

Effects of sub-clinical endometritis on ovarian follicular activity in postpartum dairy cows

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ABSTRACT

The effects of sub-clinical endometritis (scEndo) on ovarian follicular activity in pasture-fed dairy cows were determined. The uterine-health status of lactating dairy cows ($n = 169$) was assessed on day (D) 21 (± 3 days) postpartum by Metricheck. In a sub-population ($n = 47$; 32 clean, 15 scEndo), Metricheck assessment was repeated on D42 and uterine cytology undertaken on D21 and D42 [scEndo = polymorphonuclear (PMN) cells $>18\%$]. Follicular activity was assessed by ultrasound on D42 and D63. Metricheck evaluation revealed that the proportion of cows with scEndo was 15/169 (8.9%) and 4/47 (8.5%) on D21 and D42 respectively. Irrespective of the method used to determine scEndo, dominant ($P < 0.001$) but not subordinate ($P > 0.1$) follicles were larger and grew faster on D63 than on D42. Based on the D21 Metricheck but not the %PMN classification, dominant follicles of scEndo cows were larger and grew faster ($P = 0.02$) than those in clean cows. No overall effect of uterine status was found on the subordinate follicles, although these tended ($P = 0.06$) to be larger on D63 than on D42 but only in clean cows. Thus early postpartum scEndo appears to modulate the subsequent size of the dominant follicle. However, it remains to be determined whether this affects the subsequent fertility of these cows.

Keywords: follicular size; endometritis; cytology; bacteriology; dairy cows.

INTRODUCTION

The financial losses associated with uterine infection are dependent on cost of treatment, loss of milk yield and infertility (Sheldon *et al.*, 2008). Parkinson *et al.* (2007) showed a 19% lower three week submission rate and first service conception rate, and a 15% lower final pregnancy rate in the New Zealand dairy industry. For the average sized New Zealand dairy herd of 351 cows (LIC, 2008), a 10% improvement in conception rate would increase farm operating profit by NZ\$16,513. This is based on a payout of NZ\$4.50/kg milk solids and a net loss of \$500/non-pregnant cow (Burke, 2007). This additional operating profit represents an additional NZ\$192 million/year across the national dairy herd. For a European producer this cost has been estimated to be €192/cow or €1059/100 cows, equating to a direct industry cost of more than €206 million/year (Sheldon *et al.*, 2008).

Clinical and sub-clinical intra-uterine infections are associated with sub-fertility and infertility in dairy cows. This is evident by longer intervals from calving to first insemination or conception for infected animals, with more cows culled for failure to conceive within a required time frame (McDougall, 2001; LeBlanc 2002; Kasimanickam *et al.*, 2004). Cows exhibiting signs of clinical infection have lower 28 day submission rates and

lower 28 day pregnancy rates, with more culls for failure to conceive (26.3 vs 6.9%, McDougall, 2001). Few studies however have examined the effect of sub-clinical endometritis (scEndo) on fertility, although Kasimanickam *et al.* (2004) identified scEndo cows had an increased interval between parturition and pregnancy and lower conception rates.

An immune/inflammatory challenge such as that associated with a uterine bacterial infection impairs reproductive neuroendocrine function and disrupts ovarian cyclicity at multiple levels. At the level of the hypothalamus and pituitary, the oestradiol-induced pre-ovulatory luteinizing hormone surge is suppressed when bacterial endotoxin is infused into the uterus (Peter *et al.*, 1989). This is due to bacterial endotoxin or immune mediators such as the cytokines interleukin (IL)-1 or tumour necrosis factor (TNF)- α , suppressing gonadotrophin-releasing hormone (GnRH) secretion and reducing the pituitary responsiveness to pulsatile GnRH (Battaglia *et al.*, 2000; Williams *et al.*, 2001).

