Postpartum Uterine Bacterial Contamination without Clinical signs in Relation to Reproductive Performance in Dairy Cows



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Abstract: The study was performed to find out the relation between the uterine bacterial contaminations without clinical signs and postpartum (PP) reproductive performance of dairy cows. So that, uterine bacterial samples from postpartum dairy cows total (n = 44) were taken at 3rd, 15th and 30th day, and the bacterial count and score were applied. The animals were grouped to low, medium and high uterine bacterial contamination (15, 15, and 14 cows for each group respectively) according to score. Results revealed that uterine bacterial score (UBS) was decreased by the time in 3rd, 15th and 30th day (PP) for Low bacterial contamination group (5.73, 2.80, and 1.20 respectively), for Medium bacterial contamination group (7.80, 2.73, and 1.47 respectively), and for High bacterial contamination group (9.29, 6.57, and 2.21 respectively). Also, it revealed that there was a significant increase (P<0.05) in the duration of lochia in High than Low and Medium bacterial contamination groups. At 3rd day (PP), uterine location in all cows was represented in the abdominal cavity, but at 15th day (PP), uterine involution as reaching to its normal non pregnant position in pelvic cavity was delayed in High (50%) than Low (80 %,) and Medium (53.30%) UBS groups. Moreover, at 30th day. uterine location in all cows was represented in pelvic cavity. The first estrus (PP) was significantly shorter in Medium, Low than High UBS groups. The number of services per conception showed a significant increase in High than in Low and Medium UBS groups. Also, at the 90th day (PP), the conception rate was lower in High UBS group than Low UBS group and Medium UBS group. We conclude that there was a relation between postpartum uterine bacterial contamination without clinical signs especially high contamination and reproductive performance in cows.

Keywords: uterus - bacteria – postpartum - reproductive performance and cows.

INTRODUCTION

The postpartum period is considered as a non-infectious event. The reduction in uterine size and the unidirectional flow of uterine contents, as well as gradual closure of the cervix, prevent microbial contamination. However, the reality is that the uterus invasion by microorganisms to a variable extent is depending on the animal's susceptibility and the hygienic condition of the environment(Gustafsson, Kornmatitsuk, Königsson, & Kindahl, 2004). Also during the early postpartum period, multiple bacterial species

invade the uterus of cows (Rahim Ahmadi, Nazifi, & Reza Ghaisari, 2006). Uterine infection implies adherence of pathogenic organisms to the mucosa, colonization or penetration of the epithelium, and/or release of bacterial toxins that lead to the establishment of uterine disease. The development of uterine disease depends on the immune response of the cow, as well as the species and number (load or challenge) of bacteria(Azawi, 2008).

A normal postpartum cow resolves uterine infection by rapid involution of the uterus

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and cervix, discharge of uterine content, and mobilization of natural host defenses, including mucus, antibodies and phagocytic cells (Azawi, 2008). The uterine bacterial infection, bacterial products toxins, or the associated uterine inflammation may suppress pituitary LH secretion and disrupts postpartum ovarian follicular growth and function (I. M. Sheldon & Dobson, 2004). Series of studies confirmed that clinical and reproductive consequences are associated with these primary uterine pathogenic bacteria (Ahmed Elsheikh, 2013; Foeldi et al., 2008) revealed that the dairy cows which suffered severe uterine bacterial infection delayed the time taken for appearance of the first dominant follicle and had a significantly increased rate of services per conception compared to the cows with mild uterine bacterial infection. also calving interval of dairy cows with severe postpartum uterine bacterial infection was significantly longer (482.50 \pm 9.00 days) than that of dairy cows with mild postpartum uterine bacterial infection (407.10 \pm 4.80 days). (Lewis, 1997) recorded that uterine infections have negative effects on various measures of productivity in dairy cows. Although postpartum cows develop mild endometritis, most cows are able to clear pathogenic organisms that cause endometritis before any measure of productivity is affected. This study was aimed to find out the relation between the uterine bacterial contaminations without clinical signs and postpartum reproductive performance of dairy cattle.

