Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle

I. M. Sheldon¹, D. E. Noakes¹, A. N. Rycroft², D. U. Pfeiffer¹ and H. Dobson³

¹Department of Veterinary Clinical Science and ²Department of Pathology and Infectious Diseases, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield AL9 7TA, UK; and ³Department of Veterinary Clinical Science and Animal Husbandry, University of Liverpool, Leahurst, Chester High Road, Neston CH64 7TE, UK

First postpartum dominant follicles are preferentially selected in the ovary contralateral to the previously gravid uterine horn. The aim of the present study was to test the hypothesis that uterine bacterial contamination alters the location of ovarian follicle emergence and selection, and inhibits follicle growth and function. Swabs were collected from the uterine body lumen of cattle on days 7, 14, 21 and 28 after parturition. Bacteria were identified by aerobic and anaerobic culture; bacterial growth was scored semi-quantitatively and animals were categorized into standard or high bacterial contamination categories on the basis of the number of colonies detected. Follicular growth and function were monitored by daily transrectal ultrasonography, and estimation of plasma FSH, oestradiol and progesterone concentrations. There was no effect of bacterial contamination on plasma FSH concentration profiles or emergence of the ovarian follicle wave. When uterine bacterial growth scores were high on day 7 or day 21 after parturition, fewer first (1/20 versus 15/50; \( P < 0.05 \)) or second (1/11 versus 13/32; \( P < 0.05 \)) dominant follicles were selected in the ipsilateral compared with the contralateral ovary, respectively. The diameter of the first dominant follicle was smaller in animals with a high day 7 bacterial score (\( P < 0.001 \)), dominant follicle growth was slower (\( P < 0.05 \)) and oestradiol secretion was decreased (\( P < 0.05 \)). The present study provides evidence for an effect of the uterus on the ovary after parturition, whereby uterine bacteria have a contemporaneous localized effect on ovarian follicle selection and subsequent growth and function, but not on initial emergence.

© 2002 Society for Reproduction and Fertility

Introduction

After parturition, an increase in plasma FSH concentration is followed by the emergence of a wave of several follicles 4–6 mm in diameter and subsequent selection of a single dominant follicle (Beam and Butler, 1997; Crowe et al., 1998). Sequential transrectal ultrasonography has revealed that the first postpartum dominant follicle is preferentially selected in the ovary contralateral to the previously gravid uterine horn (Kamimura et al., 1993; Sheldon et al., 2002). This is important because the presence of a large follicle in the ovary ipsilateral to the previously gravid uterine horn within 4 weeks of parturition, although less frequent, is associated with improved fertility (Bonnett et al., 1993; Sheldon et al., 2000).

Suppression of folliculogenesis in the ipsilateral ovary decreases as the postpartum interval advances, concurrent with the disappearance of the corpus luteum of pregnancy, uterine involution and elimination of the ubiquitous uterine bacterial contamination after parturition (Elliot et al., 1968; Sawyer, 1995; Sheldon et al., 2000). Suppression of folliculogenesis in the ipsilateral ovary could be explained by an inhibitory local effect of the regressing corpus luteum of pregnancy or the previously gravid uterine horn or its contents (Dufour and Roy, 1985). However, the removal of the corpus luteum of pregnancy by administration of PGF₂α before parturition did not influence the growth, function or ovarian location of the first postpartum dominant follicle (Sheldon et al., 2002). In addition, the diameter of the uterine horn did not influence the ovarian location of the first postpartum dominant follicle (I. M. Sheldon and H. Dobson, unpublished).

Although the previously gravid uterine horn was not identified, in one study ovarian follicles were smaller after parturition in cattle with a uterine bacterial infection (Peter and Bosu, 1988). Furthermore, inflammatory mediators such as bacterial endotoxin and immune mediators such as cytokines disturb the hormonal interactions that control normal cyclical ovarian function (Rivest et al., 1993; Battaglia et al., 1999; Williams et al., 2001). Intracerebral or intravenous administration of inflammatory mediators disrupts GnRH release from the hypothalamus and LH secretion from the pituitary. However, a surprising inhibition of...
folicular oestradiol secretion after administration of endotoxin, in the face of adequate plasma LH concentrations, led to the suggestion of an additional effect at the ovary (Xiao et al., 1998; Battaglia et al., 2000). Despite these observations, few human or animal studies have focused on the impact of inflammatory mediators on the ovary, particularly under normal pathophysiological situations.