A localised ovarian effect is also seen where uterine infection alters follicular growth and function. Cows with high numbers of uterine pathogens on day (D) 7 postpartum had smaller-sized dominant follicles and lower plasma oestradiol concentrations (Sheldon *et al.*, 2002; Williams *et*

al., 2007). Furthermore, it has been reported that granulosa cells from recruited or dominant follicles when challenged with bacterial endotoxin *in vivo* or *in vitro* produce less oestradiol (Herath *et al.*, 2007). In addition to the reduced oestradiol output, these animals had smaller corpora lutea (CL) producing less progesterone (Williams *et al.*, 2007); the hormone crucial for implantation, recognition and maintenance of pregnancy (Spencer *et al.*, 2004). Therefore, any alteration of follicular growth and/or oestradiol production caused by bacterial infection has the potential to influence the events of ovulation, conception and pregnancy.

The experiment described in this paper is part of a project that aims to increase conception rates in New Zealand dairy cows. This is a preliminary report of the follicular and luteal dynamics found in pasture-fed dairy cows during early lactation and the impact of scEndo. An accompanying report on the blood parameters and milk production from these cows is also published in these proceedings (Green *et al.*, 2009).

MATERIALS AND METHODS

This study was approved by the Ruakura Animal Ethics Committee and conducted during August-September 2008. The uterine health status of mixed-age lactating dairy cows ($n = 169$) was assessed on D21 (± 3 d) postpartum by examination of vaginal mucus using a Metrichcek device (Simcrotech, Hamilton) which has been validated to identify the presence of uterine infection (McDougall *et al.*, 2007). Cow management was as described in the accompanying paper by Green *et al.* (2009). Animals included in the trial were multiparous, exhibited general good health and had not been treated with intra-mammary or systemic antibiotics post-calving. Cows were assigned to groups based on their Metrichcek score (0-1 = Non-infected, 2-3 = Sub-clinical, 4-5 = Clinical). Animals that exhibited signs of clinical infection were treated and excluded from the trial.

In a sub-population ($n = 47$; 32 clean and 15 scEndo), uterine endometrial samples were taken on D21 and D42 post-partum (± 3 d) for bacteriological and cytological analysis. Bacteriological samples to identify aerobic and anaerobic pathogens (Sheldon *et al.*, 2002) were collected using a cytobrush technique (Kasimanickam *et al.*, 2005; Barlund *et al.*, 2008). Bacteria were isolated in aerobic and anaerobic culture at the Animal Health Centre, Morrinsville and categorised on the basis of expected pathogenic potential within the uterus (Sheldon *et al.*, 2002; Williams *et al.*, 2007), as detailed in Green *et al.* (2009). A second cytobrush was collected and percentage of polymorphonuclear neutrophils (%PMN) determined at the Animal

Health Centre, Morrinsville. Animals were classified as scEndo if %PMN was greater than 18% (Kasimanickam *et al.*, 2005).

Follicular and luteal dynamics were evaluated by ultrasonography using a 7.5 MHz transvaginal sector probe (PieMed 200S, Netherlands) on D21, D42 and D63 in this sub-population. Prior to ultrasound scanning, cows received epidural anaesthesia with 4 ml 2% lidocaine (Bomacaine, Bomac Laboratories Ltd., Auckland). Follicular populations were mapped and the diameters of individual follicles measured using the internal callipers of the ultrasound system. The presence/absence of a CL and its location relative to the dominant follicle was recorded. On D42 and D63 only, all follicles larger than 4 mm were ablated using 19Gx1.5"BD Precision-Glide needles (Becton Dickinson, Auckland) with 25 mm Hg vacuum using an aspiration pump (Karl Storz, Germany) and four days later the diameters of the new dominant and sub-ordinate follicles were measured. In this way it was possible to gain an assessment of the relative rates of antral follicular growth.

Data analyses were as described in Green *et al.* (2009). Briefly, the data were analysed using a MIXED procedure in SAS (SAS, 1997) with cows classified as scEndo or clean based on D21 results. Change in BCS between D21 and D63 was used as a covariate in the model.

