IMPACT OF INTRAVAGINAL PROBIOTICS INOCULATION ON REPRODUCTIVE PERFORMANCE OF HOLSTEIN DAIRY CATTLE DURING TRANSITION PERIOD

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ABSTRACT

In this study we tested the hypothesis that intravaginal infusions of lactic acid bacteria (LAB) around calving would improve uterine involution and reproductive performance of dairy cows. The probiotic culture used in this study was a mixture of 2 LAB strains isolated previously from the vaginal tract of healthy pregnant heifers. Forty pregnant 2nd lactation dairy cows 2 wk before the expected day of calving were assigned to one of 2 groups, first group was the control group (C) receiving 1 mL of media only, and the 2nd group was the treated group (T) receiving 1 mL of media enriched with probiotic bacteria at 10¹⁰ to 10¹² cfu/treatment. Intravaginal infusions were performed once during wk −2, −1, +1, +2, +3, and +4 relative to parturition with probiotic bacteria. All cows were observed for reproductive performance and reproductive diseases until next conception using ultrasonography. The incidence of uterine infections in the multiparous cows were measured. Days open, conception rate at 1st, 2nd and 3rd insemination and repeat breeders (%) were calculated. Incidence of purulent and foul-smelling discharges on +1, +2, +3 and +4 wk relative to parturition and numbers of cows in the cleanup program were measured, cervix diameters (C.D) and uterine horn diameters (U.H.D) were measured at days (22-29) and days (38-45) postpartum. Ovarian structures and endometritis were determined. Results revealed that uterine and cervical involution of postpartum dairy cows were earlier in cows treated with LAB. Cows showed purulent and foul smelling discharges are fewer in number in treated group than control, also data showed that percentage of cows in the cleanup program was higher in the control group (40%) than in the treated group (20%). The averaged insemination number per conception was lesser in the treated group (2.55) than control (4). The conception rate at 1st insemination and 3rd insemination was higher in the treated group (35%, 30%) than in control one (25%, 10%), and the % of repeated breeder cows was lower in the treated group (30%) than control (40%), finally days open in the treated group (116.4) was much lower than in the control (154.72). Therefore it can be conclude that, intravaginal treatment of transition dairy cows with a mixture of lactic acid bacteria (LAB) could improve uterine involution, lower the incidence rates of uterine infections, and improve the reproductive performance.

Key words: Reproductive performance, uterine involution, Probiotics, Dairy cattle

INTRODUCTION

Transition dairy cows are susceptible to uterine infections due to the compromised immunity around calving and substantial bacterial contamination in the uterus immediately after calving. Uterine infections affect 1 in 2 dairy cows after parturition, infertility related to uterine infections has become the main rai...
efficiency is not proven and concerns about drug residue in milk and carcass, or bacterial acquisition of drug-resistance have limited their widespread use (Galvão, 2011). Presently, there are no intrauterine antibiotics approved for utilization in dairy cows in the U.S (Galvão, 2011). Only systemic cefitifur® is approved for treatment of cows with metritis. Although systemic administration of cefitifur® could lower the incidence of metritis, it does not improve the reproductive performance (Risco and Hernandez, 2003).

Infusion of povidone iodine® also has been stopped due to its ineffectiveness, impeding of phagocytic activity of leukocytes in the uterus and triggering of endometrial necrosis (Azawi, 2008).

Intramuscular PGF2α has been reported as a promising therapy in treating endometritis cows. It has a direct effect on flushing out bacteria from the uterus by stimulating myometrial contractions and enhancing immune responses (Lewis, 2003). However, a meta-analysis to previous studies demonstrated only a marginal benefit of PGF2α on reproductive performance (Burton and Lean, 1995).