The aim of the present study was to test the hypothesis that postpartum uterine bacterial contamination alters the location of ovarian follicle emergence and selection, and inhibits growth and function of the dominant follicle.

Materials and Methods

Animals

All procedures were performed under the Animals (Scientific Procedures) Act 1986 regulations for experiments on living animals, administered by the UK Home Office. In addition, experimental protocols were approved by the Royal Veterinary College Ethical Review Committee.

A dairy herd of 90 Holstein–Friesian cows, with an annual average milk yield of 6800 litres and a rolling herd mean somatic cell count of $8.4 \times 10^4$ cells ml$^{-1}$, was selected for the study on the basis of accurate farm records. All of the cows had been mated with Holstein–Friesian bulls and were pregnant. Animals with a history of Caesarean operation or the presence of vaginal lacerations, acute mastitis, lameness, abdominal disorders or other intercurrent disease were excluded from the study on the basis of daily clinical examination, to remove any confounding influence of non-uterine bacterial infection during the study period (Skinner et al., 1991; Scott et al., 1992; Horadagoda et al., 1999). Seventy Holstein–Friesian cows were included in the study during a 1 year period. No antimicrobial treatments were administered during the study.

Clinical examination

The genital tract of each cow was examined daily from day 7 to day 28 after parturition using transrectal palpation and ultrasonography with a 7.5 MHz linear array transducer (Aloka SSD 210 DXII; BCF Technology, Livingstone). The previously gravid uterine horn was identified as being longer and of greater diameter than the contralateral horn. Follicles were defined as non-echogenic (black) spherical structures with a clear demarcation between the follicular wall and antrum. A corpus luteum was defined as a grainy echogenic structure that had a well-defined border with the less echogenic ovarian stroma; in some corpora lutea there was a non-echogenic lacuna. The numbers of ovarian follicles $\geq 4$ mm in diameter and corpora lutea in each ovary were counted, and each maximum diameter was measured using the instrument’s internal callipers. When the image of the structure being scanned was not spherical, the diameter was estimated by averaging two 90° dimensions.

A dominant follicle was defined as the largest follicle in the ovary with internal diameter $> 10$ mm in the absence of other growing follicles (Dobson et al., 2000). A dominant follicle and cohorts were defined as a follicular wave (Dobson et al., 2000). The first day of dominance within a follicular wave was determined retrospectively as the day on which the dominant follicle initially exceeded 10 mm in diameter. The number of follicles in a wave $\geq 4$ mm or $> 10$ mm in diameter was based on emergence of the follicles at the same or consecutive examinations (Ginther et al., 1996). The day of ovulation was defined as the day on which the dominant follicle was last scanned before its sudden disappearance and the subsequent appearance of a corpus luteum in the same location. Luteinization was confirmed retrospectively by a subsequent increase in plasma progesterone concentration to $> 1$ ng ml$^{-1}$. A persistent follicle (follicular cyst) was defined as a follicle with internal diameter $> 10$ mm that persisted for $> 5$ consecutive days and did not ovulate (Dobson et al., 2000).

