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Changes in the preputial pH values in canines and their relation to age, cytology and the occurrence of preputial discharge.

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1. General introduction

1.1. The canine preputium in general

The prepuce is a fold of skin that covers the free end of penis in the retracted state. It consists of internal and external laminas, which are continuous at the preputial orifice. The external lamina is the skin of the outer surface. The internal lamina has lymphoid tissue and modified sebaceous glands that secrete smegma that facilitates intromission of the penis into the female vagina (Konig & Liebich, 2009).

2. Literature review

2.1. The microflora of the canine preputium

According to the literature, the normal preputial flora consists mainly of aerobic bacteria that can also be isolated from the canine semen, as well as from dogs with bacterial prostatitis, orchitis and epididymitis. In comparison to aerobic bacteria, the anaerobic ones have not been described as normal inhabitants of the canine reproductive tract.

Osborne and Lees (1995) have reported that the normal microflora of the preputium in healthy dogs, only rarely causes urinary tract infections. Moreover, the physiological flora of the lower genitourinary system has a protective effect, both by inhibiting the growth of the pathogenic microorganisms, as well as by preventing the cellular adhesions.

In a study done by Saritas (2012) normal microorganisms were isolated from the preputium of healthy dogs and the results are shown in Table 1.

Bacteria	n	Percentage
Staphylococcus species	18	36
E. coli	15	30
Proteus species	8	16
Pseudomonas species	3	6
Streptococcus species	3	6
Corynebacterium species	1	2
Bacillus species	2	4
total	50	100

Table 1: Bacteria isolated from preputial swab samples of healthy dogs (Saritas et al., 2012)

They also studied the antimicrobial susceptibility of the microorganisms, which were isolated from the canine preputium. The most effective drugs for the treatment of genital infections in male dogs were reported to be danofloxacin, amoxicillin/clavulanic acid, cloxacillin, amoxicillin and ceftiofur (Saritas et al., 2012).

Another study by Bjurstrom and Linde– Forsberg (1992) about the aerobic bacteria of genital tract in stud dogs showed that there is a similarity between the bacteria isolated from the preputium of male dogs and the bacteria isolated from genital organs of female dogs, which shared the same shelter. The main bacteria found in bitches included Pasteurella multocida, β-hemolytic Streptococci and E. coli.

In their long term research they could isolate quite similar bacteria from the preputium of stud dogs. In this study 232 samples were taken from 15 dogs and the results revealed the following bacteria (Bjurstrom and Linde- Forsberg, 1992):

- Pasteurella multocida
- β– hemolytic Streptococci
- E. coli
- Coagulase negative Staphylococci
- Staphylococcus intermedius
- Streptococcus species
- Coryneforms
- Enterococci
- Pseudomonas species
- Proteus
- Mycoplasma species were present in 80% of dogs.
- No bacterial growth was observed in 14% of samples.

A study by Doig (1981) investigated the presence of Mycoplasma and Ureaplasma species in the genital flora of male dogs. They were able to recover Mycoplasma from 85% of preputial swabs and from 72% of semen samples.

In their study dogs were categorized into four different groups:

- Group A: 11 dogs with normal fertility
- Group B: 26 infertile dogs

    - Group B.1: 20 dogs with balanoposthitis (accompanied by purulent preputial discharge)
    - Group B.2: 6 clinically healthy dogs.
- Group C: 11 clinically healthy dogs, free of genital diseases, not used for breeding
- Group D: 13 male dogs with balanoposthitis, but without breeding history

All dogs were checked for the presence of Mycoplasma and Ureaplasma species and the results can be seen in Table 2.

Groups	Cultured samples	Mycoplasma +	Mycoplasma %	Ureaplasma +	Ureaplasma %
A	11	8	73.00%	0	0
B	26	24	92	18	69
C	11	7	64	1	9
D	13	12	92	1	15

Table 2: Mycoplasma and Ureaplasma flora of the canine preputium (Doig, 1981)

The isolation rates were statistically significantly different from fertile or clinically healthy dogs in comparison to infertile ones or those having genital diseases.

Ureaplasma species could be recovered from the preputium of 69% of infertile dogs and from none of the healthy ones (Doig, 1981).

Since Mycoplasma and Ureaplasma species are difficult to grow, it makes the interpretation of the culture more complicated.

Usually the Mycoplasmas are not differentiated by the species (Root Kustritz et al., 2005). However in this study the Mycoplasma species were further differentiated.

Among the different Mycoplasma species, Mycoplasma canis was the most frequent species isolated with the second one being M. cynos.

Other species that could be recovered were: M. maculosum, M. spumans, M. edwardii and M. molare, but to a lesser extent than in females.

M. felis, M. gateae, M. bovigenitalium were present in a few preputial samples but were absent in females (Doig, 1981).

In 24% of the samples from male dogs more than one Mycoplasma species were found.

The fact that Ureaplasma species were not isolated from the preputium of dogs with normal fertility (group A), suggests that Ureaplasma plays a role in canine infertility (Doig, 1981). Summarizing this, one can say, that Mycoplasma species can be isolated from both healthy, fertile dogs as well as from dogs with genital diseases and decreased fertility.
Ureaplasma species however, are more common in dogs showing some genital problems and cannot be considered as a part of a physiological flora of the canine genital tract.

2.2. Preputial cytology

Common indications for taking a cytological sample from the preputium are discharge from the preputial opening and masses on the preputial mucosa.
Normal preputial epithelial cells appear cuboidal to columnar with a healthy nucleus.
A few polymorphonuclear leukocytes as well as macrophages are usually present in cytological samples (Root Kustritz, 2006).
The physiological smegma of the dog can be described as followed: it has a typical appearance, with high numbers of neutrophils; low numbers of rod and coccoid shaped bacteria and usually no intracellular bacteria is seen (England & von Heimendahl 2010).
If an abnormal mass is found in the preputial cavity, the cytology is evidently changed. In case of round cell tumors, like Transmissable Veneral Tumor (TVT) exfoliation is often observed. Functional Sertoli tumors that lead to an elevation in the estrogen concentration in the serum cause cornification of the preputial epithelial cells. The cytology in this case resembles a vaginal cytology sample taken from a bitch during estrus. The estrus stage of a bitch is characterized cytologically by at least 80% cornification with greater than 50% anuclear squame cells (Root Kustritz, 2006).
The epithelial cells of the vagina of a bitch can be further differentiated as (Dreimanis et al., 2012):

- Cornified cell types:
    - **Superficial cells:**
      Large (40-75 micrometer), dead epithelial cells with pyknotic nucleus. High amount of cytoplasm can be seen. The shape of the cells is folded and angular

- **Anuclear squame cells:**
  Stratified squamous epithelial cells degenerated into large, flat dead cells.