Recently, there is an increasing interest of using lactic acid bacteria (LAB) or probiotics to treat vaginal inflammatory conditions (Reid and Bruce, 2003; Ametaj et al., 2014). Nader-Macías et al. (2008) found that LAB strains isolated from vaginal tract have a strong capability of producing H2O2. H2O2-generating lactobacilli from the vaginal tract of cattle have the potential to be utilized as probiotics, among which Lactobacillus gasseri CRL1421 is reported to have the greatest capacity to inhibit Staphylococcus aureus by generating H2O2 and lactic acid (Otero and Nader-Macías, 2006). A few strains of LAB (mainly Lactobacillus fermentum) isolated from cow’s vaginal mucus have been reported to be able to inhibit the growth of Actinomyces pyogenes in vitro, a recognized pathogen isolated from matritic cows, which hold great potential to be used as probiotic product to prevent metritis (Otero et al., 2006). Pediococcus acidilactici isolated from healthy pregnant dairy cows has exhibited inhibitory effect on Lactobacillus innocua and Enterococcus faecalis by the production of pediocin (Wang et al., 2013). A previous study reported that 6 times treatment around calving with a cocktail of 3 LAB, isolated from the vaginal tract of healthy cows, lowered the incidence of purulent vaginal discharges and improved conception rates of multiparous cows (Ametaj et al., 2014), they hypothesized that lowering the number of treatments around calving from 6 times to 2 or 3 treatments might give the same beneficial effects to the cows. Therefore the objectives of this study were to test whether intravaginal treatment of transition dairy cows with a mixture of lactic acid bacteria (LAB) can lower the incidence rates of uterine infections, improve uterine involution and improve the reproductive performance.

MATERIALS AND METHODS

Vaginal samples were obtained from pregnant Holstein heifers from Sanad farm (on Cairo-Ismailia desert road). The animals that had no history of metritis infection were selected. The vulvar area was washed with povidone–iodine and water. A disposable speculum was inserted into the vagina to swab the posterior area. Samples were collected and transported to the microbiology laboratory of Ain Shams University.

Microbial strains and growth condition of the used probiotics

Three isolates were isolated from vagina of pregnant Holstein heifers revealed on 2 bacterial strains characterized and identified to be propagated and used as a probiotic supplementation. Lactobacilli isolates were grown on MRS broth (Oxoid) while Streptococci isolates were grown on M17 broth (Difco), after that the broth media incubated for 24 h at 37 °C. The strains were activated two or three times in order to obtain high biomasses in the stationary phase.

Genetic identification of isolated and tested strains

The isolates were identified according to 16sRNA in Sigma Company (Germany) by the automated sequencer (Table 1).

Table 1: The genetic identification of the tested probiotics strains.

<table>
<thead>
<tr>
<th>Probiotic strain</th>
<th>Isolation source</th>
<th>Isolation media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus farraginis strain NRIC 0676 16S ribosomal RNA gene, partial sequence, NR 041467.1</td>
<td>Pregnant heifers vaginal mucous</td>
<td>MRS</td>
</tr>
<tr>
<td>Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA gene, partial sequence, NR 113332.1</td>
<td>Pregnant heifers vaginal mucous</td>
<td>M17</td>
</tr>
</tbody>
</table>
Experimental animals and design
The probiotic culture used in this study was a mixture of 2 LAB strains isolated previously from the vaginal tract of healthy pregnant heifers. Forty pregnant 2nd lactation dairy cows 2 wk before the expected day of calving were assigned to one of 2 groups, 1st group was the control group (20 cows) receiving 1 mL of media only, and the 2nd group was the treated group (20 cows) receiving 1 mL of media enriched with probiotic bacteria at 10⁷ to 10¹² cfu / treatment. The LAB infused in the vagina of the treated cows in our experiment are commensal bacteria identified and isolated from healthy vaginal tracts of pregnant heifers as previously described by Wang et al. (2013) and Ametaj et al. (2014). Intravaginal probiotics infusions were performed once per week during wk −2, −1, +1, +2, +3, and +4 relative to parturition. All cows were observed for reproductive performance and reproductive diseases until next conception. The incidence of uterine infections in the multiparous cows were measured. Days open, conception rate at 1st, 2nd and 3rd insemination and repeat breeders % were calculated. Incidence of purulent and foul-smelling discharges on +1, +2, +3 and +4 wk were measured, cervix diameters and uterine horn diameters were measured in days (22-29) and (38-45) post-partum. The numbers of cows in the clean-up program were measured. Ovarian structures and endometritis were determined.

Research was conducted at Sanad farm. Animals used in this experiment were kept in the same conditions of housing. All cows were in the 2nd parity, data about age, health condition and reproduction have been taken from farm protocol.