Uterine swab collection and bacteriology

A transcervical guarded swab was collected from the uterine body of each cow on days 7, 14, 21 and 28 after parturition using a previously validated method (Noakes et al., 1989). The swab was transferred to a bijou bottle containing Stuart transport medium (Unipath, Basingstoke) and was cultured within 1 h of collection at the on-site bacteriology laboratory. Swabs were cultured aerobically and anaerobically on pre-equilibrated sheep blood agar (Unipath), and aerobically on MacConkey agar (Unipath). Identification of bacteria was based on the characteristics of the colony, Gram stain, morphology, haemolysis, biochemical profile (API systems, BioMérieux, Basingstoke) and other standard tests (Barrow and Feltham, 1993). Bacterial growth on the culture plates was scored semiquantitatively, dependent on the number of bacterial colonies detected on the plate: 0: no growth; 1: $< 10$ colonies; 2: 10–100 colonies; 3: 100–500 colonies; and 4: $> 500$ colonies (Noakes et al., 1991). The bacterial growth score on days 7, 14, 21 and 28 was the sum of the scores for each of the bacterial isolates, and the total bacterial growth score for each cow was the sum of the individual bacterial growth scores for all four uterine swabs. The uterine bacterial scores at each time point were used to categorize cows into categories of standard or high bacterial contamination on days 7, 14, 21 and 28. The standard category was defined as the lower 75% quartile bacterial score and the high contamination category as the upper 25%. In addition, bacteria were categorized on the basis of expected pathogenic potential within the uterus (Ruder et al., 1981; Olson et al., 1984; Farin et al., 1989; Noakes et al., 1989, 1991; Bonnett et al., 1993). The categories were: (1) pathogens known to cause endometrial lesions; (2) other recognized uterine pathogens; and (3) bacteria not recognized as uterine pathogens (see Table 1). These bacterial pathogenicity categories were used to group cows.
for the presence of the most pathogenic bacteria on days 7, 14, 21 or 28 for statistical analysis, with a fourth group in which no bacteria were isolated (Laven et al., 2000).

**Blood sampling and hormone assays**

Blood samples were collected once a day from day 7 to day 21 after parturition, from the coccygeal vein or artery into evacuated heparinized tubes (BD Vacutainer Systems, Plymouth) and transported on ice to the laboratory. Within 30 min, plasma was separated by centrifugation at 2200 g for 10 min, harvested and stored at –20°C.

Oestradiol concentration was estimated in duplicate by a previously characterized radioimmunoassay (Estradiol MAIA; Serono Diagnostics Ltd, Woking) using diethyl ether-extracted plasma (Mann et al., 1995). The mean intra-(n = 12 samples) and interassay (n = 3 assays) coefficients of variation were 8.1 and 13.1%, respectively, for a 0.9 pg ml–1 sample, and the minimum detectable quantity was 0.24 pg ml–1. Progesterone concentration was estimated in duplicate using a commercial ELISA kit (Ridgeway Science, Gloucester). The intra- (n = 10 samples) and interassay (n = 3 assays) coefficients of variation were 6.5 and 11.2%, respectively, for a 1.7 ng ml–1 sample, and the minimum detectable quantity was 0.6 ng ml–1. FSH concentration was estimated in duplicate by a previously characterized radioimmunoassay (Dobson et al., 2000). The standard used for the FSH assay was AFP 5679C RP-1. The intra- (n = 20 samples) and interassay (n = 3 assays) coefficients of variation were 3.4 and 4.7%, respectively, for a 1.2 ng ml–1 sample, and the minimum detectable quantity was 0.12 ng ml–1.

**Statistical analysis**

Data analysis was performed using SAS version 8.01 computer program (SAS Institute Inc., Cary, NC). Results are quoted as arithmetic mean ± SEM, and the level of significance was P < 0.05.

The locations of ovarian structures (first dominant follicle, first ovulation or second dominant follicle) in relation to the previously gravid uterine horn were compared using chi-squared analysis or Fisher's exact test if cells contained fewer than five observations. Logistic regression was used to examine the effect of standard or high uterine bacterial contamination, or the pathogen group, on the location of ovarian structures, and to obtain odds ratios.

Survival analysis, using Cox regression models, was used to compare time intervals from calving to appearance of the first dominant follicle, the first dominant ovulatory follicle and to ovulation, between bacterial categories, between the ovarian location of the structures or between different bacterial pathogen groups.

The numbers of follicles were compared using Mann–Whitney or Kruskall–Wallis non-parametric tests for two or more variables, respectively. Follicle diameter, plasma oestradiol and FSH concentrations were compared by repeated measurements ANOVA using a mixed model (SAS, 1997). Data from day 7 after parturition, the start of the study, to day 16, the mean day of ovulation for the first dominant follicle, were included in the analysis. Data were examined for normality using the Kolmogorov–Smirnoff test and for equality of variance using the Levene’s test. Where appropriate, data were log10- or square root-transformed to yield variance homogeneity. The explanatory variables were bacterial contamination category on days 7, 14, 21 and 28, pathogen group, location of the first dominant follicle, fate of the first dominant follicle and their interactions with time after parturition. A compound symmetry model best fitted the data, as determined using Akaike’s information criterion. Post hoc tests were performed using Bonferonni’s adjustment.

