

The Effect of Bacterial Flora on Uterine pH Values, Observed During the Estrous Cycle, Gestation and in the Cases of Clinical Metritis in Cows

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Abstract: The aim of this study was to determine the correlation between pH values for the uterine horns and uterine bacterial flora during phases of sexual cycle, pregnancy and cases of clinical metritis in the cow. Post-mortem pH values for the uterine horns of 145 cows were measured using a digital pH meter with a flexible probe. In addition, stage of the sexual cycle of the cows was evaluated by external examination and microbiology and ultrasonography. The mean pH values for *Escherichia coli* and *Staphylococcus aureus*+*E. coli* growth within the uterine horns were detected to be higher than those with *Micrococcus* sp. and Coagulase Negative Staphylococci (CNS)+*Candida* sp. growth ($p<0.05$). As the mean uterine pH value of microbial growth at estrous cycle was determined to be significantly higher than those without microbial growth ($p<0.05$), the mean pH value for uterine horns with microbial growth were determined to be higher than without microbial growth when phases of sexual cycle were collectively evaluated ($p<0.05$). In addition, mean pH values during pregnancy in the uterus with microbial growth were detected to be significantly higher than those of without microbial growth ($p<0.05$). However, the mean pH value of uterine secretions was determined as 7.25 ± 0.44 in cases of clinical metritis. In conclusion, selection of intrauterine drugs and phases of drug use in cows were deemed to be designed by considering the uterine bacterial flora and phases of sexual cycle.

Key words: Cattle, uterus, pH, estrous cycle, metritis, pregnancy

INTRODUCTION

Synchrony, the adaptation of embryo and uterine endometrium is necessary for gestation in humans and farm animals. Numerous adverse conditions such as embryo implantation disorders early embryonic death and slowing in embryo development are shaped in nonexistence of this adaptation (Barnes, 2000). Although, 40% of cattle embryos were reported to die within 3 weeks of fertilization under normal circumstances (Hugentobler *et al.*, 2008), only a limited number of studies are available reporting the intra uterine conditions for embryosurvival in the *in vivo* environment (Hugentobler *et al.*, 2004).

Important uterine pH values required for the embrionic development of cattle were reported to be affected by factors such as the applied anesthesia options, the animal's diet (Elrod *et al.*, 1993), sexual cycle days (Hugentobler *et al.*, 2004).

However, variations in intrauterine pH values were reported to have an effect on uterine contractions in the

studies performed with both rats and humans (Taggart and Wray, 1993; Parratt *et al.*, 1994, 1995). Due to the location of reproductive organs in cows, there have been a limited number of studies basing on uterine pH measurement (Hugentobler *et al.*, 2004). Determining physiological pH values in cow genital organs is important to develop new therapeutic approaches (Wehrend *et al.*, 2003). According to the research, there has been only one study performed detecting uterine pH values during interestrus phase and factors producing in interestrus interval in cattle (Wehrend *et al.*, 2003) nevertheless, there have been no studies conducted in cases of pregnancy and metritis in cows.

In addition, there has not also been even a study examining the correlation between bacteria comprising the microbial flora and uterine pH values according to the phases of sexual cycle of cows. The aim of the current study was to determine the correlation between pH values for the uterine horns and uterine bacterial flora during cyclic activity, gestation and in cases of clinical metritis in cows.

MATERIALS AND METHODS

About 145 Holstein breed cows were cut under slaughterhouse conditions after 30 days postpartum constituted the animal material of the study. The reproductive tracts of all animals were included to the study after being brought to the laboratory within 20 min maximum following the beginning of the cutting process as described by Wehrend *et al.* (2003). During the external examination each uterine was examined in general for adhesion, inflammatory changes, asymmetry between uterine horns, thickened uterine wall, palpable presence of fluid and flow and the results were noted down (Lawton *et al.*, 2000; McDougall, 2005). Outer surface of the uterus was cleaned with 1% iodine solution. Then, using a sterile scalpel blade right and left uterine horns were incised separately according to the method specified by McDougall (2005). Subsequently, sterile swab samples taken from each horn for microbiological examination were delivered to the laboratory in Stuart's transport medium (Oxoid Ltd, Hampshire, England).

