Development and Validation of a Simulation Model for Collection of Canine Vaginal Samples

Ricardo Marcos ■ Raquel Moreira ■ Sónia Macedo ■ Graça Lopes

ABSTRACT
Vaginal cytology is a widely used cytological technique mostly taught by observation, either through direct tutoring or videos. To the best of our knowledge, vaginal cytology simulators have never been assessed in veterinary medicine. Twenty-five undergraduate students with no prior experience in canine vaginal sampling were randomly assigned to two groups that practiced the procedure in either a simulator or a live animal. An inverted classroom design was followed. After observing a video tutorial, students practiced with the simulator/live animal for two classes. Three weeks later, they performed a vaginal cytology on a live animal being recorded. The videos were evaluated through an objective structured clinical examination (OSCE) by an observer blinded to the student's groups. The learning outcome was compared through OSCE pass rates and questionnaires. The simulation model was made by 3D printing and soft silicone for the vulvar labia, having pink and blue colored vaseline in the correct and incorrect locations for sampling. The model was economic and accurately replicated the female reproductive tract. It provided immediate feedback to students, who obtained pink or blue swabs from the correct and incorrect locations, respectively. Students reported that three to five or more attempts were needed to properly learn the procedure, thus justifying the need for a simulator. No differences in the OSCE pass rates were observed between the groups. The simulation model was effective for learning the vaginal cytology procedure, replacing the use of live animals. This low-cost model should be incorporated in the tool-kit of reproduction classes.

Key words: canine, cytology, collection methods, education, diagnosis, simulator, inverted classroom,

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INTRODUCTION

Vaginal cytology is an important technique in small animal clinic, and in canine reproduction to identify and monitor the ovarian cycle stage, inflammatory conditions, and neoplastic changes in the female reproductive tract.[1] Training canine vaginal sampling before the graduation of veterinary students is predominantly achieved using live models (bitches) bred for academic teaching purposes and kept in university kennels. Alternatively, animals from associations, in a context of shelter medicine programs, client-owned animals, and cadavers (nonliving animals) can also be used. All these sources provide animals like those of the real clinical practice, but they set up a non-standardized learning environment.[2] Nowadays, acquiring animals is increasingly difficult due to economic, logistical, and ethical factors.[2,3] The use of animals, even for teaching, is increasingly subject to regulatory requirements and scrutinized by animal protection groups.[4–6] Furthermore, a significant number of veterinary students desire using humane models for learning.[6,7] The general society itself is progressively concerned with the use of animals in science and this can be solved by non-animal alternatives.[6]

The Society for Theriogenology and American College of Theriogenologists identified a core curriculum for Veterinary Medicine training that includes equine, bovine, and small animals breeding soundness examination.[8] This group of competencies comprises the vaginal sampling in bitches. Simulators to be used in Animal Reproduction or Theriogenology classes have already been developed for equine and bovine species.[9–12] However, there is no model developed to be used for the bitch's vaginal sampling. A valuable simulator in this field should have a high fidelity of the female reproductive tract, be comparable to the live animal, and be able to replace their use.

The aim of this study was to develop a simulation model of the lower reproductive tract and to compare it with the use of live animals for teaching vaginal sampling to veterinary students.

METHODS

Simulation Model Development

A computed tomography scan of a normal large breed intact female dog was performed (the dog had been euthanized for reasons unrelated with this study). The normal female reproductive tract was filled by contrast through the vulva (Figure 1). Afterward, 3D image reconstruction and modeling were performed. Some adjustments were made, such as removing part of the urethra and bladder (superfluous for model development purposes), fitting the organ to a tubular shape (i.e., removing the dorsal flattening due to the rectum), making a hole in the dorsal view of the 3D model (in order to get an easy

