Vaginal cytology in queens with estrus induced with equine chorionic gonadotrophin

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Summary: In order to determine the indirect influence of several doses of equine chorionic gonadotrophin (eCG) on vaginal cytology, 42 queens were randomly allotted to four groups for treatment with 0.9% saline (control) or with 25, 40 or 60 IU/Kg of eCG injected IM. Epithelial and blood cells examined in 135 vaginal smears showed the normal cell pattern of feline estrus. Mucus was seen in all smears. However, small intermediate cells were rarely found and the presence of erythrocytes and neutrophils was considered negligible. In conclusion, the cell pattern of the vaginal epithelium of queens with estrus induced with different doses of eCG was similar to that of queens with natural estrus. Thus, we may speculate that the hormonal stimulus occurring in the vaginal epithelium of eCG-induced queens is the same as that occurring in natural estrus, or that this epithelium responds similarly to different dose of eCG. On this basis, vaginal cytology may be not an efficient tool for the assessment of the hormonal environment of queens with induced estrus.

Resumo: Para determinar a influência indirecta de várias doses de gonadotrofina coriônica eqüina (eCG) na citologia vaginal, 42 gatas foram distribuídas aleatoriamente em quatro grupos tratados com solução salina 0,9% (controle) ou com 25, 40 ou 60 UI/Kg de eCG via IM. De 135 esfregaços vaginais, foram examinadas as células epiteliais e sanguíneas, que apresentaram padrão celular normal para o estro felino. Houve presença de muco em todos os esfregaços. Por outro lado, células do tipo pequena intermediária foram raramente encontradas, e a presença de eritrócitos e neutrófilos foi considerada irrelevante. Em conclusão, o padrão celular do epitélio vaginal de gatas com o estro induzido por diferentes doses de eCG foi semelhante ao de gatas em estro natural. Assim, podemos especular que o estímulo hormonal sobre o epitélio vaginal em gatas que receberam eCG é o mesmo do das gatas em estro natural, ou que este epitélio responde da mesma maneira frente a diferentes doses de eCG. Neste sentido, a citologia vaginal pode não ser uma ferramenta eficiente para a avaliação do ambiente hormonal de gatas com estro induzido.

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¹FUNCAP fellowship
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In rats, the vaginal epithelium is highly sensitive to hormone administration, permitting a reliable endpoint assessment of hormonal imbalance (Banik and Herr, 1969; Nagaoka et al., 2002). In contrast, the indirect influence of exogenous gonadotrophins causing the steroidal hormones imbalance after ART procedures on the cellular pattern of the vaginal epithelium of cats is poorly known. Indeed, due to the sensitivity of the vaginal epithelium to steroid hormones, vaginal cytology may be an indirect parameter for the determination of the hormonal environment of gonadotrophin-induced estrus in cats. Thus, it is very important to establish reliable endpoints for the assessment of the individual hormonal milieu of queens with gonadotrophin-induced estrus queens in order to assess the consequences of ART procedures.

Thus, as the action of equine chorionic gonadotrophin (eCG) and FSH are similar concerning the releasing of estrogens, the purpose of the present study was to access the possibility of the indirect influence on the vaginal cellular profile of queens by ovarian stimulation with progressive increasing doses of eCG.

Materials and Methods

Animals and treatments

Forty-seven adult intact undefined breed cats (Felis catus) were used in this trial, 42 queens (body weight 2.5 to 4.8 kg), and five toms (3.5 to 5.8 kg). The animals were housed in individual cages (80 x 60 x 60 cm) and fed a dry maintenance cat food (Eukanuba Chiken and Rice Formula, Kitekat and Gatsy) and water ad libitum throughout the study. In addition, they were kept free in a commun solarium with sufficient material for distraction for at least four hours/day. The experiment was accomplished from February to June of 2002, in the Fortaleza city, Northeastern Brazil, situated at 3° 43’ 47” South and 38° 30’ 37” West. In this region there are few differences in number of hours per day along the year and the queens show estrous cycle during all year. In this work, the queens were maintained in natural light (approximately 12 h light / 12 h dark). The queens were randomly allotted to four groups receiving different doses of eCG (IU/kg/IM) as follows: 0 (control group), 25, 40 and 60. The estrous cycles were daily monitored, with evaluation of the peculiar signs of each phase according to previous studies (Banks, 1986; Johnston et al., 2001; Silva et al., 2001). On the fifth day of interestrus, the queens received a single injection of eCG. The control group was considered as natural estrus and received only 1 mL of saline solution (0.9%). After hormonal or placebo injection, the queens were exposed daily to the toms until male acceptance, although at that time mating was prevented by us. Twenty-four and 48 h after the first day of male acceptance by queens, four mating episodes were allowed to occur over a period of two hours. It is important to point out that this study is part of a wide trial which evaluates the effects of gonadotrophins on different reproductive parameters (data not shown). This explains the mating procedures cited above. Thus, only vaginal cytology findings will be mentioned.