Following microbiological sampling, the pH measurements of the first, medium and last 1/3 portion of each uterine horn were made. Each measurement was performed twice and the average measurements were taken. The pH measurements were carried out with an ambulant, digital pH meter (InoLab pH level 1, D-82362; Weilheim, Germany) with a flexible probe and a measuring frequency of one value per second as described by Wehrend *et al.* (2003).

The probe was rinsed with ultra-pure water between measurements and they were re-calibrated when necessary. Pregnant animals were detected by means of uterus examination. In addition, animals with purulent or mucopurulent discharge were described as with clinical endometritis via the clinical examination of the intrauterine fluid accumulations (LeBlanc *et al.*, 2002; Williams *et al.*, 2005). Morphological structures on each ovary were first examined externally and then in water bath using an ultrasound device containing 8.0 MHz linear-array transducer (100 Falco Vet, Pie Medical Equipment BV, Philipsweg 1, 6227 AJ, The Netherlands). These findings were evaluated according to the method of Noakes (2003) and Ali *et al.* (2003) and the stages of sexual cycle of cows were determined.

Swab samples obtained from uterine horns for microbiological examination were immediately cultured onto Columbia Blood Agar (Oxoid Ltd, Hampshire, England) supplemented with 7% sheep blood. Petri dishes were incubated at 37°C for 24-48 h under aerobic conditions. After incubation each different colony was examined macroscopically (colony morphology,

hemolysis) and microscopically (Gram-staining). Identification of microorganisms was performed using standard biochemical tests (Quinn *et al.*, 1999; Holth *et al.*, 2000).

Statistical analysis: The results were analyzed by one way Analysis of Variance (ANOVA), Student's t-test, Mann-Whitney test. pH values were tested for normal distribution by Shapiro-Wilks test. All values were expressed as the mean±Standard Error of Mean (SEM). A probability of $p<0.05$ was considered significant.

RESULTS

The pH values for the uterus were separately determined for each uterine horn in cases of pregnancy, clinical metritis and sexual cycle including periods of proestrus, estrus, metestrus and anestrus. No statistical difference was observed ($p>0.05$) between pH values for the right and left uterine horns when the results of bacterial growth were assessed separately as positive (sufficient microorganism growth was observed in one of the horn) and negative. Therefore, pH data obtained from the right and left uterus were assessed together for each animal to be used in subsequent analysis. When the mean uterine pH values according to the microorganisms growing were examined, we detected that pH values of especially *Escherichia coli* and *Staphylococcus aureus*+*E. coli* growing in uterine horns demonstrated a more significant increase than those of *Micrococcus* sp. and Coagulase Negative Staphylococci (CNS)+*Candida* sp. ($p<0.05$). It was determined that pH values for uterine horns were not statistically significant in terms of growing except for these microorganisms ($p>0.05$) (Table 1). When the stages of sexual cycle were evaluated separately, no significant differences were seen between the pH values for uterine horns without microbial growth ($p>0.05$). However, pH values during estrus phase were observed to be higher than those of during proestrus and diestrus phases ($p<0.05$) but during proestrus phase significantly lower than estrus and metestrus phases ($p<0.05$) when the pH values for the uterine horns with microbial growth were compared. The pH values for uterus with microbial growth during estrus phase were determined to be significantly higher than those of without microbial growth ($p<0.05$). When the total average pH values for sexual cycle phases were examined, the pH values for uterine horns with microbial growth were found to be statistically significantly higher than the ones without microbial growth ($p<0.05$). In addition, pregnant uterus pH values having microbial growth were observed to be significantly higher than uterus with no formation

Table 1: Bacterial agents isolated at different phases of sexual cycle and the correlation between pH values

