Comparison of cytological, microbiological and histopathological findings of genital tracts in cows with different degree perineal conformation disorder

EMSAL SİNEM ÖZDEMİR SALCI[1], ÖZKAN YAVAŞ[2], ÖZGE YILMAZ ARDIÇLI[3], GÜRSEL SÖNMEZ[2], SERPİL KAHYA DEMİRBİLEK[3], SENA ARDIÇLI[4], KAMİL SEYREK İNTAŞ[5]

[1] Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Bursa Uludag University, 16059-Bursa / Turkey
[2] Department of Pathology, Faculty of Veterinary Medicine, Bursa Uludag University, 16059-Bursa / Turkey
[3] Department of Microbiology, Faculty of Veterinary Medicine, Bursa Uludag University, 16059-Bursa / Turkey
[4] Department of Genetics, Faculty of Veterinary Medicine, Bursa Uludag University, 16059-Bursa / Turkey
[5] Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Near East University, Nicosia / Cyprus

SUMMARY

The aim of this study is to investigate cytological, microbiological and histopathological findings of the genital tracts in cows with different degree perineal conformation (PC) disorder. Totally, 28 cows brought the slaughterhouse were materials of the study. Information about to age, body weight, reproductive status, number of parturitions, days in milk (DIM) and the reason for slaughtering of the cows was obtained in the anamnesis learned from the owners. Before slaughtering, cows were evaluated in terms of PC and then they were divided into four groups with equal numbers of cows according to PC disorders: group I (GRI) normal; group II (GRII) mild; group III (GRIII) moderate and group IV (GRIV) severe. PC disorders of the cows were graded on a scale in terms of vulvar angle, vulvar length over ischial arch, depth of anus and perineal length. A body condition score (BCS) was also evaluated in the cows. The perineal region and vagina were inspected for vaginal discharge and vaginal mucosal appearance, respectively. Pneumovagina was classified as negative, suspicious and positive. Sterile swab samples were taken from vagina, cervix and uterus for bacteriological culture and identification. For cytological examination, smears were taken from vagina, cervix and uterus. Tissue samples were taken from vagina, cervix and uterus for histopathological examination. Statistically, Pearson's correlation, Fisher's exact tests and regression analysis were performed for all data. Clinically, characters of the vaginal discharge (serous, foamy, mucous, purulent and urine-mixed) and vaginal hyperemia increased in GRIII and GRIV. Microbiologically, as parallel to the PC disorder, *E. coli* in *Enterobacteriaceae* family was the most common bacterium in Group III and Group IV. As PC disorder in the groups increased, cytological examination findings were found to be significant, similar to clinical and microbiological examination. However, histopathological examination gave more meaningful results in groups. Statistic results pointed out that difference between the groups in terms of vaginal mucosa color, cervical and uterine microbiology was significant. In conclusion, PC disorder in cows constitutes predisposition for many genital canal diseases that may be the cause of infertility. The presence of genital canal diseases can be suspected in cattle using the PC scale. Therefore, it is appropriate to evaluate the health status of the genital canal with other diagnostic methods (microbiology, cytology and histopathology), especially in cows with PC disorder.

KEY WORDS

Cow, cytology, histopathology, microbiology, scoring of perineal conformation disorder.

INTRODUCTION

Anatomo-morphological changes in the perineal region and the form of the vulva affect the reproductive performance in large animals[1,2].

Maintaining the anatomical structures of the genital canal is important for the continuity of reproductive performance, and this can be implied clinically by the presence of ideal perineal conformation (PC)[2,3,4]. For an ideal PC in cows, anal sphincter and vulvar lips should be on the same line, and 80% of vulvar lips should form an angle of at least 80° below the baseline of the pelvic and horizontal plane[3].

If the vulvar lips angle is greater than 45° from the vertical plane, this vulva is called horizontal vulva; however, the other types are called vertical vulva[5,6].

Corresponding Author:
Emsal Sinem Özdemir Salci (ssalci@uludag.edu.tr).

Because of different factors such as increasing number of parturitions, perineal lacerations etc., if the vulvar lips and hymeneal sphincter is not sufficiently closed and the air fills into the vagina, this condition is called pneumovagina[3,6,7]. Urovagina is the collection of urine in the cranial part of the vaginal canal due to vesico-vaginal reflux[5,8]. Although urovagina is considered as a different pathology, it is also presented in severe pneumovagina cases[6].

In cows, pneumovagina is formed, if the angle of the upper vulvar commissure with the ischial arch is greater than 20 degrees; thus, the vulvar angle measurements are important both diagnosis and evaluation of the pneumovagina cases[3,6]. Particularly, in cows with pneumovagina, this angle is inclined to 40.2%, and it is reported that only 7.3% of healthy cows have this inclination. In cows with severe pneumovagina, there is a marked change in the PC. In these cows, the anus is inclined towards cranially, and the vulva is over-angled, and perineal atrophy is seen with a rate of 6.7% due to connective tissue weakness. In addition, the vulva is longer longitudinally in cows with pneumovagina[6].