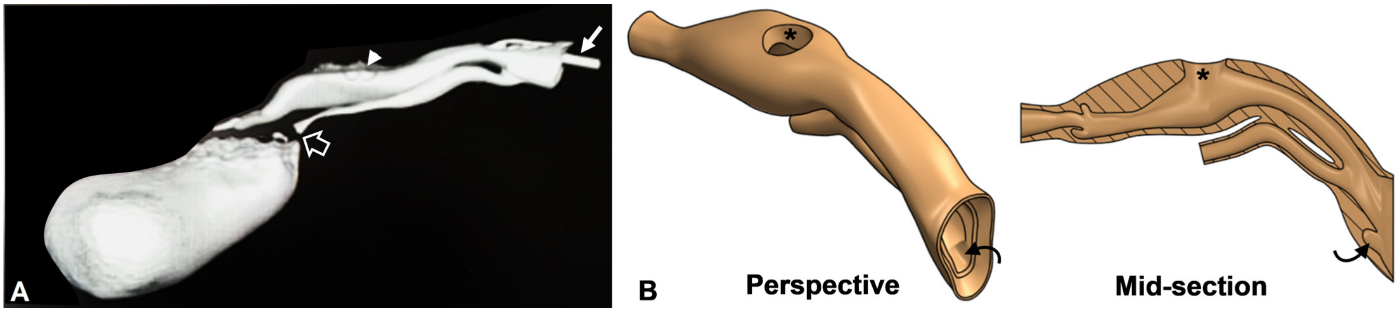

Figure 1: (A) Contrasted computed tomography of the female reproductive tract. A Folley's catether (arrow) was introduced in the vulva. The vagina was filled with contrast, even if the organ was molded by rectum (arrowhead) and some contrast escaped through the urethra to the bladder (block arrow). (B) Three dimensional model used to print the vagina in perspective and mid-section view, to disclose the interior. A dorsal opening (asterisk) was included to access the interior of the vagina. Besides the urethra, the model included all the anatomical details, including the clitoris and clitoral fossa (curved arrow)

access to the inside of the vagina), and designing the vulvar labia (Figure 1). The vaginal model was 3D printed using Thermoplastic Polyether-Polyurethane elastomer with 5% filling (Filaflex 82A, Recreus Industries, Alicante, Spain), due to its high flexibility combined with good strength and durability, except for the vulvar part. For the vulvar lips, a soft moldable silicone was used (Silicona Blanda Blanda, Herbitas, Valencia, Spain), covering 2–3 cm of the caudal vagina, to allow easier incorporation into an acrylic box. This material was chosen because it better mimics the soft consistency of vulvar labia (Figure 2).

As a support base for the 3D vaginal model, a rectangle transparent acrylic box measuring 24 cm × 17 cm × 14.5 cm was produced (Figure 3). In the caudal side of the box, a hole was made that allowed the introduction and exposure of the vulva part (Figure 3). Inside and centrally in the box, an acrylic support was attached to fix the vaginal model (Figure 3). A removable cover of artificial fur was applied to the structure to improve the visual realism and only allow visualization of the vulva (Figure 3). This adhesion was made through Velcro strips glued to the acrylic base and covering fur (Figure 3). As generally there is the need to deviate the tail in order to have access to the vulva and to improve the model's visual characteristics, a dog's tail was manually produced. This was built with small pieces of six cables of different thicknesses to imitate the caudal vertebrae and centrally joined through a moldable plastic wire (Figure 3). Finally, it was covered with synthetic fur fabric, measuring around 22 cm (Figure 3). The dog's tail was sewn to the tissue fur over the dorsocaudal area. To provide feedback to students on the performance of the vaginal cytology technique, a mixture of vaseline with food coloring (red: E122 or blue: E133, two drops per 5 g of vaseline), was placed using a long spatula in three different anatomical locations. In the cranial vaginal area (i.e., the correct location to collect the cells), a pink color mixture was placed through the dorsal orifice of the model (Figure 4). At the site of the clitoral fossa and urethral orifice (i.e., incorrect locations), blue color mixtures were placed through the caudal opening (Figure 4). The placement of the inked vaseline was verified by direct observation, through the vulvar opening and dorsal opening (Figure 1). The inked vaseline remained adherent to the model's surface for months at room temperature and needed to be refilled only after being used by ≈50 students (data not shown).

Comparison Between Traditional and Simulated Training

The fifth-year veterinary students attending the classes of Theriogenology at ICBAS – University of Porto were recruited. The study was approved by the competent local authorities, regarding the use of animals for teaching purposes ["Órgão Responsável pelo Bem-Estar dos Animais" ORBEA N°053.2/2013] and regarding the studies on human subjects [Comissão de Ética 2022/CE/P04(P382/2021/CETI)]. Students, participation

