Reproductive Physiology of the Female Cat

With special reference to cervical patency, sperm distribution and hysterography

Kaywalee Chatdarong

Department of Obstetrics and Gynaecology
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2003

Acta Universitatis Agriculturae Sueciae

Veterinaria 162

Abstract

Chatdarong, K. 2003. *Reproductive physiology of the female cat, with special reference to cervical patency, sperm distribution and hysterography.* Doctoral dissertation.
ISSN: 1401-6257, ISBN: 91-576-6395-5.

Ovarian cyclicity induces considerable changes in morphology, physiology and function of the reproductive organs in animals. This thesis aimed to study reproductive physiology of the female domestic cat, focusing on the cervix, uterus and uterine tube during different stages of the oestrous cycle, after mating and under pathological conditions. Transcervical catheterisation was performed using a specially designed catheter. Patency of the cervix and uterine motility were studied with the aids of fluoroscopy and scintigraphy by depositing contrast fluids and radiopharmaceutical medium in the cranial vagina. The relationship between cervical patency and oestrous behaviour, cornification of the vaginal cells and serum concentrations of oestradiol-17β were evaluated. Hysterography, a positive contrast study, was performed to illustrate the uterine appearance during the oestrous cycle in relation to histological characteristics of the endometrium. Immunohistochemistry using proliferating nuclear antigen (PCNA) was introduced to identify mitotic activity of the endometrial cells. Patency of the cervix to spermatozoa as well as sperm distribution after natural mating was evaluated by flushing the vagina, the uterus and the uterine tube. For the study of sperm distribution in the female reproductive tract, two methods, flushing and tissue sectioning were evaluated and compared.

Using a specially designed catheter it was possible to catheterise the cervix during interoestrus, oestrus, metoestrus and postpartum and to introduce contrast fluids into the uterus to study uterine appearance using hysterography also when the cervix was closed. The period when the cervix was patent was found to vary among individuals: the cervix was open either only during late-oestrus; during midoestrus and late-oestrus; or throughout the entire behavioural oestrus. Patency of the cervix was found to usually coincide with the maximum degree of vaginal cornification and thus, presumably is regulated at least in part by the serum concentration of oestradiol-17β. Hysterograms revealed differences in uterine luminal shape that corresponded to the histological characteristics of the endometrium in cats at various stages of the oestrous cycle, cats given medroxyprogesterone acetate (MPA) and cats with uterine pathology. Straight- and wavy-shaped uterine lumens were characteristic of the uterine horns in the inactive and follicular stages of the oestrous cycle. Coil-shaped uterine lumen appeared to be a progestagenic effect seen in the luteal, the MPA-treated and the pathological groups. A coiled uterine cavity was suggestive of endometrial hyperplasia, whereas irregular filling defects were indicative of generalised cystic changes in the endometrium. The expression of PCNA in luminal and glandular epithelial cells was observed although the mitotic activity was not related to neither stages of oestrous cycle nor uterine pathological conditions. The results from the sperm distribution study demonstrated that the cervix and the uterotubal junction (UTJ) were sperm barriers in the cat. The endometrial crypts and the UTJ functioned as sperm reservoirs before ovulation, whereas the isthmus was a sperm reservoir around the time of ovulation.

The observations determined the dynamics of the cervix, the uterus and the uterine tube in the cat in relation to ovarian activity. This thesis is the first to provide nomenclatures for describing uterine appearance in hysterograms according to the shape of the uterine horns, luminal cavity and the characteristics of the intraluminal lining, to demonstrate the PCNA expression as well as to determine the distribution of spermatozoa in the female reproductive tract of the cat after natural mating.

Key words: feline, queen, copulation, oviduct, radiography, endometrial hyperplasia

Author's address: Kaywalee Chatdarong, Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences (SLU), PO Box 7039, SE-750 07 Uppsala, Sweden (On leave from the Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand). E.mail: Kaywalee.Chatdarong@og.slu.se

To my parents and my sister

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Papers I-IV

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I.   Chatdarong, K., Lohachit, C., Ponglowhapan, S. & Linde-Forsberg, C. 2001. Transcervical catheterization and cervical patency in domestic cats. *Journal of Reproduction and Fertility Supplement* 57, 353-356.

II.   Chatdarong, K., Kampa, N., Axnér, E. & Linde-Forsberg, C. 2002. Investigation of cervical patency and uterine appearance in domestic cats by fluoroscopy and scintigraphy. *Reproduction in Domestic Animals* 37, 275-281.

III.   Chatdarong, K., Rungsipipat, A., Axnér, E. & Linde-Forsberg, C. Hysterographic appearance and uterine histology at different stages of the reproductive cycle and after progestagen treatment in the domestic cat. *Manuscript submitted for publication*.

IV.   Chatdarong, K., Lohachit, C. & Linde-Forsberg, C. Distribution of spermatozoa in the female reproductive tract of the domestic cat in relation to ovulation induced by natural mating. *Manuscript submitted for publication*.

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Abbreviations

ABC   avidin-biotin peroxidase complex
AI   artificial insemination
CH   corpora haemorrhagica
CL   corpora lutea
eCG   equine chorionic gonadotrophin
FSH   follicle stimulating hormone
FSH-P   pituitary follicle stimulating hormone
GnRH   gonadotrophin-releasing hormone
hCG   human chorionic gonadotrophin
HSA   human serum albumin particle
LH   luteinising hormone
MPA   medroxyprogesterone acetate
PBS   phosphate-buffered saline solution
PCNA   proliferative cell nuclear antigen
PMN   polymorphonuclear cell
^{99m}Tc   technetium 99m
UTJ   uterotubal junction

Introduction

Background

The domestic cat is one of the most popular pets. Considerable emphasis on the study of reproduction in this species has been generated with the increasing interest in the domestic cat as a model for research, aimed at the preservation of endangered wild felids (Wildt *et al*., 1986), and as an experimental model for at least 36 human physiological abnormalities, including inherited genetic disorders (Goodrowe *et al*., 1989). The cat is also important for studying the phenomenon of copulation-induced ovulation, a conserved mechanism. Except for the domestic cat (*Felis catus*), the Northern European lynx (*Lynx lynx*) and a few other felids, most of the 36 felid species are classified as threatened, vulnerable or endangered by the Convention on International Trade in Endangered Species (CITES, 2000).

In Sweden, the only wild felid - the lynx- is classed as vulnerable by the Swedish Red List of threatened species. The population is estimated to be between 1400 and 1800 individuals and is controlled by strictly regulated hunting to keep the number of animals to a level where they can be tolerated by the public. This number of individuals is close to the minimum that is estimated to be necessary for a favourable development of this species in Sweden (Swedish Environmental Protection Agency, Dnr 411-6644-01). Among nine species of wild felids in Thailand, one (*Felis marmorata*) is considered nearly extinct. The other eight species (*Panthera tigris, Panthera pardus*, *Neofelis nebulosa*, *Felis temmincki, Felis viverrina*, *Felis bengalensis*, *Felis chaus* and *Felis planiceps*) are considered at risk of extinction.