---

**Table 1. Categorization of bacteria, isolated by aerobic and anaerobic culture of uterine swabs from cattle, based on their potential pathogenicity**

<table>
<thead>
<tr>
<th>Bacterial category</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcanobacterium pyogenes</td>
<td>Acinetobacter spp</td>
<td>Aerococcus viridans</td>
<td></td>
</tr>
<tr>
<td>Prevotella spp</td>
<td>Bacillus licheniformis</td>
<td>Clostridium butyricum</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Enterococcus faecalis</td>
<td>Clostridium perfringens</td>
<td></td>
</tr>
<tr>
<td>Fusobacterium necrophorum</td>
<td>Haemophilus somnus</td>
<td>Corynebacterium spp</td>
<td></td>
</tr>
<tr>
<td>Fusobacterium nucleatum</td>
<td>Mannheimia haemolytica</td>
<td>Enterobacter aerogenes</td>
<td></td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>Peptostreptococcus spp</td>
<td>Klebsiella pneumoniae</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus (coagulase +)</td>
<td>Staphylococcus aureus (coagulase –)</td>
<td>Micrococcus spp</td>
<td></td>
</tr>
<tr>
<td>Streptococcus uberis</td>
<td>Proteus spp</td>
<td>Providencia rettgeri</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Providencia stuartii</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proteus spp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Propionibacterium granulosa</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staphylococcus spp (coagulase –)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>α-Haemolytic Streptococi</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Streptococcus acidominimus</td>
<td></td>
</tr>
</tbody>
</table>
high bacterial contamination categories were identical. However, 28 animals in the standard and seven in the score categories on day 7, 14, 21 or 28 were not always the individual animals comprising the standard or high bacterial score categories on days 7, 14, 21 and 28, respectively. The 75% quartile cut-off point to categorize cows into standard or high bacterial score groups for uterine swabs collected was score 6 on day 7, score 6 on day 14, score 4 on day 21 and score 3 on day 28. The mean bacterial score of the standard categories on days 7, 14, 21 and 28, respectively. The mean ± SEM bacterial growth score for each bacterial pathogenicity group is shown (Fig. 1) for uterine swabs collected on day 7, 14, 21 or 28. 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 75% quartile cut-off point to categorize cows into standard or high bacterial score groups for uterine swabs collected was score 6 on day 7, score 6 on day 14, score 4 on day 21 and score 3 on day 28. The mean bacterial score of the standard categories on days 7, 14, 21 and 28 was 2.2 ± 0.2, 2.7 ± 0.2, 1.6 ± 0.2 and 1.2 ± 0.1, respectively, and 8.4 ± 0.6, 7.7 ± 0.4, 5.5 ± 0.4 and 4.2 ± 0.2, respectively, for the high categories. The individual animals comprising the standard or high bacterial score categories on day 7, 14, 21 or 28 were not always the same. However, 28 animals in the standard and seven in the high bacterial contamination categories were identical across the four periods.

**Results**

Bacteriology

Bacteria were isolated from uterine swabs in each animal at least once during the study. More than 30 different bacteria were identified and categorized by their potential pathogenicity (Table 1). No bacteria were isolated from uterine swabs collected from 5, 4, 9 and 13 animals on days 7, 14, 21 and 28, respectively. The mean ± SEM bacterial growth score for each bacterial pathogenicity group is shown (Fig. 1) for uterine swabs collected on day 7, 14, 21 or 28. 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 75% quartile cut-off point to categorize cows into standard or high bacterial score groups for uterine swabs collected was score 6 on day 7, score 6 on day 14, score 4 on day 21 and score 3 on day 28. The mean bacterial score of the standard categories on days 7, 14, 21 and 28 was 2.2 ± 0.2, 2.7 ± 0.2, 1.6 ± 0.2 and 1.2 ± 0.1, respectively, and 8.4 ± 0.6, 7.7 ± 0.4, 5.5 ± 0.4 and 4.2 ± 0.2, respectively, for the high categories. The individual animals comprising the standard or high bacterial score categories on day 7, 14, 21 or 28 were not always the same. However, 28 animals in the standard and seven in the high bacterial contamination categories were identical across the four periods.

