Effect of pretreatment with bovine somatotropin (bST) and/or gonadotropin-releasing hormone (GnRH) on conception rate of dairy cows with ovarian cysts subjected to synchronization of ovulation and timed insemination

J. Bartolomea, J. Hernandeza, P. Sheerina, S. Luznara, D. Kelberta,b, W.W. Thatchera,c, L.F. Archbald*a,*

*Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610, USA
bNorth Florida Holsteins Inc., Bell, FL 32619, USA
cDepartment of Animal Sciences, University of Florida, Gainesville, FL 32610, USA

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Abstract

The objective of this study was to determine the effect of pretreatment with bovine somatotropin (bST) and/or gonadotropin-releasing hormone (GnRH) 7 days prior to initiation of a protocol for synchronization of ovulation and timed insemination (Ovsynch) on conception rate (CR) of cows with ovarian cysts. A total of 254 lactating dairy cows with ovarian cysts was divided into four groups (Day 0). On Day 0, cows in Group 1 (n = 61) were pretreated with 500 mg bST, s.q., and 100 µg GnRH, i.m.; cows in Group 2 (n = 73) were pretreated with 100 µg GnRH, i.m.; cows in Group 3 (n = 59) were pretreated with 500 mg bST, s.q.; and cows in Group 4 (n = 61) received no pretreatment. All cows were subjected to the Ovsynch protocol 7 days later. All cows previously received routine bST treatment every 14 days until milk production decreased to a minimum level established by the management of the herd. CR was assessed using logistic regression after adjusting for timing of concurrent bST treatment relative to Day 0, parity, season at time of insemination, and days in milk (DIM) on Day 0. CR for cows in Group 3 (12%) was significantly lower (P < 0.05) than that for cows in Group 4 (27%), and CR for cows in Group 1 (18%) and Group 2 (15%) tended to be lower (P < 0.10) than that for cows in Group 4 (27%). From the results of this study, it was concluded that bST pretreatment decreased CR, and pretreatment with GnRH, and GnRH with bST tended to...
1. Introduction

Bovine ovarian cysts are follicles that fail to ovulate at the time of estrus [1]. They are an economic problem in the dairy cow because these cows are infertile as long as the condition persists [2]. The cause of ovarian cysts is not presently known. However, some predisposing factors include age, stress, high milk production, and genetics. It appears that an important component in the pathogenesis of this condition is an absent or inappropriate release of hypothalamic gonadotropin-releasing hormone (GnRH) at the time of estrus [1]. Therefore, a therapeutic approach involves the use of exogenous GnRH which releases LH from the anterior pituitary and causes ovulation of an ovarian follicle, or luteinization of the ovarian cysts [1].

The occurrence of ovarian follicular waves has been adequately demonstrated in the healthy dairy cow [3]. Furthermore, recent research [1] has suggested that ovarian follicular waves also occur in cows with ovarian cysts; however, ovulation of follicles occurs in cows without ovarian cysts, but not in cows with ovarian cysts. It has further been suggested that the administration of GnRH to cows with ovarian cysts causes ovulation of a functionally mature follicle of an ovarian follicular wave [1]. However, even with the use of ovarian ultrasonography, it may be a clinical challenge to determine the presence of a functionally mature follicle of an ovarian wave at the time of diagnosis and GnRH treatment of cows with ovarian cysts. Therefore, it is impossible to determine the stage of an ovarian follicular wave in dairy cows with ovarian cysts when GnRH is used as a therapeutic strategy. It is speculated that this may account for the relatively low success rate in some cows with ovarian cysts following the use of GnRH. It has been demonstrated that treatment with GnRH at random stages of the estrous cycle of cows without ovarian cysts will either cause ovulation or luteinization of large follicles present in the ovary and synchronize the recruitment of a new follicular wave [4,5].

The resumption of normal follicular development requires the interaction of gonadotropins and growth hormone [6]. It has been reported that growth hormone may stimulate particular follicle populations selectively, since it inhibits the development of preovulatory follicles and stimulates the growth of the second largest follicles in heifers [7]. However, the increase in the number of follicles is correlated more closely with peripheral IGF-1 than with growth hormone [8], indicating an indirect mechanism of growth hormone action [6]. It has also been shown that bovine somatotropin (bST) can advance the follicular wave by increasing the rate of follicular atresia in cows without ovarian cysts [9,10].

It has been reported [11,12] that the initiation of the Ovsynch protocol for synchronization of ovulation and timed insemination at metestrus and late diestrus reduces pregnancy rates for this protocol in cows without ovarian cysts. The stage of the estrous cycle that appears to be the most appropriate for producing greater pregnancy rates using this
protocol is the early luteal phase [11,12]. The hypothesis of this study was that bST and GnRH administered to cows with ovarian cysts at the time of diagnosis would induce follicle development and an appropriate stage of the estrous cycle more conducive to an increased conception rate (CR) following the Ovsynch protocol.