The air in the vagina leads to vaginal irritation in cows with pneumovagina. Depending on the straining induced by this irritation, either during the passive movements of the cow or when abdominal relaxation occurs, air enters to vagina and exits from the vulvar lips spontaneously[7]. This condition causes drying in vaginal mucosa, which makes the vaginal mucosa susceptible to the infections including vaginitis, cervicitis and endometritis[1,3].

According to our best of knowledge, in the literature, there was no study in which PC was scored and investigated cytological, microbiological and histopathological changes of the genital tracts using this scale in cows. Therefore, this presented study aimed to investigate cytological, microbiological and histopathological findings of the genital tracts in cows with different degree PC disorder.

MATERIALS AND METHODS

This study was approved by Bursa Uludag University Animal Experiments Local Ethics Committee (Decision no: 2017-07 / 05).

Cows

A total of 28 cows (different age and breed) were materials of the study, which were brought to a slaughterhouse for slaughtering. Perineal region of these cows were evaluated under four groups according to PC scale as given in **Table 1** [group I (GRI) (n=7) normal; group II (GRII) (n=7) mild; group III (GRIII) (n=7) moderate and group IV (GRIV) (n=7) severe]. A body condition score (BCS) was determined in cows based on the 1-5 scale as reported previously[9].

History of the cows and PC scoring

Information about to age, body weight, reproductive status, number of parturitions, days in milk (DIM) and the reason for slaughtering of the cows was obtained from the owners. A routine clinical examination was performed, and the measurements of the perineal region including vulvar angle, vulvar length over ischial arch, depth of anus and perineal length was obtained with caliper, miter and ruler before slaughtering for scoring the PC. Following the measurements, the cows were included in their group according to the PC scale.

Clinical examinations

The perineal region was inspected in terms of vaginal discharge, anatomical structures of the vulva and any pathological changes around vulvar region. In the examination of the vagina with the speculum, mucosal appearance, presence and character of the vaginal discharge (serous, seromucous, mucous, foamy, purulent and urine mixed) were noted. The diagnosis of the pneumovagina was made according to the presence of vaginal air absorption and/or outflow by per-rectal vaginal pressure. Pneumovagina was classified as negative, suspicious and positive. While the presence of vaginal air was uncertain in suspicious cases, severe vaginal air was present in positive cases.

Macroscopic examinations

After taking sterile swap samples for cytological and microbiological examination, the presence of any macroscopic pathology related to vagina, cervix and uterus was evaluated, and any existing lesions were noted. In addition, the presence and character of the fluid in the vagina, cervix and uterus were recorded.

Microbiological examinations

Sterile swaps were used to collect samples from vagina, cervix and uterus. Vaginal samples were taken before the clinic vaginal examinations. The cervix and uterus samples were obtained immediately following to slaughter of the cows after opening of the cervix and uterus lumens with a sterile scalpel. All samples were taken to the microbiology laboratory and processed within 2 hours. The samples were cultured for bacteria and yeasts with 3 pair 5% defibrinated sheep blood and incubated at 37°C. After incubation for 24-48 h, plates were examined for growth. Colonies are stained with Gram stain. BBL™ Crystal™ biochemical tests were used for identification of colonies according to instructions of manufacturers.

Cytological examinations

Smear preparations were prepared from the samples of vagina, cervix and uterus and then they were fixed in methanol after air-drying. Following fixation, the preparations were stained with Diff-Quick. All preparations were evaluated under a light microscope (Olympus®, CX41).

Histopathological examinations

Tissue samples taken from the vagina, cervix and uterus were fixed in 10% formaldehyde solution. Paraffin blocks were prepared by going through routine tissue follow-up procedures. Sections taken from paraffin blocks with a thickness of 5 µm were stained with hematoxylin-eosin (H&E) and examined under a light microscope (Olympus®, CX41) for presence of pathological changes.

Statistical analysis

The means and standard deviations of the obtained metric results of the cows in groups were calculated.

In order to investigate any association between the selected traits and reproductive parameters in cows, multiple linear regression analysis was applied. In this context, the evaluation was performed in three steps. Initially, the best subsets regression analysis was performed to compare all possible models with respect to a specified set of predictors and to identify the best-fitting models related to the present study. The results provides the information to choose among candidate models with strongest fit for each number of predictors and the model giving the largest adjusted explained variance (R^2 adjusted)[10]. The two-way interactions between the parameters were added to the model and tested for significance. The assessment was maintained until achieving the R^2 value, which do not increase. As a final step, ultimate analysis with the selected variables determined by subsets regression was performed, and the results were expressed as regression coefficients and the corresponding statistical significance.

Correlation coefficients were estimated using the Pearson's correlation test. Correlations were classified into three groups according to levels of Pearson's correlation coefficients (PCC) generated from the analysis: low correlation (PCC<0.30), intermediate correlation (PCC=0.30-0.70), and high correlation (PCC>0.70). The differences between the groups generated by the evaluation of parameters including degree of vulvar angle, vulvar length over ischial arch, depth of anus, and perineal length (as shown in Table 1) were determined by the two-sided Fish-

Table 1 - Criteria of the perineal conformation, and grouping of the study.

