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Review

APPLICATION OF EXFOLIATIVE VAGINAL CYTOLOGY IN CLINICAL CANINE REPRODUCTION – A REVIEW

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Summary

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Vaginal cytology has many practical applications in the evaluation of both the normal and abnormal bitch. The objective of this review is to describe the use of exfoliative vaginal cytology as a diagnostic tool in clinical canine reproduction.

Key words: bitch, exfoliative vaginal cytology

INTRODUCTION

Exfoliative vaginal cytology is the most popular diagnostic method as a part of the gynaecological examination in the bitch (Dreier, 1975; Gunzel-Apel & Koivisto, 1984; Linde & Karlson, 1984; Soderberg, 1986a,b; Tammer *et al.,* 1994; Johnson, 2006; Wehrend, 2007; Trasch, 2008; Turmalaj *et al.,* 2011; Groppetti *et al.,* 2012; Leigh *et al.,* 2013; Wehrend *et al.,* 2013). It is based on determination of cyclic cellular changes occurring in the vaginal epithelium as a result of reproductive hormone levels, especially estrogens (Wright & Parry, 1989). The method is simple and useful even in physiological or pathological conditions of the bitch reproductive system, most frequently the determination of the estrous cycle stages with respect to the optimal breeding time (Turmalaj *et al.,* 2011).

PROCEDURES FOR VAGINAL CELL COLLECTION AND OBTAINING SMEARS

Vaginal cells could be collected using a sterile speculum and a saline-moistened cotton swab (Wrobel *et al.,* 1975; Wright & Parry, 1989; Gunzel-Apel, 1993). It is important to prevent contact of the swab with the vestibule, because its cells do not react as quickly to an increase in the blood estrogen concentration as the vaginal mucous membrane (Röttger, 2010).

Collection of vaginal cells may be done using only a cotton swab, without a speculum (Feldman & Nelson, 1996; Bowen, 2000; Johnston *et al.,* 2001; Kustritz,

2006; Aydin *et al.,* 2011). The cotton-tipped end of the swab is passed into the dorsal commissure of the vulva, pressed gently in the caudodorsal direction until it passes over the ischial arch and then the swab is rotated through a complete revolution in each direction and withdrawn (Aydin *et al.,* 2011). The cotton tip is lightly rolled from one end of a glass microscope slide to the other (Feldman & Nelson, 1996; Johnston *et al.,* 2001; Kustritz, 2006).

Vaginal cells may be collected by inoculation in the vaginal cavity and afterwards aspiration of sterile saline using a plastic catheter (Olson *et al.,* 1988). The liquid is applicated on a glass microscope slide, spread into a thin film and air-dried. The method is minimally invasive, but sometimes a change in cell morphology and lower absolute cell count are observed (Olson *et al.,* 1984a,b; Guyant, 1988).

The smears could be stained using a trichrome or Papanicolaou stain (Papanicolaou, 1942), but the technique is labourious (England & Concannon, 2002). Many authors reported its use after reducing some steps of the staining (Barret, 1976; Kubicek, 1978; Dumon & Morel, 1989).

New methylene blue stained smears could be viewed immediately after dye application, but red blood cells are not stained and the smears can not be stored for examination at a later time (Johnston *et al.,* 2001). Other classical staining techniques use stains such as May-Grunwald, Boehringer Mannheim, Pappenheim and Testsimplets® (Gunzel-Apel & Koivisto, 1984).

Diff-Quick® or Haemacolor® (Merck KGaA) is a rapid modified Wright-Giemsa stain easily applicable in the clinical practice. The smears are fixed with methanol and then are stained with the two solutions of Diff-Quick® stain. These smears could be stored for years and examined later (Johnston *et al.,* 2001; Chatdarong *et al.,* 2002).

Aydin *et al.* (2011) have examined vaginal smears by a direct technique comparing it with classical staining to determine the stages of the sexual cycle of the bitch and found it reliable only in detecting the estrus stage.

EVALUATION OF VAGINAL SMEARS

The evaluation of vaginal smears is performed with a light microscope at magnifications of 100 to 400×. A minimum of 10 observation fields should be examined (Theise, 2002).

