Comparison between four colpocytological examination collection techniques in cats

Comparação entre quatro técnicas de colheita para exame colpocitológico em gatas

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ABSTRACT
Colpocytology has practical applications in the feline species, such as monitoring of the estrous cycle phases, aiding in the diagnosis of hormonal disorders and diseases of the female genital tract. The aim was to compare the most efficient method for performing vaginal cytology in queens. Vaginal material...
was collected from 12 female cats, healthy, aged between one and eight years of age, with a mean weight of 3.5 kg. The queens were divided into four groups, each with a different tool for collection of vaginal cells. GIB: nylon bristles (Interdental fine brush- GUM®); GGB: gynecological brush for humans (endobrush- INLAB®); GPP: Pasteur pipette (graduated-3mL); and GS: sterile cotton swab (ABSORVE®). Cells were deposited on a microscope slide to obtain the vaginal smears and stained with rapid panoptic dye. The smear cell-based classification was performed subjectively, establishing scores from 0 to 4 (0 absence of cells, 1 very few cells, 2 few cells, 3 moderate cell numbers, 4 high cell numbers). The materials obtained were well preserved and in adequate quantity for cytological evaluation. A descriptive statistical analysis was performed to compare the collection methods. It was observed that the average swab cellularity score collected with the gynecological brush (GGB = 3.13) was higher compared to the other groups: interdental brush (GIB = 2.2), Pasteur pipette (GPP= 2.13), and swab (GS = 0.8). Although the swab is the most commonly used method in the colpocytological examination in cats, the interdental brush proved to be the most efficient method to collect samples, providing great cellular recovery and a low degree of discomfort.

Keywords: vaginal cytology, cervical brush, interdental brush, felines, Pasteur pipette, swab.

RESUMO
A colpocitologia apresenta aplicações práticas na espécie felina, como o acompanhamento das fases do ciclo estral, auxílio no diagnóstico de distúrbios hormonais e enfermidades do aparelho genital feminino. O objetivo deste estudo foi identificar o método mais eficiente para a realização das citologias vaginais em gatas. Foi colhido material vaginal de 12 gatas sem raça definida, saudáveis, com idades entre 12 meses e oito anos e peso médio de 3,5 kg. As fêmeas foram divididas ao acaso em quatro grupos utilizando diferentes instrumentos para realizar a colheita das células vaginais, sendo GIB: uma escova pequena com cerdas de nylon (escova interdental fina- GUM®); GGB: escova ginecológica utilizada em humanos (endobrush- INLAB®); GPP: pipeta Pasteur (graduada-3mL) e GS: swab de algodão estéril (ABSORVE®). Após a colheita foram confeccionados esfregaços em lámina de microscopia e corados com Panótico rápido®. A classificação com relação à quantidade de células por lámina foi feita de forma subjetiva, estabelecendo escores de 0 a 4 (0 ausência de células, 1 pouquíssimas, 2 poucas, 3 moderadas, 4 alto número de células). O material obtido em todos os grupos apresentou-se bem preservado e em quantidade adequada para a avaliação citológica. Análise estatística descritiva foi realizada para a comparação entre os grupos. Observou-se que a média do escore de celularidade dos esfregaços colhido com a escova ginecológica (GGB= 3,13) foi maior em relação aos outros grupos: escova interdental (GIB= 2,2), pipeta Pasteur (GPP= 2,13) e swab (GS= 0,8). Embora o swab seja o método mais utilizado no exame colpocitológico em gatas, a escova interdental mostrou-se como método mais eficiente na coleta das amostras, proporcionando uma grande recuperação celular, sem artefatos de técnica e baixo grau de desconforto.

Palavras-chave: citologia vaginal, escova cervical, escova interdental, felinos, pipeta pasteur, swab.