- Non– cornified cell types:
  - **Parabasal cells:**
    Small (10-20 micrometer) round cells with small amount of cytoplasm. The nucleus is round and vesiculated.
  - **Intermediate cells:**
    The cells are larger than the parabasal cells (small intermediate cells are 20-60 micrometer and big intermediate cells having the size of the cornified cells, 40-75 micrometer). The shape of the cells is round and the nucleus is round and vesiculated.

2.3. pH and microflora of the seminal fluid

The microflora of the preputial mucosa can appear in the seminal fluid, together with the normal inhabitants of penile mucosa and distal urethra, thus making the semen non sterile. The pH of dogs seminal fluid ranges between 6.3 and 6.7 (Johnston et al., 2001) which corresponds to the mean value of the preputial pH of the samples we took.

In a study done by Root Kustritz (2005) the relationship between significant bacterial cultures of the canine seminal fluid and cytology types, which were differentiated as inflammatory or non-inflammatory, was examined. In their study 95 dogs were checked in total for clinically meaningful bacterial growth and the assessment of the cytology.

The results showed that 78 dogs (82.1%) had meaningful growth of aerobic and anaerobic bacteria or Mycoplasma species.

Mycoplasma species were the most frequently isolated microbial organism in this study, but they were not further classified by their species type.

The isolated aerobic bacterial species from the seminal fluid were similar to the species that were isolated from the preputial cavity in other studies (Bjurstrom & Linde- Forsberg, 1992; Saritas et al., 2012) mentioned earlier and include the following:

- β-hemolytic Streptococcus species : 9
- Pasteurella multocida: 6
- β-hemolytic E. coli: 6

- Non-hemolytic E. coli: 6
- β-Streptococcus species: 5
- Achromobacter xylosoxidans: 1
- Actinomyces pyogenes: 1
- Bacillus species: 1
- coagulase positive Staphylococcus species: 1
- Hemophilus hemoglobinophilus: 1
- Klebsiella pneumoniae : 1
- Proteus mirabilis :1
- Pseudomonas aeruginosa: 1
- Staphylococcus intermedius: 1

The results of the cultured samples and the cytology can be seen in Table 3.

Meaningful growth	Number of samples with meaningful growth	Inflammatory cytology : number of samples	Non-inflammatory cytology : number of samples
Aerobic bacteria	27	16	11
Mycoplasma	55	21	33
Anaerobic bacteria	13	6	7
No growth	17	5	12

Table 3: Meaningful bacterial growth and the cytology of the seminal fluid (Root Kustritz et al., 2005)

The study showed that there is an association between the meaningful bacterial growth in the seminal fluid and the inflammatory cells (87.5% - true positive).
The results showed as well that 78.2 % of the samples, which showed meaningful bacterial growth, had non-inflammatory cytology (false negative) and for this reason cytological examination of the seminal fluid cannot be used for reproduction evaluation of male dogs. Microbial culture from seminal fluid should be performed, regardless of the cytology during the fertility examination of the male dogs.

The high number of the samples showed meaningful bacterial growth (82.1%), and that corresponds to the fact that the presence of aerobic bacteria and Mycoplasma species in canine lower genitourinary tract can be regarded as normal.

2.4. Disorders of the canine preputium

2.4.1. Balanophostitis

Posthitis terms the inflammation of the preputial mucosa and can occur on its own, or following an inflammation of the glans penis, the so called balanitis. Posthitis follows more frequently an already existing balanitis and often develops into a combination of both, which is then termed Balanoposthitis (England & von Heimendahl, 2010).

Until now no breed predisposition was reported and the age dependence varies according to different literatures. While the BSAVA manual states that balanoposthitis mainly affects dogs over 4 years of age other sources (Johnston et al., 2001) suggest that dogs up to 4 years of age are the main target group.

Mild Balanoposthitis is a very common condition in dogs, but except for the presence of the seropurulent exudate in the preputium, usually no other clinical signs can be observed and it only rarely serves as an indication for clinical diseases (Noakes et al, 2001).

The causes for posthits or balanoposthitis are numerous. The majority of the cases are caused by opportunistic infections with bacterial or viral agents, although they may be a part of the physiological flora of the preputium as well (Johnston et al., 2001). Other reasons include the following (England & von Heimendahl, 2010):

- Concurrent cystitis
- Urethritis
- Prostatitis
- Trauma
- Bacterial sepsis
- Lack of mucosal immunity from immunosuppressive conditions
- Atopic dermatitis
- Behavioral self- mutilation
- Rarely infection with Canine Herpes Virus (CHV) and Blastomyces.

Regarding the clinical signs, the main feature is a purulent preputial discharge, which can vary from scant white smegma to copious green pus. In some cases even bloody discharge can be observed (Nelson and Couto, 2009).
During clinical examination, a foul odor from the genital area can be detected and the manipulation of the penis is usually associated with pain, which therefore also leads to a loss of interest in bitches in case of breeding dogs.
A low degree of inflammation of the preputuial mucosa can develop into a pathological inflammation with erythematous and hyperemic mucous membranes which may be covered by caseous material (England & von Heimendahl, 2010).
In more severe cases erosions, plaques or even ulcerations can be found on the mucosal surfaces.
Penile follicles may show lymphoid hyperplasia, especially in viral balanoposthitis.
If the balanoposthitis is caused by atopic dermatitis, the dog may show concurrent pruritus on feet, ears or ventrum (Johnston et al., 2001).
The physical examination of the penis and preputial cavity is usually enough to make a first diagnosis. Inflammatory nodules, foreign materials, neoplasms and ulcerations are easily detected by the naked eye.
Samples of the discharge might be taken and cultured, but the interpretation of the results is rather difficult. The normal bacterial flora of the preputium makes it difficult to differentiate between physiological and pathological bacteria (Johnston et al., 2001).
Cytological examination is frequently used in order to establish a proper diagnosis. In case of balanoposthitis, bacteria and neutrophils are seen on the slides. As for the bacteria, heavy overgrowth of a particular type is usually revealed and in case of neutrophils both degenerate and non-degenerate forms with intracellular bacteria are seen on the slide.
If canine herpes virus is suspected, paired serum samples for titer detection or virus isolation is used to confirm or out rule the suspicion (Johnston et al, 2001).
As far as the treatment is concerned, prevention plays an important role in controlling balanoposthitis. Excessive licking of the prepuce should be eliminated and hair should be clipped around the preputial orifice to prevent contamination and irritation of the mucous membranes (England & von Heimendahl, 2010).