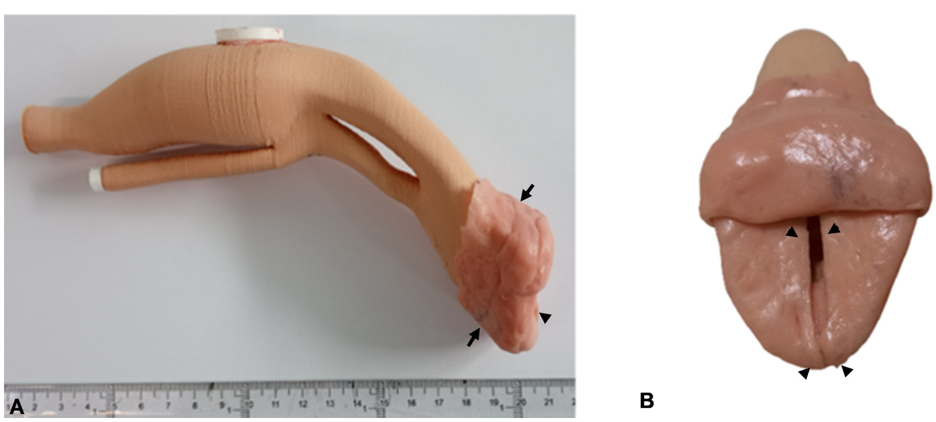

Figure 2: (A) 3D printed vagina using Thermoplastic Polyether-Polyurethane material and soft silicone to produce the vulva (arrows) and labia (arrowhead); to better evaluate the size of the piece, a rule (in centimeters) is included. (B) Detail of the vulva with the labia (arrowheads). The color and consistency of the soft silicone material resembled that of living tissues