1 INTRODUCTION
The domestic cat is characterized by particularities in its reproductive physiology, being considered seasonally polyestrous, with positive photoperiodic induced ovulation (Burke, 1986; Christiasen, 1988; Silva, 2015). With the growth of the domestic feline population, combined with the greater awareness of animal welfare, the search for qualified professional assistance for obstetric and neonatal care has increased. In this sense, colpocytology represents an indispensable resource in
the success of feline reproduction and its correct interpretation enables the analysis of hormonal status, and the detection of estrous cycle phases and reproductive disorders (Cline, Jennings, & Sojka, 1980; Richkind, 1978). However, unlike the canine species, its use is not as frequent in clinical practice. The biggest obstacle is related to the small size of the feline vagina, with a length of 20 to 30 mm, and a 10 to 15 mm vaginal vestibule, making it uncomfortable to introduce harvesting materials in this species (Silva, 2015). Another aspect is the difficulty in containing felines, as they are easily stressed outside their home habitat, especially in noisy environments, with a lot of olfactory stimulation and sudden movements.

Several techniques for obtaining vaginal cells have been described in the literature (Allen, 1995; Johnston, Kustritz, & Olson, 2001; Micheluzzi & Ostrowski, 1976; Silva, 2015). In the direct form, after cleaning the perigenital hair with physiological solution, a glass slide is pressed over the vaginal mucosa (imprinting); however, most preparations present cell deformations (Evans & Savage, 1970; Johnston et al., 2001). In the indirect form, swabs can be obtained by: sterile moistened cotton swabs or metal spatula (Allen, 1995; Christiasen, 1988; Johnston et al., 2001); aspirated from the caudal vagina using a catheter, sterile pipette, or short-tip dropper (Allen, 1995; Herron, 1977; Lein, 1988); gynecological brush (Alvarenga, 1996; Chaves, 2003) or interdental brush (Aragão, 2000; Silva, 2015).

The aim of this study was to compare four cell harvesting methods for colpocytological evaluation in domestic cats, analyzing individual advantages and disadvantages in order to identify the best cell recovery method, visualized under a microscope.

2 MATERIAL AND METHODS

This study was approved by the Institutional Ethics Committee on Animal Use (CEUA protocol no. 5609.2017.46). Twelve non-pregnant, adult female cats (*Feliscatus*) with body weights between 2.7 and 3.5 kg were evaluated. All animals underwent ovariohysterectomy in a parallel experiment, preceded by surgical risk laboratory tests, including only animals that presented normal results for the species. The material was collected only after ovariohysterectomy, and the animals were under anesthetic effect according to the routine protocols of the institution. The twelve cats were randomly divided into four groups as shown in Frame 1 and Figure 1.
Frame 1: Distribution of the groups of different materials used to collect queen vaginal cells.

| GROUPS                        | MATERIAL                                                                                                                                 |
|-------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Gynecological Brush (GGB)     | Cytological brush (endobrush-INLAB®), with white polypropylene shank, containing a conical nylon brush at one end, with dimensions of 168x3 mm (shank) and 7x4 mm (brush) (Figure 1, A). |
| Interdental Brush (GIB)       | Thin tapered interdental brush (GUM®) with small nylon bristles, dimensions 6.0 cm long and 1.1 mm in diameter (Figure 1, B).                 |
| Swab (GS)                     | Sterile cotton swabs (Absorve®), with plastic shank, measuring 13x150 mm (Figure 1, C).                                                   |
| Pasteur Pipette (GPP)         | Graduated polyethylene pipettes 3mL (Kasvi®), 160 mm long, 8 mm external diameter, and 3 mm tip diameter, obtaining cells by infusion and aspiration of sterile saline solution 0.9 % (Figure 1, D). |

Figure 1- Instruments used for the different techniques of vaginal cell harvesting. A- gynecological brush (endobrush-INLAB®), B- interdental brush (thin- GUM®), C-swab (Absorb®), D- Pasteur pipette (Kasvi®).

First, the vulva was cleaned with gauze moistened with sterile saline. The instrument used in each group was inserted into the vaginal vestibule, directed dorsally and later redirected horizontally, deepening in the cranial direction, to approximately 1.0 to 1.5 cm. In the GGB, GIB, and GS groups, clockwise and counterclockwise movements were performed to collect cells. In the GPP we used the technique described by Johnson (1994), consisting of infusion and aspiration of 1.0 mL of 0.9% sodium chloride solution. After harvesting, glass slides (Precision Glass Line®), 26 x 76 mm and 1.0
to 1.2 mm thick, were smeared. Subsequently, the slides were stained with Rapid Panoptic (Laborclin®, Pinhais, PR) and analyzed in an optical microscope by a single examiner at 100X magnification. The slide reading was standardized by dividing it into 10 fields, and cells were counted in five interspersed fields (Figure 2).