Location of events

A wave of follicular development, with the emergence of a dominant follicle, was observed in all cows within 14 days of parturition. The location of ovarian events, but not their timing, differed significantly between the ipsilateral and contralateral ovaries (Table 2). However, the number of follicles ≥ 4 mm in diameter in the first postpartum follicular wave did not differ significantly between high or standard day 7 bacterial score categories in the ipsilateral ovary (1.88 ± 0.18 versus 1.40 ± 0.22, respectively) or the contralateral ovary (2.86 ± 0.19 versus 2.95 ± 0.17, respectively).

Logistic regression for the location of the first postpartum dominant follicle indicated that the standard or high bacterial category was significant on day 7 (P < 0.05), but not on days 14, 21 or 28. The high day 7 bacterial score category was 14.4 (odds ratio) times less likely to have a first dominant follicle in the ipsilateral ovary compared with the standard category (Fig. 2a). However, the location of the first dominant follicle did not differ significantly between animals in different bacterial pathogenicity groups on day 7 after parturition.

Ultrasoundography revealed three possible fates for the first dominant follicle: ovulation (n = 48); regression followed by a second follicular wave (n = 10); or formation of a persistent follicle (n = 12). The frequency of the first dominant follicles that ovulated, regressed or persisted did not differ significantly between the standard and high bacterial categories on day 7 (37, 6 and 10 versus 11, 4 and 2, respectively), day 14 (33, 6 and 7 versus 15, 4 and 5, respectively), day 21 (36, 6 and 11 versus 12, 4 and 1, respectively) or day 28 (40, 6 and 11 versus 8, 4 and 1, respectively).

A second follicular wave was detected in 43 animals. The number of follicles ≥ 4 mm in diameter in the second postpartum follicular wave did not differ significantly between high or standard day 21 bacterial score categories in the ipsilateral ovary (2.11 ± 0.54 versus 2.34 ± 0.21) or in the contralateral ovary (2.89 ± 0.45 versus 2.44 ± 0.28). Logistic regression for the location of the second postpartum dominant follicle indicated that the bacterial category was significant on day 21 (P < 0.05), but not on days 7, 14 or 28. The high day 21 bacterial score category was 6.0 (odds ratio) times less likely to have a first dominant follicle in the ipsilateral ovary compared with the standard category (Fig. 2b). However, the location of the second dominant follicle did not differ significantly between uterine pathogenicity groups.

Timing of events

The mean interval between calving and the first dominant follicle achieving dominance was 9.2 ± 0.3 days. The calving to follicular dominance interval was similar for the day 7 high bacterial score category and the standard bacterial score cows (10.3 ± 0.6 versus 8.8 ± 0.3 days, respectively), and for the different bacterial pathogenicity groups. For animals in which the first dominant follicle ovulated, the mean interval from calving to ovulation was 15.7 ± 0.7 days. There was no significant difference in the calving to ovulation interval between cows with high or standard bacterial scores on day 7 (16.4 ± 1.4 versus 15.4 ± 0.8 days, respectively) or on day 14 (17.3 ± 1.6 versus 15.2 ± 0.8 days, respectively). An increase in plasma progesterone concentration to > 1 ng ml–1 was detected 3.2 ± 0.2 days after ovulation and did not differ significantly between bacterial score categories on days 7, 14 or 21.
The mean internal diameter of first dominant follicles increased each day between day 7 and day 16 after parturition \((P < 0.0001)\). However, uterine bacterial score, ovarian location and the fate of the dominant follicle significantly influenced follicle diameter. The diameter of the first dominant follicle was smaller in animals with a high day 7 bacterial score compared with standard score cows \((P < 0.001)\) and there was a significant interaction of bacterial score category with time \((P < 0.05, \text{Fig. 3})\). However, the influence of the bacterial pathogenicity group was not significant. The diameter of the first dominant follicle differed between follicles located in the ipsilateral ovary and follicles located in the contralateral ovary \((P < 0.05)\), and the interaction of location with time was also significant \((P < 0.05, \text{Fig. 4})\). There was no difference in follicle diameter between follicles that persisted and those that ovulated or regressed. However, between day 9 and day 13 after parturition, the first dominant follicle was smaller before regression compared with follicles that ovulated \((P < 0.05, \text{Fig. 5})\). The interaction of the fate of the dominant follicle and time was not significant.