Despite the growing popularity of the cat as a pet, most of the breeding of pedigree cats takes place within small catteries (Farstad, 2000) and a high percentage of male cats are castrated at an early age. Thus, inbreeding is a major problem both in the small populations of wild felid species and in purebred domestic cats (Axnér, 2000). Improved knowledge of the basic reproductive physiology of the female cat is instrumental for taking advantage of the techniques for assisted reproduction, and invaluable in clinical practice and diagnosis of uterine pathologies in this species.

Oestrous cycle

Ovarian activity in the female cat is dependent on photoperiod. The cat is polyoestrus and also an induced ovulator (Shille *et al*., 1979). Oestrus in free-ranging females is induced by an increase in day length, whereas decreasing photoperiod results in seasonal anoestrus. However, high ambient temperatures during summer may reduce the incidence of oestrus (Concannon & Lein, 1983; Feldman & Nelson, 1996). Ovarian inactivity during winter anoestrus is related to an elevation in circulating prolactin and melatonin in peripheral plasma (Leyva *et al*., 1984; Leyva *et al*., 1989). In cats maintained indoors, ovarian activity can be controlled by artificial light. A minimum of 10-hr artificial light, equivalent to that

of 100-watt bulb in a 4 x 4 m room, can provide oestrous cycles throughout the year (Shille & Sojka, 1995). It is possible to induce oestrus by social stimuli by either a tomcat or an oestrous female (Michel, 1993).

The female cat usually enters the follicular phase abruptly. Oestrus may be preceded by a 1-2 day period of pro-oestrus characterised by female oestrous behavioural signs but not male acceptance. The follicles develop to the vesicular stage within 24 to 48 hr prior to the first day of oestrous behaviour (Wildt & Seager, 1980). The oestrous stage ranges from 3 to 16 days (average 7 days). Behavioural changes occur often overnight from interoestrus or anoestrus and may precede changes in vaginal cornification (Shille & Sojka, 1995). A rapid growth of the follicles results in a two-fold increase in the peripheral plasma concentration of oestradiol-17β within 24 hr (Shille et al., 1979). If the queen is not mated, ovulation will not occur in most cases and the preovulatory follicles become atretic and degenerated. The cats undergo an interoestrous cyclic stage, which lasts from 2 to 19 days (average 7 days), characterised by the absence of oestrous behaviour and with basal levels of plasma oestradiol-17β concentrations (Shille & Sojka, 1995). In some oestrous periods, overlapping follicular waves cause a continuous follicular phase (Feldman & Nelson, 1996).

Artificial induction of oestrus

Pituitary follicular stimulating hormone (FSH-P) and equine chorionic gonadotrophin (eCG) are commonly used to induce follicular development in the cat. One administration of 100 iu eCG intramuscularly to anoestrous cats, followed 5-7 days later by an injection of 50 iu human chorionic gonadotrophin (hCG), produces ovulation and pregnancy results comparable to those of natural matings (Cline et al., 1980). The ovaries of the domestic cat are sensitive to over-dosing with gonadotrophins. High single doses (1000 iu) and multiple administrations of low-dose eCG result in premature luteinisation of early developing follicles (Wildt et al., 1978; Cline et al., 1980). Repeated injections of eCG may lead to the production of antibodies against FSH and luteinising hormone (LH), and a subsequent decreased response to stimulation or infertility (Swanson et al., 1995). FSH-P given in a 2-mg intramuscular daily dose for 5-7 days generally produces an oestrous behavioural response and follicular development (Wildt et al., 1978). Pregnancies have been obtained from females artificially inseminated with frozen-thawed semen following treatment with FSH-P (2 mg/day for 5 days) (Platz et al., 1978).

Mating and ovulation

A coital stimulus induces a neural firing reflex that stimulates the medial basal hypothalamus to synthesize and liberate gonadotrophin-releasing hormone (GnRH), which then stimulates the release of pituitary luteinising hormone (LH) (Robinson & Sawyer, 1987). Successful ovulation depends on the presence of mature follicles and an adequate surge of LH. Females require several days of oestrogen priming before copulation can induce a surge of LH sufficient to induce

ovulation. Although females are often sexually receptive by the second or third day of ovarian follicular growth, in some cats the amount of LH released in response to copulation may be limited until the animal reaches the fourth or fifth day of follicular growth (third or fourth day of oestrus) (Banks & Stabenfeldt, 1982). An adequate LH surge also depends on the number and frequency of copulations. LH release occurs rapidly with increases in serum concentrations evident within 5 min after mating (Johnson & Gay, 1981) and remaining elevated until 8 hr in ovulating cats, whereas the LH levels remain low in the cats that do not ovulate (Concannon *et al*., 1980).

Multiple copulation regimens such as four copulations during a 21-81 min period or ad libitum copulatory activity for 4-hrs (8-12 copulations) results in 100% successful ovulation whereas a single copulation induces an LH release sufficient to cause ovulation in only half of cats (Concannon *et al*., 1980). The greatest and the most prolonged LH releases are induced by *ad libitum* copulatory activity for 4-hrs. The intervals between copulations vary among individuals, being brief (6-12 min) shortly after the start of unrestricted male-female interaction and longer (18-61 min) toward the end of a 4-hr period (Concannon *et al*., 1980).

Although cats often copulate four times within the first hour, a single copulation on the third day of oestrus, when oestrous signs are most obvious and the oestradiol concentration is markedly high has been reported to be sufficient for an adequate LH release to induce ovulation (Tsutsui & Stabenfeldt, 1993). Ovulation occurs 25-32 hr after copulation (Shille *et al*., 1983) and can occur as late as 52 hr after the first LH peak (Wildt *et al*., 1981). Spontaneous ovulation can be observed in up to 35% of older cats housed individually with only visualization of other members in the colony (Lawler *et al*., 1993) and in young, group-housed cats (Gudermuth *et al*., 1997). The cats ovulate secondary oocytes in an all-or-none fashion depending on sufficient release of LH (Banks & Stabenfeldt, 1982).

After ovulation, the ovaries display reddish prominent corpora haemorrhagica (CH) characterised by high vascularisation. Thereafter, the developing copora lutea (CL) turn to pink or orange in colour and grow to 4.5 mm in diameter by days 12-16 after copulation (Dawson, 1946). Ovarian activity shifts from oestrogen to progesterone secretion 44-60 hr after the LH peak (Wildt *et al*., 1981). The progesterone concentration in peripheral plasma does not increase until 3-4 days after copulation (Verhage *et al*., 1976). CL of pregnancy remains functional throughout gestation, regressing at or near parturition (Verhage *et al*., 1976; Schmidt *et al*., 1983). However, in the nursing cat, the CLs remain well developed histologically until 63 days postpartum (Dawson, 1946).