Parameters	Normal (group I)	Mild (group II)	Moderate (group III)	Severe (group IV)
Degree of vulvar angle	0-10	10-30	30-55	> 55
Vulvar length over ischial arch (cm)	0-1	1-2.5	2.5-4	> 4
Depth of anus (cm)	< 4	4-6	6-8	> 8 cm
Perineal length (cm)	> 4	< 4	< 4	< 4

er's exact probability test. The statistical analyses were carried out with Minitab version 19.1.1 (Minitab, Inc., State College, PA, USA). For all statistical comparisons a probability level of $p<0.05$ was accepted as statistically significant.

RESULTS

Findings of the cows classified as PC are given in Table 2.
The cows in the study were Holstein (n=26) and Simental (n=2) breeds. Age, body weight, number of parturitions, DIM, the reason for slaughtering and BCS of the cows are given in Table 3.

Reproductive status of the cows

In GRI, all cows were open and their DIMs were >100 days. Only 6th and 7th cases in this group were repeat breeder cows. All cows in the GRII were open and the other cows' DIMs were >100, except for case 1 (DIM between 60-100 days). The 5th and 7th cases in this group were repeat breeder cows. The cows in the GRIII were open and the DIMs of the cows, except for the 6th case (DIM 60-100 days), were >100 days. The 1st case in this group was repeat breeder. The cows in GRIV were open and their DIMs were >100 days. The 6th and 7th cases in this group were repeat breeder cows (Table 3).

Clinical findings

In the inspection of the perineal region, no pathology was observed on the anatomical structures of the genital organs of the cows in groups. In the mucosal examination of the vagina, 1st and 2nd cases of GRI; 1st, 3rd and 7th cases of GRII; all cases of GRIII and GRIV had hyperemic appearance. Seromucous and serous vaginal discharge were detected in 1st and 2nd cases of GRI, respectively. Foamy mucous vaginal discharge was present in 3th case of GRII. In GRIII, foamy seromucous in the 2nd case and mucous discharge in the 1st, 6th and 7th cases were observed. Vaginal discharge characters encountered in GRIV were purulent-urine mixed in the 1st case, seromucous-urine mixed in 2nd case, and mucous in 4th case.
Pneumovagina was only observed in the cases of GRII, GRIII and GRIV; however, pneumovagina was suspected 2nd, 4th, 6th and 7th cases of GRII, because presence of the vaginal air was not clearly determined in these cases. In addition, 1st and 2nd cases of the GRIV were complicated with urovagina.

Macroscopic findings

Macroscopically, no pathological findings were found in the vagina and cervix of the cases in the groups. However, in GR I, purulent and serous discharge was observed only in the uterus of 1st and 2nd cases, respectively. In GRII, there was a mucous

Table 2 - Means and standard deviations of the parameters in the groups

Parameters	Normal (group I)	Mild (group II)	Moderate (group III)	Severe (group IV)
Degree of vulvar angle	1.85±3.28	19.42±5.28	38.14±5.33	67.28±9.35
Vulvar length over ischial arch (cm)	0.07±0.18	1.68±0.36	3.01±0.42	5.08±1.14
Depth of anus (cm)	2.05±1.57	4.92±0.76	6.58±0.49	9.85±0.95
Perineal length (cm)	5.12±0.76	4.07±0.52	3.27±0.76	2.64±0.82

Table 3 - Means and standard deviations of the parameters and the reasons for slaughtering of the cows in the groups.

Parameters	Normal (group I)	Mild (group II)	Moderate (group III)	Severe (group IV)
Age	6.01±1.52	5.07±1.78	5.64±1.10	5.85±2.11
Body weight	614.28±37.79	521.42±95.11	492.85±67.25	504.28±58.83
Number of parturitions	3±1	2.28±1.38	2.57±1.27	3.42±1.61
Days-in-milk	345.85±134.09	312±195.64	212.28±80.9	232.85±123.03
Reason for slaughtering	Infertility, udder problem, lameness	Infertility, udder problem	Infertility, lameness	Infertility, lameness
Body condition score	3.46±0.65	2.5±0.82	2.46±0.63	2.61±1.05

fluid in the uterus of the 3rd case. The uterus contents of the cases in GRIII were mucous in 3rd and 6th cases. The contents encountered in the uterus in GRIV were mucous in 2nd and 4th cases.

Microbiological findings

According to the culture results of the groups, the microorganisms obtained from the vagina, cervix and uterus are listed below.

In GR I, microbiological isolations were *Escherichia coli* (1st, 2nd, 5th and 6th cases) from vagina; *E. coli* (1st case), *Enterococcus spp.* (1st case), *Gardnerella vaginalis* (1st case), *Proteus mirabilis* (5th case) and *Aeromonas spp.* (6th case) from cervix; *E. coli* (1st case), *Gardnerella vaginalis* (6th case), *Proteus mirabilis* (1st case) and *Aeromonas spp.* (3rd case) from uterus.

In GR II, microbiological isolations were *E. coli* (1st, 2nd, 3rd, 5th, 6th and 7th cases), *Enterococcus spp.* (6th case) and *Gardnerella vaginalis* (7th case) from vagina; *E. coli* (1st, 2nd, 3rd, 4th, 6th and 7th cases) from cervix; *E. coli* (2nd, 4th and 7th cases), *Klebsiella* spp. (1st case) and *Enterobacter* spp. (3rd and 4th cases) from uterus.