Flushing of the preputial cavity with antiseptic solutions, like chlorhexidine or povidone-iodine and installing topical antibacterial drugs into the preputial cavity may help to decrease the inflammation (Nelson and Couto, 2009).
Prophylactic treatment of mild infections should be discouraged when requested by the owners, as the use of antiseptic or antibacterial preparations can lead to opportunistic infections by organisms of clinical significance, due to the loss of the normal bacterial flora in the preputial cavity (England & von Heimendahl, 2010).
In case of Mycoplasma infection, enrofloxacin is the drug of choice to eliminate the bacteria (Johnston et al., 2001).
Balanoposthitis due to atopic dermatitis can be treated with antihistamines or corticosteroids. If the background of the disease is behavioral, the trigger for the behavior should be eliminated or antianxiety drugs can be used (Johnston et al., 2001).
Castration is usually decreasing the amount of the preputial discharge.
CHV doesn't require any special treatment, as the clinical signs are usually mild and self-limiting (Johnston et al., 2001).

2.4.2. Neoplasms

In some cases male dogs are presented with an abnormal mass seen on the penis or preputial area, or an abnormal mass is detected during clinical examination. The most frequent neoplasms of the penis and prepuce are (Valenciano and Cowell, 2014):

- Round cell tumor: Transmissible Venereal Tumor
- Sertoli cell tumor: It doesn't originate from the preputium, but has an effect on its appearance and on the cytology taken from the preputial cavity.
- Rarely other tumors, like squamous papilloma, squamous cell carcinoma or different types of skin neoplasms like mastocytoma.

2.4.2.1. Transmissible venereal tumor (TVT)

Transmissible venereal tumors are sexually transmitted neoplasia of both male and female dogs.
TVT is distributed worldwide, although a higher incidence can be seen in free roaming, sexually active dogs.

In male dogs the main location is the caudal part of the penis and to a lesser extent the preputium (Nak et al., 2005; Cote, 2011).
Metastasis are rarely seen in this type of neoplasia (<5%).
Clinical signs of a TVT include serosanguineous discharge from the preputium, constant licking of the affected area, nodular lesions in the genital area and eventually masses develop on the penis or on the internal preputial lamina with cauliflower – like, papillary appearance. Nodules of various sizes and ulcerated surfaces are usually observed (Cote, 2011).
The diagnosis is based on the cytology. Being a round cell tumor, the TVT has a characteristic appearance with homogenous sheets of cells, which are round to oval in shape. They are characterized by a small amount of eosinophilic cytoplasm with cytoplasmic vacuoles and multiple mitotic figures. A prominent nucleus is located centrally (Nak et al., 2005).
Except for the tumor cells, the cytological smear contains epithelial cells, erythrocytes, neutrophils, lymphocytes and occasionally bacteria.
The recommended treatment is weekly administration of Vincristine intravenously at the rate of 0.025 mg per kg body weight for 2 to 5 weeks. Although this drug has multiple side effects, it is considered as the most effective chemotherapeutic agent for TVT treatment. The success of the treatment by Vincristine can be followed by assessing the cytology for the decrease in typical tumor cells and the increase in number of cytoplasmic vacuoles (Nak et al., 2005).

2.4.2.2. Sertoli cell tumor

Testicular tumors, like the estrogen producing Sertoli cell tumor, are common in dogs and they arise from the testicular germ cells or sex-cord stem cells.
The most important clinical finding is testicular enlargement that can be followed by atrophy of the contralateral, unaffected testicle (Cote, 2011).
These estrogen producing tumors lead to symmetric alopecia and feminization signs, like gynecomastia, pendulous preputium, loss of libido, attraction to male dogs, lethargy and sometimes redistribution of body fat (Cote, 2011).
As for the treatment, castration or at least a surgical removal of the affected testicle is the method of choice.
A study done by Dreimanis (2012) proposed to evaluate the preputial cytology as a diagnostic tool for estrogen producing testicular tumors in dogs.

In their study preputial cytology was evaluated by morphological differences of the cells that are used in vaginal smears:

- **Parabasal cells:** The cells are small and round with small amount of cytoplasm and the nuclei are round and vesiculated.
- **Intermediate cells:** large cells, roundish in shape that can appear folded or angular with round and vesiculated nuclei.
- **Superficial cells:** Large cells that are folded and angular in shape and the nuclei are small, faint and pyknotic.

The study consisted of 75 dogs divided into three groups, according to the appearance of testicular masses and their estradiol concentrations:

1. 30 control dogs that were healthy
2. 35 dogs with testicular masses and low estradiol concentrations (<40 pmol/L)
3. 10 dogs with testicular masses and high estradiol concentrations (>40 pmol/L)

Preputial swab samples were than taken from these dogs and evaluated accordingly (Table 4):

	Superficial cells - median	**Superficial cells range**
Group 1	4	0-13%
Group 2	4	2-88%
Group 3	55.5	4-100%

Table 4: Number of superficial cells according to groups (Dreimanis, 2012)

The results showed that out of the 45 dogs in groups two and three, with testicular masses, nine dogs had more than 20% superficial cells in the preputial smears.
From those nine dogs

- 6 had a Sertoli cell tumor,
- 2 had an interstitial cell tumor,
- 1 had a seminoma.

According to these findings, the sensitivity for diagnosing high estradiol concentrations (>40pmol/L) by high numbers of superficial cells in the preputial smears (>20%) was 80% and the specificity 98%.

Out of the 9 dogs that showed high numbers of superficial cells, 8 showed signs of alopecia that resolved after castration.
This finding led to 100% sensitivity for diagnosing alopecia that can be resolved by castration and 97% specificity (Dreimanis, 2012).
The mechanism leading to this finding is probably the same one as in the bitch, where during the estrus, high concentrations of estrogen cause the vaginal mucosa to thicken and to change to keratinized squamous epithelium, thus vaginal smears during estrus contain high amount of superficial cells.
According to the study performed by Dreimanis (2012) more than 20% of superficial cells are an abnormal finding in preputial smears.

In our study we examined the pH and the cytology of the male dogs preputial cavity to see if there is any connection to the presence or absence of discharge from the preputium.
Since in some cases, the preputial cytology resembles the cytology of the vagina in bitches, the next chapter will briefly describe the findings in female dogs:

2.5. The pH of the vagina in bitches

In bitches, the pH values of the vaginal secretions are influenced by the steroid hormone levels and change during different stages of the sexual cycle.
A study by Antonov (2014) showed the changes of the pH values during proestrus and estrus stages of the bitch.
Regarding proestrus and estrus the following changes in the pH value could be observed:

- **Proestrus:** 6.5 – 8.7
    - First day of proestrus : 7.4 – 8.7
    - Last day of proestrus: 6.5 – 7.7
- **Estrus:** 6.1 – 7.7
    - First day of estrus: 6.5 – 7.1
    - Ovulation: 6.5 – 6.8
    - First day after ovulation: 6.5 – 6.8
    - Second day after ovulation: 6.5 – 7.1
    - Third day after ovulation: 6.1 – 7.1

- Fourth day after ovulation: 6.5 – 7.9

During insemination canines have vaginal deposition of semen and therefore it is important that there will be a correspondence between the vaginal and seminal pH, so there will be a better chance of survival for the semen in the female genital tract (Antonov et al., 2014). During the favorable insemination time, which is 2 days after ovulation, the pH of the vagina is very close to the pH of the semen: 6.5-7.1.

