Stress and Uterine Bacterial Flora in Dairy Cows Following Clinically Normal and Abnormal Puerperium

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Abstract. ACTH challenge tests for the assessment of stress on the first and third week postpartum and bacteriologic examination of uterine swabs on the second and fourth week, were conducted in dairy cows following clinically normal (n=12) and abnormal (n=21) puerperium. Postpartum ovarian activity and uterine involution were also investigated. The basal plasma cortisol concentrations (mean $\pm$ S.E.) during the first and third week postpartum were 3.1 ± 0.9 ng/ml and 3.4 ± 1.1 ng/ml in normal cows, and 6.5 ± 1.9 ng/ml and 4.9 ± 1.2 ng/ml in abnormal cows, respectively. The mean basal plasma cortisol values of abnormal cows were elevated and significantly higher ($P < 0.05$) during the first week postpartum. Bacterial organisms were isolated from the uterus of 33.3% of normal and 61.9% of abnormal cows during the second week postpartum and in 33.3% of normal and 23.8% of abnormal cows during the fourth week. Resumption of ovarian activity was neither affected by puerperal disorders nor uterine bacterial infection. Uterine involution at 4 weeks postpartum was completed in all cows with normal puerperium and delayed in 23.8% of abnormal cows. The results indicate that cows with abnormal puerperium had significantly higher basal cortisol levels, and had higher occurrence of uterine bacterial infection during the second week when compared with normal cows.

Key words: Stress, Uterine infection, Puerperium, Cows.

(*J. Reprod. Dev.* 43: 157–163, 1997)

Puerperal complications such as dystocia, retained placenta and milk fever in dairy cows are considered as stressful conditions that influence the subsequent reproductive function. Stress is believed to cause the synthesis and release of adrenal glucocorticoids [1] which are capable of markedly diminishing an animal's defense against infection [2]. Glucocorticoids may increase the susceptibility of the uterus to infection through their

anti-inflammatory actions [3]. It has been further reported that if the defense mechanisms of the reproductive tract are impaired or weakened, bacteria may colonize in the uterus and lead to the development of uterine infection [4]. The bacterial content of the postpartum bovine uterus had been described in numerous studies [5–13]. The cortisol levels in the peripheral blood had also been accepted as a reliable physiological endpoint for determining response to stress or to any disease of the adrenal cortex [1]. However, no study has ascertained the degree of stress and uterine bacterial

contamination in postpartum dairy cows. In this study, the degree of stress and the uterine bacterial flora in dairy cows following clinically normal and abnormal puerperium were investigated. Possible effects of abnormal puerperium and uterine bacterial infection on ovarian activity and uterine involution were also evaluated.

Materials and Methods

A total of 33 Holstein-Friesian cows from the dairy herd of the Rakuno Gakuen University in Ebetsu, Hokkaido, Japan were used in this study. There were 12 primiparous and 21 pluriparous cows, with ages ranging from 2 to 9 years and a mean of 3.8 years. At calving, the cows were in good nutritional condition, with an average body condition score of 3.3 (1-emaciated; 5-obese), based on the categories described by Weaver [14]. The cows were fed grass silage, hay and concentrates, a ration meeting the nutritional recommendations of the National Research Council [15]. All the animals were kept tied in stanchions and were turned out into an outdoor paddock every morning for 2 h.

Reproductive examinations (visual inspection, vaginoscopy, palpation per rectum and ultrasonography) to determine the presence of postpartum disorder (e.g. dystocia, retained placenta and purulent vaginal or cervical discharges), were performed weekly during the first month after calving. Individual cow health records were also evaluated for the occurrence of other postpartum diseases (e.g. milk fever and fatty liver disease). The degree of uterine involution was determined according to criteria adapted from Buch *et al.* [16] and Johanns *et al.* [17]. Gross uterine involution at 30 days postpartum was described as normal when the uterine horns were located on the pelvic floor, had equal or similar size, were 4 cm or less in diameter, and had normal tone and consistency.

The degree of stress in the postpartum dairy cows was determined by measuring the basal cortisol levels and the adrenocortical response to ACTH on the first and third week after calving. Twenty-five i.u. of ACTH (Cortrosyn® Inj., Daiichi Pharmaceutical Co.) were administered intramuscularly. About 5 to 10 ml of blood was collected via coccygeal venipuncture into heparinized test tubes 15 min and immediately before ACTH injection (0 min) and 60 min after the injection. Blood

samples were centrifuged at 1,580 g for 15 min immediately after collection. The plasma was stored at -20°C until analysed for cortisol concentrations. Plasma cortisol was determined through a double antibody enzyme immunoassay according to the method described by Nakao *et al.* [18]. The sensitivity of the enzyme immunoassay was 25 pg/tube. The recovery rate of cortisol in the assay was $105.6 \pm 14.4\%$. The intra-assay coefficients of variation in plasma with low and high cortisol levels were 7.7% and 8.8%, respectively. The inter-assay coefficients of variation were 4.5% and 13.8%, respectively. The criteria for the classification of basal and the type of adrenocortical response to ACTH were adapted from Nakao and Grunert [19, 20]. In their studies, peak adrenocortical response was observed 1 h after ACTH injection. The basal cortisol levels were classified as either normal (<5 ng/ml) or elevated (≥ 5 ng/ml). The type of adrenocortical response to ACTH was defined as: 1) low response if peak cortisol levels were <15 ng/ml, 2) medium response if between 15 and 50 ng/ml, and 3) high response if >50 ng/ml.