In GR III, microbiological isolations were *E. coli* (1st and 5th cases), *Klebsiella* spp. (1st case), *Candida* spp. (2nd case) and *Staphylococcus* spp. (7th case) from vagina; *E. coli* (1st, 2nd, 3rd and 4th cases), *Enterococcus* spp. (2nd case), *Proteus mirabilis* (5th case) and *Klebsiella* spp. (6th and 7th case) from cervix; *E. coli* (1st, 2nd, 3rd, 4th, 6th and 7th cases), *Aeromonas spp.* (4th case) and *Klebsiella* spp. (5th case) from uterus.

In GR IV, microbiological isolations were *Streptococcus bovis* (1st case), *E. coli* (1st, 2nd, 3rd, 4th and 5th cases), *Enterobacter* (3rd case), *Aeromonas* (6th case) and *Proteus mirabilis* (7th case) from vagina; *Klebsiella* spp. (2nd case), *E. coli* (3rd, 5th and 6th cases) and *Enterococcus spp.* (7th case) from cervix; *E. coli* (in all cases) from uterus.

Cytological findings

In GRI, only 1st case was cytologically positive, and plenty of polymorph nuclear cells (neutrophils) were seen in the examination of the samples taken from the vagina, cervix and uterus. No sign of inflammation was determined in the examinations of the samples of vagina, cervix and uterus taken from all cases in GRII. Cytological examinations in GRIII revealed numerous epithelial cells in uterine samples of 4th case, severe polymorph nuclear cell infiltration in vaginal samples of 6th case, and severe inflammatory cells in vagina (Figure 1) and uterine (Figure 2) samples of 7th case. In GRIV, a small number of neutrophils and lymphocytes in the vaginal samples of the 1st case. And in 4th case, only a few lymphocytes were found in the samples taken from uterus.

Histopathological findings

In GRI, epithelial desquamation in vaginal mucosa, and diffuse, moderate mononuclear cell infiltration in lamina propria were observed in 1st case, which was indicating to signs of vaginitis. Diffuse, moderate mononuclear cell infiltrations were detected in the lamina propria of cervix (cervicitis). In the uterus, desquamation of mucosal epithelial cells, and diffuse, severe mononuclear and polymorph nuclear cell infiltrations in lamina propria were noted, which was pointing out signs of endometritis (Figure 3). In 2nd case, only in the cervix, mild mononuclear cell infiltration in the lamina propria was present.

In GRII, focal, mild mononuclear cell infiltration was observed in the vaginal mucosa of 2nd and 7th cases, which was compatible with vaginitis. In 1st, 2nd, 3rd, 4th and 7th cases, desquamation in the mucosal epithelium, focal, multifocal or diffuse, mild to moderate mononuclear cell infiltrations were noted in the lamina propria of the uterus (endometritis).

In GRIII, chronic, granulomatous vaginitis findings (central caseification necrosis and calcification, macrophages and Langhans type multinucleated giant cells surrounded by connective tissue) were observed in 2nd case (Figure 4). In 3rd, multifocal, mild to moderate mononuclear cell infiltrations in the lamina propria of the cervix and uterus were noted. These findings pointed out the cervicitis and endometritis in this case. In 4th case, degeneration and desquamation in the glandular ep-

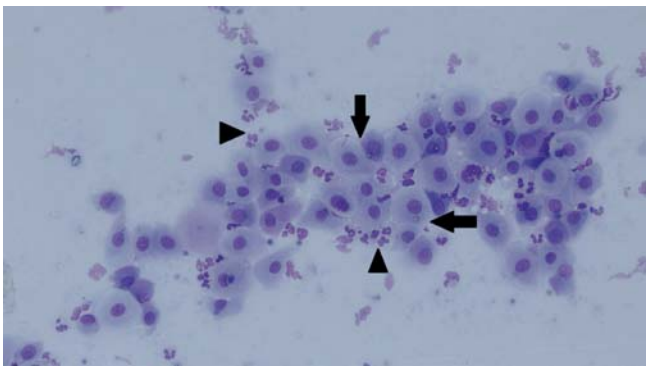

Figure 1 - This cytological view of case 7 in GRIII points out polymorph nuclear cells (arrow heads) and epithelial cells (arrows), which is obtained from vagina, (Diff-Quick, X 200).

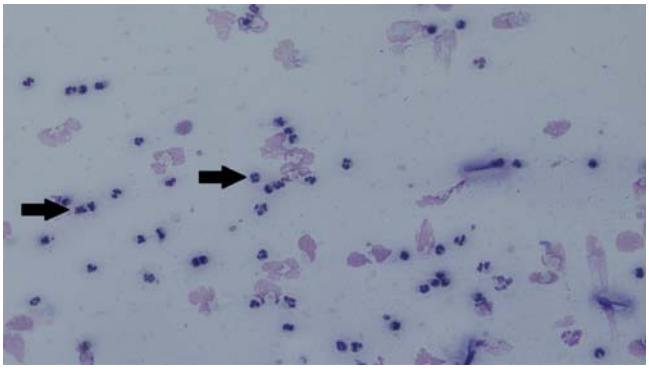

Figure 2 - Arrows demonstrate polymorph nuclear cells on cytological view of uterus in case 7 of GRIII, (Diff-Quick, X 200).

