



## Prevalence of cervicitis in dairy cows and its effect on reproduction



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### ABSTRACT

The objective of this study was to determine whether cervicitis in dairy cows is an independent disease or occurs concomitantly with inflammation of the uterus, and to clarify possible effects of cervicitis on reproductive performance. Dairy cows ( $n = 416$ ) from 33 dairy farms were examined by rectal palpation and vaginoscopy between 42 and 50 days postpartum. Inclusion criteria for this study were absence of abnormal vaginal discharge and abnormalities of the uterus (fluctuation) at rectal palpation. Cervicitis was diagnosed when the second cervical fold was swollen and prolapsed with (C2) or without (C1) reddening. Cytobrush samples from the uterus ( $n = 370$ ) and the cervix ( $n = 402$ ) were collected, and the percentage of neutrophils in the uterus (PMNU) and the cervix as indicators of inflammation (threshold:  $\geq 5\%$ ) was determined. In addition, endometrial biopsies for histology were collected, 300 of which were suitable for evaluation. Cervicitis (C1/C2) was diagnosed in 253 of 416 (60.8%) of cows. Of these, the prolapsed cervical mucosa was hyperemic (C2) in 29.1% of cases. Of 370 available uterine cytology samples, 221 cows had a clinical cervicitis; however, 170 (76.9%) had PMNU less than 5%. Of 300 uterine histologic examinations, 82 (27.3%) did not reveal any abnormalities; the remaining cows either had uterine inflammation and/or degenerative uterine changes such as endometriosis and angiosclerosis. Furthermore, of 300 biopsied animals, 184 revealed a cervicitis (C1/C2); however, 30.4% of these animals had no histopathologic uterine findings. For further analysis, only animals either without histopathologic findings and normal uterine cytology or with solely endometritis (defined as PMNU  $\geq 5\%$  and/or positive histopathology of the uterine tissue) were evaluated ( $n = 157$ ). Of these, 95 cows had cervicitis. Unexpectedly, 63 of 95 (66.3%) cows had cervicitis without endometritis. With regard to reproductive performance, days to first service were not affected by cervicitis. Number of days open in animals with cervicitis but without endometritis tended to be lower than in cows with cervicitis plus endometritis ( $P = 0.092$ ). Also, number of days open relative to percentage of neutrophils greater than 5% was lower when the cervical compared to the uterine mucosa was affected ( $P < 0.05$ ). Total conception and pregnancy rates of animals 200 days into lactation decreased significantly in cows with severe cervical inflammation (C2). In conclusion, the results of this study suggested that cervicitis occurs independent of endometritis, and a higher degree of cervicitis is associated with poorer reproductive performance.

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## 1. Introduction

Good fertility is a prerequisite for a high-producing dairy cow. In recent years, the main focus has been on diseases of the uterus and their effects on reproductive performance. Therefore, basic research on inflammation and/or degenerative endometrial diseases is well advanced [1–4]. An often unnoticed structure is the cervix uteri. It is a self-contained organ in the reproductive tract, which represents the anatomic and functional barrier between the vagina and the uterus [5]. However, little is known about inflammation or bacterial contamination of the cervix and the subsequent influence on reproduction in dairy cows. In the past 5 years, there have been three basic studies on the histologic structure of the bovine cervix [6] and its role in dystocia [7] and the postpartum involution process [7,8]. Studies on inflammation of the cervical canal date from the 70s, but these were carried out on slaughtered animals or animals repeatedly treated for sterility. Only one recent study investigated the prevalence of cervicitis and its effect on days to conception in dairy cows [9].

Therefore, the aim of this study was to determine the prevalence of cervicitis in Lower Saxon dairy herds and to evaluate the relationship between inflammation of the cervix and the endometrium. Furthermore, possible effects on fertility were analyzed to evaluate the importance of cervicitis in dairy cows.

## 2. Material and methods

### 2.1. Animal care and approval

Study design, examination of cows, uterine cytobrush, and biopsy samples were approved according to guidelines for ethical animal treatment by the local authority (LAVES Az: 06A421).

