March 2015 Vol. 22 No. 1 35-39
Journal of Northeast Agricultural University (English Edition)

Dynamic Analysis of Nitric Oxide and Total Oxidant Capacity in Cow Uterine Secretion with Subclinical Endometritis

Song Xue, Li De-jun, Feng Guo-feng, Li Bei, and Liu Yun-feng*

College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, China

Abstract: Subclinical endometritis is a physiological inflammation that serves to clear persistent contaminants from the uterus. To investigate the alteration of antioxidant, such as vitamin E (VE) and vitamin C (VC), total oxidant capacity (TOC) and nitric oxide (NO) in cows with normal and subclinical endometritis (SCE), we examined the concentrations of NO, VC and VE, TOC and polymorphonuclear neutrophils (PMN) percentage in uterine secretion. The cows were divided into two groups, normal (n=20) and subclinical endometritis (SCE, n=60), based on endometrial cytology (presence of PMN≥5%). Uterine secretion and blood were collected as described previously. Griess reaction was used to determine the concentration of NO. The concentrations of TOC, VC and VE were detected by a commercially available assay kit. The results showed that the concentrations of NO, TOC and PMN percentage were significantly higher (P<0.01, P<0.05, and P<0.01, respectively) in uterine secretion with SCE compared to those from normal; however, the levels of VC and VE were significantly lesser (P<0.01). In conclusion, the concentrations of NO, TOC, VC, VE and PMN percentage differed between normal and SCE cows. Meanwhile, the relationship between the concentration of NO and PMN percentage from uterine secretion in cows with subclinical endometritis were positively correlated. Consequently, these alterations in NO, TOC, VC, VE levels and PMN percentage contributed to as a diagnostic index of the uterine inflammation, with the aim to increase the reproduction of the cows and the decrease economic losses.

Key words: cow, subclinical endometritis, nitric oxide, antioxidant, total oxidant capacity

CLC number: S823 **Document code:** A **Article ID:** 1006-8104(2015)-01-0035-05

Introduction

Subclinical endometritis (SCE), as one of the most important disorders in dairy cows during the post-partum period, impacts reproduction (Sheldon *et al*., 2006; Dubuc *et al*., 2010) and causes economic losses (Azawi, 2008). Earlier diagnose and treatment of SCE play an important role in controlling clinical endometritis, yet the index of the early diagnosis are still not clear (Ahmadi *et al*., 2005; Gilbert, 2005; Ribeiro *et al*., 2013; Mohammad *et al*., 2014).

Polymorphonuclear neutrophils (PMN) in uterine secretion is an indicator reflecting uterine health state (Kasimanickam, 2004). Excessive amounts of neutrophilic granulocytes, macrophages, lymphocytes, eosinophilic granulocytes and various epithelial cells of the uterine tissue in uterine fluid are considered as a response to prevent exogenous pathogenic bacterium in the part of inflammation of the uterus (Singh, 2008). In addition, the level of pro-inflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin

Received 20 October 2014
Supported by Funding (RCB22) from the Doctoral Research Foundation of Northeast Agricultural University (2012); the Postdoctoral Fund of Heilongjiang Province (LBH-Z11239)
Song Xue (1989-), female, Master, engaged in the research of nutrition regulation and metabolism disease. E-mail: songxue521521@163. com
* Corresponding author. Liu Yun-feng, associate professor, engaged in the research of nutrition regulation and metabolism disease. E-mail: liuyunfeng@ neau.edu.cn

(IL) 1β, IL-6, IL-8 and some other molecules such as nitric oxide (NO) are increased during the period of infections (Sheldon *et al*., 2001; Li *et al*., 2010; Loyi *et al*., 2013). As an inflammatory mediator, NO which synthesized by macrophages causes smooth muscle relaxation and mediates cytoimmunity and inflammation toxicity. Excess NO is produced during inflammation as a primer defense system (Subandrio *et al*., 2000). Study showed that NO content is increased during inflammation diseases (Rawlingson, 2003; Abdorrahman *et al*., 2005). In addition, substances derived from oxidation of NO, such as peroxynitrite, changed antioxidant balance of the bacteria (Onur *et al*., 2010).

In the process of inflammation, pro-inflammatory cytokines and cytotoxic radicals which are released from macrophages and granulocytes inhibit cellular metabolic pathways and lipid peroxidation (Ewa *et al*., 2012). Several studies showed that increase in lipid peroxidation during endometritis decreases the levels of some antioxidant molecules, such as vitamin E (VE) and vitamin C (VC), which lead to an increase in oxidative stress (Kankofer *et al*., 2005; Lorraine and Stacey, 2009). Oxidative stress is a result of unbalance between oxidant and antioxidant levels (Lykkesfeldt and Svendsen, 2007; Onur *et al*., 2010), occurs different pathological events, such as mastitis, metritis, and retained fetal membranes during the periparturient period in cows (Lykkesfeldt and Svendsen, 2007; Lorraine and Stacey, 2009). Due to membrane lipid peroxidation and oxidative stress, mammalian tissues such as cellular can be damaged by the accumulation of reactive oxygen species (Lorraine and Stacey, 2009). Therefore, earlier diagnosis and treatment of the endometritis, especially SCE, are important to minimize economic losses. Antioxidant is used to treat mastitis in goats, bovines and mares, during the reproductive state (Abdorrahman *et al*., 2005; Eyassu *et al*., 2007; Lorraine and Stacey, 2009); however, few reports show the change of the antioxidant in SCE.

Thus, with the aim to reveal the early diagnosis index of the subclinical endometritis, the levels of NO, VC and VE, total oxidant capacity (TOC) and PMN percentage from uterine secretion in cows were examined.

Materials and Methods

Experimental animals

Totally 80 Holstein cows from Dairy Tarm were enrolled in this study (Sarkar *et al*., 2006; Kasimanickam *et al*., 2004). All the Holstein cows were routinely examined once between 28 and 33 days after calving, included inspection of the vulva, tail, and perineum, vaginoscopy, and transrectal palpation of the cervix, uterus, and ovaries. Uterine discharge was classified as clear mucus, mucus with flecks of pus, mucopurulentand orpurulent according to the criteria described previously (Sarkar *et al*., 2006; Kasimanickam *et al*., 2004). Briefly, normal cows with no abnormal uterine discharge were selected according external inspection and vaginoscopy. SCE was diagnosed by endometrial cytology and histopathology. The cows were divided into two groups, which included 20 normal cows and 60 cows with SCE. Blood and uterine secretion were collected at the same time of inspection. None of cows had other diseases requiring systemic treatment.

Sample collection

Uterine secretion was collected as described previously (Sioutas *et al*., 2008). In brief, uterine washings were collected aseptically using two ways 18 gauges. Sterilized stainless steel catheters were fixed in the uterine horn with 10-15 mL air, then withdrew introducer, 40-50 mL of washing fluid (sterile PBS pH 7.0) was infused into the uterine horn and mixed with the intrauterine contents by massaging the uterus per rectum. 40 mL uterine washings were centrifuged at 1 000 g for 10 min, and the supernatant was frozen at –80℃ until all the samples were collected.

The percentage of PMN was counted with a minimum of 100 cells at 400 magnification.

Blood sample from vena cervicalis was collected