have been used in the treatment of COF. However, there were only few studies about the treatment of COF occurring after puerperium with progesterone inserts and the results were inconsistent (Douthwaite and Dobson 2000, Zulu et al. 2003, Ambrose et al. 2004, Iwakuma et al. 2008).

Thus, the aim of the present study was to investigate the influence of ovarian cysts occurring after puerperium on fertility and the effect of their treatment with progesterone releasing device on reproductive performance in dairy cows.

Materials and Methods

The study was carried out on 76 Polish Holstein-Friesian cows from 3 herds in North-East Poland. The number of cows in the herds ranges from 60 to 120. The cows were 3 to 6 years of age, the milk yield ranged from 6000 to 8000 L per year. The cows were kept in loose-housing barns and fed total mixed ration based on grass silage, maize silage, concentrate, and vitamin and mineral supplements.

The herds participated in the herd health program (Barański et al. 2005). The herds were visited at 14-day intervals. Cows with anestrus above 60 days p.p. were examined using rectal palpation and ultrasonography (Honda HS 1500V, linear probe, 5 MHz, Honda Electronics Ltd, Japan). COFs were defined as follicular structures with a diameter > 2.5 cm in the absence of a corpus luteum at two repeated examinations. At the day of COF diagnosis blood samples were collected from the coccygeal vein to measure progesterone (P_4) concentration. The blood samples were centrifuged and stored in -25°C to the day of assay by RIA according to the method described by Hoffmann (1977). On the basis of the wall thickness measurement and progesterone concentration at the first examination, the COFs were differentiated into follicular (wall < 3 mm, P_4 < 1 ng/ml) and luteal (wall > 3 mm, P_4 > 1 ng/ml) cysts.

The experimental group (GP) consisted of 23 animals with COF. The cows were treated with PRID Delta (Ceva Animal Health, Poland), containing 1.55 g of progesterone, for 7 days and PGF 2α was administered 24 h before removing the device. Fifteen cows with

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Table 1. Disappearance rate and estrus rate in cows with COF treated with PRID Delta (GP) or non-treated animals (GC) after the first, second and all treatments/examinations.

Group	Disappearance rate % (n/n)			Oestrus rate % (n/n)		
	1	2	cumulative	1	2	cumulative
GP n = 23	39.1 (9/23)	26.1 (6/23)	95.7 (22/23)	34.8 (9/23)	26.1 (9/23)	91.3 (9/23)
GC n = 15	40.0 (6/15)	20.0 (3/15)	93.3 (14/15)	33.3 (5/15)	20.0 (3/15)	86.7 (13/15)

Table 2. Reproductive performance in cows with COF treated with PRID Delta (GC) or non-treated (GC) and in control cows without COF (GH).

Variables	GP n = 23	GC n = 15	GH n = 38
First service conception rate % (n/n)	21.7 ^b (5/23)	26.7 ^{ab} (4/15)	55.6 ^a (21/38)
Second service conception rate % (n/n)	21.7 (5/23)	13.3 (2/15)	23.7 (9/38)
Cumulative conception rate % (n/n)	65.2 ^b (15/23)	73.3 ^b (10/15)	89.4 ^a (34/38)
Services per conceptions	3.91 ^b	4.53 ^b	1.56 ^a
Days open (days)	183.3 ^b	277.6 ^c	114.5 ^a
Culling rate % (n/n)	34.8 (8/23)	33.3 (5/15)	13.1 (5/38)

Values within the same row with different letters differ significantly at $p < 0.05$

COF were left untreated and served as a control group (GC). The cows with COF were matched to healthy cows without COF (GH) of the same herd, with the same age and calving season.

After removal of PRID Delta, the cows were observed for oestrus three times daily by the owners. After detection of oestrus the cows were artificially inseminated. Pregnancy was diagnosed by ultrasonography between days 35 and 45 after insemination. The cows without oestrus signs within 14 days after treatment were re-examined. If COF persisted, the cows were treated again with PRID Delta. The treatment was repeated maximum three times.

Disappearance rate of the COFs and oestrus detection rate were calculated for the treated and non-treated cows with COF. Moreover, the following reproductive performances were calculated for the cows with COFs (GP, GC) and control cows (GH): first service conception rate, second service conception rate, cumulative conception rate, services per conceptions, days open and culling rate.