Artificial induction of ovulation

Other than natural mating, stimuli, including direct vaginal stimulation with a vaginal swab or a glass rod (Greulich, 1934) and administration of hCG (Wildt & Seager, 1978; Cline *et al*., 1980) or GnRH (Chakraborty *et al*., 1979) can induce ovulation. Ovulation stimulated with hCG or GnRH results in comparable numbers of released oocytes, regardless of whether administered in a natural or

FSH-P induced oestrus (Goodrowe & Wildt, 1987). Excessive doses can result in ovarian hyperstimulation and degeneration of oocytes. Dosages of 75-100 iu hCG give better results than 200 iu hCG as demonstrated by fewer degenerated oocytes (Goodrowe *et al.*, 1988). More mature oocytes are obtained by administering 100 iu hCG on day 3 rather than on days 1 or 2 of natural oestrus (Donoghue *et al.*, 1993). A combined regimen of eCG and hCG has been used to induce oestrus and ovulation in domestic cats and has resulted in the birth of kittens following laparoscopic artificial insemination (Howard *et al.*, 1992).

Pseudopregnancy

If ovulated oocytes are not fertilised or pregnancy fails for other reasons, the female cat undergoes a short luteal phase or "pseudopregnancy". The cat differs from other canivores in that the luteal phase of the non-pregnant cat is only approximately one half the duration of the normal gestation period (Feldman & Nelson, 1996). Pseudopregnancy in the cat is not associated with behavioural changes or lactation as in the dog. Plasma progesterone reaches maximum levels around 20-25 days after mating (Verstegen, 1998) and begins to decline gradually from day 25 to reach basal values around 30-40 days after the first copulation (Verhage *et al.*, 1976; Shille & Stabenfeldt, 1979; Shille & Sojka, 1995).

The anatomy of the cervix and cervical patency

Ovarian cyclicity induces considerable changes in morphology, physiology and function of the reproductive organs. The cervix, the uterus and the uterine tubes are target organs affected directly by hormonal changes during the various stages of the oestrous cycle. The cervix in the cat is oriented horizontally in straight alignment with the vestibule and vagina. The vestibule-vaginal junction (the so-called cingulum) and the anterior vagina are narrow and non-distensible, and the vagina narrows towards the cervix (Crouch, 1969; Watson & Glover, 1993; Shille & Sojka, 1995). Examination of the vagina is only possible with the cat under general anaesthesia and with a 3-mm cystoscope equipped with a device for dilating the vaginal lumen (Shille & Sojka, 1995). Watson & Glover (1993) and Swanson & Godke (1994) report the length of the combined vestibule and the vagina to be 40 mm and 49.9±1.1 mm. The portio vaginalis uteri can be reached only with ≤1-2 mm wide catheter (Watson & Glover, 1993). The prominent dorsal median postcervical fold and the fornix located ventrolateral to the external cervical opening (Crouch, 1969) renders transcervical catheterisation difficult. The anatomical construction of the vagina and cervix in the cat tends to direct a catheter into the vaginal fornix, resulting in unsuccessful transcervical catheterisation (Hurlbut *et al.*, 1988).

The cervix appears to be an important barrier for spermatozoa after both natural mating and artificial insemination. Intravaginal insemination with fresh semen results in conception rates of only 42.9% (3/7 cats) (Sojka *et al.*, 1970) and 10.6% (6/56 attempts) with frozen-thawed semen (Platz *et al.*, 1978). Conception rates of 50% (9/18 cats) (Howard *et al.*, 1992) and 80% (8/10 cats) (Tsutsui *et al.*, 2001)

have been reported when laparoscopic intrauterine insemination and unilateral intrauterine horn insemination with fresh semen was used.

In the dog, results from artificially inseminated semen deposited in the vagina are inferior compared to semen deposited in the uterus (Linde-Forsberg *et al*., 1999: Linde-Forsberg, 2001). The number of fresh spermatozoa required for successful intrauterine insemination in the cat is 8×10^6 (Tsutsui *et al*., 2001) whereas to obtain similar results 80×10^6 spermatozoa are required for intravaginal insemination (Tanaka *et al*., 2000). Since surgical intrauterine insemination is not permitted in some countries, transcervical intrauterine insemination would be an alternative non-invasive method for improving the success rate of assisted feline reproduction, and is a potentially useful non-surgical technique for diagnosis and therapy of uterine diseases. However, proper devices and skilled knowledge for performing transcervical catheterisation in the cat are limited. Only two reports of transcervical catheterisation in the cat have been published and these were conducted for embryo transfer (Hurlbut *et al*., 1988; Swanson & Godke, 1994).

Cervical dynamics during the oestrous cycle have been studied in the bitch (Silva *et al*., 1995; Verstegen *et al*., 2001) but have not been reported for the cat. Using vagino-uterographic contrast studies, the cervix of the bitch was seen to close 6.7±1.4 days (Silva *et al*., 1995) and 6.9±1.1 days (Verstegen *et al*., 2001) after the estimated time of the LH peak, when most bitches are still in oestrus. Thus, cervical closure is suggested to be a limiting factor for reproductive success after natural mating or intravaginal insemination in the dog.

Sperm transport through the cervix and distribution in the female reproductive tract

The function of the cervix in restricting the entry of fluids, particles or spermatozoa into the uterus has been widely studied in several species but information for cats is not available. The cervix functions as a main barrier to spermatozoa for species with vaginal deposition of semen at natural breeding, such as primates, rabbits and ruminants. The tomcat appears to deposit semen in the vagina during natural mating (Blandau, 1973; Watson & Glover, 1993). Cat spermatozoa, therefore, have to pass through the cervix in order to reach the site of fertilization in the ampullary region of the oviduct (Van Der Stricht, 1911).

Diagnostic imaging, such as positive contrast radiography and scintigraphy are used to demonstrate the transport of radiopaque fluid and radioactive particles through the cervix into the uterus. The transport of radiopaque fluid from the vagina through the cervix has been demonstrated in the rabbit (Akester & Inkster, 1961) and the dog (Linde, 1978; Silva *et al*., 1995; Verstegen *et al*., 2001). With the use of hysterosalpingo-radionuclide scintigraphy (HERS), radiopharmaceutical particles (^{99m}Tc labelled human albumin spheres) have been shown to migrate from the vagina into the peritoneal cavity in women (Iturralde & Venter, 1981). With the use of scintigraphy, radiolabelled rabbit spermatozoa inseminated vaginally have been observed in the uterus within 5 min (Bockisch, 1993). During oestrus, dead spermatozoa (Noyes *et al*., 1958) and inert radioactive polystyrene

microspheres (Glover & Patterson, 1963) placed in the vagina have also been shown to enter the uterus in rabbits.