MATERIALS AND METHODS

Animals and samples collection: The present study was carried out at two farms in Sharkia and Damietta Provinces, Egypt during the period from January 2014 to May 2015. A total number of 44 cows aged from 3-5 years at puerperium stage (22 from each farm) were used. Uterine (endometrial swabbing) samples were taken on days 3, 15 and 30 postpartum using a transcervical guarded swab (consists of a small cotton piece wrapped around one ends of a rod sheathed in a metal guard tube). The animals were grouped to low, medium and high uterine bacterial contamination (15, 15, and 14 cows for each group respectively) according to uter-

ine bacterial count and score.

Bacteriological examination: Uterine bacterial count was performed according to (Cain, Hanks, Weis, Bottoms, & Lawson, 2013). Sterile test tubes were labeled as follows: 10^{-2} , 10^{-3} , 10⁻⁴, 10⁻⁵, and so on. Nutrient agar plates (OX-OID. CM0085) were labeled as follows: 10⁻¹. 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, and so on. 1:10 dilution of the sample was prepared. 9 ml of sodium chloride 0.9% w/v was inserted in each test tube. By using a new sterile pipette, 1 ml of the 10⁻¹ tube was transferred into the tube labeled 10⁻². By using a new sterile pipette, 1 ml of the 10⁻² tube was transferred into the tube labeled 10⁻³. this step was repeated for the next dilutions. By using a new sterile pipette, 1 ml of the 10⁻¹ tube was transferred to the agar plate labeled 10⁻¹, and the liquid was spread thoroughly and evenly over the surface of the plate using a sterile disposable spreader. By using a new sterile pipette, 1 ml of the 10⁻² tube was transferred to the agar plate labeled 10⁻², and the liquid was spread thoroughly and evenly over the surface of the plate using a sterile disposable spreader, and this step was repeated for the next agar plate. Finally, agar plates were inverted and incubated at 37°C for 24-48 hours. Colonies of the plate having 30 to 300 colonies were counted (Lee, 2009). The Colony- Forming Unit / Milliliters (CFU/ml) can be calculated using the formula: CFU/ml = (No. of colonies x dilution factor) / volume of culture plate. A bacteriological score varying 1 to 10 was assigned to these bacteria, which corresponded respectively to table (1).

Table (1): Bacterial counts and their suggested scores

Bacterial count (CFU/ml)	Score
$< 10^2$	1
$10^2 \text{to} < 10^4$	2
$10^4 \text{to} < 10^6$	3
$10^6 \text{to} < 10^8$	4
$10^8 \text{to} < 10^{10}$	5
$10^{10} \text{to} < 10^{12}$	6
$10^{12} \text{to} < 10^{14}$	7
$10^{14} \text{to} < 10^{16}$	8
$10^{16} \text{to} < 10^{18}$	9
$10^{18} \text{ to} < 10^{20}$	10

An ascending arrangement of animals was done according to means of uterine bacterial score then grouping animals into 3groups: low (15 cows), medium (15 cows) and high (14 cows) bacterial contamination groups. The mean uterine bacterial score (UBS) for each animal was calculated as follow: (3rd day UBS +15th day UBS +30th day UBS) / 3

Detection of postpartum reproductive performance: Anatomical location of uterus in abdominal and pelvic cavity was detected by rectal palpation in 3rd, 15th and 30th days postpartum (Saut et al., 2011). For lochia discharge, cows were observed after parturition for recording duration of lochia. For first estrus postpartum, cows were observed for detection of estrus behavior, the interval from calving to first estrus was recorded (Habib, Bhuiyan, & Amin, 2010). Cows which come in estrus were inseminated. Non-return cows were examined for pregnancy by ultrasonography or by rectal examination 60 days after insemination. Number of services per conception was calculated by dividing the number of conceptions with the number of inseminations (Habib et al., 2010). Conception rate was calculated according to (Overton, 2009), conception rate was done at three periods, within 60 days, within 61-90 days and <90 days according to (Miah, Salma, & Hossain, 2004; Schefers, Weigel, Rawson, Zwald, & Cook, 2010).

