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FACTORS AFFECTING TREATMENT RESPONSE TO OVSYNCH PROTOCOL IN HOLSTEIN DAIRY COWS SUFFERED FROM CYSTIC OVARIAN DISEASE

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ABSTRACT

The objective of this study was to evaluate the efficacy of using Ovsynch synchronization protocol as a treatment tool for cows affected with ovarian cysts. In addition, the effects of season, parity, days in milk (DIM) and level of milk production in 100 days on the treatment response were also investigated. On the day of cyst diagnosis (Day 0), 96 lactating Holstein dairy cows with cystic ovarian disease (COD) were treated with Busrelin® (GnRH), Cloprostenol® (PGF2aα) on Day 7, Busrelin® on Day 9, and TAI on 10th day (Ovsynch protocol). Pregnancy was diagnosed between 42 and 48 days after timed artificial insemination (TAI). Cows were classified according to the milk production level into high producers (above average) and low producers (below average); according to parity into primiparous (first lactation) or multiparous (second or more lactations); according to season into hot and cold season and according to DIM into below 100 DIM and above 100 DIM. Data for conception rate (CR) were analyzed using SPSS statistical software. The treatment of COD with Ovsynch protocol resulted in acceptable CRs regardless the cyst type. Although the investigated factors exerted no significant influences on the treatment response, cows treated in cold season tend to become pregnant more than cows treated in hot season. Primiparous cows were more likely to become pregnant than multiparous cows. It appears that high-producing cows respond better to treatment compared to low-producing cows. It was concluded that the Ovsynch-based TAI protocol can be successfully used for the treatment of ovarian cysts in lactating dairy cows and produce an acceptable CR that not significantly influenced neither by season, parity, and milk yield nor by days in milk.

Keywords: *Cystic Ovarian Disease; Ovsynch protocol; dairy cows.*

INTRODUCTION

COD in cows is characterized by persistent anovulatory follicular structures with a diameter of more than 25 mm on one or both ovaries in the absence of CL for at least 10 days and accompanied with interrupted or abnormal estrous cycles (Lopez-Diaz and Bosu, 1992; Peter, 1997; Garverick, 1997). Although the exact causes of ovarian cysts are not presently known, lack of release or inappropriate release of GnRH at the time of estrus appears to be an important factor in development of ovarian cysts (Garverick, 1997). Moreover, the occurrence of ovarian cysts is closely associated with milk production; as it is more common in high-producing cows (Roberts, 1971; Johnson *et al.*, 1966; Bartlett *et al.*, 1986; Lopez-Gatius *et al.*, 2002). However, there are many predisposing factors for ovarian cysts (Melendez *et al.*, 2003) and it has also been stated that this condition is hereditary (Roberts, 1971).

COD is classified as either follicular cysts (FC), which are thin-walled containing little or no luteal tissue, or luteinized cysts, which are thick-walled containing much luteal tissue (Peter, 1997; Kesler and Garverick, 1982; Kawate *et al.*, 1990). It has been suggested that luteinized cysts diagnosed clinically may include a cystic corpus luteum (CL), which is a CL with a large fluid-filled central cavity (Kim *et al.*, 2004). The incidence of ovarian cysts in dairy cows ranges from 6 to 30% (Casida and Chapman, 1951; Bierschwal, 1966; Morrow *et al.*, 1966; Whitmore *et al.*, 1974; Britt *et al.*, 1977; Kesler and Garverick, 1982). Although it occurs frequently during late lactational periods (Bartlett *et al.*, 1986), it is generally greatest between 30 and 60 days postpartum

(Menge *et al.*, 1962; Morrow *et al.*, 1966; Whitmore *et al.*, 1974; Erb and White, 1981). COD is a serious cause of reproductive failure because it occurs frequently and the affected cows are infertile (Kesler and Garverick, 1982). Furthermore, ovarian cysts are a major reproductive disorder responsible for economic loss in the dairy industry (Bartlett *et al.*, 1986; Borsberry and Dobson, 1989).