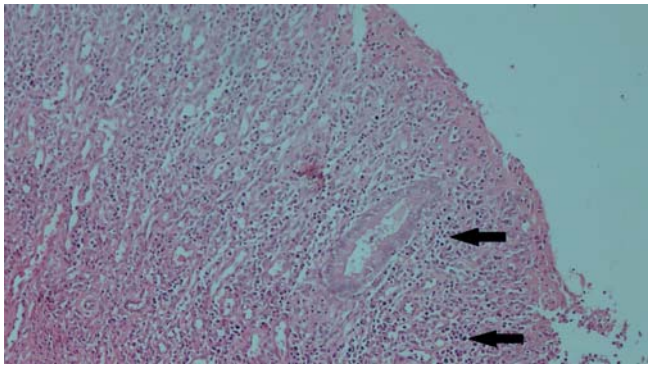

Figure 3 - This microscopic view shows the endometritis in case 1 of GRI: mononuclear and polymorph nuclear cell infiltrations (arrows) in the lamina propria of uterus, (H&E, X 100).

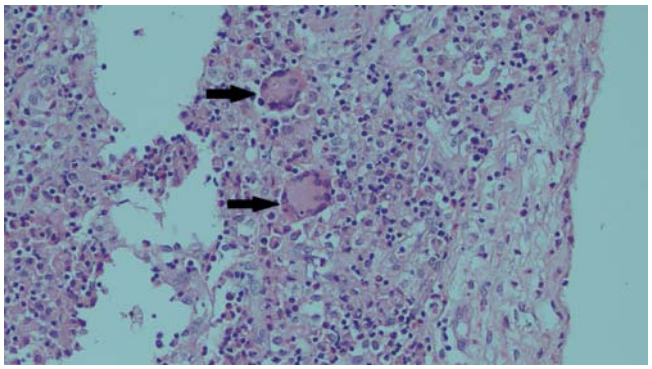

Figure 4 - Chronic granulomatous vaginitis finding of the case 2 in GRIII. Arrows points out Langhans type multinucleated giant cells, (H&Eosin, X 200).

ithelium, diffuse, severe mononuclear cell infiltrations and periglandular fibrosis were observed in the lamina propria of the uterus. In 6th case, a large number of free erythrocytes were found in the lamina propria and submucosa in the uterus. In addition, abundant iron-laden macrophages (hemosiderinophage) were noteworthy in these areas. In 7th case, hyperplasia in the epithelial layer of the vaginal mucosa, and diffuse, severe and mostly mononuclear cell infiltrations in the lamina propria were observed.

In GRIV, in 1st case, multifocal, severe mononuclear cell infiltration was noted in the lamina propria and submucosa of the vagina (vaginitis). In 2nd case, mild mononuclear cell infiltration was observed in the lamina propria of the vagina and cervix, and there were also multifocal to diffuse mononuclear cell infiltrations in the periglandular areas and lamina propria of the uterus. Diffuse moderate mononuclear cell infiltration was present in the uterus of 4th case. Focal, mild mononuclear cell infiltrations were observed in the vagina and cervix of case 7.

Statistical analysis findings

Regression coefficients and statistical significance of parameters (number of parturition, BCS, body weight, DIM and age on vaginal mucosa color, vaginal discharge, histopathology, cytology, microbiology and macroscopic discharge in the uterus) were given in Table 4.

Correlations:

There is a positive correlation between vaginal discharge and vaginal cytology (R=0.548, p=0.003), cervical histopathology (R=0.431, p=0.022) and the presence of macroscopic discharge in the uterus (R=0.775, p=0,000).

A positive correlation was observed between vaginal cytology and vaginal histopathology (R=0.420, p=0.026), cervical cytology (R=0.471, p=0.011) and uterine cytology (R=0.533, p=0.003).

Table 4 - Regression coefficients and statistical significance (p) of the parameters.