The objective of this study was to determine the effect of treatment of cows with ovarian cysts with GnRH and bST 7 days prior to the initiation of a protocol (Ovsynch) for synchronization of ovulation and timed insemination.

2. Materials and methods

This study was conducted in a large dairy herd (approximately 3000 milking cows) in north central Florida. The herd was visited weekly on a reproductive herd health program, and all reproductive, health and management records were computerized using a cow-side computer program. The milking herd was divided into eight different lots based on production criteria. Cows from each of the lots were used during the period of study. The cows were milked three times daily and were kept on shaded lots between milking. This study used 254 lactating dairy cows diagnosed with ovarian cysts using clinical findings obtained by palpation of the uterus and ovaries per rectum. Cows were diagnosed with ovarian cysts if there was no corpus luteum (CL), and multiple follicles (>20 mm in diameter) were present on one or both ovaries in the absence of uterine tonicity [1,13–15]. Previously published criteria were used to determine presence of a CL [16]. Between 60 and 63 days postpartum, all cows in this herd were subjected to concurrent treatment with bST (500 mg, s.q.; Posilac, Monsanto Co., St. Louis, MO) every 14 days until milk production decreased to a minimum level established by the management of the herd.

At the initiation of the study (Day 0), cows were sequentially divided into four experimental groups. Cows in Group 1 (n = 61) were pretreated with bST (500 mg, s.q.; Posilac, Monsanto Co.) and GnRH (100 µg, i.m.; Cystorelin, Rhone Merieux Inc., Athens, GA); cows in Group 2 (n = 73) were pretreated with GnRH as for Group 1; cows in Group 3 (n = 59) were pretreated with bST as for Group 1; and cows in Group 4 (n = 61) received no pretreatment. On Day 7, all cows in the study were treated with GnRH (100 µg, i.m.; Cystorelin, Rhone Merieux Inc.), one luteolytic dosage of PGF2α (25 mg, i.m.; Lutalyse, Upjohn Company, Kalamazoo, MI) 7 days later, GnRH (100 µg, i.m.) 2 days later, and then time-inseminated approximately 16 h after the second treatment with GnRH.

Pregnancy was determined by palpation of the uterus per rectum approximately 45–50 days after artificial insemination using techniques and procedures previously described [16]. Conception rate was defined as the percentage of cows that became pregnant as a result of timed insemination.

When cows were placed in the study (Day 0), the interval (days) between last routine bST treatments and Day 0 was determined (1–3, 4–6, 7–14 days; concurrent bST treatment). In addition, parity, days in milk (DIM), and time of year at insemination (season) were also recorded on Day 0.

Baseline comparisons for parity (1, 2, 3+), season (October to February, March to September), DIM (78–122, 123–160, 161–240, 241–651), and routine bST treatments were carried out after treatment assignment to establish comparability of groups by use of a
chi-square test (Table 1). The CR of cows in each group was the main outcome of interest and was compared among groups by logistic regression. All the variables were forced into a logistic regression model to adjust for confounders residuals. The backward elimination procedure based on the likelihood-ratio test was used to select the final model [17].

3. Results

The backward elimination test in the logistic regression model indicated that the interaction term between bST and GnRH ($P < 0.10$; Fig. 1) and parity ($P < 0.03$) was associated with conception. Since the interaction tended to be significant, the main effect for bST and GnRH pretreatments were not considered and CR was compared between cows with ovarian cysts (Group 4; control) and cows with ovarian cysts pretreated with either GnRH (Group 2), bST (Group 3) or both GnRH and bST (Group 1) prior to initiation of the Ovsynch protocol.

The odds ratio, 95% confidence intervals (CI) and $P$-value for CR of cows with ovarian cysts in each group adjusted for parity are shown in Table 2. The CR for cows with ovarian cysts in Group 3 (12%) was significantly lower (OR = 0.3; 95% CI = 0.1–0.9; $P = 0.03$) than the CR of cows with ovarian cysts in Group 4 (27%). The CR for cows with ovarian cysts in Group 1 (18%) tended to be lower (OR = 0.5; 95% CI = 0.2–1.2; $P = 0.10$) than that of cows with ovarian cysts in Group 4 (27%). The CR for cows with ovarian cysts in Group 2 (15%) tended to be lower (OR = 0.4; 95% CI = 0.2–1; $P = 0.06$) than that of cows with ovarian cysts in Group 4 (27%).
Fig. 1. Interaction ($P < 0.10$) between pretreatment with bST and GnRH 7 days before the Ovsynch protocol in lactating dairy cows with ovarian cysts.