Statistical analysis was performed using the Statistical Package for Social Sciences version 22.0 (SPSS for Windows 22.0, Inc., and Chicago, IL, USA). Data are represented in mean ± standard deviation values. Duncan's test was performed for comparing values between the groups. P<0.05 was considered to be significant

RESULTS

Uterine bacterial score (UBS): Table (2) and Fig. (1) showed that at 3^{rd} day, mean uterine bacterial score (UBS) was significantly smaller (P<0.05) in Low bacterial contamination group

(5.73±0.23) and Medium bacterial contamination group (7.80±0.24) than High bacterial contamination group (9.2 9±0.22). At 15th day, mean UBS was significantly smaller (P<0.05) in Low (2.80 ± 0.20) and Medium (2.73 ± 0.21) than in High bacterial contamination group (6.57±0.25) but there was no significant variation between Low and Medium bacterial contamination groups. At 30th day, mean UBS was significantly smaller (P < 0.05) (1.20 ± 0.11) and Medium (1.47 ± 0.13) than in High bacterial contamination (2.21±0.21) but there was no significant variation between Low and Medium bacterial contamination groups.

Table (2): Uterine bacterial score (M±SE) in Low, medium, and high bacterial contamination groups.

Character	NI.	Uterine bacterial score (UBS)			
Groups	No	3 rd	15 th	30^{th}	Means
		day	day	day	ivicans
Low bacterial contamination	15	5.73 ± 0.23	2.80± 0.20 ^a	1.20± 0.11 a	3.24± 0.05 ^a
Medium bacterial contamination	15	7.80 ± 0.24	2.73± 0.21 ^a	1.47± 0.13 ^a	4.00± 0.08 ^b
High bacterial contamination	14	9.29 ± 0.22	6.57± 0.25 ^b	2.21± 0.21 b	6.02± 0.20 °

The different superscript letters mean significantly differed at P < 0.05

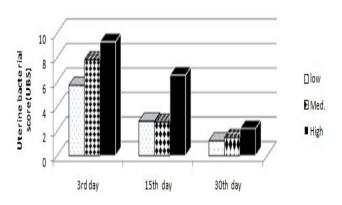


Fig.(1): Uterine Bacterial score in Low, Medium, and High bacterial contamination groups.

Effect of postpartum uterine bacterial contamination on reproductive performance: Duration of lochia, as presented in Table (3) and Fig. (2), revealed that the duration was significantly shorter (P<0.05) in Low (12.60±0.36 days) and Medium (13.53±0.35days) than in High bacterial contamination group (17.71±0.49 days). Although Low bacterial contamination group was shorter than Medium bacterial contamination group, this difference was not significant. First estrus postpartum, data obtained in Table (3) and Fig. (3) revealed that the elapsed time from parturition to first estrus was significantly shorter (P<0.05) in Medium $(51.07\pm2.00 \text{ days})$, Low (52.93 ± 1.93) days) than in High bacterial contamination group (63.64±5.63 days), but there was no significant variation between Low and Medium bacterial contamination group. The results of services per conception presented in Table (3) and Fig. (4) revealed that the number of services per conception was significantly lower (P<0.05) in Low (1.67 ± 0.19) , Medium (1.93±0.15) than in High bacterial contamination group (3.14±0.21) although the number of services per conception in Low (1.67±0.19) was lower than that for Medium bacterial contamination group (1.93±0.15), this difference was not significant. Despite the uterine location of tested cows which is founded in Table (4) and Fig. (5), it was observed that at 3rd day postpartum 100% of uterine location in Low, Medium and High bacterial contamination groups was represented in abdominal cavity. However at 15th day postpartum, the uterine location in 20%, 46.70% and 50% of cows in Low, Medium, and High bacterial contamination groups, respectively were represented in abdominal cavity. While the uterine location in 80%, 53.30% and 50% of cows in the same previous groups was represented in pelvic cavity respectively at the 15th day postpartum. Meanwhile at the 30th day postpartum, 100% of uterine location in Low, Medium, and High bacterial contamination groups was represented in pelvic cavity.

