Available online at www.sciencedirect.com

SciVerse ScienceDirect

Theriogenology 77 (2012) 570–578

Theriogenology

www.theriojournal.com

Effects of polymorphonuclear neutrophile infiltration into the endometrial environment on embryonic development in superovulated cows

M. Drillich[a,b], D. Tesfaye[c], F. Rings[c], K. Schellander[c], W. Heuwieser[a], M. Hoelker[c,*]

[a] *Clinic for Animal Reproduction, Freie Universität Berlin, Berlin, Germany,*
[b] *Clinic for Ruminants, Section of Herd Health Management, Vetmeduni Vienna, Vienna, Austria*
[c] *Institute of Animal Science; Animal Breeding and Husbandry Group, University of Bonn, Bonn, Germany*

Received 23 November, 2010; received in revised form 22 August, 2011; accepted 30 August, 2011.

Abstract

Recent studies on bovine uterine disorders have demonstrated that endometrial infiltration with polymorphonuclear neutrophils (PMN) in the postpartum period or at the time of breeding negatively affects reproductive performance. The objective of the present study was therefore to analyze the effect of endometrial PMN infiltration on superovulation outcome. Cows were synchronized and superovulated receiving a total of three artificial inseminations within 24 h. Endometrial cytologic samples were collected by cytobrush technique at first artificial inseminations (AI) (d −1) and before embryo flush (d 7). Embryos were recovered by uterus flushing at Day 7 and evaluated for total cell number and apoptotic cell index. A total of 425 embryos were flushed out of 48 superovulated cows. The PMN dynamics from first AI to flushing had a significant effect on flushing outcome. Significant differences in terms of number of palpable corpora lutea (14.1 vs 7.2) and transferable embryos (8.8 vs 1.9) were found between cows with PMN proportions increasing from zero (0%) at AI to positive proportions (> 0%) at flushing (group PMNZP) and cows with higher endometrial PMN proportions decreasing to lower but still positive proportions from AI to flushing (group PMNHL). Moreover, cows classified to PMN class zero at first AI flushed a significant higher number of total embryos (10.3 vs 6.9) and transferable embryos (6.8 vs 3.7) compared to cows of PMN class positive at first AI ($P > 0.05$) in our study. Considering a significant interaction effect between PMN class at first AI and flush ($P < 0.05$), PMN class at first AI (d −1) correlated significantly with number of total flushed and transferable embryos only in combination with a positive PMN class at flush (d 7). Likewise, PMN class at flush (d 7) beard a significant effect on total number of flushed embryos only when classified to PMN class zero at first AI. Collectively, the present work is the first study that demonstrated a significant relationship between endometrial PMN infiltration at first AI as well as PMN dynamic from first AI to time of flush and superovulation outcome.

Keywords: Polymorphonuclear neutrophils; Superovulation; Dairy cows

1. Introduction

With continuous improvement in dairy cattle for the last 50 years, there is a dramatic increase in average milk yield, which may be associated with a continuous decline in fertility [1]. It is generally accepted that fertilization rate is up to 90% and that early embryonic

* Corresponding author. Tel.: +49 2223 917288; fax: +49 2223 917282.
E-mail address: michael.hoelker@itz.uni-bonn.de (M. Hoelker).

0093-691X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2011.08.033

loss in the first 40 d after breeding accounts for 30 to 40% of total losses after fertilization [2,3].

Because a healthy uterus is the prerequisite for excellent fertility, postpartum uterine diseases have been described as a leading cause for reproductive failure in dairy cattle [4,5]. In the last years the impact of subclinical endometritis on fertility has been the objective of several studies [5–7]. Subclinical endometritis is defined by the presence of polymorphonuclear neutrophils (PMN) in the endometrium of clinically healthy cows [8]. Endometrial samples can be obtained with a small brush, described as cytobrush-technique by Kasimanickam et al in 2004 [6] or by low-volume flushing of the uterus [9,10]. Although it is generally accepted that the infiltration of PMN is diagnostic for subclinical endometritis in the postpartum period [8], a threshold value for the proportion of PMN as indicative of subclinical endometritis is still under discussion [5–7].