There are different vaginal cell types (Ehlers, 2000). According to most authors, the cells from the vaginal wall are differentiated into basal, parabasal, intermediate, superficial and squamous cells (Rieck & Kratzheller, 1955; Schutte, 1967; Dreier, 1975; Guyant, 1988; Maneke, 2002; Theise, 2002; Johnson, 2006).

It is generally accepted that vaginal cell types in the smear are related to the stage of the estrous cycle (Bell *et al.,* 1973), which makes the vaginal exfoliative cytology a valuable add-on test in reproductive clinical diagnostics (Christie & Bell, 1973; Ehlers, 2000).

Basal cells are the smallest cells (10–20 μm) in the vaginal wall. They are occupied almost entirely by the nucleus and are rarely identified in the smears because they are on the basement membrane (Olson *et al.*, 1984a; Wright & Parry, 1989).

Parabasal cells are small (15–25 μm), round or ovoid with marginal big nucleus. Occasionally, they contain cytoplasmic vacuoles. Parabasal cells may also contain neutrophil granulocytes in the cytoplasm

and then are called metestrus cells, although they can occur in other cycle stages (Wright & Parry, 1989) or vaginitis (Olson *et al.,* 1984b).

Intermediate cells exhibit great variations in diameter, so they are differentiated as small (20 μm) and large type (30 μm) (Rieck & Kratzheller, 1955; Dreier, 1975; Olson, 1989; Maneke, 2002). Both types have a well shaped nucleus. Small intermediate cells are round to elliptical, but may also have polygonal outline whereas the large type has an irregular and angulated cytoplasmic border (Christie *et al.,* 1972). Intermediate cells have a prominent nucleus. The large type is sometimes confused with superficial cells because they are of similar size (Johnston *et al.,* 2001).

Superficial cells are large cells with a diameter ranging between 30 μm (Christie *et al.,* 1972) and 75 μm (Concannon & Digregorio, 1986). They have irregular or angulated borders and dark, pyknotic or faint nucleus. Superficial cells attain their maximum at the time of estrogen peak (Rieck & Kratzheller, 1955; Christie *et al.,* 1972; Dreier, 1975; Concannon & Digregorio, 1986; Johnston *et al.,* 2001; Maneke, 2002; Johnson, 2006).

Squamous cells are large cornified superficial cells which underwent degeneration to become dead anucleated cells (Simmons & Olson, 1989). They usually stain dark blue-purple during the estrus (Johnston *et al.,* 2001).

Besides the vaginal cells, other types of cells observed in vaginal smears are red blood cells; neutrophil granulocytes (leukocytes); bacteria; tumour cells; clitoral fossa epithelial cells; spermatozoa; giant trophoblastic cells and debris (Maneke, 2002).

CLINICAL APPLICATION OF VAGINAL EXFOLIATIVE CYTOLOGY

Determination of optimum time for breeding or artificial insemination

Fertility in the bitch is considered to be of great socio-economic importance (Leigh

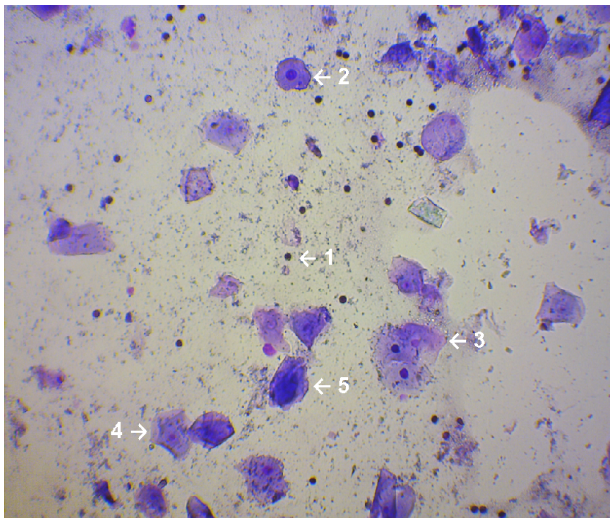

Fig. 1. Vaginal smear of a bitch in proestrus: 1 – red blood cell; 2 – small intermediate cell; 3 – large intermediate cell; 4 – superficial cell; 5 – squamous cell (original, Haemacolor®, ×100).