Table (3): Reproductive performance in Low, Medium and High bacterial contamination groups.

Groups	No	Duration of lochia (days)	Day of first estrus	services per con- ception
low bacterial contamination	15	12.60 ± 0.36^{a}	52.93±1. 93 ^a	1.67± 0.19 ^a
Medium bac- terial contam- ination	15	13.53 ± 0.35 ^a	51.07± 2.00 a	1.93± 0.15 ^a
High bacterial contamination	14	17.71 ± 0.49 b	63.64± 5.63 b	3.14± 0.21 ^b

The different superscript letters mean significantly differed at P < 0.05.

Table (4): Percentage of abdominal and pelvic location of uterus at P.P period in Low, Medium and High bacterial contamination groups

		Postpartum uterine location						
Groups	No	3 rd day		15 th day		30 th day		
		Abdominal	Pelvic	Abdominal	Pelvic	Abdominal	Pelvic	
Low bacterial contamination	15	15 (100%)	0 (0%)	3 (20%)	12 (80%)	0 (0%)	15 (100%)	
Medium bacterial contamination	15	15 (100%)	0 (0%)	7 (46.70%)	8 (53.30%)	0 (0%)	15 (100%)	
High bacterial contamination	14	14 (100%)	0 (0%)	7 (50%)	7 (50%)	0 (0%)	14 (100%)	

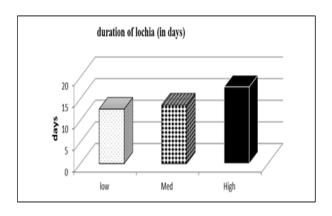


Fig. (2): Duration of lochia (in days) in Low, Medium and High bacterial contamination groups.

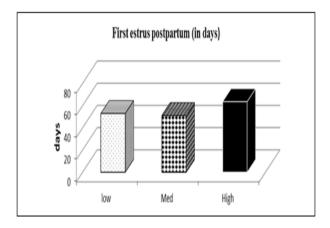


Fig. (3): First estrus postpartum (in days) in Low, Medium and High bacterial contamination groups.

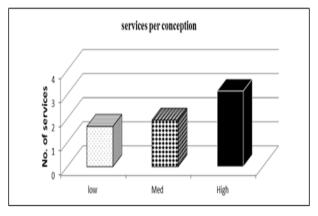


Fig. (4): Number of services per conception in Low, Medium and High bacterial contamination groups.

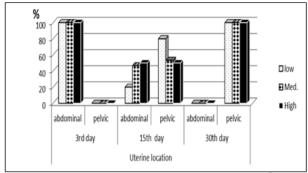


Fig. (5): Percentage of abdominal and pelvic location of uterus at P.P period in Low, Medium and High bacterial contamination groups.

46.67% of cows in Low bacterial contamination group were conceived from 1st service versus 20.00% in Medium bacterial contamination group. However, 40.00% of cows in Low bacterial contamination group, 66.67% in Medium bacterial contamination group, and 21.43% in High bacterial contamination group were conceived from 2nd service. It was observed that 13.33% of cows in Low and Medium bacterial contamination groups were conceived from 3rd service versus 42.86% in High bacterial contamination group. It was also observed that 35.71% of cows in High bacterial contamination group were conceived from 4th service. Moreover, data obtained in Table (6) and Fig. (7) revealed that within 60 days postpartum, the percentage of conceived cows was higher in Low bacterial contamination group (40%) than in Medium bacterial contamination group (20%), and there was no conception in High bacterial contamination group in this period. However, within 61-90 days postpartum, the percentage of conceived cows were (40%) in Low bacterial contamination group, (53.33%) in Medium bacterial contamination group, versus (7.14%) in High bacterial contamination group. Moreover, about (20%) of Low bacterial contamination group, (26.67%) of Medium bacterial contamination group, and (92.86%) of High bacterial contamination group required more than 90 days postpartum to be conceived.