The localisation of spermatozoa in the female reproductive tract provides information both on cervical patency to spermatozoa and on the functions of certain regions of the reproductive tract. Structures acting as sperm barriers or as sperm reservoirs after insemination or natural mating have been defined in many species of animals. The cervix is the primary barrier for spermatozoa in animals with vaginal semen deposition at natural breeding, whereas the uterotubal junction (UTJ) serves to further restrict sperm access to the uterine tubes (Scott, 2000). The cervix, the UTJ and the lower isthmus are reported to be sites for sperm storage in the cow (Hunter *et al*., 1991) and the pig (Flechon & Hunter, 1981; Mburu *et al*., 1997) and the uterine tube, regardless of region, forms a functional sperm reservoir *in vitro* in the bitch (Pacey *et al*., 2000). However, there are no reports on sperm distribution in the cat.

Uterine morphology examined by hysterography and histology

Changes in uterine morphology are also dependent on ovarian activity during the various stages of the oestrous cycle. Laparoscopy has been used to observe changes in the uterus in the cat (Wildt & Seager, 1980) but diagnostic imaging is an alternative, non-invasive, technique. Few diagnostic imaging studies have been conducted to demonstrate the normal reproductive organs of the cat due to their diminutive size. Using radiography, with the aid of a paddle to compress the caudal abdomen, the postpartum uterus can only be observed for up to six days after parturition (Ferretti *et al*., 2000).

Hysterography as a method for diagnosing uterine disorders has been reported in the dog (Funkquist *et al*., 1985), but little data is available on the hysterographic appearance in the cat. Hysterographic appearances of cats in oestrus and interoestrus have been revealed in only one previous study (cited by Johnston *et al*., 2001). The physiological variations in uterine morphology as seen in the hysterograms should be confirmed by a histological study: a thorough understanding of the physiological changes is a prerequisite for accurate diagnosis of pathological changes in the uterus. The histological characteristics of the endometrium in cats have been described during oestrus (West *et al*., 1977); early pregnancy (Roth *et al*., 1995); pseudopregnancy (Boomsma *et al*., 1991); as well as the histopathological appearance of endometrial hyperplasia, a common uterine disorder in cats over five years and in cats given exogenous progestagens (Dow, 1962; Lawler *et al*., 1991; Potter *et al*., 1991).

Degeneration and regeneration are common features involved in epithelial development, which can be defined by the mitotic activity of the cells. Proliferative cell nuclear antigen (PCNA) is commonly used to determine endometrial hyperplasia and adenocarcinoma in humans (Ito *et al*., 1993). Thus, immunohistochemical staining of the endometrial epithelial cells with PCNA might be a useful tool for defining degrees of endometrial proliferation. Mitotic figures in the endometrium during normal oestrous cycle in cats have been

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described (Dawson & Kosters, 1944), but examination using PCNA has not been performed in cats.

Aims of the study

The overall aim of the present work was to increase knowledge about reproductive physiology in the female domestic cat. Special attention was directed to the morphology and function of the cervix, the uterus and the uterine tubes during different stages of the oestrous cycle, after natural mating, as well as under pathological conditions. The specific aims of the study were to:

- develop techniques for studying cervical patency and uterine appearance of the female tubular genital tract;

- examine patency of the cervix to fluids, particles and spermatozoa;

- demonstrate the distribution of spermatozoa in the female tubular genital tract after natural mating and evaluate techniques used for studying sperm transport; and

- describe differences in the hysterographic appearance during various stages of the oestrous cycle, after MPA treatment and under pathological conditions, and to relate these findings to the histological features of the endometrium.

Materials and Methods

Animals and general management

One hundred and twenty-one (121) female domestic cats were included in the studies (Table 1). Thirty of the female cats and one male cat were research colony cats (Papers II & IV). Ninety-one of the female cats were privately owned and submitted for routine spaying at the Small Animal Hospital, Chulalongkorn University, Bangkok, Thailand (Papers I & III). Six of the 30 female colony cats aged 6-9 years were housed in a group under an artificial light schedule (16L: 8D) at the Department of Obstetrics and Gynaecology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden (Paper II). Twenty-four of the 30 female colony cats were maintained in a group with a male cat in a separate wire enclosure adjacent to the females at the Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand (Paper IV). The colony cats kept in Thailand were exposed to an artificial light schedule (14L: 10D), in addition to ambient light available from the windows. All colony cats were fed a commercial diet and had free access to water.

Table 1. *Numbers of colony and privately owned female cats in each experiment by stage of oestrous cycle, MPA-treatment and pathological conditions*

Paper	Colony cats	Privately owned cats					
	Follicular stage	Inactive stage	Follicular stage	Luteal stage	Postpartum stage	MPA treated	Pathological conditions
I (n=17)		9	3	2	3		
II (n=6)	6						
III (n=74)		20	9	18	12	12	3
IV (n=24)	24						

Assessment of the reproductive cycle

Stage of reproductive cycle was assessed by reproductive history (Papers I-IV), behavioural observations (Papers II & IV), vaginal cytology (Papers I & II), hormonal assays (Papers II-IV), ovarian examination during ovariohysterectomy (Papers I, III & IV), and histological sections of the ovaries (Paper III). The cats were considered to be in behavioural oestrus when they exhibited oestrous behaviours, such as calling, rubbing, rolling, lordosis, treading of the hind legs and crouching to the floor. Vaginal cytology was performed using a 2-mm diameter cotton swab (Förbandsmaterial AB, Partille, Sweden) moistened with physiological saline to obtain cells from the dorsal wall of the cranial vagina. The vaginal cells were smeared onto a glass slide and stained with Hemacolor (E. Merck, Darmstadt, Germany). A vaginal smear with a clearing of the background,

a reduction of cellular debris and a proportion of superficial cells of 80% or more was considered as an oestrous smear.

Blood was collected via cephalic venipuncture at the time of examination (Paper II) or ovariohysterectomy (Papers III & IV). The serum was separated by centrifugation and stored at -20˚C until assayed. The hormonal analysis was performed at the Department of Clinical Chemistry, Swedish University of Agricultural Sciences, Uppsala. The serum was analysed for oestradiol-17β by radioimmunoassay using enhanced luminescence (Amerlite Estradiol 60-assay, Ortho-Clinical Diagnostics, Amersham, UK) (Papers II-IV). Progesterone was determined by luminescence immunoassay (Immulite Progesterone, Diagnostic Products Corporation, LA, USA). The inter- and intra-assay coefficients of variation for oestradiol-17β were 30.5% and 17.4% at 7.1 pmol/L; 8.6% and 2.3% at 47.0 pmol/L; and 19.7% and 8.0% at 128.3 pmol/L. The minimal assay sensitivity of oestradiol-17β was 4.2 pmol/L. The inter- and intra-assay coefficients of variation for progesterone were 13.2% and 2.5% at 2.3 nmol/L; 6.7% and 1.2% at 24.6 nmol/L; and 3.6% and 1.7% at 54.2 nmol/L. The minimal assay sensitivity of progesterone was 0.6 nmol/L.

The ovaries were examined after ovariohysterectomy for the presence or absence of follicles and CH or CL. Activity of CL was assessed by the degree of vacuolation of the luteal cells in the histological sections of the ovaries, indicating cell degeneration according to Dawson (1941) (Paper III).