The pH of the seminal plasma of dogs ranges between 6.3 and 6.7 and it possesses a buffering capacity, which makes it possible to neutralize small changes in the pH (Antonov et al., 2014). The same study also revealed that some pathological processes in the genital organs of the bitch may lead to changes in the vaginal pH, due to the influence of the vital activity of microorganisms, inflammatory secretions and hormonal changes in the body. Nevertheless additional investigations are needed to prove this statement.

2.6. Vaginal cytology in bitches

The cell types that can be found in smears, taken from the vaginal mucosa, change during the estrous cycle of the bitch. This is due to changing levels of steroid hormones, such as progesterone and estrogen.

The major cell types during the different stages of the estrous cycle are as followed (Root Kustritz, 2006):

- **Proestrus:** During proestrus an increasing number of cornified epithelial cells are seen. The number of polymorphonuclear (PMN) cells decreases.
- **Estrus:** During this stage of the cycle at least 80% cornification is observed, with greater than 50% anuclear squame cells.
- **Metestrus:** With the beginning of metestrus an abrupt return to complete non–cornification and an influx of PMN cells is seen.
- **Anestrus:** This stage of the estrous cycle is characterized by a scant number of epithelial cells, all of which are non– cornified.

2.7. Vaginal microflora

A study of Laurusevicius (2008) reported that the vaginal tract of the bitches is a non-sterile environment and houses a high number of various organisms, any of which can become an opportunistic pathogen under certain conditions.

Olson (1986) stated in their study that about 60% of clinically healthy bitches contain bacteria in the cranial vagina and in up to 90% of bitches bacteria is present in the caudal part of the vagina.

In their study 46 vaginal samples were cultured with the following results:

- Staphylococcus species: 57.6%
- Escherichia coli: 15.1%
- Pasteurella species: 15.1%
- Streptococcus species: 9.1%
- Pseudomonas aeruginosa: 3.1%

In their study, Laurusevicius (2008) also found out that the number of bacteria corresponds to the stages of the estrous cycle:

- Proestrus: Bacteria were isolated from 28.6% of the bitches.
- Estrus: Bacteria were isolated from 12.2% of the bitches.
- Metestrus: Bacteria were isolated from 2% of the bitches.
- Anestrus: Bacteria were isolated from 24.5% of the bitches.

If we compare those findings to the results of the study of Antonov (2012) regarding the pH of the vagina of the bitch, we can see that during proestrus, under conditions of a higher pH (6.5 – 8.7), there is a higher amount of aerobic bacteria (28.6%) in comparison to the estrus stage, when the vaginal pH is lower (6.1 – 7.7) and the aerobic bacteria is found less frequently (12.2%).

Hirsh and Wiger (1977) showed in an earlier study, that higher amount of bacteria can be isolated from bitches with reproductive disorders, than from healthy animals.

3. Aim of the study

The purpose of this study was to determine if there is a connection between the preputial pH and the appearance of the smegma, as well as the effect of the pH and the presence of the preputial discharge on the cytology. In addition we wanted to see if the age of the dog has any influence on the pH values and appearance of the preputial discharge.

Bibliography searches revealed that only a few reports were made regarding the cytology and microflora of the canine preputium and no information is available describing the normal pH range of the dogs' foreskin.

4. Materials and methods

Animals

The pH measurements and cytology samples were taken from the preputium of 25 dogs. The dogs were brought to the clinic for castration and the majority originated from animal shelters located in different parts of Hungary, as part of a program initiated by the government, “1000 dogs”.

There was no possibility to follow up the dogs, as they arrived in the afternoon, were castrated the following day and returned to the shelter later the same day.

The history of the dogs was very limited as well. Regarding the age of the dogs that were sampled it ranged from 7 months to 8 years. The vast majority of the sampled dogs were of mixed breeds.

Measurement of the pH

To measure the pH Duotest indicator paper was used. To gain more precise results, three different indicator papers were used in regards to the following pH ranges:

- 3.5 – 6.8
- 5 – 8
- 7 – 10

Sample taking

A dog was positioned in standing position or in lateral recumbency. Using gloves, the preputial orifice was gently opened. A strip of pH paper, approximately 2 cm in length, was inserted into the distal preputium and held there for about 30 seconds, to allow the paper to have contact with the internal preputial sheaths from both sides.

By comparing the changes in the color of the indicator paper and comparing it to the appropriate table of the strip used, the pH values were noted.

As the determination of pH is qualitative, for more precise results 2 ranges of the indicator paper were used most of the time, especially in case of a borderline or inconclusive result.

In order to take cytology samples a long cotton swab was used, which was first moistened with physiological saline (0.9%) and then administered into the preputial orifice and advanced proximally up to the penile base. The sample was taken by circular movements on the internal lamina of the preputial wall.
The swab was then rolled onto a glass slide, air dried and stained.

Staining

For staining the air dried slides the Diff-Quick method with the following reagents was used:

- Diff-Quick Fixative:
    - Triatylmethane Dye, 100% pure dye content in Methyl alcohol, 1.8 mg/liter
- Diff-Quick Solution 1:
    - Xanthene Dye, 100% pure dye content, buffer and Sodium azide (0.01%) as preservative, 1g/liter.
- Diff-Quick Solution 2:
    - Thiazine Dye mixture, 100% pure dye content (0.625 g/liter Azure A and 0.625 g/liter Methylene blue) and buffer, 1.25 g/liter.

Procedure

After air drying the slide it was covered with the fixative for 30 seconds. Afterwards the excessive fixative was drained and the slide was dipped into the first solution five times, each time for approximately one second.
Again the excessive solution was drained and the slide was dipped into the second solution 10 times, again each time for approximately one second.
As a final step the slide was rinsed with tap water and allowed to air dry before it was examined under the light microscope.

Evaluation

Preputial discharge

The dogs were evaluated whether they were presented with discharge or discharge was absent. If discharge was present they were further categorized:

- +: low amount of discharge was present at the preputial opening.

- ++: medium amount of discharge was present at the preputial opening.
- +++: high amount of discharge was present at the preputial opening.

pH values

As described before, the pH was measured by inserting pH paper into the preputial opening and the discoloration of the paper was compared to the appropriate table.