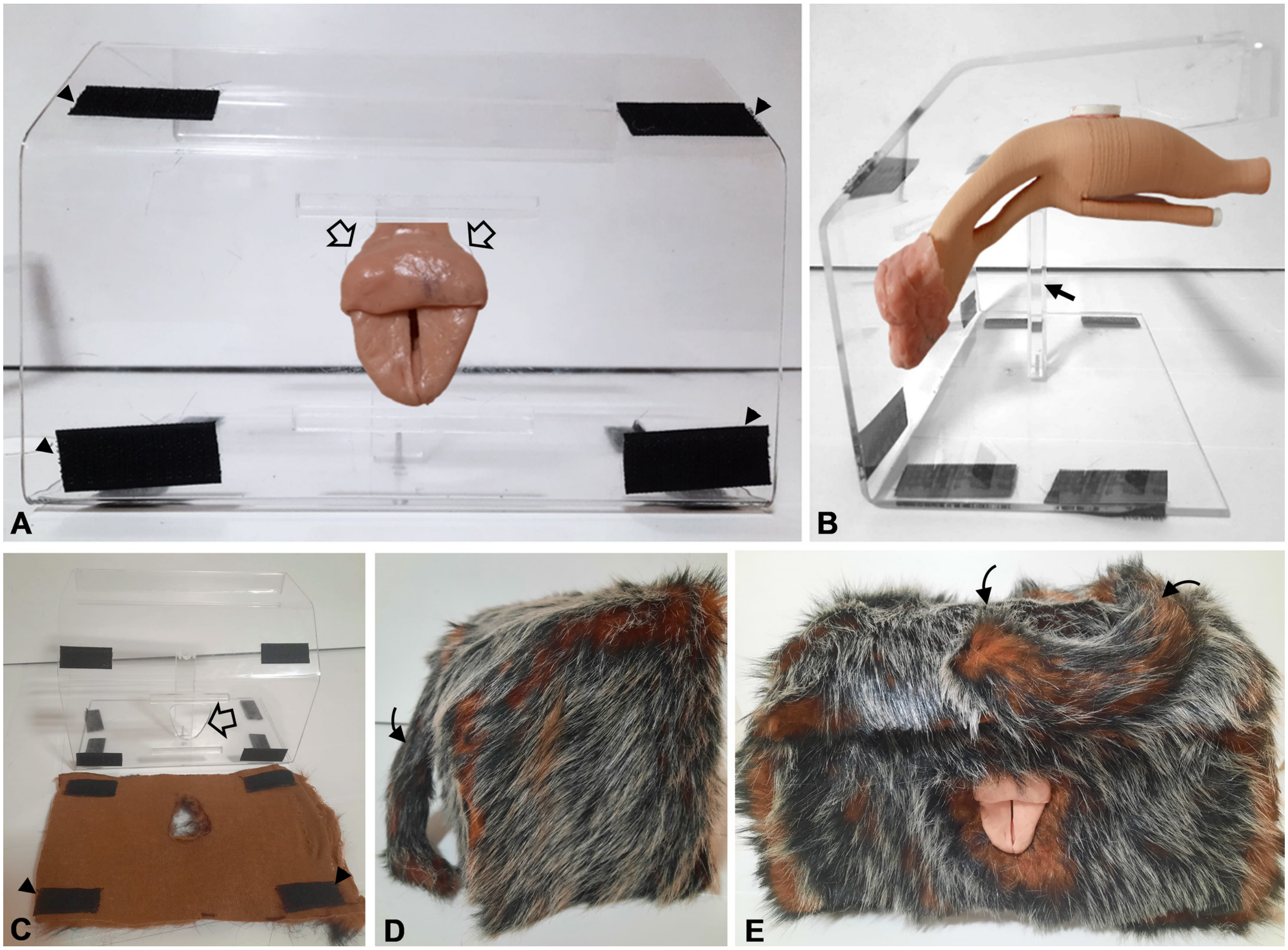

Figure 3: (A) Acrylic support for the model, in which a central hole was made (block arrow); the vulva is displayed for illustrative purposes. Velcro strips (arrowheads) were inserted in the support. (B) Lateral view of the support. It included a vertical column (arrow) that supported the printed vagina. (C) Artificial fur was cut with the exact measures of the box and Velcro strips (arrowheads) were inserted on it. (D) The acrylic support is all covered with artificial fur. To better mimic the live animal, a tail (curved-arrow) was manually constructed. (E) After building the model, only the vulva was seen from the outside (when the tail [curved-arrow] was lifted)

was voluntary, as they could choose not to participate without failing the course. An informed consent at the beginning of the study was signed, allowing data analysis of the questionnaires and video recording during the procedures.

Classes followed an inverted design (in-class flip with stations) and students were randomly divided into two groups (Figure 5). Firstly, students watched a small video on the vaginal sampling technique, explaining the procedure and the most

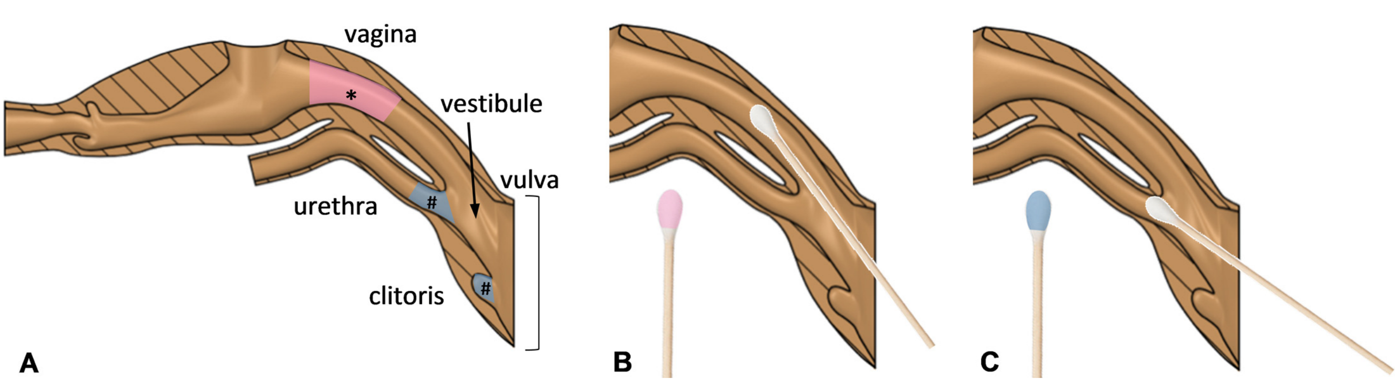

Figure 4: (A) Mid-section view of the 3D printed model showing the area covered with pink-colored vaseline, corresponding to the correct location for sampling the cells, and the areas covered with blue-colored vaseline. (B) When students introduced the swab in a correct angle, a pink colored swab was obtained (detail). (C) On the contrary, when students introduced the swab with an incorrect angle, a blue swab was obtained (detail)