Uterine swabs for bacteriological examination were obtained on the second and fourth week postpartum using a double-sheathed apparatus. In collecting uterine swabs, the culturing technique described by Studer and Morrow [21] was followed. The swab, with its plastic sheath, was immediately brought to the laboratory after collection for routine isolation and identification of aerobic and facultative anaerobic bacteria [22].

Ovarian function, with emphasis on the interval from calving to first ovulation, was evaluated through weekly palpation per rectum and ultrasonographic examination, together with three times weekly milk progesterone levels. Milk progesterone concentrations were determined through a double antibody enzyme immunoassay method described by Nakao *et al.* [23]. The sensitivity of this assay method was 10 pg/tube. The average recovery rate was 103.7%. The intra-assay and inter-assay coefficients of variation were 0.8% and 13.2%, respectively. The day of first ovulation was defined as 3 to 4 days prior to the appearance of progesterone levels 1.0 ng/ml or more [24], together with the ultrasonographic observation of corpus luteum formation.

Statistical analysis of the data consisted of Chi-square test for the comparison of the types of basal

cortisol levels and adrenocortical response to ACTH, and ANOVA F-test for the cortisol values and the interval to first ovulation.

Results

Puerperal complications were observed in 21 of 33 cows (63.6%). The following postpartum disorders, in descending order, were observed either alone or in combination: purulent vaginal or cervical discharge (18), retained placenta (10), dystocia (2), milk fever (2), vulvar or vaginal laceration (2), pyrexia (2), and fatty liver disease (1). All cases of metabolic disorders were associated with reproductive abnormalities.

The average basal and peak cortisol levels during the first and third week postpartum in cows with normal and abnormal puerperium are shown in Fig. 1. During the first week postpartum, the basal cortisol levels (mean $\pm$ S.E.) in normal and abnormal cows were 3.1 ± 0.9 ng/ml and 6.5 ± 1.9 ng/ml, respectively. For the third week, the corresponding values were 3.4 ± 1.1 ng/ml and 4.9 ± 1.2 ng/ml, respectively. The cows with abnormal puerperium had elevated and significantly higher

($P < 0.05$) mean basal cortisol level during the first week postpartum. Further evaluation of the basal cortisol levels showed elevated values in 42.9% and 33.3% of abnormal cows, during the first and third week postpartum, respectively. Elevated basal cortisol values were seen in 25% of normal cows during the same periods. The average peak adrenal responses to ACTH were not significantly different between normal and abnormal cows during the first and third week postpartum.

The examination of the types of peak adrenocortical response to ACTH during the first week postpartum revealed low response in 33.3%, medium in 50%, and high in 16.7% of normal cows (Fig. 2). For abnormal cows, the respective values were: 19.1%, 57.1% and 23.8%. During the third week postpartum, the peak adrenal responses were low in 25%, medium in 66.7% and high in 8.3% of normal cows and for abnormal cows the corresponding

Fig. 1. Adrenocortical response to ACTH during the first and third week postpartum in dairy cows with normal and abnormal puerperium.

Fig. 2. Types of peak adrenocortical response to ACTH during the first week postpartum in dairy cows with normal and abnormal puerperium.

values were 14.3%, 57.1% and 28.6% (Fig. 3). There were more abnormal cows with high peak adrenal response during the first and third week postpartum although this was not statistically significant.

Bacteria were isolated from the uterus of 21 (63.6%) of 33 postpartum cows. The bacteria isolated during the two sampling periods from the uterus of normal and abnormal cows are shown in Tables 1 and 2, respectively. On the second week postpartum, 4 of 12 (33.3%) normal and 13 of 21 (61.9%) abnormal cows were found positive for bacterial isolates. While on the fourth week, 4 of 12 (33.3%) normal and 5 of 21 (23.8%) abnormal cows were positive. The three common bacterial isolates from the uterus were *Actinomyces pyogenes*, *Streptococcus* spp. and *Escherichia coli*. It was also noted that the most common bacterial isolate (45%) from cows with purulent vaginal or cervical discharge was *Actinomyces pyogenes*.

Fig. 3. Types of peak adrenocortical response to ACTH during the third week postpartum in dairy cows with normal and abnormal puerperium.

Evaluation of the interval to first ovulation (mean $\pm$ S.E.) showed no significant difference for cows with normal (17.9 ± 1.9 days) and abnormal (18.7 ± 1.6 days) puerperium. At 4 weeks postpartum, uterine involution was found to be normal in all the cows (100%) with normal puerperium, while in cows with abnormal puerperium, 5 cows (23.8%) had delayed uterine involution.