Ultrasound examination
Done by Unit of Radiology and Ultrasonography, Animal Reproduction Research Institute. Ultrasound scanning for reproduction have been performed starting from 22 to 45 days postpartum through two periods. First period was 22–29 days, 2nd was 38–45 days postpartum. In this study it is been used diagnostic ultrasound electronic linear scanner. Examination technique is similar to standard transrectal manual examination. After removing feces from the rectum, we placed probe intra-rectally to obtain image of the uterus using portable, battery operated and B-mode ultrasound scanner. To obtain data of uterine horn diameter and cervix diameter, we have used, “freeze” mode and after that „caliper“ option at the edges of viewed tissues to get precise data about searched uterine dimensions. Obtained data about tissue dimensions were calculated. A Sonosite ultrasound (Sonosite, M- turbo, USA) fitted with (5-10)-MHz, L52x linear-array probe was used to obtain images of the cervix, uterine body and horns, follicles, and corpora lutea on ovaries of all cows according to (Melendez et al., 2004).

Statistical analysis
The various data were subjected to ANOVA procedure for a complete randomized design and performed according to Snedecor and Cochran (1982). Analysis of variance, by Duncan’s test using, and least significant difference were applied to the data to test for differences between treatments using a computer program ‘COSTAT’. Significance was declared at P = 0.05. A trend was considered to exist if 0.05 < P < 0.10.

RESULTS
Incidence of purulent and/or fowl smelling discharges
Results showed that, cows showed purulent and fowl smelling discharges are fewer in no. in treated group than control, although no. of cows showed difficult labor and/or retained placenta was higher in the treated group (Table 2).

Table 2: Incidence of purulent or fowl smelling discharges

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cows no.</th>
<th>No. of cows showed dystocia and/or retained placenta</th>
<th>No of cows with purulent and/or fowl smelling discharges</th>
<th>Incidence of purulent and/or fowl smelling discharges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>2</td>
<td>12</td>
<td>60%</td>
</tr>
<tr>
<td>Treatment</td>
<td>20</td>
<td>4</td>
<td>4</td>
<td>20%</td>
</tr>
</tbody>
</table>

Ultrasound scanning for reproductive tract (22–29 day postpartum)
The results showed that, the cervical diameter, right horn and lift horn diameter decreased in the treated group than control, statistically there is no significant differences between treatment and control (P<0.05), only the average of cervix diameter tend to be significantly (P<0.09) lesser in the treated group than control. Cows in treated group showed lower percent of endometritis and pyometra than control group (table 3 and figure 3).
Table 3: Ultrasound scanning for reproductive tract (22-29 day postpartum)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cervix diameter (average) Cm Mean±SD</th>
<th>Right horn diameter (Average) Cm Mean±SD</th>
<th>Lift horn diameter (Average) Cm Mean±SD</th>
<th>Cows showed endometritis (E.)</th>
<th>% of endometritis</th>
<th>Cows showed pyometra</th>
<th>% of Cows showed pyometra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.67±0.45</td>
<td>3.2±1.8</td>
<td>3.08±1.91</td>
<td>6/19</td>
<td>31.6</td>
<td>1</td>
<td>5.26</td>
</tr>
<tr>
<td>Treatment</td>
<td>3.41±0.43</td>
<td>2.53±0.48</td>
<td>2.54±0.39</td>
<td>1/16</td>
<td>6.25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sign.</td>
<td>0.09</td>
<td>0.16</td>
<td>0.27</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Ultrasound scanning for reproductive tract (38-45 day postpartum)

Results showed that the cervical diameter, right horn and lift horn diameter were decreased in the treated group than control, statistically, there is no significant differences among treatments (P<0.05), also treated group showed lower percent of endometritis, pyometra and ovarian cysts than control (Table 4, figure 1 and 2).