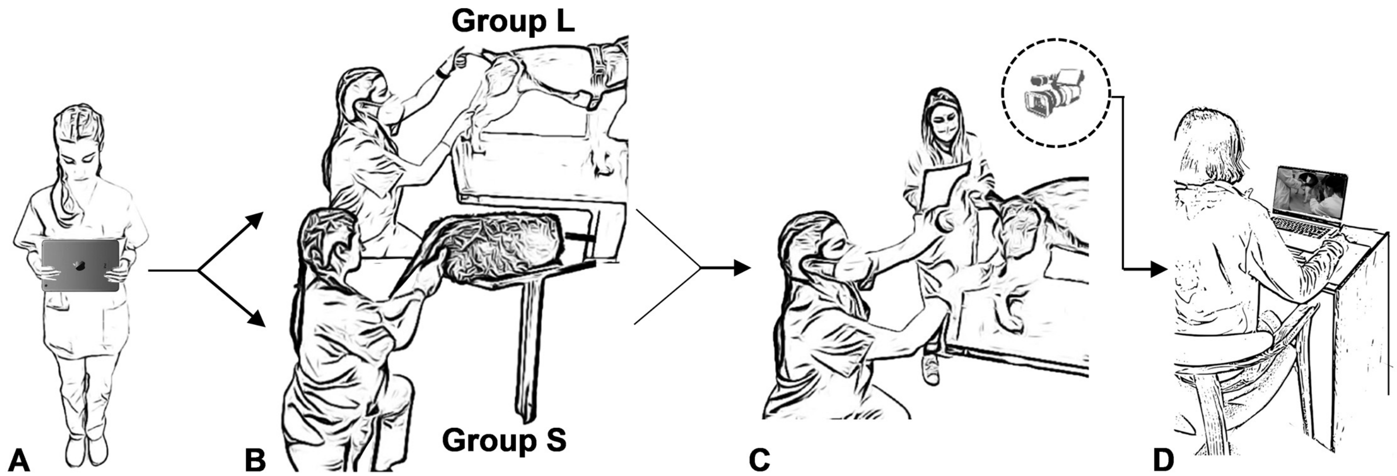

Figure 5: (A) The activity followed an in-class flip with stations design, in which students started by watching a video of the procedure. (B) Students were randomly divided in two groups: those that practiced with the simulator (Group S) and those that worked with live animals (Group L). (C) An examination procedure with a live animal was performed, which was video-recorded. (D) The procedures on the videos were assessed by filling an objective structured clinical examination scoresheet. The external observer was blinded to the previous type of training of students

frequent errors, namely the placement of the clitoris and urethral opening. Briefly, both groups were instructed to open the vulvar lips, insert the moistened cotton swab at the dorsal commissure of the vulva, so as to avoid the ventral clitoral fossa, and then up and over the pelvic brim and into anterior vagina.[8] Group L students were also instructed that if by mistake the swab was introduced in the fossa clitoris, the bitch would show signs of discomfort (vocalize/whine, struggle) and the swab would not progress. Group S students were instructed that the color of the swab would allow a self-assessment on the correct versus incorrect site of sampling collection. Then one group practiced the vaginal sampling procedure only in the simulator (Group S from simulator), whereas the other group practiced on live bitches (traditional teaching method, Group L from live animals). Groups S and L practiced the technique during two classes of 3 hours, but with minor differences. Due to animal welfare reasons, group L students only practiced the procedure twice, once during the first class and once 2 weeks after; this restriction was due to the limited number of existing bitches (four to five per class). Contrasting to this, group S students could freely practice the procedure, without any restriction on the number of trials. The model was fitted to group activities (Figure 6): one student handled the tail, while others worked with the model, collecting the material and getting immediate feedback on a correct or incorrect technique by the color of the swabs (pink and blue swabs, respectively) (Figure 6). Afterward, students practiced the extension of the inked vaseline material in the glass slide, as it would occur in the real activity (Figure 6). At the end of the second class, a questionnaire (Supplemental File 1) was given to each student.

Three weeks after the end of training, an evaluation of the vaginal sampling technique was performed in a bitch. All the parameters to be evaluated had been previously practiced, by all evaluated students, using one or another model. Since the teaching staff at ICBAS (R.M., R.M., and G.L.) had helped during classes, being aware of which students practiced at one or another model, a fully unbiased evaluation could not be assured. Therefore, it was decided to video record the evaluation procedure, so that an external evaluator (S.M.), blinded to the previous training model, assessed the videos and filled an objective structured clinical evaluation (OSCE) scoresheet (Supplemental File 2). This evaluator assessed if students performed all the steps of the vaginal sampling collection technique, that is, moistened the cotton swab, separated the vulvar labia, introduced the cotton swab at an angle of 45° and rolled the swab against the vaginal surface, and finally removed the swab.

Statistical Analysis

A statistical software (SPSS26 [IBM, Armonk, USA]) was used. The answers to the questionnaires of the two groups of students and OSCE scores were compared with the Mann–Whitney U test.