Table (5): Conception rate of cows in low, medium and high uterine bacterial contamination groups.

Groups	No	Conception rate %			
Groups	110	1 st service	2 nd service	3 rd service	4 th service
Low bacterial contamination	15	7(46.67%)	6(40%)	2(13.33%)	0(0%)
Medium bacterial contamination	15	3(20%)	10(66.67%)	2(13.33%)	0(0%)
High bacterial contamination	14	0(0%)	3(21.43%)	6(42.86%)	5(35.71%)

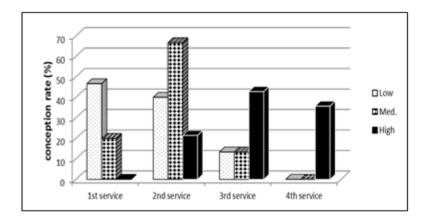


Fig. (6): Conception rate (%) of cows in low, medium and high uterine bacterial contamination groups.

Table (6): Number and percentage of conceived cows within different postpartum periods in Low, Medium and High bacterial contamination groups.

Groups	No	Number and percentage of conceived cows				
Groups	INO	Within 60 days	Within 61 - 90 days	<90 days		
Low bacterial contamination	15	6 (40%)	6 (40%)	3 (20%)		
Medium bacterial contamination	15	3 (20%)	8 (53.33%)	4 (26.67%)		
High bacterial contamination	14	0 (0%)	1 (7.14%)	13 (92.86%)		

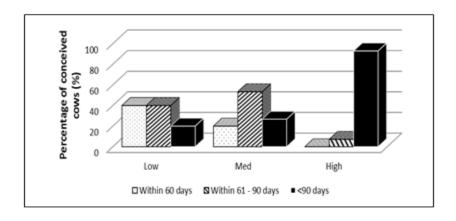


Fig. (7): Percentage of conceived cows within different postpartum periods in Low, Medium and High bacterial contamination groups.

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DISCUSSION

The reproductive performance of the breeding herd is critical due to the operation's ability to produce income. Income generation is affected not only by the absolute number of females that conceive but also by the timing and distribution of the pregnancies (Engelken, Trejo, & Voss, 2007). In the present study, mean uterine bacterial score was decreased by the time in 3rd, 15th and 30th day postpartum for low bacterial contamination group (5.73, 2.80, and 1.20 respectively), medium bacterial contamination group (7.80, 2.73, and 1.47 respectively), and high bacterial contamination group (9.29, 6.57 and 2.21 respectively). Decreasing of the bacterial score by the time in our results was supported by (I. Sheldon, Noakes, Rycroft, Pfeiffer, & Dobson, 2002) who reported that the ranges of total bacterial growth scores were 0-15, 0-12, 0-10, and 0-9 for days 7, 14, 21, and 28, respectively. The postpartum period is considered as a non-infectious event. The reduction in uterine size and the unidirectional flow of uterine contents, as well as the gradual closure of the cervix, prevent microbial contamination. However, the reality is that the uterus invasion by microorganisms to a variable extent is depending on the animals susceptibility and the hygienic condition of the environment (Gustafsson et al., 2004). Also during the early postpartum period, multiple bacterial species invade the uterus of cows (Rahim Ahmadi et al., 2006). A normal postpartum cow resolves uterine infection by rapid involution of the uterus and cervix, discharge of uterine content, and mobilization of natural host defenses including mucus, antibodies, and phagocytic cells (Azawi, 2008). Series of studies confirmed that clinical and reproductive consequences are associated with these primary uterine pathogenic bacteria (Foeldi et al., 2008).