Vaginal cytology

Ten to 15 minutes after the last mating, vaginal cells were obtained by gently passing a sterile cotton-tipped swab into the vaginal canal followed by a quick 180° rotation. The cells were transferred to one or two slides, air dried, and immediately fixed with 100% ethanol. The smears were stained with hematoxylin and eosin, modified (Mattos et al., 2001b). A total of 135 vaginal smears were obtained from the four different treatments on the second and third days of permitted mating. Twenty-six slides were prepared for the control group, 36 for the 25 IU group, 34 for the 40 IU group, and 39 for the 60 IU group.

Evaluation of vaginal cells

Smears were examined under a light microscope at magnifications of X32, X100, X400 and X1000. Two-
hundred epithelial cells from each slide were evaluated and classified according to previous studies (Concannon et Digregorio, 1986; Shille et al., 1979; Mattos et al., 2001b) as: parabasal (PB), small intermediate (SI), large intermediate (LI), nucleated superficial (NS) and anucleated superficial (AS). Blood cells, mainly erythrocytes and neutrophils, as well as the presence of the mucus and cellular debris were also recorded.

Statistical analysis

All data were analyzed by SAS. Cell type data were log transformed and were reported as mean ± SEM. The effect of the different doses of eCG on the vaginal cell profile was analyzed by General Linear Model (GLM). Data for the control group and treated groups were compared by Dunnett’s test. Student’s t test was performed to compare each treatment as well as the mean of all groups on the first and second days of mating acceptance. Values were considered statistically significant when p<0.01.

Results

Figure 1 shows that there was no difference in the percentage of epithelial vaginal cells from the vaginal smears obtained for each treatment. Similarly, no differences in cell type were detected between the first and second day of mating within each group or when the mean for all groups were compared (Figure 2). Few parabasal cells and small and large intermediate cells were present, in contrast to nucleated and anucleated superficial cells. No cell debris were found in any smear. In contrast, mucus and spermatozoa were detected in all smears. Concerning blood cells, neutrophils were not found in any smear and only one erythrocyte was seen on one slide obtained on the second day of mating from the 40 IU group. Thus, the presence of neutrophils and erythrocytes was considered negligible in this trial.

The elapsed time, in days, between the application of the treatment and the mating acceptance was of 18.03 ± 7.69 for control group; 2.68 ± 1.12 for 25 UI group; 2.89 ± 1.36 for 40 UI; and 2.37 ± 1.65 for 60 UI.

Discussion

Exogenous gonadotropin administration to cats causes several pathological alterations such as altered cyclicity, exacerbated estrous behavior, follicular cysts, feline mammary fibroadenoma, pyometra, and other alterations (Dresser et al., 1987; Silva et al., 2001). On this basis, we speculated that gonadotropin would have an indirect action on the differentiation of vaginal epithelial cells observed by vaginal cytology. However, our findings rejected this hypothesis since each gonadotropin dose yielded the same results (Figure 1). In this respect, examination of the cell profile by vaginal cytology during the estrous cycle may be an inefficient tool for the evaluation of the hormonal environment in cats with induced estrus.

In this trial, the profile of the epithelial cells detected on vaginal smears for feline estrus was similar to the findings reported by other authors for feline natural cycles (Mower et al., 1975; Herron, 1977; Mills et al., 1979; Shille et al., 1979; Toniolo et al., 1995; Nascimento and Lopez, 1999; Aragão and Ferreira, 2000; Johnston et al., 2001). According to Johnston et al. (2001), no cell debris were found. The presence of mucus and spermatozoa in all smears has also been reported in previous studies (Mower et al., 1975; Herron, 1977).

The vaginal epithelial profile was the same for the first and second day of mating (Figure 2), suggesting that collection of a vaginal smear with a swab and mating do not alter exfoliated cells. In addition, the interval of one day during estrus also caused no changes in cell profile. Interestingly, some cat breeders believe that mating causes vaginal bleeding which is necessary for conception. However, in the present trial as well as in all the available scientific literature the erythrocytes were absent (Mower et al., 1975; Herron, 1977; Shille et al., 1979; Mills et al., 1979; Nascimento and Lopez, 1999; Johnston et al., 2001). In addition, according to Johnston et al. (2001), excluding anestrus and the pregnancy/pseudopregnancy phases of the estrous cycle, white blood cells are usually absent or rarely found in vaginal smears from queens. In addition, in contrast to canine vaginal smears, no erythrocytes are observed in queens during proestrus/estrus.

In conclusion, the cellular pattern of the vaginal epithelium of queens with eCG- ovarian stimulation at the schedule and dose used in the present study was similar to that of natural estrus. Thus, the indirect hormonal stimulus of the vaginal epithelium was the same, even with progressive increasing doses of eCG.

Acknowledgements

The authors wish to thank FUNCAP and CNPq for fellowships, and EFFEM do Brasil® for supplying Kite Kat® food. We also wish to thank Dr. Manuel Odorico de Morais, Departamento de Farmacologia, Universidade Federal do Ceará (UFC), and Margarida Maria de Lima Pompeu, Núcleo de Medicina Tropical – UFC, for facilities.

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