RESULTS

A total of 25 fifth-year students enrolled in this study, 12 and 13 students in groups S and L, respectively. Gender and age distribution were similar in the groups, with a clear female gender preponderance (92% and 85% females in groups S and L, 22–26 years old in both groups). At the OSCE evaluation, students from both groups performed all the steps. No statistically significant differences were noted at the OSCE scoresheet performed through the unbiased video evaluation (Figure 7).

Regarding data collected by the questionnaire, the majority of students had zero or little experience (up to five times) on performing vaginal sampling collection (92% and 54% of students in groups S and L had never done the procedure) (Figure 8A). The majority of students considered that three to five attempts were the ideal (67% and 54% of students in groups S and L, respectively) to learn the procedure, but some students considered that more attempts were needed (>5 was deemed necessary by 25% and 46% of students in groups S and L, respectively). Only one student (Group S) considered that two attempts were ideal (Figure 8B). Likewise, when students reported which parameters would increase their success in performing a vaginal sampling, most selected a greater number of attempts (Table 1). This option was immediately followed by a higher number of models, but only in group L. Students reported that the higher number of models and the possibility to repeat the procedure were two major advantages of the model.

Figure 6: (A) Practice with models during classes. Students are working in groups, lifting the tails of models (arrows) or giving support. (B) Detail of the procedure in the model. A student is repeating the procedure, after having some swabs from wrong locations (cardinal); another student is lifting the tail (arrow). (C) After retrieving the swab, the material was placed over glass slides. At the end of the class, the swabs could be gathered to compare those from correct (asterisk) and incorrect locations (cardinal); in this case 3:2 ratio was achieved. (D) Practice with live animals during classes: while one student is doing the procedure, another is lifting the tail (arrow)

Table 1: Students' answers from anonymous questionnaires to the question "Indicate which parameters would increase the success in performing the vaginal cytology procedure; please select all the applicable options"

Parameters that would increase the success	Group S ($n = 12$)	Group L ($n = 13$)
More time for performing the procedure	1 (8)	1 (8)
Greater number of training attempts	9 (75)	12 (92)
Larger number of models available	2 (17)*	10 (77)*
Different type of training models	7 (58)	8 (62)
Support and feedback by the teacher	5 (42)	7 (54)
Support and feedback from classmates the teacher	2 (17)	2 (15)

Note: Number of students that selected that answer and percentage, respecting the number of students in each group, in parenthesis.
*Significant differences.

The color of the swabs was an important feature for Group S students, as it granted self-assessment on the proper technique. If students got a blue swab (incorrect sampling location), they repeated the procedure until achieving a pink one, corresponding to vaginal sampling.

DISCUSSION

Best practices in teaching practical skills should target active learning from students, with deliberate practice of skills, set in a safe environment.[13] Simulation-based strategies generally hit those targets and have been increasingly used in veterinary faculties.[2,6,14–16]

To the best of our knowledge, the present study reported the first developed vaginal cytology model, being one of few studies directly comparing simulation-based with animal-based practice. In most studies, simulation has been compared to other teaching strategies, such as theoretical approaches (e.g., using videos) or comparisons among simulators have been performed.[17] Nowadays, the drive in veterinary pedagogy is to use alternatives to live animals in teaching. Therefore, various