Cytology

The cytological examination was performed under a light microscope. To get an overview first a lower magnification (x10) was used followed by a higher magnification (x40) to observe details.

The samples were evaluated for the appearance of neutrophils, epithelial cells, bacteria and mucus filaments and then categorized according to the following scheme:

- +: low amount of cells/mucus filaments
- ++: medium amount of cells/mucus filaments
- +++: high amount of cells/mucus filaments

5. Results

	Name	ID No.	pH	Age (months)	Discharge	Breed
1	Pamacs	228498	6.8	24	-	Golden Retriever
2	Jim	235052	8	12	-	Mongrel
3	Blacky	235053	6.5	48	++	Mongrel
4	Avis	235055	6.9	12	-	Mongrel
5	Patrick	235054	5.6	24	++	Staff. Terrier
6	Dylan	235233	6.8	9	-	Mongrel
7	Maike	235234	5.6	15	++	Mongrel
8	Franklin	235235	7.1	41	++	Mongrel
9	Turbo	235236	7.3	22	-	Mongrel
10	Dugo	235335	6.3	7	-	Mongrel
11	Hector	234504	7.3	72	-	Mongrel
12	Balint	234501	6.5	36	+++	Mongrel
13	Vespa	234502	6.8	18	++	Mongrel
14	Pemsli	235799	6	7	-	Mongrel
15	Bobo	235712	6.8	24	++	Mongrel
16	Hugo	235710	7.4	12	-	Mongrel
17	Bingo	235870	7	17	+	Mongrel
18	Ormester	235867	6.9	24	+	Mongrel
19	Ringo	236261	6.5	72	+++	Mongrel
20	Onyx	236262	6.8	18	+	Mongrel
21	Miki	236264	7.9	42	+	Mongrel
22	Ordog	239798	6.5	18	-	Mongrel
23	Chippy	239799	5.6	96	+	Yorkshire Terrier
24	Benjamin	235196	6.8	10	-	Mongrel
25	Gusto	235201	6.5	24	-	Mongrel

Table 5: pH values of the preputium of male dogs and the appearance of preputial discharge

5.1. pH results

The pH results of the preputial swab samples can be seen in Table 5. According to these findings the mean, median, standard deviation and standard error of the mean were calculated (Table 6).

Mean	6.728
Median	6.8
Standard deviation	0.6188
Standard error of the mean	0.1238

Table 6: Mean, median, standard deviation and the standard error of the mean of the preputial pH of the sampled dogs.

In order to determine if there is a connection between the pH value and the appearance of the preputial discharge, the sampled dogs were divided into two groups: Group 1 consisted of 12 dogs without preputial discharge and group 2 consisted of 13 dogs that showed preputial discharge to various degrees.

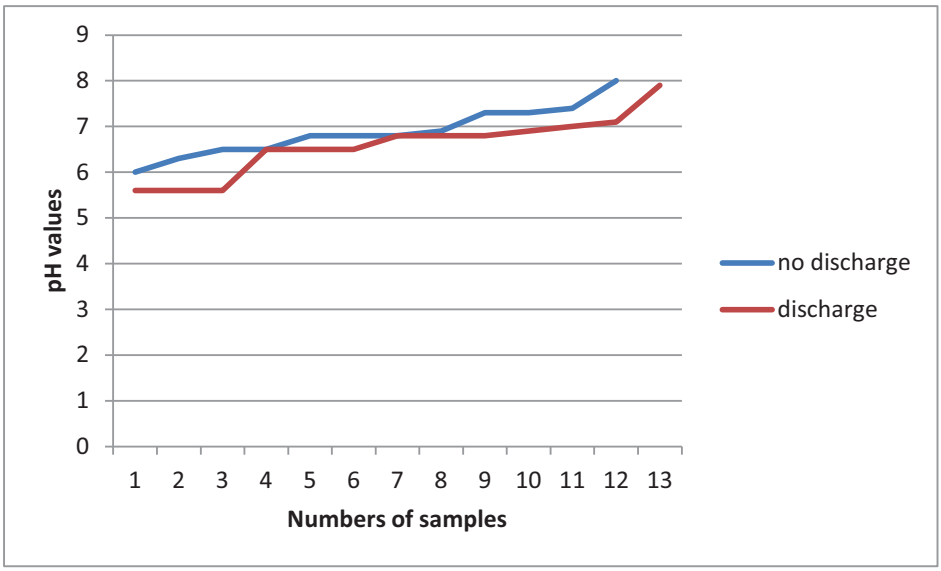

Figure 1: The appearance of preputial discharge in relation to the pH values

As a next step the mean, median, standard deviation and standard error of the mean were calculated for each group.

The results of group 1 can be seen in the following table:

Mean	6.883
Median	6.8
Standard deviation	0.5474
Standard error of the mean	0.158

Table 7: Mean, median, standard deviation and standard error of the mean of the preputial pH in dogs without preputial discharge

The results of group 2 were as followed:

Mean	6.585
Median	6.8
Standard deviation	0.6669
Standard error of the mean	0.185

Table 8: Mean, median, standard deviation and standard error of the mean of the preputial pH in dogs showing preputial discharge

The two groups were then compared in a two-sample t-test (two tails test, with 95 % of confidence level). This test assesses whether the means of two population means are statistically different from each other. The t-test gives the probability that the difference between the two means is caused by chance. In case of a t-value higher than the critical t-value, the difference is 'significant', and we can conclude that the difference is not caused by chance.

The difference in the pH values was found to be not statistically significant; with the t-value being 1.225 and the p– value 0.87961. The critical value in our case is 2.068, which is higher than the t-value of our calculations.

In order to see if the preputial discharge can be linked to the age, the same calculations were made, according to the age of the dogs in months.

For group 1 the results were as followed:

Mean	19.08 months
Median	12 months
Standard deviation	17.8
Standard error of the mean	5.138

Table 9: Mean, median, standard deviation and the standard error of the mean of the age of dogs without preputial discharge**.**

And for group 2 with preputial discharge:

Mean	36.54 months
Median	24 months
Standard deviation	24.12
Standard error of the mean	6.689

Table 10: Mean, median, standard deviation and the standard error of the mean of the age of dogs with preputial discharge.

This time, the difference between the means of the two groups was on the border of being statistically significant; with the t-value being 2.07 and the p– value was 0.02517. The difference is visualized in the following graph.