Parameter		NP	BCS	BW	DIM	Age	NP × BCS	BCS × DIM
Vaginal Mucosal Color		+0.298	+0.28		-0.001569		-0.135	
	p	0.17	0.277		*0.005		*0.032	
Vaginal Discharge		+0.077		0.00016	-0.001620	-0.132		
	p	0.626		0.885	*0.043	0.3		
Vaginal Histopathology		-0.231	+0.067	-0.00151	+0.00170	+0.054	+0.054	-0.000642
	p	0.673	0.853	0.472	0.941	0.751	0.556	0.369
Vaginal Cytology		+0.001	+0.088	-0.00074	+0.00027	-0.097	+0.0449	-0.000355
	p	0.383	0.489	0.628	0.308	0.442	0.503	0.494
Vaginal Microbiology		+0.251	+0.025	+0.00151	+0.00008	-0.086	-0.096	-0.000168
	p	0.938	0.177	0.436	0.588	0.661	0.262	0.796
Cervical Histopathology		-0.004	+0.539	-0.00097	+0.00197	+0.115	-0.0251	-0.001032
	p	0.642	0.324	0.566	0.253	0.408	0.732	0.080
Cervical Cytology		-0.025	-0.037	+0.000489	-0.000181	+0.0143	+0.0008	-0.000004
	p	0.788	0.723	0.592	0.637	0.847	0.983	0.989
Cervical Microbiology		+0.134	+0.066	-0.00137	+0.00040	+0.056	-0.0652	+0.000093
	p	0.801	0.67	0.489	0.459	0.73	0.452	0.889
Uterine Histopathology		+0.0476		-0.003	+0.000522			
	p	0.5		*0.021	0.465			
Uterine Cytology		-0.141	-0.31	+0.00039	-0.00086	-0.142	+0.0901	+0.000127
	p	0.538	0.921	0.833	0.545	0.359	0.276	0.840
Uterine Microbiology		+0.0439	-0.0029	+0.045				
	p	0.541	*0.036	0.797				
Macroscopic Uterine Discharge		-0.0022	+0.031		-0.001441			
	p	0.973	0.762		*0.023			

NP: Number of parturitions, **BCS**: Body condition score, **BW**: Body weight, **DIM**: Days in milk, *: Statistically significant effect observed in the regression model. Models were selected according to the best subsets regression analysis.

There is a positive correlation between cervical histopathology and cervical cytology (R=0.413, p=0.029) and the presence of macroscopic discharge in the uterus (R=0.592, p=0.001), cervical microbiology and uterine microbiology (R=0.503, p=0.006).
There is a positive correlation between the presence of macroscopic discharge in the uterus and uterine histopathology (R=0.0549, p=0.002).
There is a negative correlation between body weight and uterine histopathology (R=-0.430, p=0.022) and uterine microbiology (R=-0.496, p=0.0007).

Comparisons between groups:

There was a statistically significant difference between the groups in terms of vaginal mucosa color (p=0.004), cervical microbiology (p=0.032) and uterine microbiology (p=0.006).
As PC disorder became more severe, the contamination of the cervix (p=0.032) and the uterus (p=0.006) with bacteria of fecal origin increased. Moreover, the colors of the vaginal mucosa were more hyperemic (p=0.004).

DISCUSSION

Reproductive problems and infertility cause economic losses in dairy cows[7,11]. Reproductive problems in cows are multi-factorial, and although endometritis and metritis are the most important pathological conditions of cows' reproductive tracts, vaginitis and cervicitis should be evaluated to determinate the cause of the infertility[6,12,13]. Bacterial contaminations of the reproductive tracts lead to vaginitis and cervicitis, which are resulted from PC disorder, pneumovagina, urovagina and traumatic recto-vulvar injuries, and then this bacterial contamination result in ascending infective uterine pathologies that responsible to early embryonic death, placentitis, abortion and premature newborns[6,8,14]. PC disorder can be congenital or acquired, and it results from perineal atrophy and rectovaginal lacerations in various degrees, which predispose the cows to endometritis and other genital canal diseases[6]. According to our literature review, there is no study where PC has been classified in cows; thus, in this study, PC has been classified in the cows with different parameters (degree of vulvar angle, vulvar length over ischial arch, depth of anus and perineal length). The results obtained by clinical examinations (body condition, pneumovagina, vaginal inspections, etc.) and the cytological, microbiological and histopathological findings of the vagina, cervix and uterus in cows to be slaughtered were grouped according to this classification.
As mentioned above, PC disorder may occur due to some general reasons including age, number of parturitions and low body condition score[6,8]. Abnormalities of the pelvic angle and cranioventral displacement of the vulva following parturition are the other causes of the PC disorder[4,6,8]. PC disorder is a reason of the infertility; thus, considering to the vulvar angle (VA) and vulvar length, surgical correction could be planned on the perineal region to prevent diseases (pneumovagina, urovagina and etc.) in horses[2,15,16] and in cow[3,8]. As stated above, in this study, a scale was determined using perinal region conformation such as vulvar angle, vulvar length over ischial arch, depth of anus, and perineum length in the evaluation of pneumovagina, urovagina and the other infectious diseases, which cause infertility in cows.