Induction of oestrus and ovulation (Paper IV)

Oestrus in 24 female cats was induced by an intramuscular administration of 100 iu eCG (Folligon, Intervet International Inc., Boxmeer, The Netherlands) during anoestrus or interoestrus. Successful oestrous induction was assessed by signs of oestrous behaviour during 5-7 days after the eCG administration. Ovulation was induced by natural mating with a male cat four times within one hour. Successful intromission was assessed by the expression of a characteristic vocalisation by the female cat, subsequent disengagement of the male and typical female post-coital behaviour (Axnér & Linde-Forsberg, 1998). Ovulation was confirmed by the presence of CH and CL observed after surgery and a rise in serum progesterone concentration.

Sedation and anaesthesia

Food was withheld for at least 8 hr before sedation and anaesthesia. Sedation was performed using 100-120 µg kg^{-1} bw medetomidine HCl (Domitor, Orion Pharma AB Animal Health, Espoo, Finland) intramuscularly (Paper II). At the end of the examination, Atipamezol HCl (Antisedan, Orion Pharma AB Animal Health, Espoo, Finland) was administered intramuscularly in half the volume of the previously given dose of medetomidine. The cats were pre-medicated with 0.04 mg kg^{-1} bw atropine sulphate (Atropine A.N.B. Laboratories, Bangkok, Thailand) (Paper I). General anaesthesia was induced with an intramuscular injection of 3 mg kg^{-1} bw xylazine HCl (Rompun, Bayer Korea, Seoul, Korea) and 10 mg kg^{-1}

bw ketamine HCl (Calypsol, Gedeon Richter, Budapest, Hungary (Paper I); Keta-hameln, Astrapin Pharma GmbH & Co.Kg, Hameln, Germany (Papers III & IV)).

Investigation of the cervix

Transcervical catheterisation

A transcervical catheter consisting of an inner and an outer polypropylene tube was developed to fit the anatomy of the cat vagina and cervix (Paper I). The outer, vaginal, catheter was designed from a polypropylene urinary catheter (2.8 mm diameter) (Clay Adams, Sparks, USA), by closing one end with heat, and making a new opening 8 mm from the tip. A 3.5 French tomcat catheter (Sherwood, St.Louis, USA) 140 mm in length and 1-mm in diameter was used as an inner, cervical, catheter. The new opening in the vaginal catheter was fashioned in such a way that it directed the inner catheter towards the cervical opening.

With the anaesthetised cats lying in lateral recumbency, transcervical catheterisation was performed by inserting the outer catheter into the vagina until no further cranial movement could be achieved. The inner catheter was thereafter inserted through the outer catheter and pushed blindly through the cervix into the body of the uterus (Papers I & II). The success of the transcervical catheterisation was assessed during surgery when the tip of the catheter was palpated in the body of uterus and green food colour mixed with 1 ml of 200 000 units penicillin G (Penomycin M and H Manufactering, Bangkok, Thailand) was seen in the uterus (Paper I). Transcervical catheterisation was performed in cats at different stages of the oestrous cycle to assess the success rate of the method (Paper I) and to introduce a contrast medium into the uterus to perform hysterography (Paper III).

Assessment of cervical patency

Cervical patency to contrast fluid and a radiopharmaceutical medium

The cervical patency was examined in 17 privately owned cats in various stages of oestrus at the time of routine ovariohysterectomy. The exact day of the oestrous cycle was not known for all cats (Paper I). Cervical patency was studied in a non-ovulatory oestrous cycle in six colony cats examined at 2-day intervals at early oestrus, mid-oestrus, late oestrus and during interoestrus (Paper II). Additional examinations were performed after late oestrus until the contrast fluid remained in the vagina. Cervical patency was assessed using radiography (Papers I & II), fluoroscopy (Paper II) and scintigraphy (Paper II). Diatrozate 76% (Urografin, Schering AB, Berlin, Germany) (Paper I) or Iohexol 300 mg Iodine/ml (Omnipaque, Nycomed AB, Roskilde, Denmark) (Paper II) were used as the contrast medium. Radiopharmaceutical medium was prepared by reconstituting a vial of human serum albumin particle (HSA) with 2 ml of 400 MBq eluted technetium 99m (^{99m}Tc). To localise the position of the uterus in relation to the kidneys and other internal organs, 0.5 ml eluted ^{99m}Tc (50 MBq) was administered intravenously.

The contrast fluid and the radiopharmaceutical medium were infused slowly through a 3.5-mm French tomcat catheter placed in the cranial vagina with the cats

lying in lateral recumbency (Paper I), or in dorsal recumbency and with the hindquarters elevated at an angle of approximately 15-degrees (Paper II). Radiographs ((Picker, Picker International Inc., Cleveland, USA) (Papers I & III) or Siemens-Elema, München, Germany (Paper II)) were taken in lateral projection after 0.5 ml Urografin was infused into the cranial vagina and the hindquarters of the cats were elevated for 5 min (Paper I) or in ventrodorsal projection at 1-, 3- and 5 min after Omnipaque infusion (Paper II). Additional lateral exposures were taken as required. Fluoroscopic recordings (Siemens-Elema, München, Germany) were taken at the time of Omnipaque infusion (Paper II). During mid-oestrus and interoestrus, the cats were examined with a gamma camera (Picker SX-300; Picker International Inc., Cleveland, USA) equipped with a LEGP collimator and a dedicated computer system (NUD, Nuclear Diagnostics, Stockholm, Sweden) (Paper II).

Through the tomcat catheter, 0.2 ml of the radiopharmaceutical medium (^{99m}Tc-HSA) (40 MBq) was introduced into the cranial vagina. The cervix was defined as open when the contrast fluid or the radiopharmaceutical particles was seen to enter the uterus. The cervix was determined as closed when the contrast fluid or the radiopharmaceutical medium remained in the cranial vagina. The relationship between cervical patency to the contrast medium and oestrous behaviour, cornification of the vaginal cells and the serum oestradiol-17β concentration were evaluated (Paper II).

Cervical patency to spermatozoa (Paper IV)

The ovaries and reproductive tracts of 24 female cats were surgically removed at 30 min (n=6), 3 hr (n=6), 48 hr (n=6) and 96 hr (n=6) after natural mating. The females ovariohysterectomised at 30 min were mated only once, whereas the other females were mated four times in one hour. The reproductive tracts were divided into seven segments on each side: infundibulum, ampulla, isthmus, UTJ, cranial and caudal uterine horn, and uterine body. The vagina and the lumina of the segments from one side were flushed with 0.5 ml phosphate-buffered saline solution (PBS). The contralateral side of the reproductive segments was fixed immediately without prior flushing and these fixed reproductive segments were then further processed for routine histology. The differences in the numbers of spermatozoa in the vagina and each reproductive segment were evaluated.