Effect of postpartum bacterial contamination on reproductive performance in dairy cows, duration of lochia, and the greatest flow of lochia occurs during the first 2–3 days; by 8 days it is reduced, and virtually disappeared by 14–18 days postpartum (**Noakes** *et al.*, **2001**). Data revealed that duration of lochia was significantly shorter (P < 0.05) in low and Medium groups than that reported in high bacterial contamination group. First estrus postpartum is the time between the date of calving to the date of first subsequent estrous, this period is required for resumption of ovarian activity and uterine involution. It refers to the reproductive efficiency of an individual because the shortest the post-partum heat period the highest the calf production in their lifespan (Habib et al., 2010).

Our finding revealed that the elapsed time from parturition till the appearance of first estrus was significantly (P < 0.05) shorter in Medium and Low than that found in High bacterial contamination group. The results were confirmed by (Ahmed & Elsheikh, 2013) who stated that the sever postpartum uterine bacterial infection in dairy cows significantly extended the time taken for the resumption of the first estrus compared to the dairy cows suffered mild postpartum uterine bacterial infection. In addition, (I. Sheldon et al., 2002) observed that uterine bacterial infection, bacterial products toxins or the associated uterine inflammation may suppress pituitary LH secretion and disrupt postpartum ovarian follicular growth and function. In the same line, (Ahmed & Elsheikh, 2013) stated that a severe postpartum uterine bacterial infection in dairy cows significantly delayed the time taken for the appearance of the first dominant follicle as compared to dairy cows suffered mild postpartum uterine bacterial infection.

The study revealed that the number of services per conception was significantly (P < 0.05) lower in low bacterial contamination group and Medium bacterial contamination group than that found in high bacterial contamination group. These results came in the same line with (Ahmed & Elsheikh, 2013) who reported that

the mean rate of service per conception for the dairy cows with sever postpartum uterine bacterial infection was significantly higher than that of the dairy cows with a mild uterine bacterial infection. Number of services per conception (all cows) is a better index for evaluating conception in the herd because it includes all inseminations, whether the cow finally became pregnant or not (Fodor & Ózsvári, 2015) Uterine location and involution as measured by the time taken to reach the uterus to non- pregnant size, position and consistency was completed by day 24.1 ± 4.7 (range - 18 to 34 days). (Abeywansa, Abeygunawardena, Jayatilaka, 1991) results agree with our finding which revealed that at 3rd day uterine location for each cow in all groups was represented in abdominal cavity while its location at 30th day was represented in non-pregnant position in pelvic cavity. Also (Saut et al., 2011) reported that at 3rd day postpartum, uterine location for each cow was represented in abdominal cavity, and 58% of the cows evaluated by rectal palpation was located within the abdominal cavity at 14th day pp while at 28th day pp the uterus of all cows was within the pelvic cavity. Also, our results at 15th day postpartum indicated that the uterine location in 80 %, 53.30%, and 50 % of cows in Low, med, and High bacterial contamination groups were represented in pelvic cavity, respectively. These results indicate that high bacterial growth density delay uterine involution, and delaying involution by the effect of bacteria was supported by (Dobson-Hill, 2009) who reported that most cows are able to eliminate bacterial contamination during puerperium. However, 10 to 17% of cows are unable to do this. In these cows, the bacteria persist, cause infection and inflammation, and delay uterine involution.