Table 4: Ultrasound scanning for reproductive tract (38-45 day postpartum)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cervix diameter (average) Cm Mean±SD</th>
<th>Right horn diameter (Average) Cm Mean±SD</th>
<th>Lift horn diameter (Average) Cm Mean±SD</th>
<th>Cows showed endometritis (E.)</th>
<th>% of Endometritis</th>
<th>Cows showed pyometra</th>
<th>% of pyometra</th>
<th>Ovarian cyst</th>
<th>% of Ovarian cyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.6±0.55</td>
<td>2.91±1.168</td>
<td>2.77±1.91</td>
<td>5/17</td>
<td>29.4%</td>
<td>1/17</td>
<td>0.06%</td>
<td>2/17</td>
<td>1.2%</td>
</tr>
<tr>
<td>Treatment</td>
<td>3.31±0.49</td>
<td>2.54±0.48</td>
<td>2.33±0.39</td>
<td>2/16</td>
<td>12.5%</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Sign.</td>
<td>0.16</td>
<td>0.22</td>
<td>0.27</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 1: Ultrasound image showing cervix diameter (38-45 days postpartum)
Reproductive parameters
Results showed that percentage of cows in the cleanup program was higher in the control group (40%) than in the treated group (20%), the averaged insemination no. per conception was decreased in the treated group (2.55) than control, conception rate at 1st insemination and 3rd insemination was higher in the treated group (35%, 20%) than control (25%, 10%), the no. of repeat breeder cows was lower in the treated group (30%) than control (40%), finally, days open in the treated group (116.4) was much lower than in the control group (154.72) with no significant (P<0.05) between the two groups as shown in table 5.
**Table 5: Effect of intravaginal probiotics inoculation on reproductive performance**

<table>
<thead>
<tr>
<th>Group</th>
<th>No of cows in the cleanup program before 1st insem.</th>
<th>No of insems/conception</th>
<th>Conception rate at 1st insem.</th>
<th>Conception rate at 2nd insem.</th>
<th>Conception rate at 3rd insem.</th>
<th>% of repeat breeders (more than 3 inseminations/conception)</th>
<th>Days open Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8/20 (40%)</td>
<td>4</td>
<td>25%</td>
<td>25%</td>
<td>10%</td>
<td>8/20 (40%)</td>
<td>154.72±138 (15 cows)</td>
</tr>
<tr>
<td>Treatment</td>
<td>4/20 (20%)</td>
<td>2.55</td>
<td>35%</td>
<td>15%</td>
<td>20%</td>
<td>6/20 (30%)</td>
<td>116.4±42.13 (20 cows)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The results of this study revealed that intravaginal administration of LAB confers a health benefit to the reproductive tract against bacterial infections of dairy cows, results revealed that cows showed purulent and fowl smelling discharges were fewer in number in treated group than control, also the percentage of cows in the cleanup program was higher in the control group (40%) than in the treated group (20%), although number of cows showed difficult labor or retained placenta was higher in the treated group which refers to the prophylactic and treatment effect of the used vaginal probiotics. These results agreed with a previous study reported that 6 doses around calving with a cocktail of 3 LAB isolated from the vaginal tract of healthy cows lowered the incidence of purulent vaginal discharge of multiparous cows (Ametaj et al., 2014). According to (Williams et al., 1995) time interval from parturition to the end of involution lasts 23 – 42 days. In the same study it was found that the greatest number of cows (51, 4%) completed involution during period from 29 to 35 days postpartum. The most intensive involution changes are developing up to 25 days postpartum, when uterine horn diameter is 20 – 40 mm and both horns are approximately same size (Leslie 1983). In research of Leslie (Leslie 1983) uterine involution in nursing cows has ended already after 15–25 days postpartum. In this study greatest number of cows has ended uterine involution in time period from 22–29 days. Results in table 3 and 4 also declared that treated group had better reproductive involution than control although the non-significance shown between treatments. Cervical diameter, right horn and lift horn diameter decreased in the treated group than control which confirm earlier uterine involution in the treated group, also treated group showed lower percent of endometritis, ovarian cysts and pyometra which confirm the prophylactic and treatment effect of the used vaginal probiotics.