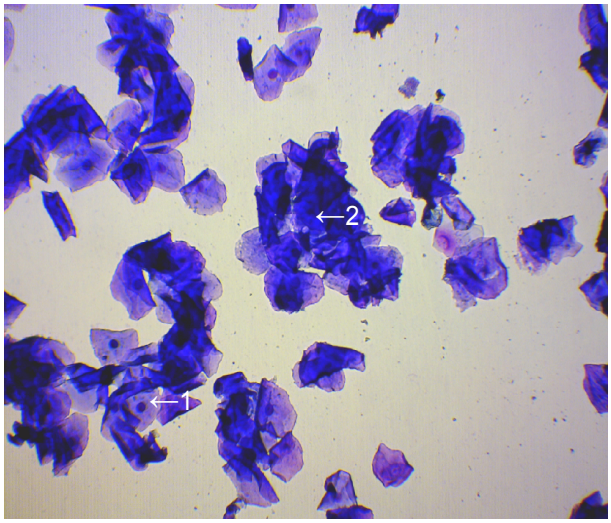

Fig. 2. Vaginal smear of a bitch in estrus: 1 – superficial cell; 2 – squamous cell (original, Haemacolor®, ×100).

et al., 2013). The majority of the bitches presented with history of infertity are in fact fertile (Concannon *et al.,* 1989). The most common cause of conception failure with reported incidence between 40 and 50% is mistimed breeding time (Zoldag *et al.,* 1993; Johnston *et al.,* 1994).

A gradual shift from parabasal and intermediate to superficial cells occurs during the proestrus. Also, large numbers of red blood cells are present (Fig. 1). The optimum time for natural breeding or artificial insemination is the estrus stage, when the percentage of superficial and squamous cells (Fig. 2) in vaginal smears is above 80% (Simmons, 1970; Johnston *et al.,* 2001; Srinivas *et al.,* 2004). Breeding or insemination should be done in a two or three days interval until diestrus occurs, as recognised by the appearance of parabasal cells, neutrophils (Simmons, 1970; Srinivas *et al.,* 2004) and the number of superficial and squamous cells decreases by at least 20% (Holst & Phemister, 1974) (Fig. 3). Conception rates after a single mating are above 95% if breeding occurs 3 to 10 days before the onset of cytologic diestrus. The detection of the first day of cytologic diestrus allows determining whether the breeding was done at the appropriate time (Johnston *et al.,* 2001).

Exfoliative vaginal cytology is additionally applied in cases of silent heat, i. e. "white heat", without a vulvar haemorrhagic discharge in the presence of superficial and squamous cells in vaginal smears (Wright & Parry, 1989; Gunzel-Apel, 1993).

Predicting the whelping date

If breeding or insemination are done at the optimum time for ultimate conception rates, it is possible to predict with a great accuracy the time of whelping if a series of vaginal smears are taken and the first day of cytologic diestrus is determined. The anticipated time of whelping is 56–58 days, most commonly (93% of bitches) 57 days after the onset of cytologic diestrus (Holst & Phemister, 1974; Johnston *et al.,* 2001).

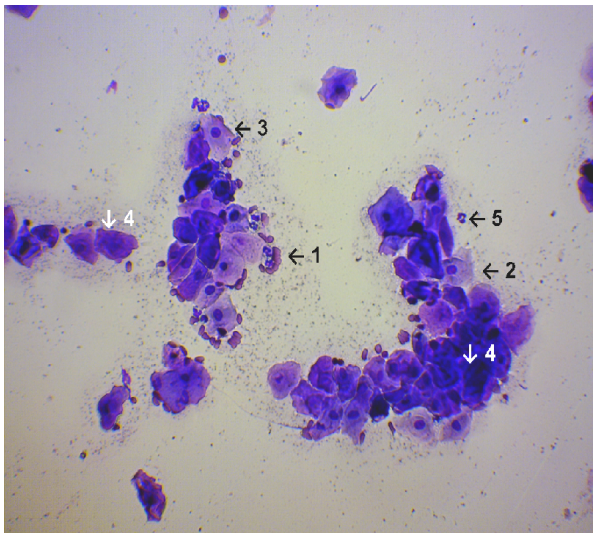

Fig. 3. Vaginal smear of a bitch on the first day of diestrus: 1 – red blood cell; 2 – small intermediate cell; 3 – large intermediate cell; 4 – squamous cells; 5 – neutrophil granulocyte (original, Haemacolor®, ×100).