Investigation of sperm distribution in the female reproductive tract (Paper IV)

Sperm recovery by flushing

Eighteen female cats were mated four times in one hour with the same male cat and were ovariohysterectomised at 3 hr (n=6), 48 hr (n=6) and 98 hr (n=6). Six females were mated only once and were submitted for ovariohysterectomy 30 min later. Each reproductive tract was divided into seven segments. The infundibulum was separated at the end of the conical shaped tubal segment. The ampulla was differentiated from the isthmus by its convoluted shape and slightly larger diameter. The UTJ comprising 0.3 cm of the tip of the uterine horn and 0.2 cm of

the caudal isthmus was excised. The remainder of the uterine horn was divided into two equal segments, defined as the cranial and the caudal uterine horn. The body of the uterus was divided longitudinally into an equal left and right part. The vagina and the lumen of each segment of one side were flushed with 0.5 mL PBS.

The flushings were collected into separate plastic Eppendorf vials. Centrifugation at 1500 g for 5 min concentrated the spermatozoa in the flushings. Approximately half the volume of the supernatant was discarded. Of the resuspended samples, 5μL was placed on a glass slide, covered with an 18x18 mm cover slip and examined under a phase contrast microscope at x100 magnification to calculate total numbers of spermatozoa in the flushings from each segment and from the vagina.

Sperm numbers from tissue sections

After flushing, the seven flushed segments and the corresponding contralateral non-flushed segments were immersed in 3% neutral buffered formalin solution. Each segment was cut transversely into four equal parts, embedded in a paraffin block and sectioned to a thickness of 5-μm. Every fifth serial section was mounted and stained with haematoxylin and eosin. From each segment, 40 sections were chosen for counting of sperm numbers under a light microscope at 400x magnification.

Evaluation of techniques used for studying sperm distribution

Flushing and tissue sectioning techniques for examining sperm numbers in the female reproductive segments were evaluated by comparing sperm numbers obtained from flushed and non-flushed tissue sections.

Investigation of the uterus

Assessment of uterine morphology

Hysterography

The uterine appearance of 80 cats in inactive (n=20), follicular (n=15), luteal (n=18) and postpartum (n=12) stages of the oestrous cycle, cats treated with MPA (Depo-gestin, A.N.B. Laboratory, Bangkok, Thailand) (n=12) or with uterine pathological lesions (n=3) were assessed using a positive contrast study (Papers II & III). The cats were positioned in dorsal recumbency and the hindquarters elevated at an angle of approximately 15-degrees (Paper II) or 30-degrees (Paper III). When the cervix was shown to be functionally open, the infusion of Omnipaque into the cranial vagina was continued until the endometrial lining was observed (Paper II). Transcervical or intrauterine catheterisation was performed to introduce the contrast fluid into the uterus in cats with closed cervix (Paper III). A volume of 2-3 ml Omnipaque was infused until an efflux of the contrast was observed in the vagina (Papers II & III). Radiographic images were taken ventrodorsally and laterally immediately after the contrast infusion was completed (Paper III), and ventrodorsally at 1-, 3- and 5 min after the uterine horns were

entirely filled with the contrast medium as observed with fluoroscopy (Paper II). The hysterographic appearances of cats in different groups were described (Papers II & III).

Nomenclatures and measurements to describe the hysterographic appearances were established based on assessment of the shapes of the uterine horns and luminal cavity and the characteristics of the intraluminal lining. The shape of the uterine horns was classified as being straight or curved and that of the luminal cavity as being straight, wavy or coiled. The intraluminal lining was classified as being smooth, or as irregular when the lining of the uterine horns showed filling defects in the contrast medium. The degree of coiling and waviness were expressed as a ratio between the number of waves or coils per 2 cm of uterine length. The amplitude of the coiling was defined as the distance between peaks of the contralateral coil (Paper III).

Ultrasonography (Paper II)

During oestrus in six of 80 female cats, ultrasonography of the uterus was performed once with ultrasound (Apogee CLA, Interspec Ins., Ambler, USA) and a 5-10 MHz changeable convex array probe (Apogee CLA, Interspec Ins., Ambler, USA). The endometrial appearances were described. The diameter of the uterine horns was measured, as were the cystic formations in the endometrial wall.

Histology and immunohistochemistry (Paper III)

After hysterography 74 of 80 cats were ovariohysterectomised and the uteri measured for length and diameter. Subsequently, the uteri were fixed in 10% buffered formalin solution, embedded in paraffin, sectioned and stained with haematoxylin and eosin and the endometrium was evaluated histologically for lesions in the luminal epithelial, subepithelial and glandular layers. An image analyser (Microphot-FXA, Nikon Inc., Tokyo, Japan) equipped with a computer system (Easy Image Measurement, Bergström Instrument AB, Solna, Sweden) was used for measuring endometrium thickness, myometrium thickness, luminal epithelial cell height and glandular epithelial cell height.

The endometrial cell activity in cats at various stages of the oestrous cycle, cats treated with MPA and cats with uterine pathological conditions was investigated by immunohistochemical labelling with avidin-biotin peroxidase complex (ABC), according to the procedures described previously (Simoes *et al*., 1994). Non-specific binding sites were decreased by incubating the tissue with 10% bovine serum albumin (Fluka, Buchs, Switzerland): deparaffinised and rehydrated sections of the uteri were incubated with monoclonal anti-mouse proliferating cell nuclear antigen (PCNA) (Dako, Glostrup, Denmark) (1:200). Thereafter, sections were treated with biotinylated rabbit anti-mouse IgG antibody (Dako, Glostrup, Denmark) (1:400). The immunostaining was developed with 0.05% 3,3-diaminobenzidine tetrahydrochloride in 0.01 M Tris-HCl, pH 7.6 (DAB) (Sigma-Aldrich, St. Louis, USA) and counterstained with Mayer's haematoxylin. The proliferation index (PCNA index) of the luminal and glandular epithelium was calculated from the percentage of nuclear positive immunoreactive cells from 500 cells in five randomly selected fields.

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Assessment of uterine motility

Fluoroscopy and scintigraphy (Paper II)

Movement of the uterus was observed with fluoroscopy and scintigraphy in a non-ovulatory oestrous cycle in six cats. Fluoroscopy was performed at 2-day intervals at early oestrus, mid-oestrus and late oestrus and fluoroscopic recordings were taken continuously from completion of the Omnipaque infusion into the cranial vagina to 5 min after the contrast medium filled the uterine horns. Scintigraphy was performed once during mid-oestrus. Simultaneous with the ^{99m}Tc-HSA infusion, dynamic hysteroscintigraphy was obtained with a gamma camera at 1-frame/sec in 128 x 128 x 16 matrix for 180 sec. The other 30 sec dynamic images (1 frame/sec) were taken at 5 and 10 min after ^{99m}Tc-HSA infusion. The intrauterine transcornual movement of the contrast fluid and the radiopharmaceutical medium was described according to the contraction pattern of the uterus.