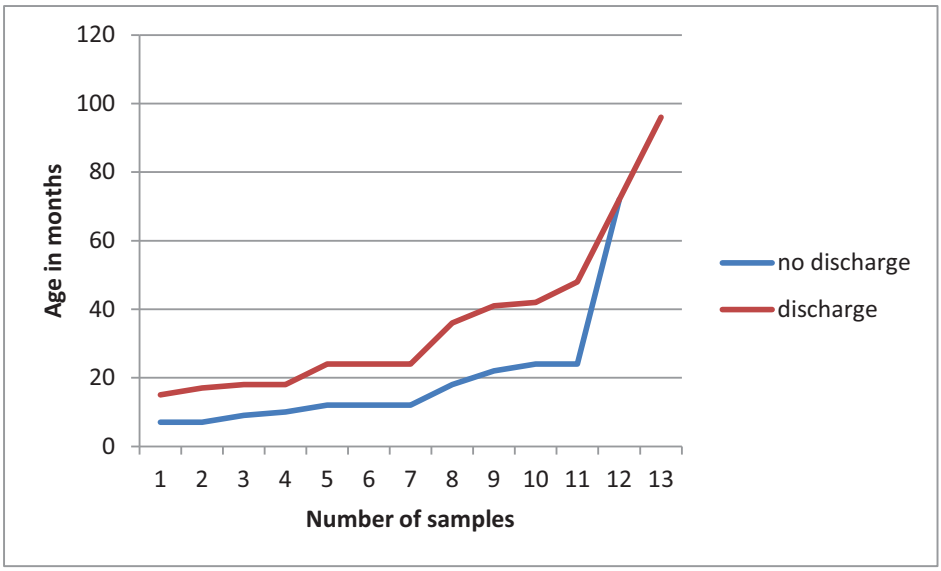

Figure 2: Appearance of preputial discharge in relation to the age in months

In addition we wanted to see the values of the pH according to the age of the dogs. The figure below shows that there is no correspondence between the age and the pH values recorded.

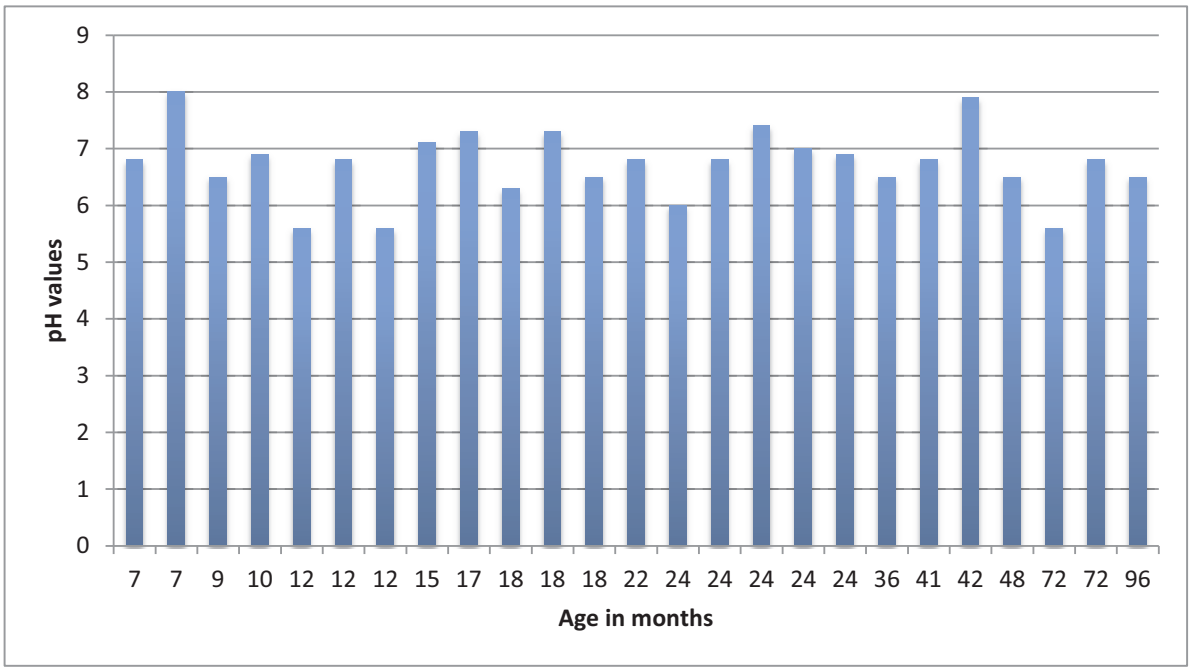

Figure 3: pH values in relation to the age in months

5.2. Cytology

The evaluation of the slides revealed various numbers of neutrophils, epithelial cells and some mucous shreds. Only on few slides bacteria was visible, which appeared as coccoid in shape. Whereas neutrophils and epithelial cells were seen on all of the slides, mucus shreds appeared only in 8 out of 25 samples and bacteria was found on only 5 slides.

No intracellular bacteria could be seen on any of the slides.

The detailed cytological evaluation of the slides can be seen in Table 11.

A few pictures below will demonstrate the findings of the cytological examination.

Name	ID No.	Neutrophils	Epithelial cells	Bacteria	Mucous filaments	
Pamacs	228498	+++	+			
Jim	235052	+	++			Figure 5
Blacky	235053	++	+	+		
Avis	235055	+	+	+		
Patrick	235054	+++	+			Figure 6
Dylan	235233	+	+			
Maike	235234	++	+			
Franklin	235235	+-	+		+	
Turbo	235236	++	++		+	
Dugo	235335	++	+		+	
Hector	234504	+	+		+	
Balint	234501	++	+		+	Figure 4
Vespa	234502	++	++		+	
Pemsli	235799	+++	++	+		Figure 7
Bobo	235712	+	+		++	
Hugo	235710	++	+			
Bingo	235870	++	+	+		
Ormester	235867	+++	+	+		
Ringo	236261	++	++			
Onyx	236262	++	+			
Miki	236264	+	+			
Ordog	239798	++	+			
Chippy	239799	+	++			
Benjamin	235196	++	+			
Gusto	235201	+	+		+	