Table 2
Conception rate, adjusted odds ratio (AOR), 95% CI, and $P$-value for cows in each group

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Conception %</th>
<th>AOR</th>
<th>95% CI</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (bST + GnRH)</td>
<td>11/61</td>
<td>18</td>
<td>0.5</td>
<td>0.2–1.2</td>
<td>0.10</td>
</tr>
<tr>
<td>2 (GnRH)</td>
<td>11/73</td>
<td>15</td>
<td>0.4</td>
<td>0.2–1.0</td>
<td>0.06</td>
</tr>
<tr>
<td>3 (bST)</td>
<td>7/59</td>
<td>12</td>
<td>0.3</td>
<td>0.1–0.9</td>
<td>0.03</td>
</tr>
<tr>
<td>4 (Control)</td>
<td>17/61</td>
<td>27</td>
<td>1.0</td>
<td>Referent</td>
<td>NA</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10/94</td>
<td>11</td>
<td>0.4</td>
<td>0.2–0.9</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>16/71</td>
<td>23</td>
<td>1.0</td>
<td>0.7–2.1</td>
<td>0.97</td>
</tr>
<tr>
<td>3+</td>
<td>20/89</td>
<td>23</td>
<td>1.0</td>
<td>Referent</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA: not applicable.

The CR of cows with ovarian cysts with one lactation period (11%) was significantly lower ($P < 0.02$) than that for cows with ovarian cysts with 2, or more than 3 lactation periods (23 and 23%, respectively). Cows with ovarian cysts with one lactation period had an increased risk of non-pregnancy (OR = 0.4; 95% CI = 0.2–0.9; $P = 0.02$).

4. Discussion

The hypothesis of this study was that administration of bST and GnRH to cows with ovarian cysts at the time of diagnosis would induce an appropriate stage of the estrous cycle conducive to an increased CR using a protocol for synchronization of ovulation and timed insemination. However, the results of this study did not support this hypothesis. In fact, the results showed that pretreatment with only bST significantly reduced CR, while pretreatment
with GnRH, and GnRH and bST combined tended to reduce CR in cows with ovarian cysts concurrently treated with bST and subjected to the Ovsynch protocol.

Cows in the dairy herd of the present study were concurrently treated with 500 mg bST every 14 days, beginning between 60 and 63 days postpartum, and continuing until milk production decreased to a minimum level established by the manager of the herd. It is speculated that the interval from concurrent treatment with bST to the initiation of the study (Day 0), and the total dose of bST administered to cows in this study may be important factors influencing CR. However, previous research [15] has shown that there was no effect of interval from the last treatment with bST to the day of initiation of a protocol for synchronization of ovulation and timed insemination on CR in cows with ovarian cysts.

In the present study, cows in Groups 1 and 3 received an additional dose of 500 mg bST on Day 0. Therefore, it is tempting to suggest that this additional bST treatment of cows in Groups 1 and 3 may be associated with the reduced CR observed in this group of cows. A previous report [18] has shown that cows without ovarian cysts which were supplemented with high doses of bST experienced decreased conception and had reduced fertility. In addition, long-term infusion with rbST modified the growth and function of the first wave dominant follicle resulting in development of multiple estrogen-active follicles [19].

It was expected that pretreatment with GnRH would induce a new follicular wave and 7 days later a dominant follicle would be present in the ovaries to be ovulated following the first GnRH treatment of the Ovsynch protocol. However, the results of this study indicated that pretreatment with GnRH 7 days prior to the Ovsynch protocol tended to decrease CR. Since daily ovarian ultrasonography was not done in this experiment, it is difficult to explain why the pretreatment with GnRH was not effective in increasing CR in cows with ovarian cysts. Perhaps the 7 day period prior to the beginning of the Ovsynch protocol was not long enough for a follicle to reach an ovulatory size. Recent research has shown that optimum ovulatory capacity is reached when follicles are around 12 mm in diameter [20].

Interestingly, it has been reported that the release of LH in response to GnRH is increased in lactating dairy cows supplemented with bST [21], and that the combination of bST and gonadotropins is effective in causing greater follicular development and ovulation. It has also been reported that the administration of growth hormone to hypogonadotropic anovulatory women significantly decreased the dose and frequency of human menopausal gonadotropin (hMG) required for induction of ovulation, and increased the percentage of successfully treated patients [22]. However, in the present study, CR in cows of Group 1 that received an additional dose of bST and GnRH was decreased. In addition, pretreatment with both bST (Group 3) and bST with GnRH (Group 1) decreased CR; this effect of bST was evident in either the presence or absence of GnRH.

From the results of this study, it was concluded that bST pretreatment decreased CR, and pretreatment with GnRH, and GnRH with bST tended to decrease CR in lactating dairy cows with ovarian cysts concurrently treated with bST and subjected to the Ovsynch protocol.

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References