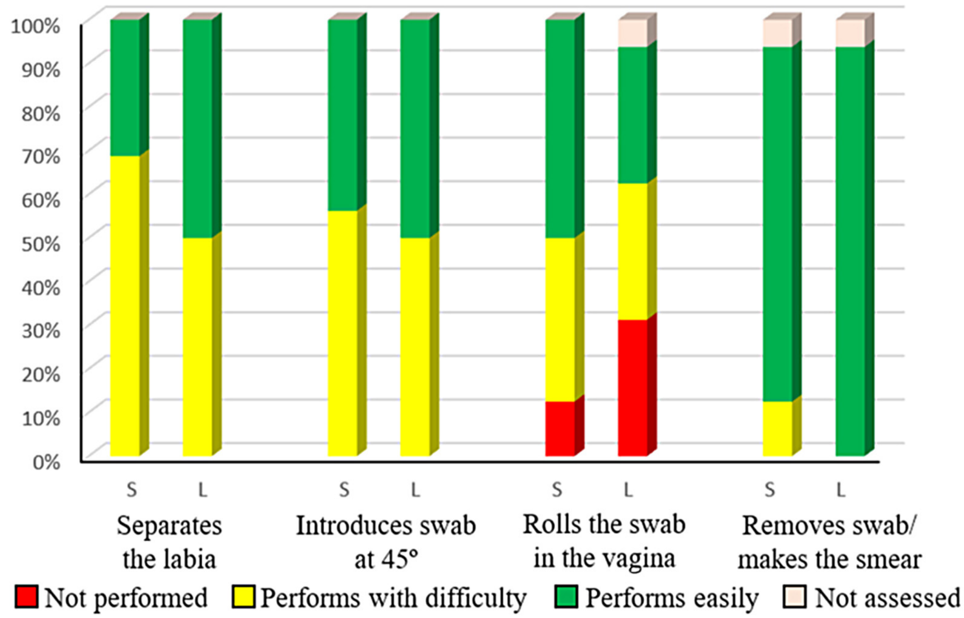

Figure 7: Objective structured clinical examination pass rates from students who practiced in the simulator (S) and in the live animal (L), assessed through the videos by an evaluator blinded to the previous practice of students. No differences in the various steps of the procedure were noted. "Not assessed" refers to procedures that were not included in the video files

simulators for gynecological examination have been developed and assessed, mostly for large animals,[9–12] but none existed for vaginal cytology in the bitch. Commercial simulation models of the canine female reproductive tract already exist (e.g., Gyndog, Veteducators GmbH – Vetiqo, Berlin, Germany), allowing the gynecological examination via speculum or endoscope of the female reproductive tract and also practicing artificial insemination by transcervical catheterization. However, these are specialized procedures restricted to veterinarians devoted to small animal reproduction. Contrasting with this, vaginal sampling is a generalized procedure, a skill that should be mastered by any small animal practitioner. The construction of the model was simple and economic. The 3D printed vaginal costed $34 US, and only 8 g of soft silicone was needed for the vulvar labia (costing less than 1 USD), meaning that the model costed less than $100 US (the acrylic box [Figure 3] was the most expensive item). Vulvar labia were relatively easy to make and no special skills were needed, since modeling the soft silicone was as simple as modeling play dough.

The three more important features of simulation-based strategies are feedback, deliberate practice, and curriculum integration.[18] While all forms of feedback are important for deeper learning, it has been shown that feedback by the simulator[2] and after the simulation[19] are the most relevant. Complex simulators may provide feedback by haptic methods or electronic devices, but herein feedback was achieved straightforwardly, since immediate feedback was provided by the color of the retrieved ink. This granted self-assessment for students, who repeated the procedure until getting the right color. Feedback from colleagues is also relevant, since teaching others is always useful for solidifying a skill and improves knowledge retention[19]; this occurred in our classes, since students relied on their classmates' opinions during training in groups (Figure 6). As to terminal feedback, it was provided during the examination with live animals by the presence of the facilitator (teacher).

Deliberate practice is another cornerstone among simulators, being the strongest predictor of a skill level.[20] The ultimate