The primary therapies for bovine FC include either use of GnRH analogue or exogenous progesterone (Peter, 1997; Kesler and Garverick, 1982; Kawate *et al.*, 1997). In addition, cows with luteinized cysts can be successfully treated with PGF2α when correctly diagnosed (Peter, 1997). Treatment with GnRH induces luteinzation of cystic structures or initiates ovulation of other follicles from the cystic ovary (Kesler *et al.*, 1981; Cook *et al.*, 1990; Jou *et al.*, 1999; Douthwaite and Dobson, 2000), and the resultant luteinized cysts or CL undergo spontaneous regression. Thus, a single GnRH treatment has been the standard treatment for cows with ovarian cysts (Bierschwal *et al.*, 1975; Seguin *et al.*, 1976; Nakao *et al.*, 1992; Osawa *et al.*, 1995), however, a large proportion (25–39%) of cows with ovarian cysts treated with GnRH did not respond (Cantley *et al.*, 1975; Nakao *et al.*, 1992; Osawa *et al.*, 1995; Tebble *et al.*, 2001). Recently, the Ovsynch protocol has been applied to treat dairy cows with ovarian cysts (Crane *et al.*, 2006; Gundling *et al.*, 2009). The objective of this study was to determine the efficency of ovsynch protocol as a treatment tool for COD and if the treatment response is affected with season, parity, milk yield and DIM. This treatment response was expressed as the CR.

MATERIALS and METHODS

Experimental animals

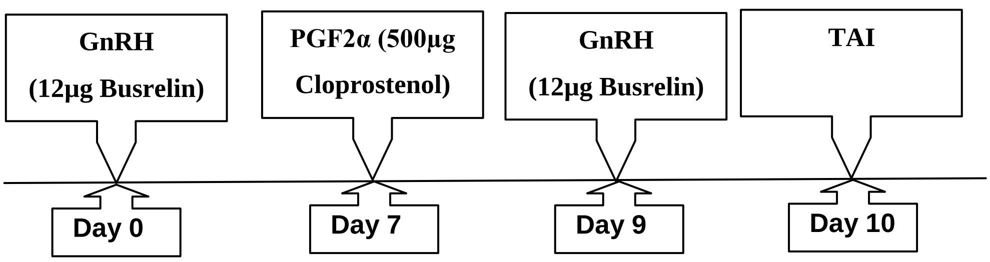
The present study was performed over the period from October 2013 to November 2014 in a well managed private Holstein herd located in Northwestern Egypt (Juhayna Dairy farm "Milky's"). This dairy herd was on a reproductive herd health program and all reproductive health and management records were computerized (Afifarm system, SAE, Afikim). Cows in the farm were milked thrice daily and were fed a balanced TMR according to National Research Council recommendations (NRC, 2001). Cows were routinely vaccinated against BVD, IBR, bovine respiratory synctial virus, parainfluenza, leptospirosis, campylobacteriosis, clostridiosis. The voluntary waiting period (VWP) used in this dairy herd was 45 days.

The diagnosis of COD was based on transrectal palpation of the ovaries and uterus. The criteria used on palpation per rectum were the presence of a large cystic structure on the ovary (>25 mm in diameter), absence of CL on both ovaries (Crane *et al.*, 2006).

Treatment and insemination protocol

Cystic cows (n=96) were treated through application of ovsynch protocol as follow: on days zero (days of cyst diagnosis) the cows received 12µg Busrelin® (GnRH, MSD, USA); on 7th day the same cows received 500µg Cloprostenol® (PGF2α, MSD, USA); on 9th day 12µg Busrelin® was injected to the same cows as shown in figure 1. All hormone injections were administered i.m. All cows were bred by fixed TAI 20±4 hours after the second Busrelin® (GnRH) injection.

Figure 1: Ovsynch protocol



Data collection

For each individual cow, data concerning to 100 day total milk yield (range 2322-7571, mean, 4078 Kg), parity (range 1-7) and days DIM (range 46-396 day) were recorded. Results of the first insemination were recorded to determine the CR. animals were classified according to parity into primiparous (n=27) and pleuriparous, (n= 69) according to milk production into high (above 4078.468 Kg ,n=52) and low (less than 4078.468 Kg, n=44) producers (in relation to average herd production level in 100 DIM), according to DIM at cyst diagnosis into cows less than 100 DIM (n=49) and more than 100 DIM (n= 47). Season of treatment was classified as hot (n=29) and cold (n=67) season.

Pregnancy diagnosis

Pregnancy was determined by rectal palpation of the uterus and its contents 42 to 48 days post-insemination according to (Zemjanis, 1970). CR was defined as the percentage of cows that conceived to the TAI.

Statistical analysis

Data for pregnancy were analyzed using Student T test of the (SPSS, 2007). Statistical significance was declared at P<0.05. Both T and P values were included.