Discussion

In the present study, a greater degree of stress in cows with abnormal puerperium were shown by the elevated and significantly higher mean basal cortisol levels during the first week postpartum. Further classification of the basal cortisol levels into normal and elevated, and the peak adrenocortical responses to ACTH into low, medium and high also showed the tendency for cows with abnormal puerperium to have more cases with high cortisol values during the first and third week postpartum. Most studies on adrenocortical function in postpartum cows showed highest cortisol values at the time of calving and a return to basal values by 0.5 to 3 days postpartum [25–27]. It was sug-

Table 1. Bacteriological findings in uterine swabs of dairy cows with normal puerperium (n=12)

Bacteria	No. of isolates		Total No.	(%)
	2nd wk.	4th wk.		
<i>Streptococcus</i> spp.	1	3	4	36.4
<i>Escherichia coli</i>	2	1	3	27.2
<i>Actinomyces pyogenes</i>	2	0	2	18.2
<i>Pseudomonas aeruginosa</i>	0	1	1	9.1
<i>Bacillus</i> spp.	0	1	1	9.1

Table 2. Bacteriological findings in uterine swabs of dairy cows with abnormal puerperium (n=21)

Bacteria	No. of isolates		Total No.	(%)
	2nd wk.	4th wk.		
<i>Actinomyces pyogenes</i>	7	3	10	45.4
<i>Streptococcus</i> spp.	3	1	4	18.2
<i>Escherichia coli</i>	2	1	3	13.6
<i>Proteus mirabilis</i>	2	0	2	9.1
<i>Enterococcus faecalis</i>	1	0	1	4.5
<i>Micrococcus</i> spp.	1	0	1	4.5
<i>Proteus vulgaris</i>	1	0	1	4.5

gested that except for the surge at calving, plasma concentrations of glucocorticoids did not differ between late gestation and early lactation and that there was no modification in adrenal responsiveness [27]. However, several studies on cows with dystocia reported cortisol concentrations that were higher than the average value during normal parturition [26, 28]. Nakao and Grunert [20] investigated the adrenocortical response in cows with dystocia and found that on day 1 postpartum, those that required caesarean section after unsuccessful extraction or total fetotomy had higher basal and ACTH-stimulated plasma cortisol concentrations than those in cows with normal parturition.

Several researchers reported that from 85% to 96% of the bovine uteri contained bacterial flora in the early postpartum period [5, 6, 17]. The lower occurrence found in the present study is closer to that reported by Fredriksson *et al.* [9] who found 49% of the cows positive at about 2 weeks postpartum. Fredriksson and his group had attributed the differences in incidence to various factors such as breed, environment, management procedures and sampling techniques.

It was also observed in the present study that during the second week postpartum, cows with abnormal puerperium had higher incidence of uterine bacterial infection than those with normal puerperium. This agrees with the observation that cows with postpartum disorders have a greater tendency for bacterial infection [10, 29]. On the fourth week, a decreased incidence of uterine bacterial infection was observed in cows with abnormal puerperium. It was noted that about a third of the cows with normal puerperium had bacterial flora during the second and fourth week postpartum. Although these cows did not have clinical signs of uterine infection, it could indicate the proportion of normal cows that would be susceptible to uterine bacterial contamination.

It is generally considered that the bacterial flora of the postpartum cow uterus follow a predictable transient pattern [10] and that the highest rate of bacterial isolation appears at 2 weeks followed by a gradual decline to a constant rate by 7 weeks [5, 8].

The three common bacterial organisms isolated in the present study were similar with those reported by Studer and Morrow [7] and Torres *et al.*

[12]. *Actinomyces pyogenes* was also reported by Noakes *et al.* [11] as the most common bacterial isolate from postpartum cows. The possible role of *A. pyogenes* infection in cases of postpartum endometritis [6, 7, 30] and the pathogenic synergism with gram negative anaerobes in severe cases of postpartum uterine infections had been suggested [5, 9, 11, 13].

The effects of stress and the occurrence of uterine bacterial infection were most apparent during the early postpartum period. This could indicate that the increased susceptibility of the uterus to bacterial infection occur during the time when postpartum cows are under a greater degree of stress.

It has already been reported elsewhere [9, 29] that postpartum complications and uterine bacterial infections delay the involution of the uterus. Similar findings were observed in this study. Some authors had reported a delayed onset of ovarian activity in cows with endometritis when compared with healthy cows [31, 32], however, a similar study by Dohmen *et al.* [13] found no relationship between bacterial findings and ovarian activity. The present findings tend to support the observations made by the group of Dohmen. It should be recognized that ovarian activity and uterine involution are reported to be influenced by various factors. It may be necessary to evaluate these factors and their intricate relationships as was done by Zain *et al.* [33].

In conclusion, cows with abnormal puerperium had significantly higher basal plasma cortisol levels during the first week postpartum and higher incidence of uterine bacterial infection during the second week than normal cows. Onset of ovarian activity was apparently affected neither by puerperal disorders nor uterine bacterial infection, whereas uterine involution at 4 weeks postpartum was delayed in about a fourth of abnormal cows.

Acknowledgment

E. B. Torres was supported by a research fellowship from the Japan Society for the Promotion of Science. The authors wish to thank the staff of the dairy farms of the Rakuno Gakuen University for their help and cooperation in carrying out this experiment.

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