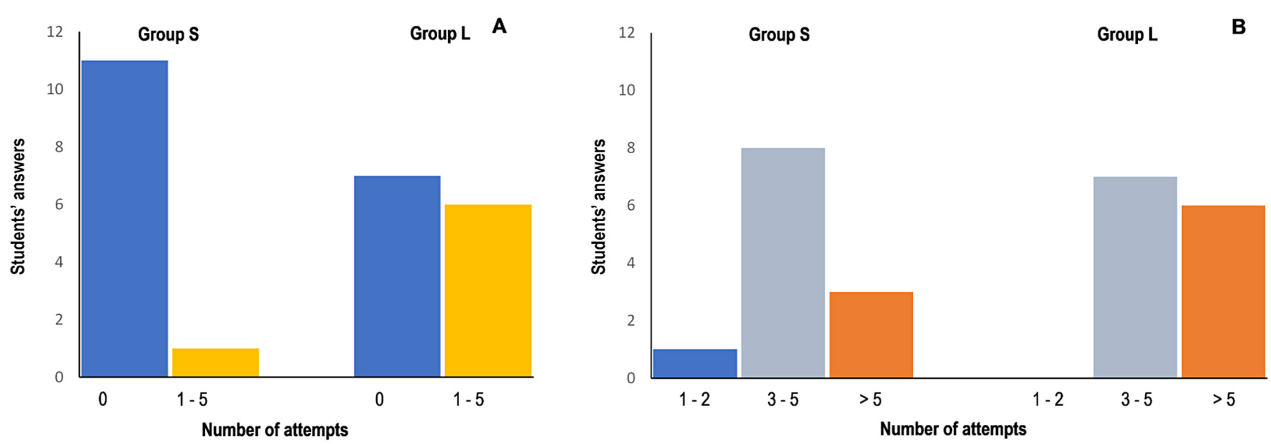

Figure 8: Answers from students that practiced in the simulator (S) and in the live animal (L) to the questions (A) "What was your previous experience on canine vaginal sampling?" and (B) "How many attempts are needed for a proper learning of the canine vaginal sampling?"

goal of practical classes at the university is to provide the expertise level on specific tasks, including vaginal sampling. In accordance with this, students considered that three to five attempts, or even more, were needed to properly learn the procedure, and among various parameters, the majority of students considered the higher number of attempts (i.e., deliberate practice) as the most important for increasing their success (Table 1). Inevitably, such a repetition of procedures collides with animal welfare, since vaginal sampling is uncomfortable for non-estrous bitches, and that was the reason behind the two attempts restriction of Group L. Notably, most students considered such number as insufficient. Even if overall students performed well in the OSCE evaluation, Group L students were probably less confident than those who had practiced more. In order to increase practice with live animals, a higher number of bitches were needed in classes, so that more repetitions per student could be allowed. However, this not only collides with the increasing concern of modern societies toward the use of animals in science,[6] but also poses financial pressure to universities from kennel costs. Furthermore, traditional teaching relying on live animals or in hospital cases usually provides limited opportunities for students to practice the collection of samples. In order to circumvent the costs and ethical issues, some faculties use cadavers to practice several technical procedures. Although students could repeat vaginal cytology in cadavers, the typical anatomical characteristics of a live bitch may not be maintained due to the decomposition and thawing processes. In contrast with this, the simulator allowed deliberate practice in a comfortable and well-controlled environment that provided a valuable teaching moment. As proved from the OSCE evaluation, the simulator was as effective for learning the vaginal sampling procedure as a live animal. Students could spend as much time as they needed in each stage of the model, according to their individualized training. It should be noted that simulators surpass the use of cadavers in terms of validation, reliability, and transfer of skills to clinical practice, especially in basic techniques.[21] It is well known that deliberate practice can be increased through increasingly difficult challenges. In this vein, the model developed herein can be adapted by fitting a smaller vagina, mimicking a vaginal cytology in a toy breed. After successfully practicing in larger models, students will further practice in those smaller ones.

Curriculum integration is another fundamental requisite of simulators. Herein, the simulation activity was carried out during the practical classes of Theriogenology, being framed within teaching the estrous cycle in the bitch. Using a simulation model has several advantages in this regard, as it allowed integrating theoretical and practical elements. For instance, the model could be adjusted in clinical cases of the estrous cycle and vaginitis. When dealing with real patients at the clinical practice, it is highly unpredictable when opportune pedagogical cases will appear.[22] During an entire semester, students may well miss a case of estrus or vaginitis. The model could be further improved by including the clinical history of animals and the microscopical observation of vaginal samples, by using virtual slide, thus creating an immersive simulation experience. It has been shown that these latter are comparable to traditional slides for teaching cytology to students.[23] It would be interesting to further investigate its use in an immersive simulation strategy that would include the collection of samples and basic diagnostic procedures.

Our study has some limitations, mainly related to learning outputs. Firstly, we failed to assess skills maintenance. This could have been achieved by repeating the OSCE evaluation after 6 months or in the following year,[24] using a similar checklist. Secondly, we included a limited number of students. Despite obtaining promising results, it would be interesting to evaluate the use of the model in a large population of students, ideally in a multicenter study. Thirdly, the model does not allow confirmation of successful sampling collection by microscopic observation of cells, as it is possible in the bitch. Finally, we performed no pre-test to assess any prior experience on the vaginal sampling procedure and assumed *a priori* that groups were similar on this parameter (as it turned out to be from the answers to the questionnaires).

In conclusion, we have developed a vaginal sampling simulator and proved that it is comparable to the use of live animals to practice the collection of samples. Therefore, live animals can effectively be replaced by this model for learning the vaginal sampling technique. This model continues the settled path of using non-animal alternatives in teaching veterinary students and it may prompt a wider and more refined use of cytology as a diagnostic tool among veterinary practitioners.

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