Proinflammatory factors, e.g., interleukins, tumor necrosis factor, have been found elevated in endometrial tissue in cows with subclinical endometritis [11,12]. Hill and Gilbert [13] have demonstrated in cultured embryos the negative effect of inflammatory products on embryo quality. Sheldon et al [14] postulated that lipopolysaccharide produced by E. coli negatively affects ovarian function, resulting in disturbed steroidogenesis, lower peripheral progesterone concentrations, and long luteal phases.

Most studies on bovine subclinical endometritis have analyzed the effects of elevated proportion of PMN at the end of the postpartum period, not at insemination. Only one study investigated the effects of PMN infiltration in the endometrium at the time of artificial inseminations (AI) on first service conception rate [15]. Although the authors concluded that the percentage of PMN at the time of insemination interacts with subsequent fertility, it is still poorly understood by which mechanisms endometrial functions as well as embryonic development are affected.

The use of different exogenous hormones and the timing of programs to induce superovulation have been investigated extensively [16–18]. Less attention has been paid on the maternal effects on maintenance of pregnancy in the early stage. To our knowledge, no studies have been published about the effects of subclinical endometritis or the presence of PMN in the endometrium on the outcome of superovulation programs. The objective of the present study was therefore to analyze the effects of endometrial PMN infiltration on embryonic development after superovulation.

2. Materials and methods

The study was conducted on a commercial dairy farm in Germany. Holstein cows were housed in a free-stall barn with slotted floors and cubicles, lined with rubber mats. Herd average milk yield was 9,260 kg (4.08% fat, 3.48% protein) per cow per year. Cows were fed a total mixed ration.

2.1. Superovulation protocol

Primiparous Holstein cows (n = 48) without signs of clinical endometritis, ie vaginal discharge diagnosed by vaginoscopy, were subjected to a superovulation protocol beginning 30 to 45 d postpartum as shown in Fig. 1. Presynchronization was performed by adminis-

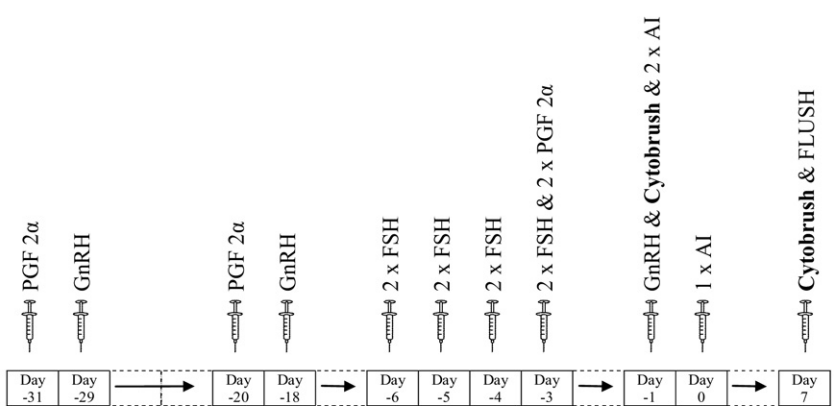

Fig. 1. Synchronization of donor animals by intramuscular injection of $PGF_2\alpha$, twice within 11 days. Two days after $PGF_2\alpha$ treatments, all animals received GnRH. Twelve days after the second GnRH injection, heifers received the first of eight consecutive FSH-injections over 4 days. Two $PGF_2\alpha$ treatments were performed 72 and 84 h after the initial FSH injection. Finally, 48 h after the first $PGF_2\alpha$ application, ovulations were induced by giving GnRH simultaneously with the first of a total of three artificial inseminations (AI) within a 24 h interval.