RESULTS

In the current study, we aimed at evaluating the response of dairy cows with COD to treatment by Ovsynch protocol and examining some factors which may modify this response. Concerning to effects of season on response of cows to treatment, CR in cows treated during winter was non-significantly higher (P>0.05) than that of cows treated during summer (34.84±5.86 vs. 24.14±8.08%, resp.). Regarding to parity, primiparous cows recorded non significant higher (P>0.05) CRs (37.04±9.47%), compared to pleuriparous cows (29.41±5.56%). Concerning the milk yield, cows producing above herd average responded slightly better to treatment as they showed slightly higher CR (33.33±6.66%), compared to low producers (30.23±7.08). As regards to DIM cows treated beyond 100 DIM had numerically (P>0.05) higher CR (36.73±6.95%), in comparison to those treated after 100 DIM (26.08±6.54%) as shown in the table.

The table: Factors affecting the response of cows with ovarian cyst to treatment by Ovsynch protocol in Holstein cows.

Factor	Classes	Cows (n)	CR	T value	P value
Season	Hot season	29	24.14±8.08	1.03	0.30
	Cold season	67	34.84±5.86		
Parity	Primiparous	27	37.04±9.47	0.72	0.47
	Pleuriparous	69	29.41±5.56		
Total 100 days milk (◆) (kg)	Above average	52	33.33±6.66	0.32	0.75
	Below average	44	30.23±7.08		
DIM (days)	Above 100 DIM	47	26.08±6.54	1.11	0.26
	Below 100 DIM	49	36.73±6.95		
Total (overall CR)		96	30/66 (45.45±8.12)	--	--

-Values of conception (CR) are expressed as mean ± SE.
-(◆) Average total milk in 100 DIM, (4078.468 Kg).

DISCUSSION

The present study assessed whether an Ovsynch-based TAI protocol could be used as an efficient tool for the treatment of ovarian cysts in lactating dairy cows. In addition, effects of season, parity, milk yield and DIM at cyst diagnosis on the treatment response were also evaluated. In the present study, cows diagnosed with ovarian cysts after the VWP (45 days) were treated by Ovsynch protocol. The choice to treat animals without differentiating between FC and luteal cysts is in a measure, due to the difficulty to perform a certain diagnosis. It is probable that the combination of application of PGF2α on the 7th day and GnRH on the 9th day was more effective. According to Moreira *et al.* (2000), cows treated according to Ovsynch protocol respond to the treatment more intensively than treatment with PGF2 or GnRH separately. Moreover, the two types of cysts may be considered as different forms of the same disorder (Vanholder *et al.*, 2006). In particular, luteal cysts are believed to be FC in later stages (Garverick, 1997). Interestingly, it has been reported that following treatment with GnRH, ovarian cysts may luteinize, but they never ovulate (Garverick, 1997). In addition, the use of GnRH to treat ovarian cysts showed the presence of a CL and the cystic structure 7 days after treatment (Bierschwal, 1966; Ambrose *et al.*, 2004) indicating that the CL formed from ovulation of an ovarian follicle, and not the existing ovarian cyst.

The comparison among results obtained in different studies could be quite difficult. Difficulties relay on several experimental variabilities such as ovarian cyst definition, criteria for animals enrollment, type of drugs used for treatment, dosages and protocols and criteria for treatment response evaluation. The overall mean CR in cows with ovarian cysts subjected to the Ovsynch protocol in the present study (45.45±8.12) and in other studies were lower than normal CR (~ 60%) that observed in heathy cows without ovarian cysts (Momcilovic *et al.,* 1998; Pursley *et al.,* 1995). Furthermore, the obtained CRs in all groups coincide with that reported by Fricke and Wiltbank (1999) (47.6%) but are higher than those in previous reports in which conventional Ovsynch was used (18.3%, Crane *et al.,* 2006 and 23.1%, Gundling *et al.,* 2009).

Concerning the effect of season on the treatment response, in spite of the non significant differences among seasons (P>0.05) the cows treated during cold season were more likely to conceive as compared to those treated during hot season. These results came in accordance with (Badinga *et al.,* 1985) who reported that, pregnancy rate in dairy cows declines in summer months due to heat stress. In normal cows not suffering from ovarian cyst, heat stress during summer season has a negative effect on CR. Therefore, it seems logic for cows treated during summer season to have lower CR when compared to those treated during winter. Furthermore it has been demonstrated that, ovarian follicles are susceptible to heat stress (Badinga *et al.,* 1993). The preovulatory follicle is a key component of the reproductive system and impairment of its development during thermal stress may affect other reproductive events, such as gonadotrophin secretion (Gilad *et al.,* 1993) and subsequent development of both the CL (Howell *et al.,* 1994; Wilson *et al.,* 1998) and the embryo (Putney *et al.,* 1989). In addition, it appears that high ambient temperature may decrease the plasma LH concentration in cows (Wise *et al.,* 1988), which is needed for full development of the dominant follicle (Gong *et al.,* 1996).