**Plasma oestradiol concentration**

Plasma oestradiol concentration increased between day 7 and day 16 after parturition \((P < 0.0001)\). In addition, there were significant interactions of day \(\times\) day 7 bacterial category \((P < 0.05, \text{Fig. 3})\), and day \(\times\) fate of the first dominant follicle \((P < 0.05, \text{Fig. 5})\). There was no significant effect of the bacterial pathogen group or the location of the first dominant follicle \((P > 0.05, \text{Fig. 4})\). On days 15 and 16, plasma oestradiol concentrations were lower in animals with a high day 7 bacterial score \((\text{Fig. 3})\). Oestradiol concentration was also lower between day 12 and day 15 in animals in which the first dominant follicle regressed compared with animals in which the follicle ovulated or persisted \((\text{Fig. 5})\).

**Plasma FSH concentration**

Plasma FSH concentration between day 7 and day 16 after parturition differed significantly between days after parturition \((P < 0.0001)\). However, there were significant interactions of day \(\times\) day 7 bacterial category \((P < 0.05, \text{Fig. 3})\), and day \(\times\) fate of the first dominant follicle \((P < 0.05, \text{Fig. 5})\). There was no significant effect of the bacterial pathogen group or the location of the first dominant follicle \((P > 0.05, \text{Fig. 4})\). On days 15 and 16, plasma FSH concentrations were lower in animals with a high day 7 bacterial score \((\text{Fig. 3})\). FSH concentration was also lower between day 12 and day 15 in animals in which the first dominant follicle regressed compared with animals in which the follicle ovulated or persisted \((\text{Fig. 5})\).
parturition ($P < 0.05$, Figs 3, 4 and 5). FSH concentration was highest on day 7, decreased between day 8 and day 12, and subsequently increased between day 13 and day 16. Additional significant variables were the fate of the first dominant follicle ($P < 0.01$, Fig. 5) and the interaction of day $\times$ fate of the dominant follicle ($P < 0.01$). However, plasma FSH concentration did not differ significantly between animals with standard or high bacterial scores on day 7 (Fig. 3), between bacterial pathogenicity groups, or between animals in which the first dominant follicle was located in the ipsilateral or contralateral ovary (Fig. 4).

![Graph](http://example.com/graph1.png)

Fig. 3. (a) Diameter of first dominant follicle, (b) plasma oestradiol concentration and (c) plasma FSH concentration between day 7 and day 16 after parturition for cows in which there was standard (○, $n = 50$) or high (■, $n = 20$) uterine bacterial contamination on day 7. Values are mean ± SEM. Within a day, values differ between bacterial contamination categories: $^aP < 0.05$; $^bP < 0.01$; $^dP < 0.001$.

![Graph](http://example.com/graph2.png)

Fig. 4. (a) Diameter of first dominant follicle, (b) plasma oestradiol concentration and (c) plasma FSH concentration between day 7 and day 16 after parturition for cows in which the dominant follicle was located in the ipsilateral (■, $n = 16$) or contralateral (○, $n = 54$) ovary. Values are mean ± SEM. $^b$Within a day, values differ between ovary groups ($P < 0.05$).
Discussion

The present study provides evidence that after parturition uterine bacterial contamination has a contemporaneous effect on ovarian folliculogenesis. Spontaneous uterine bacterial contamination influenced the location of ovarian dominant follicle selection, but not follicle wave emergence. In addition, uterine bacterial contamination suppressed dominant follicle growth and function.