Bacterial species	Proestrus	Estrus	Metestrus	Diestrus	Pregnant	Metritis	Mean uterus pH
<i>Micrococcus</i> sp.	4	-	-	-	-	-	6.71±0.20 ^a
CNS+ <i>Candida</i> sp.	-	-	-	4	-	-	6.73±0.32 ^{ab}
<i>Candida</i> sp.	-	-	-	10	8	-	7.00±0.27 ^{abc}
<i>Arcanobacterium pyogenes</i>	-	-	-	-	-	8	7.02±0.25 ^{abc}
<i>S. aureus</i> + <i>Candida</i> sp.	-	4	4	-	-	-	7.08±0.52 ^{abc}
CNS	2	2	2	20	-	-	7.20±0.40 ^{abc}
<i>Bacillus</i> sp.	-	-	-	-	6	-	7.32±0.10 ^{abc}
Non-hemolytic <i>Streptococcus</i> sp.	-	-	10	-	2	-	7.32±0.42 ^{abc}
<i>Candida</i> sp.+ Non-hemolytic <i>Streptococcus</i> sp.	-	-	4	-	-	-	7.33±0.13 ^{abc}
<i>S. aureus</i>	2	6	4	-	-	2	7.39±0.58 ^{bc}
<i>E. coli</i>	-	4	10	-	-	-	7.48±0.32 ^c
<i>S. aureus</i> + <i>E. coli</i>	-	4	-	-	-	2	7.49±0.23 ^c

^{a,b,c}: Significant difference between values symbolized with different letters within the same column (p<0.05)

Table 2: The mean pH values for uterine horns with and without microbiological growth during phases of sexual cycle, pregnancy and clinical metritis

Phases of sexual cycle	n	With microbial growth	n	Without microbial growth	p
Proestrus	8	6.84±0.44 ^a	16	6.98±0.34 ^a	p>0.05
Estrus	20	7.51±0.38 ^b	6	6.95±0.40 ^a	p<0.05
Metestrus	34	7.23±0.44 ^{bc}	16	7.01±0.37 ^a	p>0.05
Diestrus	34	7.05±0.36 ^{abc}	120	7.05±0.38 ^a	p>0.05
Mean	96	7.19±0.44	158	7.03±0.37	p<0.05
Pregnant	16	7.27±0.27 ^{bc}	8	6.71±0.55 ^a	p<0.05
Metritis	12	7.25±0.44 ^{bc}	-	-	-

^{a,b,c}: Significant difference between values symbolized with different letters within the same column (p<0.05); Insignificant difference between groups (p>0.05)

of microbial growth when the gestational status was evaluated (p<0.05). The mean pH values for uterine horns were detected as 7.25±0.44 even if different micro-organisms were isolated from cases of clinical metritis (Table 2).

DISCUSSION

As far as we know, this is the first study investigating the factors produced in cow uterus in cases of sexual cycle, pregnancy and metritis and how uterine pH values were affected. No statistical difference between the pH values for right and left uterine horns of each cow was detected and the data from both uterine horns belonging to the same animal were evaluated in the current study. This is consistent with the report of Hugentobler *et al.* (2004). A mean uterine pH value of 6.62 was recorded post mortem in cows at interestrus intervals (Wehrend *et al.*, 2003). Kane *et al.* (2002) reported that uterine pH value was not statistically affected in heifers fed diets containing different proportions of protein and showed an alteration of pH values in heifers ranging from 7.00-7.05.

In the current study we compared the uterine pH values, according to the phases of sexual cycle in cow uterine with no microbial growth. Lower uterine pH values were obtained during proestrus and estrous cycles but pH values increased slightly during metestrus and anestrus cycles. These findings did not exhibit statistically significant differences. We measured mean uterine pH value of 7.03. Nevertheless, the mean value of

pH (7.19) in the uterine with microbial growth increased significantly. Uterine pH values were found high especially in the uterine with *S. aureus* and *E. coli* growth.

Valero *et al.* (2009) reported that the optimal pH value required for the growth of *S. aureus* was 6.5 yet they also had fairly high growth ability in the pH interval ranging from 7.0-7.5. The growth capability of *E. coli* was determined to decrease at low pH values of 4.0 and to increase also at high pH values of 8.0 (Presser *et al.*, 1997; Yuk and Marshall, 2004). Additionally in the investigation performed on cows, occurrence of differences were observed in ion (Hugentobler *et al.*, 2007) and intrauterine energy substrate alterations such as glucose, lactate and pyruvate (Hugentobler *et al.*, 2008) on the 6th, 8th and 14th days of the sexual cycle. These differences developing according to the cycle phase have been considered to have influenced bacterial growth. The effects of these substances found in the uterine in different densities on the bacterial flora are required to be investigated.