Statistical analyses

Data generated in Papers III & IV were analysed with the Statistical Analysis Systems software (Vers. 8, SAS Institute Inc, Cary, NC, USA). Analysis of variance (ANOVA) was applied to quantitative data using general linear model (GLM). Normal distribution of residuals from ANOVA was tested using the UNIVARIATE procedure. Variables that were not normally distributed were transformed into 10-logarithms or inverse numbers (Paper III). The Tukey-Kramer test was used to compare mean values and the FREQ procedure was used to analyse the frequency distribution of hysterographic features and histological characteristics in each group of cats (Paper III). Differences in mean scores between groups for categorical data in which the normality could not be obtained after transformation were analysed by the NPAR1WAY procedure (Wilcoxon's rank-sum test) (Papers III & IV). A p-value ≤ 0.05 was considered as statistically significant.

Results

Clinical observations and hormonal analyses

The duration of natural oestrous behaviour of six cats ranged between 5 and 8 days, and the duration of the maximum vaginal cornification ranged between 4 and 8 days (Paper II). Onset of oestrous signs of 24 induced cats was observed on Days 3-7 after eCG administration (Paper IV). The eCG treatment induced an average of 10.4±6.3 mature follicles ($\geq$2 mm) per cat (n=24) (Paper IV). The mean serum concentration of oestradiol-17β during interoestrus was 13.2 pmol/L (range 8-26 pmol/L) (n=22) (Papers II & III). Natural behavioural oestrus was first detected when serum concentration of oestradiol-17β was greater than 80 pmol/L (n=6) (Paper II): peak serum concentration of oestradiol-17β occurred within 4 days after the onset of oestrous behaviour (Paper II). A mean oestradiol-17β concentration of 47.7 pmol/L (range 14-113 pmol/L) was observed in cats in which mature follicles were present in the ovaries ($\geq$ 2 mm in diameter) without knowing the accurate day of oestrus (n=2) (Paper III). The means and ranges of oestradiol-17β concentrations analysed from cats in a natural oestrous cycle (Paper II) and an induced oestrous cycle (Paper IV) are illustrated in Table 2. Oestradiol-17β concentrations approached basal levels on Day 8 of natural oestrus whereas oestrous behaviour subsided from Day 5 (2/6 cats), Day 6 (1/6 cats), Day 7 (2/6 cats) or Day 8 (1/6 cats) (Paper II).

Table 2. *Oestradiol-17β concentrations (pmol/L) in serum of cats in natural and in induced oestrus. Means (ranges)*

Day of oestrous cycle	Numbers of cats	Serum concentration of oestradiol-17 β (pmol/L)	
		Natural oestrus	Induced oestrus
2	6	125.5 (85-180)	-
3	12	-	119.2 (53-180)
4	6	91.2 (30-180)	-
5	6	-	84.7 (20-180)
6	6	36.8 (20-62)	-
7	6	-	25.7 (8-42)
8	4	21.8 (17-30)	-
10	2	23.0 (18-28)	-

On Day 3 of the eCG-induced oestrus, four intromissions within one hour successfully induced ovulation in 12 females (Paper IV). In 12 cats with an eCG induced oestrus, 123 of 140 mature follicles were ovulated as confirmed by the presence of CH or CL (87.9%) (Paper IV). Progesterone was at basal level on Day 3 of eCG-induced oestrus (n=12) (Paper IV), the level was still basal at 48 hr after mating (Day 5 of oestrus), except in one cat with a high serum progesterone level (46.5 nmol/L). At 96 hr after mating (Day 7 of eCG-induced oestrus), serum progesterone was 74.4 nmol/L (range 31.2-127.2 nmol/L) (n=6) (Paper IV).

Techniques used for the studies

Transcervical catheterisation and cervical patency

The distance from the vulva to the cranial vaginal fornix of the cats was assessed as being 45-60 mm when measured with the aid of the 3.5 French tomcat catheter (Paper II). Urografin (0.5 ml) deposited vaginally through a tomcat catheter was not observed entering the uterus, even in oestrous cats when they were lying in lateral recumbency (Paper I). When the cats were positioned in dorsal recumbency with the hindquarters elevated at an angle of 15-degrees, passage of the Omnipaque and ^{99m}Tc-HSA was observed with fluoroscopy and a gamma camera during oestrus (Paper II).

Slow infusion of the contrast medium was necessary to prevent backflow. When the cervix was functionally open, a continued infusion of 2-4 ml of Omnipaque, including an amount of 0.5-1.0 ml backflow, would fill the uterine horns (Paper II). A similar volume of Omnipaque (2-3 ml) was sufficient to fill the uterine horns using transcervical catheterisation, with or without cervical manipulation through laparotomy or intrauterine deposition using an intravenous catheter at the time of surgery (Paper III).

Sperm recovery techniques (Paper IV)

There were no differences in sperm numbers in the tissue sections between the flushed and the non-flushed segments of the reproductive tract in the cats ovariohysterectomised at 30 min, 3 hr, 48 hr and 96 hr after mating ($p > 0.05$). During early sperm transport (at 30 min and 3 hr after mating), more spermatozoa were recovered by flushing than observed in the tissue sections, whereas, after ovulation (at 48 hr and 96 hr after mating) more spermatozoa were observed in the tissue sections than were recovered by flushing.

Success of transcervical catheterisation

Transcervical catheterisation with the specially designed catheter was successful in 20/29 cats in the inactive stage of the oestrous cycle; 9/12 in the follicular stage: 9/20 in the luteal stage; 15/15 in the postpartum stage; 12/12 cats treated with MPA; and 2/3 cats with pathological lesions in the uterus (Papers I & III). In 32 cats, blind transcervical catheterisation was successful in 9/29, 5/12, 4/20, 7/15, 5/12 and 2/3 cats, in the respective groups (Papers I & III). Blind insertion of the inner catheter without manipulation of the cervix through laparotomy was successful in 35.2% of cases (32/91 attempts) (Papers I & III).

Cervical patency during oestrus

Cervical patency to contrast fluid and a radiopharmaceutical medium (Paper II)

During interoestrus, Omnipaque or ^{99m}Tc-HSA remained in the vagina. In some of the oestrous stages when the cervix was open, vaginally deposited contrast fluid or

radiopharmaceutical medium was rapidly transported through the cervix into the uterus. The cervical patency dynamics varied among the cats. In 3/6 cats, transcervical transport of the Omnipaque was identified in all stages of oestrus; in 1/6 cats during mid-oestrus, late oestrus and 1 day after oestrus; and in 2/6 cats only during late oestrus.

The patency of the cervix in all six cats occurred after the peak and during declining levels of serum oestradiol-17β, the concentration ranging from ≥180 to 17 pmol/L. The cervix was open at the time of maximal cornification of the vaginal cells in 13/16 observations (>80%). The closure of the cervix, characterised by the Omnipaque remaining in the vagina, occurred following the end of the behavioural oestrus and the phase of maximum cornification of the vaginal epithelium in 5/6 cats. In the remaining cat, the cervix was still open after this stage, on Day 8 of oestrus. Rapid transportation of particles from the vagina through the cervix into the uterus in 3/5 cats during mid-oestrus was determined by scintigraphic examination.