**Figure 2**- Parameter used to count cat vaginal cells. The slide was divided into 10 fields, and the reading was standardized into five interspersed fields.

The classification of number of smear cells was performed subjectively, establishing scores from 0 to 4, being 0: absence of cells, 1: very few cells, 2: few cells, 3: moderate number of cells, and 4: high number of cells, as described by Saltiel, Gutierrez, de Buen-Llado and Sosa (1987). A descriptive analysis of the data was performed using the Sigma Plot 12.0 software.

### 3 RESULTS

All slides were suitable for cytological evaluation regarding quantitative and qualitative aspects. Graph 1 shows that the mean cellularity score of the smears obtained with the GGB was higher than the other methods, followed by the GIB and GPP, with the GS demonstrated the lowest recovery.
Clusters were identified formed by stacked cells in smears from the GGB, presenting a layered arrangement with higher cell density (Figure 3). Similarly, in the GIB, it was possible to recover an adequate amount of material, with intact cells arranged along the smear, both singly and juxtaposed (Figure 4).

**Figure 3**- Photomicrography of queen vaginal cells, recovered by the gynecological brush technique, stacked cells with layered arrangement and high cell density can be observed.

**Figure 4**- Photomicrography of queen vaginal cells recovered by the interdental brush technique, intact cells can be observed, with a cellular overlap.
The material collected from the GPP was similar, in terms of cell recovery, to the GIB, but with greater cell distribution, allowing more individualized visualization of the cells and giving a clearer and more homogeneous appearance to the slide (Figure 5). Smears from the GS were lower in both quantity and quality. The presence of technical artifacts and lower cell recovery suggests vaginal cell adherence to cotton fibers (Figure 6). The comparative analysis of the results is shown in Table 1.

Figure 5- Photomicrography of queen vaginal cells recovered by the Pasteur pipette technique, a distortion-free smear with greater cell homogeneity can be observed, without clustering.
**Figure 6** - Photomicrography of queen vaginal cells recovered by the swab technique, the artifact technique can be observed, in which vaginal cells were adhered to cotton.

**Table 1**: Comparative analysis of queen colpocytology performed by four techniques for obtaining cells with regard to score and cell characteristics.

| GROUP | SCORE | CHARACTERISTICS OF CELLULARITY | Smear Aspect | Technique Artifacts |
|-------|-------|--------------------------------|--------------|---------------------|
| **GGB** | High | Layered cell arrangement, stacking and high cell density | Dense | Absent |
| **GIB** | Medium | Cells arranged singly or in layered form | Dense | Absent |
| **GPP** | Medium | Higher cell dispersion, allowing homogeneous visualization and individual characterization of cells without formation of cell clusters and overlap | Clear and homogeneous | Absent |
| **GS** | Low | Low cell recovery, presence of technique artifacts | Distorted and dense | Present |

GGB: gynecological brush group; GIB: interdental brush group; GPP: Pasteur pipette group; GS: swab group.

**4 DISCUSSION**

Nowadays, assisted reproduction in felines is a growing perspective and the use of efficient instruments for collecting vaginal cells can provide more efficient diagnoses. In the 1990s, the swab was the most commonly used resource for vaginal smears of female dogs and cats in routine veterinary practice (Johnson, 1994; Toniollo et al., 1995). The swab is easy to perform and easy to handle, low cost, promotes good smear quality, reduces the possibility of vaginal trauma, and facilitates access to the deepest parts of the vagina (Toniollo et al., 1995). Its use is no longer recommended in human cytology due to deficient cell uptake (Alves, 1999; Kavak, Eren, Pekin, & Kiillu, 1995). For Bourke,
Mills and Barnes (1997), the greatest cellular distortion is related to the adherence of the cells to the cotton fibers, the pressure applied to the endometrial epithelium at the time of material collection, and the pressure applied to roll the swab on the glass slide.