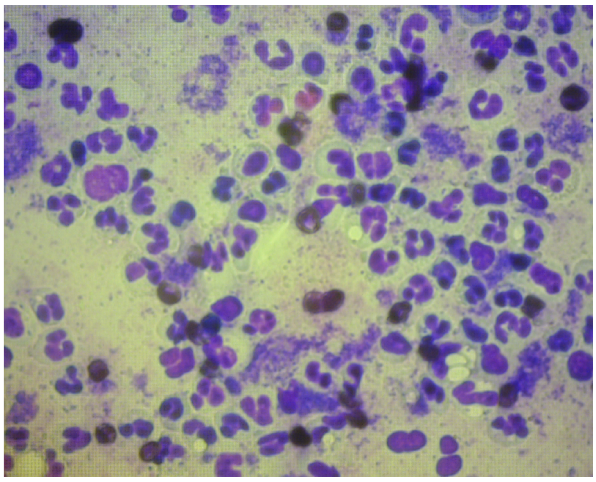

Fig. 4. Vaginal smear from a bitch with vaginitis (original, Haemacolor®, ×400).

Vaginitis

The vaginal smear in dogs with vaginitis (Fig. 4) usually consists of polymorphonuclear leukocytes, with or without bacteria (Johnson, 1991).

Ovarian cysts and tumours

Bitches with ovarian cysts or granulosa cell tumour have hormonal imbalances such as excessive estrogen production, exhibiting prolonged estrus, vulvar swelling,

dermatological changes and cystic endometrial hyperplasia with persistent serosanguineous discharge. Vaginal cytology is characterised with more than 80–90% superficial and squamous cells, similarly to the normal estrus stage, so abdominal ultrasonography is required (Koivisto *et al.,* 2011; Tavasoli & Solati, 2011).

Ovarian remnant syndrome

Ovarian remnant syndrome is defined as the presence of functional ovarian tissue left in the abdomen in a previously ovariohysterectomised bitch. The clinical presentation is characterised by signs of proestrus and estrus, including vulvar swelling and haemorrhagic discharge and behavioural changes, such as flagging (Wallace, 1991). Due to the estrogen produced by the remnant, vaginal exfoliative cytologic examination may reveal cornified epithelial cells, similar to normal proestrus and estrus stages (Stone *et al.,* 2003).

Mismating

Vaginal cytology is the best evaluation method for diagnosing a mismated bitch. Spermatozoa could be found in vaginal smears for 24–36 h after mating (Fig. 5). Lack of sperm does not eliminate mating (Srinivas *et al.,* 2004). It could be impossible to find spermatozoa because there are none present if a mating did not take place or there was in fact a copulation but no sperm have been recovered.

Vaginal tumours

The transmissible venereal tumour is the most frequently diagnosed neoplasm in the bitch using vaginal exfoliative cytology (Srinivas *et al.,* 2004). Vaginal smears (Fig. 6) contain copious amounts of erythrocytes, neutrophils, lymphocytes, parabasal, intermediate cells and round-to-ovoid shaped cells containing intracytoplasmic vacuoles (Antonov, 2015).

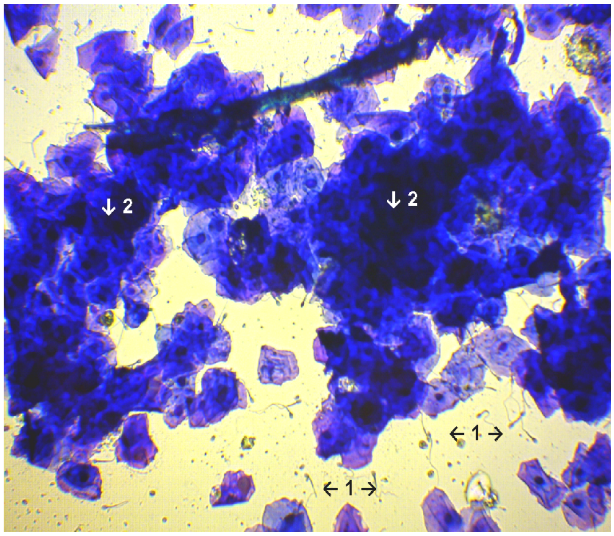

Fig. 5. Vaginal cytology of a bitch an hour after mating: 1 – spermatozoa; 2 – squamous cells (original, Haemacolor®, ×100).