RESULTS

The proportion of cows with scEndo using the Metrichcek classification on D21 was 15/169 (8.9%) and on D42, 4/47 (8.5%). The incidence of self-resolving scEndo cows by D42 was 11/15 (73%). Classification via %PMN was undertaken on 43 and 46 cows on D21 and D42 respectively. In the sub-population, 16/43 cows (37%) and 3/46 cows (6.5%) were classified as having scEndo by %PMN on D21 and D42 respectively. The incidence of self-resolving scEndo cows by D42 was 13/16 (81%). Only 50% agreement was found between the two methods of classification. Consequently, only 8/15 cows determined scEndo by Metrichcek were also determined by %PMN and a further eight cows were identified from the remaining sub-population as having scEndo.

The bacterial species isolated from uterine samples taken on D21 in the sub-population ($n = 47$) of clean and scEndo cows are summarised in Green *et al.* (2009). By D42, in agreement with the high self-resolve rate found in these cows, the number of different species present and their incidence had decreased dramatically. Isolation of bacterial species known to cause uterine infection was similar between clean and scEndo cows in the present study.

TABLE 1: Mean \pm standard error of mean of the follicle size and growth of dominant and subordinate follicles on day 42 and day 63 postpartum based on uterine status classified by Metrichheck or %PMN in 47 cows diagnosed as either having sub-clinical endometritis (scEndo) or not (clean) on day 21 postpartum.

Follicle type	Parameter	Day 42		Day 63		Uterine status	P value	
		Clean	scIUI	Clean	scIUI		Day	Interaction
Metrichheck classification								
Dominant	Size (mm)	9.5 \pm 0.5	10.0 \pm 0.7	11.1 \pm 0.5	12.7 \pm 0.7	0.02	0.001	0.41
	Growth/4 days (mm)	5.3 \pm 0.5	6.0 \pm 0.7	7.1 \pm 0.5	8.6 \pm 0.7	0.02	0.001	0.41
Subordinate	Size (mm)	7.0 \pm 0.4	8.1 \pm 0.5	7.9 \pm 0.4	7.6 \pm 0.5	0.36	0.64	0.11
	Growth/4 days (mm)	3.0 \pm 0.4	4.1 \pm 0.5	3.9 \pm 0.4	3.6 \pm 0.5	0.36	0.64	0.11
%PMN classification								
Dominant	Size (mm)	9.2 \pm 0.5	9.9 \pm 0.7	11.4 \pm 0.5	11.7 \pm 0.7	0.28	0.001	0.71
	Growth/4 days (mm)	5.2 \pm 0.5	5.9 \pm 0.7	7.4 \pm 0.5	7.7 \pm 0.7	0.28	0.001	0.71
Subordinate	Size (mm)	7.0 \pm 0.4	7.7 \pm 0.5	7.9 \pm 0.4	7.7 \pm 0.5	0.60	0.27	0.26
	Growth/4 days (mm)	2.9 \pm 0.4	3.6 \pm 0.5	3.9 \pm 0.4	3.7 \pm 0.5	0.60	0.27	0.26

The percentage of animals determined, via the presence of CL, to have returned to cyclicity on D21, D42 and D63 were 42.6%, 82.2% and 88.4% respectively. Using the D21 Metrichheck classification, 20/47 (42.6%) had CL present on D21; of which 17/32 (53%) were clean and 3/15 (20%) scEndo cows. On D42, 37/45 (82.2%) cows had CL present; 26/31 (84%) clean and 11/14 (79%) scEndo cows. On D63, 38/43 (88.4%) cows had CL present; 27/30 (90%) clean and 11/13 (85%) scEndo cows. Similar values were found using the %PMN classification (P.J. Back, Unpublished data).

Irrespective of the method used to determine scEndo, dominant follicles were larger and grew faster on D63 than on D42 (9.6 \pm 0.5 versus 11.9 \pm 0.5 mm, $P < 0.001$). This effect was not evident in subordinate follicles (7.5 \pm 0.4 versus 7.8 \pm 0.4 mm, $P > 0.1$). In the clean cows, subordinate follicles tended to be larger ($P = 0.06$) on D63 than on D42 (Table 1). Based on the D21 Metrichheck classification, dominant follicles of scEndo cows were larger and grew faster ($P = 0.02$) than follicles in clean cows. This was not evident when analysed based on PMN classification. No overall effect was found on subordinate follicles using either classification method. However using the Metrichheck classification, on D42 only, subordinate follicles of clean cows tended ($P = 0.08$) to be smaller than those in scEndo cows.