Table 11: Cytological evaluation of the collected samples

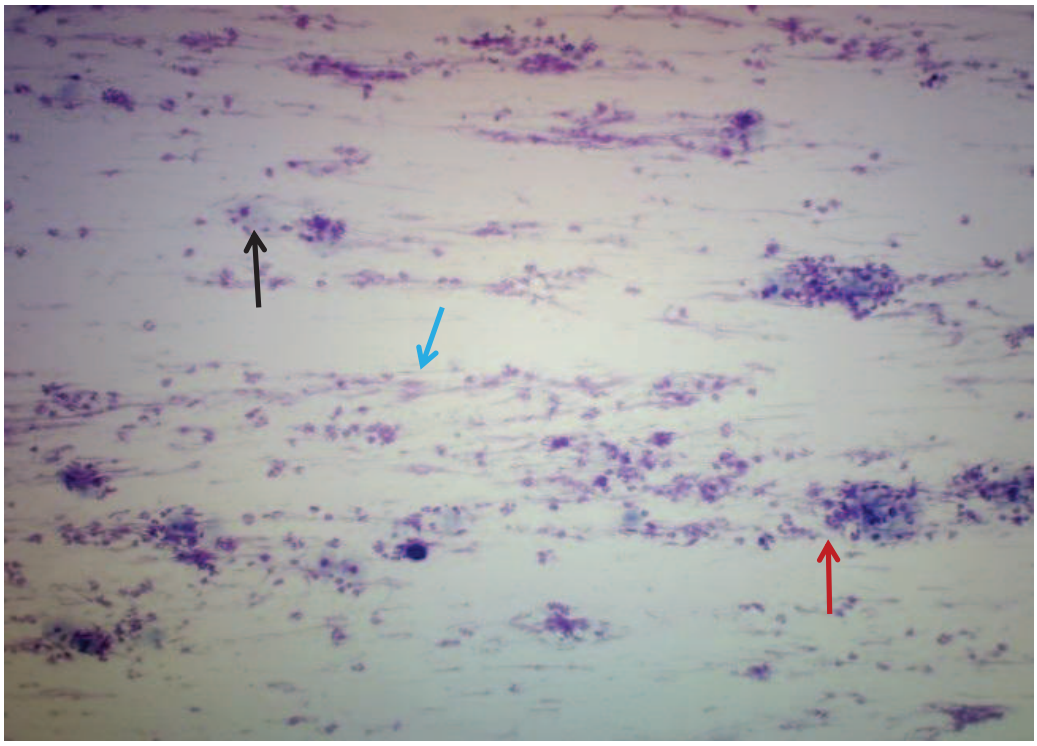

Figure 4: Cytology sample with low amount of epithelial cells (black arrow), medium/low amount of neutrophils (red arrow) and mucus shreds (blue arrow)

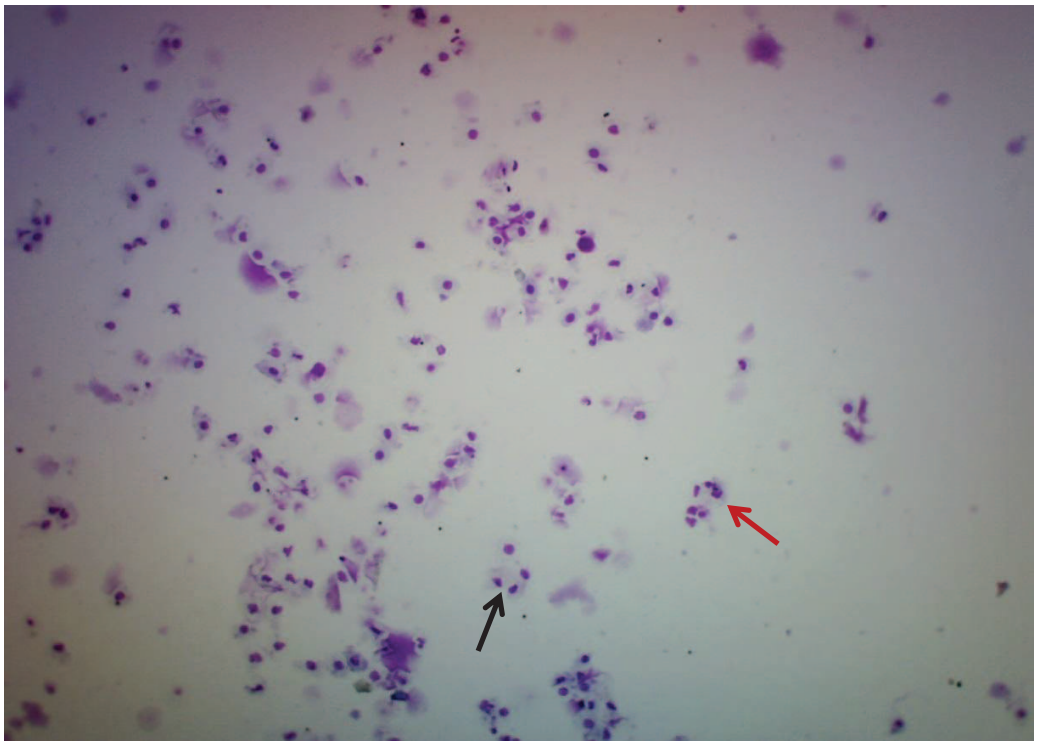

Figure 5: Cytology sample with medium amount of epithelial cells and low amount of neutrophils

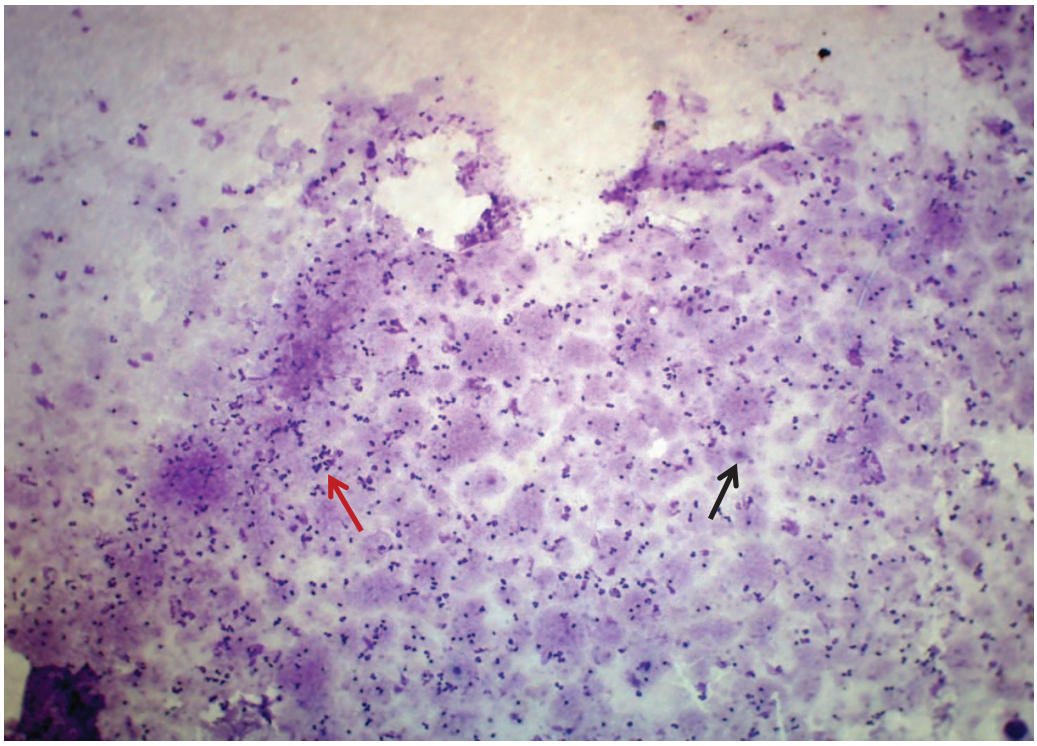

Figure 6: Cytology sample with high amount of neutrophils and low amount of epithelial cells**.**