Bacterial infections of the uterus are commonly present in the postpartum dairy cows, which are associated with histological lesions and inflammation of the uterine tissue (Sheldon et al., 2006). The lower incidence rates of metritis and total uterine infections in the treated group obtained in this study confirm previous finding that intravaginal LAB lowers purulent vaginal discharges in the treated cows (Ametaj et al., 2014). Lactobacillus spp., such as L. rhamnosus GG, L. rhamnosus GR-1, L. fermentum RC-14, and L. acidophilus are well-known for their ability to maintain and restore a normal vaginal microflora and therefore have been used to prevent and treat urogenital infections in women (Reid et al., 2001; Gardiner et al., 2002; Reid et al., 2003). Lactobacillus spp. has been administered directly in the vagina attenuating or treating symptoms of vaginal infections (Reid et al., 1995; Hilton et al., 1995). This treating effect can be explained that infusion of LAB in the vaginal tract of cows increased concentrations of IgA in the vaginal mucus. Secretory IgA (sIgA) is recognized as the most important mucosal immunoglobulin of mucosal tissues, there are reports demonstrating that commensal bacteria can stimulate the production of IgA with the involvement of local epithelial cells and dendritic cells (DCs) (Thomas and Versalovic, 2010). In addition, Boullier et al. (2009) found that sIgA was able to dampen the inflammation at mucosal tissues. The LAB-treated cows had greater concentrations of sIgA in the vaginal tract on wk 0 (immediately after calving), +1 and +2. This was probably due to the invasion of pathogenic bacteria into the reproductive tract during this period, as Kaila et al. (1992) found that Lactobacillus could promote the development of sIgA specific-antibody producing cells and therefore enhance the secretion of local sIgA in the presence of pathogenic bacteria.
The averaged insemination no. per conception was less in the treated group than control, also treated group had better conception rate at 1st insemination and 3rd insemination than control group, these results were in agreement with (Ametaj et al., 2014). Who reported that 6 doses around calving with a cocktail of 3 LAB isolated from the vaginal tract of healthy cows improved conception rates of multiparous cows. The no. of repeat breeder cows decreased in the treated group than control, finally days open in the treated group was much lower than control and agreed with (Deng et al., 2015) who found that two doses vaginal infusion of LAB decreased the number of days from calving to conception than control by 40 d (110 vs. 150 d).

CONCLUSION

It can be conclude that, intravaginal treatment of transition dairy cows with a mixture of lactic acid bacteria (LAB) can lower the incidence rates of uterine infections, improve uterine involution and improve the productivity of reproduction.

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REFERENCES


تأثير التلقح المهبلي لبكتريا البروبيوت ك على الأداء التناسلي لأبقار الهولشتين الحلية

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في هذه الدراسة تم اختبار فرضية ما إذا كان استخدام بكتريا حمض اللاكتيك مهilia من الممكن يكون لها تأثير إيجابي على الآداء التناسلي وتحسين التلقح المهبلي (العديد الرحم لجهاز الطبيعي بعد الولادة) في الأبقار الحلية. وكان البروبيوت المستخدم في هذه الدراسة مخلوطاً من 2 ملاعق علبة من قناع البكتريا مع ملحية جيدة وتم تلقيحها بعد أربعين يوم من المجموعة الثانية. 한ماي تأثير قناع الولادة وتم تقصيمهم عشوائياً إلى مجموعتين، الصحافة الضيقة (20 بقرة) ، المجموعة الثانية (20 بقرة)، تم تحضيرها بـ 1 مللي media وفقاً، و المجموعة الثانية (20 بقرة)، تم تحضيرها بـ 1 مللي media وفقاً، و المجموعة الثانية (20 بقرة)، تم تحضيرها بـ 1 مللي media وفقاً، و المجموعة الثانية (20 بقرة)، تم تحضيرها بـ 1 مللي media وفقاً، و المجموعة الثانية (20 بقرة)، تم تحضيرها بـ 1 مللي media وفقاً، و المجموعة الثانية (20 بقرة)، تم تحضيرها بـ 1 مللي media وفقاً، و المجموعة الثانية (20 بقرة)، تم تحضيرها بـ 1 مللي media وفقاً، و المجموعة الثانية (20 بقرة)، تم تحضيرها بـ 1 مللي media وفقاً، و المجموعة الثانية (20 بقرة)، تم تحضيرها بـ 1 مللي media وفقاً، و المجموعة الثانية (20 بقرة)، تم تحضيرها بـ 1 مللي media وفقاً، و المجموعة الثانية (20 بقرة)، تم تحضيرها بـ 1 مللي media وفقاً، و المجموعة الثانية (20 بقرة)، تم تحضيرها بـ 1 مللي media وفقاً، و m_elgarhi2005@yahoo.com

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