Elrod *et al.* (1993) recorded the mean uterine pH value of 6.83 in a study performed on cows at estrus phase but Elrod and Butler (1993) found the value of 6.87 during the same phase. In a study performed on swine the mean pH value of 7.24 for uterine horns was determined during the pre-ovulatory period (Smiljakovic *et al.*, 2008). In this study, the pH values of 6.98 and 6.95 were respectively determined at phases of proestrus and estrus in the uterine with non-microbial growth which is consistent with above studies. Uterine pH value (7.51) in the uterine with microbial growth was determined to increase

significantly especially at estrous phase in comparison to the phase of proestrus (6.84) ($p < 0.05$). No published data have been available on the intrauterine alteration of directions of ion and energy substrates during the phases of proestrus and estrus at sexual cycle in cows. The possibility of the contribution of microorganisms such as *S. aureus* and *E. coli* isolated from the uterine in large amounts at estrus phase to the increase in pH value have been considered as the reason of why pH value was high only in the uterine horns with microbial growth.

Hugentobler *et al.* (2004) found no difference in uterine pH values between days 6 and 8 of the estrous cycle in heifers underwent anesthesia with thiopentone only. They found a mean uterine pH value of 6.96 during this cycle. Elrod *et al.* (1993) and Elrod and Butler (1993) found mean uterine pH values of 7.13 and 7.09, respectively in 7 days postestrus cows fed a balanced diet. Uterine horns pH value measured during the post-ovulation period was determined to lower to 6.87 in a study performed on swine (Smiljakovic *et al.*, 2008). Similar to the above-mentioned studies, the mean pH values of 7.01 and 7.05 were respectively determined at phases of metestrus and diestrus in the uterine with non-microbial growth in the current study as well.

The correlation between uterine pH value and uterus contractions have been emphasized in numerous studies performed on humans and rats (Taggart and Wray, 1993; Parratt *et al.*, 1994, 1995).

In a study performed by Taggart and Wray (1993) the average of pH values of uncontracted rat uterine in the relaxed state was measured as 7.18 and increasing contraction was determined as pH value increased. In a study performed during human pregnancy it was determined that acid shift in the uterine pH value decreased uterine contraction however, alkaline shift increased the contraction (Parratt *et al.*, 1994, 1995). Parratt *et al.* (1995) detected the mean value of uterine pH (7.15) in the pregnant human group significantly higher than that of the non-pregnant group (7.07).

Researchers have been explaining the difference between pH levels as the elevation in the uterine pH value up to 7.26 towards the last phase of pregnancy which causes uterine contractions in humans. A study performed on the uterine pH values during gestation in the cow is not available. In the current study the mean pH values during various phases of gestation in the cow without and with microbial growth were found as 6.71 and 7.27, respectively.

In uterine horns without microbial growth, no difference was determined between uterine pH values at gestation and uterine pH values during sexual cycle in non-pregnant cows in this study but alteration of pH

during pregnancy was not examined since gestation periods of the cow were not exactly detected. Whether alteration of uterine pH occurred during gestation and at delivery in cows should also be investigated via studies similar to the ones performed on humans.

CONCLUSION

The mean pH values for uterine with microbial growth were determined to be significantly higher than the mean pH values for uterine without microbial growth. Although, mean pH values did not develop significant differences in the uterine without microbial growth during estrous cycle and gestation, significant differences were determined especially at estrus phase in the uterine with microbial growth. There was a rise in the uterine pH values of *S. aureus* and *E. coli*. Consequently, alteration of the uterine pH value according to the bacterial flora suggests benefits of the choice of drugs for intrauterine applications and importance of drug implementation phases. Implementation of drugs which are effective especially in basic mediums during the estrus phase of sexual cycle has been considered to yield better results.

ACKNOWLEDGEMENTS

The researchers would like to thank Dr. S. Pamuk (Department of Food Hygiene and Technology, Afyon Kocatepe University) for her contribution on the pH measurements.

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