DISCUSSION

The aim of this preliminary trial was to determine follicular and luteal activity in pasture-fed dairy cows during early lactation and the impact of scEndo. The present study found a low prevalence of scEndo, ~9% at D21, with a high self-resolve rate (73%) by D42 post-partum. Detailed discussion

relating to both scEndo prevalence and the bacterial species identified is summarized in the related paper by Green *et al.* (2009).

Examination of the results for the follicular and oestrous activity, suggests a potential delay in the return of scEndo cows to ovarian cyclicity by D21 postpartum, although by D42 and D63, little or no difference is apparent in agreement with the high self-resolve rate identified by D42. Despite the majority of the scEndo cows being classified as clean by D42 and D63, a significant and surprising increase in the scEndo follicular size and rate of follicular growth on D63 was identified compared to those of the D21 clean cows. Limited data are available on the effect of uterine infection on follicular size and growth rates. Previous studies report that dominant follicular size and rates of growth were decreased in animals with a high bacterial load on D7 postpartum (Sheldon *et al.*, 2002; Williams *et al.*, 2007). However, there are several major points of difference in the way these studies were conducted compared to the present study. In previous studies measurements were taken during the first two follicular waves after parturition, from D7–28 postpartum, when these animals were identified as having a clinical not sub-clinical infection. Sampling was conducted in the current study around the earliest time postpartum that cows would be mated on either D42 or D63 and the majority of the cows with scEndo at D21 postpartum had self-resolved. Thus, in self-resolved previously infected cows, there appear to be long-term consequences on the dynamics of ovarian follicular growth and it may well be that the larger dominant follicles do not equate to being of better quality.

Perry *et al.* (2007) in beef heifers found that the size of the ovulatory follicle at the time of insemination significantly influenced pregnancy

rates and embryonic/fetal mortality. Ovulation of follicles <10.7mm or >15.7mm were less likely to support pregnancy than follicles that were 12.8 mm in diameter. Direct comparison to follicular size in the current study is not possible, as the size at ovulation was not recorded. However Perry *et al.* (2007) also found the effects of serum oestradiol concentrations and behavioural oestrus on pregnancy rate appear to be mediated through ovulatory follicle size, thus proposing that management practices that optimize ovulatory follicle size may improve fertility. Although the data from the current study suggests that early postpartum scEndo has surprisingly long-term influences on the subsequent size of the dominant ovarian follicles, it remains to be determined if this affects the resumption of fertility in these cows. Follicular size alone however, is unlikely to be the best indicator of oocyte quality. Measurement of intra-follicular factors may show a greater correlation to subsequent fertility. Consequently, determination of the steroid and metabolite concentrations of follicular fluid collected in the present study is planned.

Isolation of bacterial species known to cause uterine infection were similar between clean and scEndo cows in the current study. Sheldon *et al.* (2002) tested the influence of the pathogenicity group on follicle size and this was not significant, whereas the effect of bacterial load was. It is not possible to compare the severity of infection and its impact on follicle size and growth as bacterial load was not determined in the present study. Studies in sheep on the effect of bacterial endotoxins on follicle and luteal dynamics provide evidence of carry-over effects into the subsequent cycle (Battaglia *et al.*, 2000). Endotoxin interrupted the preovulatory oestradiol rise thus blocking or delaying the luteinizing hormone surge and shortened the luteal phase resulting in reduced progesterone production. The occurrence of scEndo can therefore affect several key stages of the reproductive cycle, as the production of a viable embryo requires ovulation of a competent oocyte, adequate progesterone production by the CL and a receptive uterine environment (Green *et al.*, 2005).

In summary, despite uterine infection being subclinical and the majority of cows self-resolving before D42 around the time of rebreeding, follicle dynamics in the early postpartum dairy cow were altered. Further work is planned to determine if this results in detrimental effects on the subsequent fertility and to investigate possible mechanisms responsible for compromising oocyte quality prior to ovulation.

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