### 2.2. Animals and study design

A total of 416 Holstein Friesian dairy cows from 33 dairy farms in Lower Saxony, Germany were examined between August 2006 and May 2007. The average herd size was 85 (19–156) animals per farm, with an average milk yield of 9416 (7493–10977) kg milk per year. The parity of cows ranged from 1 to 10. Depending on herd size, farms were visited for reproductive examinations every 2 to 8 weeks.

### 2.3. Clinical examinations

Cows were examined by rectal palpation and vaginoscopy between 42 and 50 days postpartum. Transrectal palpation served to assess uterine size and symmetry of the uterine horns as well as uterine fluctuation according to Grunert [10]: uterus retractable and horn diameter less than 2 cm (score 1), 2 to 5 cm (score 2), or greater than 5 cm (score 3), uterus not retractable but greater curvature palpable (score 4), uterus not retractable and greater curvature incompletely palpable (score 5), and uterus not retractable and greater curvature poorly outlined (score 6). After the vulva was cleaned with dry paper towels, a tube speculum and flashlight were used for vaginoscopy. Form

and color of the *Portio vaginalis cervicis* were evaluated. Vaginal discharge was categorized as clear or translucent mucus (0), mucus with flecks of white or off-white pus (1), exudate containing less than 50% purulent material (2), exudate containing greater than 50% purulent material (3; 4, modified). Inclusion criteria for further examinations were absence of abnormal vaginal discharge and abnormalities of the uterus (fluctuation) at rectal palpation. Cows that did not meet these criteria were excluded from the study. Cervicitis was diagnosed when the second cervical fold was swollen and prolapsed with (C2) or without (C1) reddening. Cervices without abnormality were designated as C0 (Fig. 1). All examinations were made by the same skilled veterinarian.

### 2.4. Cytology

Cytobrush samples from the endometrium ( $n = 370$ ) and the cervical mucosa ( $n = 402$ ) were collected using a modified swab collecting instrument (MERKT, Leisegang GmbH, Berlin, Germany) with an attached sterile brush (Cytobrush Plus GT, Medscand Medical, Malmö, Sweden). Using rectal guidance, the cytobrush was directed through the cervix, then exposed and rolled into the endometrium or cervical mucosa, and then covered again with a protective sheath. Once outside the vagina, the cytobrush was rotated on a paint-coated microscopic slide and fixed with Merckofix (Merck KGaA, Darmstadt, Germany). Smears were evaluated under a microscope at 400 $\times$  and 1000 $\times$  magnification. A total of 200 cells were counted and differentiated. Subsequently, the percentage of polymorphonuclear neutrophils (PMN) was determined by evaluation of endometrial and cervical cells and PMN. To assess the repeatability of PMN estimates, slides were counted and differentiated by two independent persons. The correlation between the two individual persons was high ( $r = 0.86$ ;  $P < 0.0001$ ). Positive cytology was defined as PMN greater than or equal to 5%, and PMN less than 5% as negative cytology.

### 2.5. Endometrial biopsy

A total of 416 animals were biopsied using a Kevorkian biopsy instrument (Hauptner, Solingen, Germany), and 300 were suitable for evaluation. A remaining 116 samples could not be evaluated due to material artifacts and collecting difficulties. Usable specimens were immersed in a 4% neutral phosphate-buffered formaldehyde solution for 24 hours and then embedded in Paraplast (Vogel, Gießen, Germany) using a tissue embedding machine (Shandon Hypercenter XP, Frankfurt, Germany). For histology, sections that were 3 to 4  $\mu\text{m}$  thick were produced and stained with hematoxylin. Sections were evaluated [11,12] using an Olympus Light Microscope (Olympus Europa GmbH, Hamburg, Germany).

### 2.6. Reproductive performance

The influence of cervicitis and endometritis on reproduction indices was investigated. Indices included: days to first service, days open (period between calving and conception), first service conception rate (number of

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