Regarding the cow's parity, despite no significsnt differences were detected, primiparous cows responded better to treatment than pleuriparous cows in the current work. In agreement with the current study, several authors reported higher CRs after Ovsynch and TAI in primiparous than in older cows (Stevenson *et al.,* 1996; Cartmill *et al.,* 2001; Tenhagen *et al.,* 2001; Peters and Pursley, 2002). Other studies did not demonstrate this effect (Jobst *et al.,* 2000; Klindworth *et al.,* 2001). This runs in accordance with the work reported by Crane *et al.* (2006) and this may be attributed to the lower incidence of other diseases such as mastitis and lameness in primiparous cows. These diseases have detrimental effects on production and fertility. In the same concern, Chebel *et al.* (2004) reported that multiparous cows were 13% less likely to conceive than primiparous cows, and this might be partially explained by the higher incidence of postparturient diseases compared to primiparous cows (14.9% versus 6.2%; P < 0.001). Diseases during the early postpartum period are known to affect reproductive performance of lactating dairy cows (Gröhn and Rajala-Schultz, 2000). Therefore, it is possible that older cows experienced lower CR because they were at a higher risk for periparturient problems known to affect fertility. Possible reasons for better fertility in primiparous cows include a reduced risk of metabolic disorders in early lactation (Erb and Gröhn, 1988; Gröhn and Rajala-Schultz, 2000). The effect of parity on CR after Ovsynch has been discussed controversially (Stevenson *et al.,* 1996; Jobst *et al.,* 2000; Cartmill *et al.,* 2001; Klindworth *et al.,* 2001; Tenhagen *et al.,* 2001; Peters and Pursley, 2002). However, these studies were either carried out on one farm or with a limited number of animals per farm. Studies on the effect of parity on fertility found results ranging from lower CRs in primiparous than in mature cows (Stevenson and Call, 1988; Diskin, 1996; Wathes *et al.,* 2001) to higher odds for pregnancy in primiparous cows (Hillers *et al.,* 1984; Thompson *et al.,* 1996; Gröhn and Rajala-Schultz, 2000). Numerous studies found no differences in reproductive performance associated with parity (Fonseca *et al.,* 1983; Hansen *et al.,* 1983).

As regards to the association between total 100 days milk yield and the treatment response, CR following Ovsynch treatment protocol of cows with ovarian cyst was non significantly (P>0.05) higher(33.33±6.66%) in high producing cows than in low producing cows (30.23±6.66%). Interestingly, Crane *et al.* (2006) reported that high yielding cows with ovarian cysts became pregnant more often following ovsynch treatment than low yielding cows with ovarian cysts. Moreover, it has been reported that, cows without ovarian cysts that had above average milk production had a greater pregnancy rate in response to the Ovsynch protocol than similar cows with lower than average milk production (Peters and Pursley, 2002). However, there are other studies that disagreed with the hypothesis that milk production and fertility are negatively correlated. These studies suggested that there are other factors that have a greater influence on fertility than milk production (Lucy, 2001). It is generally accepted, notwithstanding the genetic component, that an increased level of feeding is associated with an increase in milk production. In this connection, it is interesting to note a recent report (Adamiak *et al.,* 2005) that demonstrated the effects of level of feeding on oocyte quality. This indicated that the effects are dependent on the body condition of the animal. Furthermore, it has been reported that, low milk producing cows often have lower dry matter intake and are at greater risk to be anestrous and experience low fertility compared to high-producing cows (Staples *et al.,* 1990; Lucy *et al.,* 1992). In addition, it is also possible that cows with lower production are also experiencing a higher incidence of lameness, mastitis, and peri-parturient disorders which could negative affect both fertility and milk production. Moreover, it has been speculated that improved reproduction in high producing cows probably reflects better feeding, healthier cows, and improved reproductive management (Lucy, 2001). It has been previously suggested that higher milk yield of dairy cows enhances metabolism of sex steroid hormones in the liver (Fricke and Wiltbank, 1999; Sangsritavong *et al.,* 2002), and thus milk yield may affect ovulation synchronization and subsequent CRs. On the contrary, it has been reported that increased milk production is associated with a decrease in cow's fertility (from 65% to 40%) during the last 40 years (Lucy, 2001). Furthermore it has been reported that increased milk production is associated with an increased incidence COD in the early postpartum period (Roberts, 1971; Johnson *et al.,* 1966; Bartlett *et al.,* 1986; Lopez Gatius *et al.,* 2002). Moreover, high producing dairy cows are less likely to spontaneously recover compared to low producing cows (Lopez Gaitus *et al.,* 2002). Another study found that cows with FC produced an average of 379 kg more milk in the first 90 days postpartum, and 438 kg more by 305-day milk than their non cystic herdmates (Johnson *et al.,* 1966).