Different diagnostic methods including vaginal examination, cytology and biopsy have been used to evaluate healthy status of the genital tracts[13,17,18]. In cases of pneumovagina, air suction to the vagina and fecal contamination of the genital tracts is possible[11]. The diagnosis of pneumovagina is made by the detection of the airflow into the vagina (or outflow from the vagina) and the presence of foamy vaginal discharge or mucosal hyperemia in the examination of the vagina with the speculum[1,6]. Evaluation of the vaginal secretions is not always a reliable diagnostic method for all parts of genital canal infections, because vaginal, cervical and uterine disease can be seen together or alone[13]. If there is a microbial contamination, purulent vaginal discharge can be seen in clinical examination[7]. On the other hand, in a slaughter study, it is reported that mucous, bloody mucous, mucopurulent and hemorrhagic character vaginal fluids do not always pointed out the uterine infections[13]. In this presented study, while serous and seromucous vaginal discharges were observed in cows with normal PC, the presence of foamy and mucous character discharge was determined in GRIII and IV due to the presence of pneumovagina, because PC disorder was more severe in these groups. Particularly, in cases had severe PC disorder (pneumovagina and urovagina), there were purulent and mucous discharges mixed with urine. But, statistically no difference was found in groups.
Hyperemic appearance in the vaginal mucosa was also more common in cases with severe degree PC disorder. Depending on the urine accumulated in the vagina, inflammation in the cervix and vagina, and endometritis may be formed by contact of the cervical urine accumulated[4,5,8]. If vesicovaginal reflux is permanent, endometritis may result in periglandular fibrosis[5]. Urovagina accompanied by pneumovagina, causes endometritis, leading to infertility problems in cows[4,5,8]. Macroscopically, as in the presented study (case 1 in GRI), it is possible to encounter genital canal pathologies in cows with normal PC. Because PC disorder became moderate and severe in GRIII and GRIV, macroscopically different contents (purulent, serous, mucous, etc.) were encountered in the uterus. Moreover, macroscopic findings in the uterus were compatible with microbiological and histopathological findings.
The genital system microflora in cattle shows a very dynamic structure consisting of aerobic, facultative anaerobic and anaerobic bacteria[19,20]. Vagina also forms a natural habitat for many saprophytic microorganisms that can be opportunistic pathogens[21]. Microbiologically, different species has been reported in the genital canal of cows. Particularly, reported bacteria are in vagina; *Bacillus spp., E. coli, Enterococcus, Pediococcus, Lactobacillus*[20], in cervix; *Enterococcus faecalis*[13] and in uterus; *E. coli, Streprococcus bovis, Streprococcus uberis, Enterococcus* spp., *Proteus mirabilis, Aerococcus spp., Trueperella* spp. *Klebsiella* spp., *Moraxella* spp., *Staphylococcus* spp.[6,11,22]. Additionally, *Enterobacteriaceae* are the dominant microorganisms in the vaginal flora[20]. In the presented study, only *E. coli* was seen in the vagina of cows with normal PC (GRI). In the cervix and uterus, *E.coli, Enterobacter* spp, *Proteus* spp, *Aeromonas* spp. and *Gardinella vaginalis* were encountered.
Ascending bacterial infections of the reproductive tracts are most important causes for infertility in cows, and uterine contaminant bacteria are nonspecific and belong to a great number of bacterial species[11,13]. In cases where pneumovagina is complicated with urovagina, the genital canal becomes susceptible

to secondary infections[4,5,8]. Moreover, if there is a failure of physical barriers such as pneumovagina and urovagina, pathogen bacteria introduce the uterus[11]. It is reported that the number of fecal origin bacteria is higher in the uterus of pneumovagina cows[6,11].

E. coli, Streptococcus bovis, Streptococcus equinus and other different bacteria have been identified in the bacteriological examination of samples taken from the uterus in cows with pneumovagina[11]. *Enterococcus* spp., *Staphylococcus* spp. and *E. coli* are the other detected genital canal microorganisms in slaughtered cows[13].

In this study, as the degree of PC disorder increased in cows, microorganism flora difference was observed in the cervix and uterus. Most of these microorganisms were of fecal origin. In cows with moderate and severe PC disorder (GRIII and GRIV), the frequency of microorganism in the genital canal was higher than the cows with normal PC due to the effect of possible pneumovagina.

The dominant microorganism seen in cows with PC disorder was *E. coli* from *Enterobacteriaceae* family.

Bacteriological examination of the genital canal cannot provide meaningful and reliable information unless it is evaluated together with cytological and histological examination[23]. Cytological examination is an effective, easy-to-perform and reliable method in terms of clinical practice[18,23]. However, cytological examination of the genital tracts is not commonly used method in the diagnosis of the reproductive disorders of the cows[12,18]. Cell types encountered in cytological examination are; epithelial cells, granulocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils[12,13,17,18]. In a post-mortem study, it has been reported that vaginitis, cervicitis and endometritis are detected according to cytological and histopathological findings[13].

In the presented study, polymorph nuclear cell infiltration and inflammation cells were evaluated histopathologically. Since cytological findings are not sufficient in the evaluation for PC disorder, cytological findings should be evaluated together with microbiological and histopathological findings. In the cytological and histopathological examination of the uterus, findings such as mononuclear cell infiltration are important for the diagnosis of endometritis[17,18].

However, for the definitive diagnosis of endometritis, endometrial biopsy, which is not applicable in clinical conditions, should be preferred[17,18,23]. Since the presented study was carried out on the post-mortem material, the examination of the genital canal can be done easily by histopathological examination. When the cases in the groups were evaluated, vaginitis and endometritis were more prominent in the cows with PC disorder. Therefore, it was obvious that histopathological examination gave more meaningful results.

CONCLUSIONS

In conclusion, PC disorder in cows is predisposing factor for many genital canal diseases that may cause of infertility. As planned in this study, the presence of genital canal diseases can be suspected in cattle using the PC scale.

Therefore, it is appropriate to evaluate the health status of the genital canal with other diagnostic methods (microbiology, cytology and histopathology), especially in cows with PC disorder.