As in the current study, previous publications have shown that the gynecological brush was more effective at cell recovery and integrity than smear swabs in women (Alves, 1999; Kavak et al., 1995) and mares (Bourke et al., 1997), as well as the lower cost, easy handling, and lower degree of swab friction discomfort. However, the greater cell recovery obtained by the gynecological brush can be attributed to the greater invasiveness of the technique, which in turn may cause greater discomfort to the unaided or anesthetized cats, as the gynecological brush originally meets the proportion of the adult human vagina, which is 6.86 to 14.79 cm in length, 4.7 to 6.3 cm in width, and 2.39 to 6.45 cm in diameter of the introitus (Pendergrass, Reeves, Belovicz, Molter, & White, 1996), while the domestic cat vagina is 20 to 30 mm long with a lumen of only 1 mm in the anterior vagina consisting of non-flexible tissue (Silva, 2015; Watson & Glover, 1993).

The brush measurement (7x4 mm) is similar to the erect penis of the tom (5.1 +/- 0.5 mm wide), and the brush bristles can promote vaginal stimulation similar to penile spicules, inducing ovulation, as already described in previous studies by Schmidt (1986) and Verstegen (1998). As the queens in this study were under anesthetic effects at the time of collection, it was not possible to evaluate the degree of discomfort generated by the technique. However, it is evident that this discomfort exists, and anesthesia could facilitate the procedure in the most reactive animals. Nonetheless, while stress and discomfort-induced cortisol decreases hypothalamus-pituitary-gonadal axis function (White & Porterfield, 2013) the use of certain anesthetic protocols, including ketamine, also leads to reduced LH tonic secretion by suppressing ovulation (Johnson & Gay, 1981).

Thus, an alternative to reduce the discomfort caused by the gynecological brush is the use of smaller brushes, such as interdental brushes, which can be conical or cylindrical, with a wide variety of brands, prices, and sizes in the market, combined with a personalized, specialized, and skilled service, adopting gentle but firm handling, essential for the queens to feel safe and favorable to the procedure.

When comparing the harvesting methods using the interdental brush and the swab in queens, Aragão (2000) observed that the interdental brush provided results similar to the gynecological brush used in women regarding cell recovery and obtaining cleaner smears and fewer artifacts. These findings also coincide with those of the present study, which obtained similar results regarding cell preservation, quantity, and arrangement. In addition, the smaller size made the interdental brush a more suitable option for feline species, providing less discomfort than the gynecological brush, even with the cats under anesthetic recovery.
However, it is worth remembering that the presence and contact of bristles with the vagina wall does not nullify the sensory stimulation to ovulation, although to a lesser extent, when compared to the gynecological brush. Thus, the use of the Pasteur Pipette (or similar instrument, such as a dropper or catheter), commonly used in rats (Cora, Kooistra, & Travlos, 2015; Sharma & Sharma, 2016), could be the most appropriate colpocytological method so as not to induce ovulation.

In the current study, the technique used with the Pasteur Pipette was the same as that used by Johnson (1994), infusing 1.0 mL of 0.9% saline solution followed by immediate aspiration. The smears obtained demonstrated similar results, with greater cell recovery compared to the swab, without the formation of clusters, giving the slide a clear and organized appearance. Thus, use of the pipette should be considered when it is intended to avoid visualization of cell stacking and ovulation induction, as it does not provide sensory stimulation to the walls of the vaginal mucosa. However, the good tolerance of animals to the technique can be attributed to the fact that they are under an anesthetic protocol. In the absence of this, it is possible that discomfort may occur due to the containment, presence of liquid in the vagina of the queen, and even the temperature of the solution.

Therefore, before choosing a single method for colpocytology in cats, it is important to have information on all the techniques and their indications, so as not to interfere with the objectives of reproduction, especially with regard to ovulation induction or suppression. Consideration should also be given to the technique that causes the least discomfort, individual temperament, and concomitant use of sedation or anesthesia protocols.

5 CONCLUSION

The use of a swab proved to be the least recommended technique, due to the low cell recovery and greater presence of artifacts, as well as the gynecological brush, due to the greater discomfort due to its proportions. On the other hand, the interdental brush, with a variety of sizes, commercially available shapes, and easy handling, proved to be the most practical and suitable for the delicate anatomy of the feline external genitalia, especially in cases where sedation and/or anesthesia and reproductive management are unnecessary.

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