As regards to DIM, despite no significant differences (P>0.05), cows treated before 100 DIM had numerically higher CR (36.73) in comparison to those treated after 100 DIM (26.08). These result came in agreement with Crane *et al.* (2006) who reported no significant association between DIM and treatment response of ovarian cysts in lactating dairy cows using the Ovsynch protocol. The cows before 100th day post-partum responded more intensively than the cows after 100th days post-partum. This supports the assumption about the ovarian sensitivity, since at that time the milk production culminated, which was probably the dominant factor (Lopez-Gatius *et al.*, 2002). In addition, cows remains not conceived after 100 days pstpartum may suffered from reproductive problems other than COD such as subclinical endometritis. On the contrary, other studies have shown that cows receiving TAI early postpartum (<75 DIM) had a lower pregnancy rate than cows receiving TAI later during lactation (Pursley *et al.,* 1997). Furthermore, pregnancy rates were 37, 42, and 48% at first, second, and third TAI, suggesting a positive relationship between DIM at TAI and pregnancy rate (Pursley *et al.,* 1997). Moreover, Navanukraw *et al.* (2004) reported that, Ovsynch cows receiving TAI later during lactation tended (P=0.18) to have a greater CR (43.1%) than Ovsynch cows receiving TAI earlier during lactation (31.9%).

CONCLUSION

In conclusion, the Ovsynch-based TAI protocol can be successfully used for the treatment of FC in Holstein lactating cows and produce an acceptable CRs that are not significantly influenced neither by season, parity, milk yield nor by days in milk.

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العوامل التي تؤثر على استجابة أبقار الهولستين الحلابة والمصابة بمرض تحوصل المبايض للعلاج بنظام توافق التبويض

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أجريت هذه الدراسة لتقييم مدى إمكانية استخدام نظام توافق التبويض كعلاج للأبقار الهولستين الحلابة والمصابة بتحوصل المبايض وكذلك قياس مدى تأثر درجة الاستجابة للعلاج ببعض العوامل مثل: موسم الفحص والعلاج، عمر الأبقار (عدد الولادات)، معدل إنتاج اللبن وطول الفترة من الولادة وحتى التشخيص والعلاج. تم إجراء هذه الدراسة في إحدى المزارع التابعة لشركة " جهينـة " وتسمى (مزرعة ميلكيز) في الفترة من نوفمبر ٢٠١٣ وحتى أكتوبر ٢٠١٤. وقد أجريت هذه الدراسة على عدد ٩٦ بقرة هلوستين حلاب والتي تعاني من تحوصل المبايض، وقد خضعت هذه الأبقار لنظام علاجي يعرف باسم " توافق التبويض " وفيه يتم حقن الأبقار بهرمون الجونادوتروبين (GnRH) في اليوم الأول (اليوم ٠) وبعده بسبعـة أيام (اليوم ٧) يتم حقن الأبقار بهرمون البروستاجلاندين (PGF2α) وبعده بيومين (اليوم التاسع) يتم حقن الجرعة الثانية من هرمون الجونادوتروبين (GnRH) وبعد حوالي ١٨-٢٠ ساعة أي في اليوم العاشر تلقح كل الأبقار اصطناعيا. وقد أظهرت هذه الدراسة نتائج إيجابية بعد استخدام هذا النظام من العلاج بناءاً على معدلات الخصوبة المقبولة والعالية نسبيا. كما أظهرت عدم تأثر استجابة الحيوانات للعلاج بالتغير في الموسم وكذلك عمر الحيوان وكمية إنتاج اللبن في ١٠٠ يوم، كذلك عدم تأثره بطول أو قصر فترة ما بعد الولادة وبناءاً على تلك النتائج يمكن استخلاص أن نظام توافق التبويض يعتمد عليه كعلاج فعال للأبقارالحلاب التي تعاني من تحوصل المبايض ويوصى باستخدامه بغض النظر عن نوع الحويصلات.