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References

1. England. G.C.W. (2005). Fertility and obstetrics in the horse. 3rd ed., 117-120, Blackwell Publishing, UK.
2. Robinson N.E., Sprayberry K. (2009). Current therapy in equine medicine. 6th ed., 764-765, Saunders, USA.
3. Dehghani S.N., Yavari M., Kafi M. (2011). Treatment of pneumovaginitis in dairy cattle by caslick operation. Res Opin Anim Vet Sci, 1(6): 349-351.
4. Youngquist R.S., Braun W.F.Jr. (1993). Abnormalities of the tubular genital organs. Vet Clin North Am Food Anim Pract, 9(2): 309-322.
5. Gautam G., Nakao T. (2009). Prevelance of urovagina and its effect on reproductive performance in Holstein cows. Theriogenology, 71:1451-1461.
6. Goncagul G., Seyrek Intas K., Kumru I.H., Seyrek Intas D. (2012). Prevalence and accompanying signs of pneumovagina and urovagina in dairy cows in the Southern Marmara region. Tierärzt Prax Ausg G Grosstiere Nutztiere, 40(6): 359-366.
7. Demet Ö. (2010). İneklerde pnömovagina olgusu; önemli bir döl tutmama sorunu. Dicle Üniv Vet Fak Derg 1(1): 26-28.
8. Ay S.S., Fındık M. (2011). İneklerde ürovaginanın nedenleri ve sağaltım seçenekleri. Vet Hekim Der Derg 82(1): 63-68.
9. Ferguson J.D., Galligan D.T., Thomsen N. (1994). Principal descriptors of body condition score in dairy cattle. J Dairy Sci, 77: 2695-2703.
10. Chatterjee S., Simonoff J.S. (2013). Handbook of regression analysis. 30-34, John Wiley & Sons, USA.
11. Goncagul G., Seyrek-Intas K., Kumru I.H., Ozakin C., Ozdemir Salci E.S., Weiss R., Prenger-Berninghoff E. (2012). Bacterial infertility and ascending uterine infections with respect to pneumovagina and urovagina in cows. Res Opin Anim Vet Sci, 2(12), 583-586.
12. Brodzki P., Brodzki A., Kurek L., Marczuk J., Tatara M.R. (2015). Endometrial cytology at luteal and follicular phases of the ovarian cycle in cows. Ann Anim Sci, 15(1): 107-117.
13. Casarin J.B.S., Martini A.P., Trentin J.M., Fiorenza M.F., Pessoa G.A., Barros S.S., Rubin M.I.B. (2018). Bacteriological, cytological and histopathological evaluation of the reproductive tract of slaughtered cows. Pesq Vet Bras, 38(1): 53-58.
14. Pouret E.J.M. (1982). Surgical technique for the correction of pneumo- and urovagina. Equine Vet J, 14(3): 249-250.
15. Caslick E.A. (1937). The vulva and the vulvo-vaginal orifice and its relation to genital health of the thoroughbred mare. Cornell Vet, 27(2): 178-187.
16. Pascoe R.R. (1979). Observations on the length and angle of declination of the vulva and its relation to fertility in the mare. J Reprod Fertil Suppl, 27: 299-305.
17. Kasimanickam R., Duffield T.F., Foster R.A., Gartley C.J., Leslie K.E., Walton J.S., Johnson W.H. (2004). Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology, 62(1-2):9-23.
18. Prieto M., Barrio M. Quintela L.A. Perez-Marin C.C., Becerra J.J., Vigo M., Diaz C., Cainzos J., Prieto A., Fernandez F.I., Martinez D., Herradon P.G. (2012). Validation of a simple method for the interpretation of uterine cytology in cows. Vet Med-Czech, 57(7): 360-363.
19. Otero C., Saavedra L., Silva de Ruiz C., Wilde O., Holgado A.R., Nader-Macias M.E. (2000). Vaginal bacterial microflora modifications during the growth of healthy cows. Lett Appl Microbiol, 31(3): 251-254.
20. Wang Y., Ametaj B.N., Ambrose D.J., Ganzle M.G. (2013). Characterisation of the bacterial microbiota of the vagina of dairy cows and isolation of pediocin-producing Pediococcus acidilactici. BMC Microbiol, 13:19.
21. Panangala V.S., Fish N.A., Barnum D.A. (1978). Microflora of the cervico-vaginal mucus of repeat breeder cows. Can Vet J, 19(4): 83-89.
22. Fuentes B.M., Arias L.A.Q., Gonzales J.J.B., del Sol L., Feijoo J.E.M., Punal J.L.G., Lopez M.B., Lago A.P., Cao J.M.D., Rodriguez G.F., Herradon P.J.G., Martinez A.I.P. (2017). Agreement between postmortem endometrial cytology, biopsy and bacteriology in culled dairy cows. Anim Reprod, 14(4): 1024-1033.
23. Hemberg E., Lundeheim N., Einarsson S. (2005). Retrospective study on vulvar conformation in relation to endometrial cytology and fertility in Thoroughbreed mares. J Vet Med A Physiol Pathol Clin Med, 52(9): 474-477.