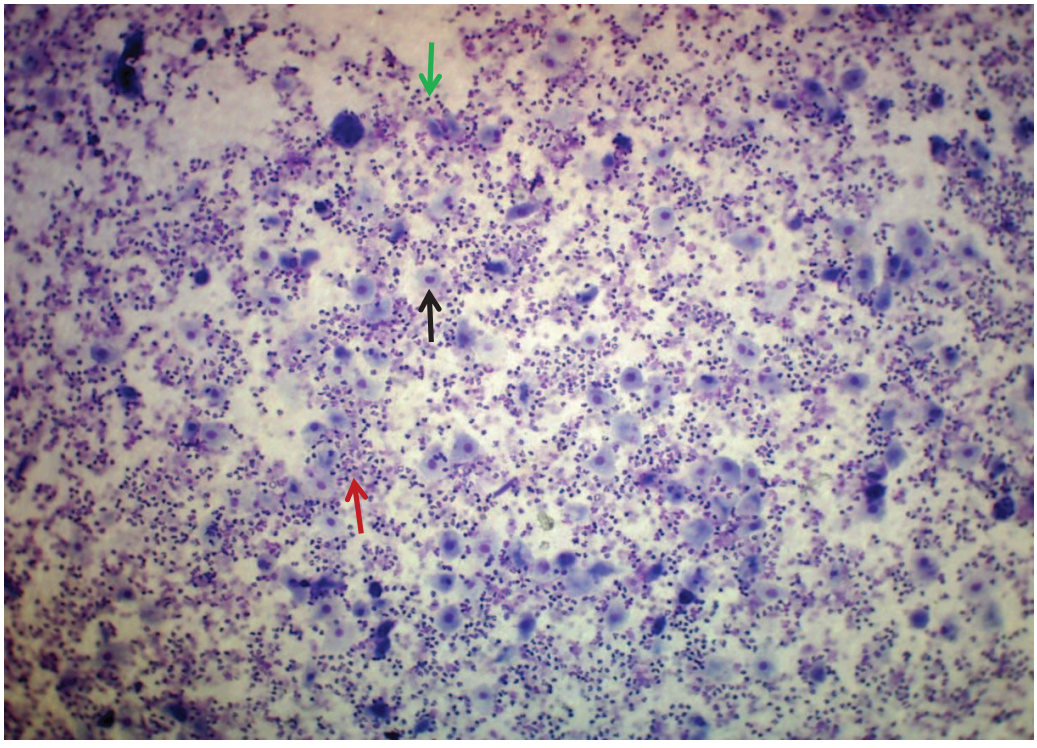

Figure 7: Cytology sample with medium amount of epithelial cells, high amount of neutrophils and some coccoid bacteria (green arrow).

6. Discussion

Correlation between the pH value and the presence of discharge

The range of the pH was rather big although only 25 samples were taken. The values ranged from a minimal value of 5.6 to a maximal being 8. The overall mean pH value of the preputial cavity was 6.728.

In dogs without preputial discharge, the mean value was 6.883 and in dogs showing various degree of discharge, the mean value was 6.585

Our aim was to see, if there is any correlation between the pH values and the discharge of the preputium in male dogs.

Since our calculation showed that the t-value is much smaller than the critical t-value, no such correlation could be observed.

As only intact male dogs were sampled, we cannot know if the castration affects the pH or the appearance of preputial discharge. Further studies might be advised to determine the effect of castration on pH and cytology.

Correlation between the presence of discharge and age

In case of the preputial discharge in relation to age, our calculations showed that the t– value is almost identical to the critical t– value which is 2.068, and the p-value is under 0.05, which shows that the difference could not be caused by a chance. There is a chance that if a higher number of samples were taken, the difference would be statistically more significant. Since the shelters do not always know the precise age of the dogs and often estimate it, it may have affected the precision of the calculations.

Since no literature is currently present about the normal pH values of the canine preputium and their connection to preputial discharge, the comparison to other studies was not possible.

Correlation between the pH values and the age

According to our results a connection between the age of the dogs and the pH values could not be seen. It looks from our samples that the pH is not related to the age, but the majority of the dogs examined were younger than 4 years of age, thus more samples from older dogs should be evaluated in order to make a conclusion.

Cytology

The cytological examination revealed that epithelial cells, neutrophils, coccoid bacteria and mucus shreds are present in the canine preputium.

According to our findings, no correlation between the pH and the cell types seen on the slide could be proven.

There was no effect of preputial discharge on the cytological samples as well.

We did not find any abnormalities on the slides, like cornified cells to suggest Sertoli cell tumors; neither did we see characteristic round cells to propose the presence of Transmissible Venereal Tumors.

All the bacteria seen during the cytological evaluation appeared as coccoid and no intracellular bacteria was visible.

We could not see that the pH influences the cytology, as it does in the bitches during different stages of the estrous cycle (Root Kustritz, 2006; Antonov, 2014).

Unfortunately in this study we were not able to examine the microbiology of the preputium of the sampled dogs as it was too expensive. If microbiology would have been performed, we could have checked if the microorganisms are changing in different pH ranges, if dogs from different shelters have different microorganisms in their preputial flora as well as comparing the microflora of male and female dogs coming from the same shelters.

7. Summary

In this study the pH value of the preputial cavity of dogs was measured and evaluated whether it has an effect on the appearance of the preputial discharge and the cytology samples. Furthermore we wanted to see if a link between the preputial pH and the age of the dogs exists.

Regarding the pH, it was not possible to compare the findings of our study to other literature, as no research was made till now about the normal pH range of the canine preputial cavity. Our results revealed, that the age is most likely correlated to the presence of the preputial discharge, but a higher amount of dogs needs to be examined, especially older ones, to draw a definite conclusion.

The cytology sample findings coincided with previous studies (Root Kustritz, 2006; England & von Heimendahl, 2010) and our samples showed variable amounts of epithelial cells, neutrophils, in some cases mucous shreds and rarely coccoid bacteria.

Due to the fact that no microbiological examinations were made, it was not possible to link the pH to different microflora of the preputium, thus it is advisable to study the effects of the preputial pH further by taking and evaluating microbiological samples.

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