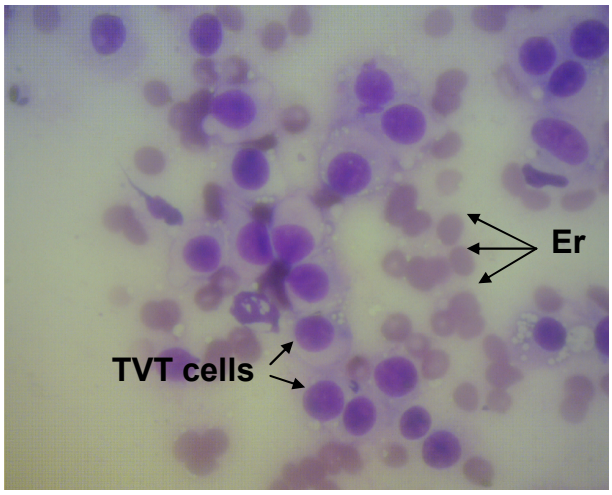

Fig. 6. Vaginal smear of a bitch with transmissible venereal tumour (TVT). Er – erythrocytes (original, Haemacolor®, ×400).

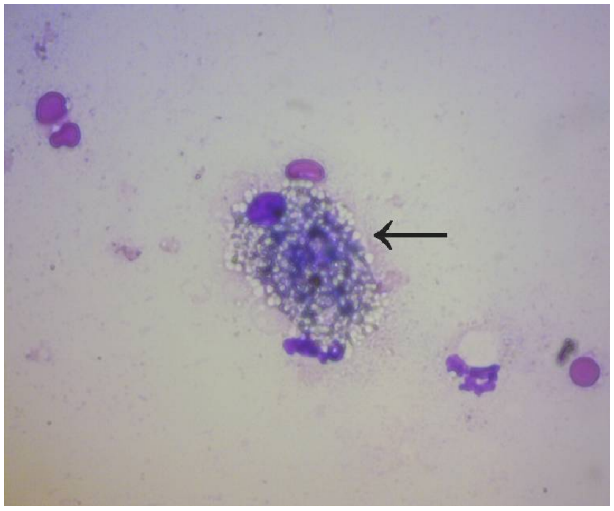

Fig. 7. Trophoblast polynucleated cell in a vaginal smear from a bitch with subinvolution of placental sites (arrow) (original, Haemacolor®, ×400).

Subinvolution of placental sites

Subinvolution of placental sites (SIPS) occurs when the involution of the uterus is delayed and bitches have haemorrhagic uterine discharge that passes from the vulva for several weeks after whelping (Johnston *et al.,* 2001). Normally foetal trophoblasts in bitches may be found in the upper loose connective tissue of the lamina propria for the first 2 weeks after whelping (Al-Bassam *et al.,* 1981). In

bitches with SIPS these trophoblastic cells do not degenerate and continue to invade the endometrium or even the myometrium, which causes vascular damage to blood vessels and failure of normal endometrial blood vessel thrombus formation (Johnston, 1986). These trophoblast cells are polynucleated and heavily vacuolated and could be observed in the vaginal smears from bitches with SIPS (Dickie & Arbeiter, 1993) (Fig. 7).

CONCLUSION

Vaginal exfoliative cytology is a very useful technique because of its simplicity, accessible equipment and the possibility of getting rapid results in clinical canine reproduction. It could be used as a valuable add-on to any reproductive diagnostics in the bitch. Although it is a routine method, there are still some uninvestigated issues, for example related to the effect of copulation and artificial insemination on the dynamics of canine vaginal cell populations.

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