A postpartum transient increase in plasma FSH concentration preceded the emergence of the first postpartum follicular wave and subsequent selection of a dominant follicle during the following phase of decreasing FSH concentrations (Beam and Butler, 1997; Crowe et al., 1998). Similarly, in the present study, after parturition a wave of follicles ≥ 4 mm in diameter emerged and a first dominant follicle was selected in each animal irrespective of the severity of uterine bacterial contamination. Plasma FSH concentrations did not differ between standard or high bacterial score categories, and there was no effect on the number of follicles ≥ 4 mm in diameter that emerged in each ovary. However, similar to previous observations, fewer follicles ≥ 4 mm in diameter emerged and fewer first dominant follicles were selected in the ovary ipsilateral to the previously gravid uterine horn (Kamimura et al., 1993; Sheldon et al., 2002). During the second follicular wave after parturition, there was no difference between the ovaries for the number of follicles ≥ 4 mm in diameter emerging, although fewer second dominant follicles were selected in the ipsilateral ovary. These observations support our previous findings that the effect on folliculogenesis in the ipsilateral ovary decreases with increasing time after parturition, in parallel with uterine involution and disappearance of the corpus luteum of pregnancy (Sawyer, 1995; Sheldon et al., 2000). However, removal of the corpus luteum of pregnancy before parturition did not affect the asymmetric distribution of dominant follicles between the ovaries (Sheldon et al., 2002). Thus, it is possible that uterine bacterial contamination after parturition may determine the location of dominant follicle selection.

In the present study, when uterine bacterial growth scores were high on day 7 or day 21, few first or second dominant follicles were selected in the ipsilateral ovary, respectively. These observations indicate that uterine bacterial contamination may have a localized effect preventing dominant follicle selection in the ipsilateral ovary. Similarly, cows with retained fetal membranes, which are likely to be associated with increased uterine bacterial contamination, had less follicular activity in the ipsilateral ovary, although the presence of dominant follicles was not identified (Risco et al., 1994). The effect of uterine bacterial contamination appeared to be short term. The uterine bacterial scores on days 7 and 21 were contemporaneous with the time of first and second dominant follicle selection on about days 9 and 20, respectively. Bacterial scores at other times were not significant. Furthermore, the individual animals comprising the high score categories on days 7 and 21 were not exactly the same.

There are two potential mechanisms by which uterine bacterial contamination could have a localized effect on ovarian folliculogenesis. Firstly, bacterial load or the induced inflammatory response may differ between the two uterine horns. Alternatively, the concentration of inflammatory mediators reaching the ipsilateral ovary may be higher than the concentration reaching the contralateral ovary because
of the greater blood flow to and from the gravid uterine horn (Ford et al., 1979). Most animals had bacterial contamination of the uterus within 2 weeks of parturition, so it was not possible to determine whether the inflammatory response was the sole mechanism for the disparity in the pattern of folliculogenesis between the two ovaries.

The precise mechanism by which inflammatory mediators could influence ovarian events has not been determined. However, intravenous or intracerebral infusion of endotoxin or the cytokine interleukin 1 (IL-1) disrupts the follicular phase of the oestrous cycle in several species (Peter et al., 1989; Rivest et al., 1993; Battaglia et al., 1999). A contemporaneous effect of inflammatory mediators was to be expected, as there is acute inhibition of hypothalamic GnRH release and pituitary LH secretion (Rivest et al., 1993; Williams et al., 2001). However, these neuroendocrine mechanisms do not fully explain the observations of a localized effect on the ipsilateral ovary in the present study, although evidence has been provided for a direct effect of inflammatory mediators at the ovary by suppression of oestriadiol secretion in the presence of adequate plasma LH concentrations (Xiao et al., 1998; Battaglia et al., 2000).

In addition to the effect of the high day 7 uterine bacterial score on dominant follicle location, follicles grew more slowly and produced lower plasma oestriadiol concentrations. A significant difference in plasma oestriadiol concentration between dominant follicles in the standard or high day 7 bacterial score categories was not detected until days 15–16, when the difference in follicle diameter was maximal. The effect of uterine bacterial contamination on follicle growth and function could be a centralized effect mediated by disruption of LH secretion, or a direct effect on the ovary (Battaglia et al., 2000). Decreased plasma LH concentration reduced the growth rate and oestriadiol secretion of dominant follicles after follicle selection (Ginther et al., 2001). However, in the present study, the effect on follicle growth rate was evident at a diameter of < 8.5 mm, the diameter for deviation between dominant and subordinate follicles (Ginther et al., 1999). Furthermore, although inflammatory challenge disrupts ovulation by perturbing the LH surge, the proportion of dominant follicles ovulating was not affected by spontaneous uterine bacterial contamination in the present study.