Conception rate represents the ratio of the number of conceptions to the number of services and is expressed as a percentage (Habib et al., 2010) Reproductive efficiency can be increased in farm animals by decreasing the interval from parturition to conception

(Wettemann, 1980). Data revealed that at first service, 46.67% of cows in Low bacterial contamination group were conceived versus 20 % Medium bacterial contamination group while there was no conception for High bacterial contamination group. Also, it was observed that by third service all animal in Low bacterial contamination group and Medium bacterial contamination group were conceived, while by the fourth service all animal in High bacterial contamination group were conceived. Also, results revealed that within 60 days postpartum the percentage of conceived cows was higher in Low bacterial contamination group than in Medium bacterial contamination group. At the day 90 postpartum, most of the animals in Low bacterial contamination group (80%) and Medium bacterial contamination group (73.33%) were conceived versus only 7.14% in High bacterial contamination group, and the highest percentage of these animals (92.86%) need more than 90 days to conceive. This finding agreed with (I. M. Sheldon & Dobson, 2004) who stated that In cattle, postpartum contamination of the uterine lumen is ubiquitous, and persistence of pathogenic bacteria commonly causes clinical disease. The consequences are subfertility associated with delayed ovulation after parturition, a persistence of the corpus luteum once it forms, and lower conception rates. Also, (Gitonga, 2010) recorded that management and biological factors that may prolong the calving to conception interval are including; delayed resumption of postpartum ovarian activity, poor heat manifestation or detection, and presence of postpartum problems.

It was concluded that there was a relation between the high contaminations without postpartum clinical signs appearance and reproductive performance, so farms need more hygienic conditions or methods of treatment to maintain dairy farms economically viable.

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التلوث البكتيريّ الرحميّ بعد الولادة دون علامات سريرية وعلاقتها بالأداء التناسلي في الأبقار الحلوب توفيق محمد بركات، جمال شوقى*، محمد عبد الرحمن رجب

قسم التوليد والتناسل والتلقيح الاصطناعي، كليه الطب البيطري، جامعة الزفازيق، الزفازيق، مصر

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المستغلص: أجريت الدراسة لمعرفة العلاقة بين الملوثات الجرثومية الرحمية دون وجود علامات سريرية والأداء التناسلي بعد الولادة (PP) في الأبقار الحلوب. تم أخذ مسحات رحمية من الأبقار بعد الولادة (ن = 44) في اليوم الثالث والخامس عشر والثلاثين، وتم تطبيق عدد البكتيريا والنتيجة. تم تقسيم الحيوانات حسب التلوث البكتيري الرحمية (UBS) انخفضت بحلول والثلاثين، وتم تطبيق التوالي) وفقاً لدرجة النقييم. أظهرت النتائج أن درجة البكتيريا الرحمية (UBS) انخفضت بحلول الوقت في اليوم الثالث والخامس عشر والثلاثين (PP) لمجموعة التلوث الجرثومي المنخفض (5.73، 20.0 و 1.20 على التوالي)، لمجموعة التلوث المتوسطة (PS) محموعة التلوث المتوسطة (PS) محموعات التلوث الجرثومي المنخفض (5.73، 20.0 و 2.21 على التوالي)، لمجموعة التلوث المتوسطة (PS) و 2.00 و 2.00 و كالمجموعة عالية التلوث الجرثومي على التوالي)، لمجموعة والمتوسطة في اليوم الثالث (PP) وكان موقع الرحم (الانكماش الرحميّ) في جميع الأبقار في تجويف البطن. ولكن في اليوم الثلاثين لوحظ موقع ولكن في اليوم الثلاثين لوحظ موقع ولكن في اليوم الثلاثين لوحظ موقع في مجموعة عالية (PS) والمنخفضة والمتوسطة (PS) أقصر بكثير في مجموعتين UBS المنخفضة والمتوسطة عنه في مجموعة عالية (UBS). ووجد أن عدد التلقيحات لكل حمل به زيادة معنوية في مجموعة عالية من مجموعة على UBS المنخفضة ومجموعة متوسطة UBS. في اليوم (PP) كان معدل الحمل أقل في مجموعة الجرثومي بعد الولادة دون علامات سريرية ومجموعة متوسطة SUB. الأذاء النتاسلي في الأبقار.

الكلمات المفتاحية: رحم - بكتيريا - فترة بعد الولادة - الأداء النتاسلي - أبقار.

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