The cystic disappearance rate and the oestrus detection rate were defined as the percentage of cows without COF and as the percentage of cows with oestrus signs 14 days after the first, second and all three treatments/

examinations, respectively. First service conception rate was defined as the percentage of the animals pregnant after the first insemination, second service conception rate as the percentage of the animals pregnant after the second insemination, cumulative conception rate as the percentage of the animals pregnant after treatment 1 to 3. Services per conception were defined as the number of inseminations per conception. Days open describe the interval from parturition to conception. The culling rate was defined as the percentage of cows removed from the herd due to infertility.

The statistical analysis of data was performed by Mann-Whitney U test using GraphPad Prism version 7.00 (GraphPad Software, San Diego, CA, USA). The levels of significance was considered as $p < 0.05$.

Results

The cumulative disappearance rate of the COFs and oestrus rate were slightly higher in cows treated with PRID Delta (GP) than in non-treated animals (GC) (95.7% vs. 93.3 and 91.3% vs. 86.7%, respectively), but the differences between both groups were not significant ($p > 0.05$; Table 1).

Table 3. Cumulative disappearance rate and estrus rate in cows with follicular or luteal COF treated with PRID Delta (GP) or non-treated (GC).

Variables	GP		GC	
	Follicular COF	Luteal COF	Follicular COF	Luteal COF
Cumulative disappearance rate (%)	92.9 (13/14)	100 (9/9)	100 (7/7)	87.5 (7/8)
Cumulative oestrus rate (%)	92.8 (10/14)	88.9 (5/9)	85.7 (5/7)	87.5 (6/8)

Table 4. Reproductive performance in cows with follicular or luteal COF treated with PRID (GP) or non-treated (GC).

Variables	GP		GC	
	Follicular COF n = 14	Luteal COF n = 9	Follicular COF n = 7	Luteal COF n = 8
First service conception rate (% n/n)	14 (2/14)	33 (3/9)	29 (2/7)	25 (2/8)
Second service conception rate (% n/n)	21.4 (4/14)	11.1 (1/9)	14.3 (1/7)	12.5 (1/8)
Cumulative conception rate (% n/n)	71 (10/14)	55.5 (5/9)	71 (5/7)	62.5 (5/8)
Services per conceptions	3,79	4,11	4,43	4,63
Days open (days)	170	213,25	205	338
Culling rate (% n/n)	29 (4/14)	51 (4/9)	29 (4/7)	38 (3/8)

The cows with COF, both treated with PRID Delta (GP) and non-treated (GC), had worse fertility performances compared to cows without COF (GH) (Table 2). Cumulative conception rate was significantly ($p < 0.05$) lower in the cows with COF treated with PRID Delta (65.2 %) and in non-treated (GC) (73.3 %) than in the GH (89.4 %). Services per conception were significantly ($p < 0.05$) higher in cows with COF than in cows without COF (3.91, 4.53 and 1.56, respectively). The days open were also significantly ($p < 0.05$) higher in cows with COF than in control cows (183.3 days, 277.6 days and 114.4 days, respectively). Culling due to infertility was higher in cystic cows (34.8% and 33.3%), but there were no significant ($p > 0.05$) differences in comparison to control group (GH) (13.1%).

The cows with COF treated with PRID Delta had similar conception rates like non-treated cows with COF (cumulative conception rate 65.2% vs. 77.3%; $p > 0.05$). The number of services per conception was 3.91 in the cows with COF treated with PRID Delta and 4.53 in the non-treated animals ($p > 0.05$). The days open were significantly ($p < 0.05$) lower in cows treated than in non-treated (183.3 days vs. 277.6 days). There were no differences in the culling rate between treated and non-treated cows with COF (34.8% vs. 33.3%; $p > 0.5$).

The disappearance rate and oestrus rate were simi-

lar in the cows with luteal and follicular cysts ($p > 0.05$; Table 3). Cumulative conception rates, days open and culling rates tended to be better in the cows with follicular COFs than in the cows with luteal COFs, but the differences between both types of cysts were not significant ($p > 0.05$; Table 4).