Although there were small differences in diameter between dominant follicles in the ipsilateral and contralateral ovaries before dominance, subsequent follicle diameters were similar. In addition, follicle function as determined by plasma oestriadiol concentrations was similar for both ovaries, and the interval from parturition to ovulation was similar. These observations indicate that dominant follicles in the ipsilateral ovary were probably at least as functionally competent as follicles in the contralateral ovary.

Important uterine pathogenic bacteria are associated with more severe clinical disease, increased endometrial inflammation and reduced fertility (Farin et al., 1989; Bonnett et al., 1993). In particular, Arcanobacterium pyogenes, Fusobacterium necrophorum and Prevotella spp act synergistically to cause more severe clinical signs (Ruder et al., 1981; Olson et al., 1984). In the present study, although there was an effect of bacterial load on folliculogenesis, the importance of uterine bacterial pathogens was not significant. This finding indicates that the inflammatory response to the amount of bacterial contamination is probably more important than the presence of particular bacterial species.

In conclusion, there was no effect of uterine bacterial contamination on plasma FSH concentration and ovarian follicle wave emergence. However, spontaneous bacterial contamination of the postpartum uterus influenced the ovarian location for dominant follicle selection. Contemporaneous high bacterial contamination was associated with fewer first and second dominant follicles in the ovary ipsilateral to the previously gravid uterine horn. In addition, dominant follicle growth was slower and oestriadiol secretion was reduced in the presence of high bacterial contamination. The results of the present study provide evidence for a localized effect of uterine bacterial contamination on the ovary after parturition.

This study was supported by the Royal College of Veterinary Surgeons (Wilson) Scholarship in Production Animal Medicine, and a Royal Veterinary College Internal Grant to I.M.S. The authors thank Professor Mac Johnston for access to the dairy herd, and Angelika Von Heimendahl, Soujun Li, and Mark Whalley for assistance with sample collection. They also thank Vicky Harrison, Hilary Purcell and Jean Routley for technical assistance with hormone assays, and Maggie Bushnell for bacteriology. They acknowledge NIAMDD, Bethesda, MD for FSH assay reagents.

References


Crowe MA, Padmanabhan V, Mihn M, Beitins IZ and Roche JF (1998) Resumption of follicular waves in beef cows is not associated with periparturient changes in follicle-stimulating hormone heterogeneity despite major changes in steroid and luteinizing hormone concentrations. Biology of Reproduction 58:1445–1450


Dufour JJ and Roy GL (1985) Distribution of ovarian follicular populations...
in the dairy cow within 35 days after parturition Journal of Reproduction and Fertility 73 229–235
Elliot L, McMahon KJ, Gier HT and Marion GB (1968) Uterus of the cow after parturition: bacterial content American Journal of Veterinary Research 29 77–81
Farin PW, Ball L, Olson JD, Mortimer RG, Jones RL, Adney WS and McChesney AE (1989) Effect of Actinomyces pyogenes and gram-negative bacteria on the development of bovine pyometra Theriogenology 31 979–989
Noakes DE, Wallace L and Smith GR (1991) Bacterial flora of the uterus of cows after calving on two hygienically contrasting farms Veterinary Record 120 440–442
Peter AT and Bosu WTK (1988) Relationship of uterine infections and folliculogenesis in dairy cows during early puerperium Theriogenology 30 1045–1051
Peter AT, Bosu WTK and DeDecker RJ (1989) Suppression of preovulatory luteinizing hormone surges in heifers after intrauterine infusions of Escherichia coli endotoxin American Journal of Veterinary Research 50 368–373
Skinner JG, Brown RA and Roberts L (1991) Bovine haptoglobin response in clinically defined field conditions Veterinary Record 128 147–149

Received 28 November 2001.
First decision 6 February 2002.
Accepted 4 March 2002.