Discussion

Our study showed that COFs occurring after 60 days p.p. had negative impact on fertility of dairy cattle. They decrease conception rate, increase the number of days open and culling rate because of infertility. These results are in agreement with other data on the influence of ovarian cysts occurring after the puerperium on fertility in dairy cows (Lopez-Diaz and Bosu 1992, Gröhn et al. 1997, Fourichon et al. 2000). The time of COF formation seems to have an influence on reproductive performance in dairy cows. COF diagnosed during the puerperium do not seem to affect the fertility in cows (Fourichon et al. 2000, Gossen and Hoedemaker 2006). In the post-partum period the self-cure rate of COF is particularly high (Garverick 1997).

In this study, the cumulative disappearance rate of the cysts and oestrus rate did not differ between the cows with COF treated with PRID (GP) and non-treated

(GC) animals. The treatment of the cows with PRID Delta for 7 days had no effect on conception rates, number of services per conception and culling rate due to infertility, however days open were significantly lower than in non-treated animals (183.3 days vs. 277.6 days; $p < 0.05$). The significant reduction in the number of days open indicates the usefulness of COF treatment after the puerperium with PRID Delta.

The need for treatment of COF is controversial because spontaneous recovery rate is high (Garverick 1997, Grunert 1999). Some studies showed that the treatment of COF had no positive effect on fertility performance (Jou et al. 1999, Prasse 2007, Kruse et al. 2016). However, it has been found that the treatment of ovarian cysts was economically more sound than waiting for spontaneous cyst regression (De Vries et al. 2006).

At the time of diagnosis there were 21 follicular (55.3%) and 17 (44.7%) luteal cysts. Similarly, Douthwaite and Dobson (2000) and Zulu et al. (2003) reported that about 40 % of COFs after puerperium were luteal. In contrast, Hasler et al. (2004) and Gaebler et al. (2015) found that all or more than 90 % of ovarian cysts during puerperium were follicular. This difference could be related to luteinisation of follicular cysts over time (Garverick 1997, Lopez-Gatius and Lopez-Bejar 2002, Peter 2004, Vanholder et al. 2006).

There were no differences in disappearance and oestrus rates, as well as in reproductive performances between the follicular and luteal cysts. Thus, the differentiation between two types of ovarian cysts seems not to be necessary in case of treatment with progesterone insert.

There have been only few studies on the treatment of COF after puerperium with progesterone releasing devices. Douthwaite and Dobson (2000) treated cows with ovarian cysts > 60 days p.p. and obtained for cows with both types of cysts overall pregnancy rate of 50% after three inseminations, however, the first service pregnancy rate was only 18% for cows with follicular cysts and 28% for cows with luteal cysts. The calving to conception interval for cows with follicular cysts that had been treated with a PRID did not differ from that for their healthy untreated herdmates. In contrast, the cows with luteal cysts tended to take longer to conceive again than their healthy untreated herdmates. In the study of Zulu et al. (2003) the first conception rate was 20% and overall conception rate 60% for both types of cysts after PRID treatment > 40 days p.p. The recovery rates, conception rates and days to conception did not differ between follicular and luteal cysts. Iwakuma et al. (2008) evaluated the efficacy of 7-day insertion of CIDR combined with prostaglandin $F_2\alpha$ at CIDR removal in the treatment of COF > 40

days p.p. The conception rates within 60 days after CIDR removal were above 50%. The average interval between CIDR removal and conception was 24 days. There were no significant differences in reproductive parameters between cows with $P_4 > 1$ ng/ml and $P_4 < 1$ ng/ml.

Progesterone treatment using a progesterone releasing device had a high rate of therapeutic effectiveness in cows with COF during puerperium (Kim et al. 2004, Gaebler et al. 2015). In embryo donor beef cows that developed COF after superovulation, progesterone treatment resulted in oestrus and ovulation in all cows within 10 days of CIDR withdrawal (Todoroki et al. 2004).

In our study we compared group of the treated animals (GP) with the control group consisted of healthy animals (GH) and with group of untreated animals (GC) with COF which allowed to appropriately compare the results obtained.

In conclusion, the present results confirm the negative impact of COF after puerperium on fertility in dairy cows. The cows with COF had inferior reproductive performance compared with the cows without COF. Furthermore the results indicate the usefulness of PRID Delta for the treatment of COF occurring after the puerperium regardless of cysts type. The treatment with PRID Delta led to significant reduction in the number of days open in the cows with follicular and luteal cysts.

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