Cervical patency to spermatozoa (Paper IV)

The cervix was found to act as a sperm barrier in the cat. At 30 min after natural mating, only one quarter $(1.1 \times 10^4 / 4.5 \times 10^4)$ of the mean total number of spermatozoa recovered from the reproductive tract by flushing was found in the uterus and the uterine tubes. Differences in sperm numbers ($p<0.05$) were found between the vagina and the uterine segments including the UTJ flushed at 30 min and 3 hr following mating (n=12). The total number of spermatozoa recovered by flushing throughout the reproductive tract at 30 min after one mating did not differ from that at 3 hr after the fourth mating ($p<0.05$). Spermatozoa were still detected in the vagina in 5/6 cats at 48 hr after mating and 1/6 cats at 96 hr after mating.

Localisation of spermatozoa in the female reproductive tract (Paper IV)

There was a considerable variation among cats both within and between groups in the numbers of spermatozoa recovered by the flushing procedure and from the tissue sections. The numbers of recovered spermatozoa decreased with time after mating ($p<0.05$). The highest proportion of recovered spermatozoa at 30 min, 3 hr, 48 hr and 96 hr after mating was detected in the vagina. Before ovulation (determined at 30 min and 3 hr after mating), the majority of spermatozoa were found in the vagina and the uterine segments: after ovulation (determined at 48 hr and 96 hr after mating), higher numbers of spermatozoa were present in the uterine tubal segments than before ovulation. During the time before ovulation, sperm numbers recovered by flushing from the vagina, the uterine segments (including the UTJ) and the uterine tubal segments differed ($p<0.05$).

In the histological sections, the majority of spermatozoa were located in groups in the epithelial crypts, with only a few residing in the lumina of the reproductive tract. There were no differences in numbers of recovered spermatozoa in the tissue sections between the flushed and the non-flushed segments of the reproductive

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tract in any of the four groups of cats ($p>0.05$). At 30 min and 3 hr following mating, the majority of the spermatozoa were found in the uterine segments (including the UTJ) ($p<0.05$), whereas at 48 hr and 96 hr they were mostly located in the uterine tubal segments ($p<0.05$). Higher sperm numbers were detected in the ampulla and isthmus after ovulation than before ovulation ($p<0.05$). However, in the isthmus, the sperm numbers recovered by both flushing and counting in tissue sections, were highest at 48 hr after mating ($p<0.05$).

Uterine morphology assessed by hysterography and ultrasonography

With positive contrast techniques, the uterine appearance in cats at various stages of the oestrous cycle, in cats treated with MPA, and in cats with pathological lesions in the uterus, differed (Papers II & III). Thus, criteria for hysterographic descriptions were defined. Straight shape of the uterine horns with a straight luminal cavity and a smooth inner luminal contour was indicative of cats in the inactive stage of the oestrous cycle, whereas a wavy luminal shape of the uterine horns was characteristic for cats in the follicular stage of the oestrous cycle. In the luteal stage and during the various phases of luteal activity, the luminal cavity of the uteri varied in shape between being straight, irregular wavy and coiling. A coil-shaped uterine lumen seen in the MPA-treated and pathological groups was suggested as characteristic for a progestagenic effect as observed in some cats in the luteal group. In the colony of old cats kept in Uppsala, Sweden (Paper II), the hysterograms examined during oestrus were described as a spiral- (2/6) and a tight spiral- (2/6) uterine horn based on degrees of waviness of the uterine cavity whereas coiled luminal shape of uterine horns with irregular filling defects was defined as a corkscrew appearance (Paper II). Ultrasonography demonstrated a few 1 mm cystic changes in the endometrial wall of one of the cats showing spiral-shaped uterine horns with a smooth intraluminal lining and in both of the cats that had a tight spiral-shaped uterus with a smooth inner contour. Multiple 1-2 mm cystic endometrial changes were observed in both of the cats with a corkscrew appearance of the uterus and distinct irregular filling defects. Waviness and coiling of the uterine lumen was related to a proliferation of the endometrial glands, whereas irregular filling defects in the contrast medium were indicative of endometrial cystic changes as confirmed by the histological study.

Uterine morphology assessed by histology and immunohistochemistry (Paper III)

The histological characteristics of the endometrium in cats during various stages of oestrus and in cats with pathological conditions differed. The endometrium of cats in the inactive and postpartum stages of the oestrous cycle was characterised by a single luminal and glandular epithelial cell lining. Pseudostratification of the luminal epithelial cells was characteristic for the endometrium during the follicular stage of the oestrous cycle. During the luteal stage, both the luminal and glandular epithelium were pseudostratified. Hyperplastic changes of the luminal and

glandular epithelium were the predominant characteristics of histopathological features seen in the MPA-treated and pathological groups.

Length of the uterine horns measured from gross specimens was found greatest in the luteal stage of the oestrous cycle although the differences observed between the stages of the oestrous cycle and between normal and pathological uterine horns were not significant. The outer diameters of the uterine horns measured from gross specimens and from hysterograms between groups of cats were not significantly different (p>0.05). The luminal diameters measured from hysterograms decreased in correspondence to the increase of endometrial and myometrial thickness measured microscopically. The endometrium thickness was greatest in the pathological group whereas the myometrium was thickest in the MPA treated group. The luminal epithelial cells were highest in the follicular stage whereas the glandular epithelial cells were highest in the luteal group.

The PCNA immunostaining cells showed a distinct brown nuclear positive colouration in both luminal and glandular epithelium, indicating mitotic activity in these cells. Between the groups of cats, the percentage of the positive cells in the luminal and glandular epithelium did not differ (p>0.05). A large variation in the PCNA index was noted within the groups of cats.

Uterine motility assessed by fluoroscopy and scintigraphy (Paper II)

Under the fluoroscope, uterine contractions were observed in both ascending and descending directions, flushing the Omnipaque back and forth between the uterine horns and causing reflux of contrast fluid out of the uterine horns through the cervix into the vagina. In most observations, the contrast fluid remained in the uterus for 5 min after infusion. Fluoroscopy revealed frequent and strong uterine contractions. The dynamic images of the hysteroscintigraphy displayed a migration of ^{99m}Tc-HSA into both uterine horns immediately after deposition in the cranial vagina. Movement of the radiopharmaceutical medium within and between the uterine horns was observed. The radioactivity remained during the entire 10 min observation period. In one cat after 3 min, more ^{99m}Tc-HSA was retained in the left uterine horn than was retained in the right uterine horn.

General discussion

Clinical findings

Female cats enter the follicular phase abruptly. A rapid rise of oestradiol-17β secreted by growing follicles at the onset of the follicular phase induces distinct changes in reproductive behaviour, vaginal cornification and opening of the cervix. Ovarian activity is recognised initially by an overt sexual behaviour: the female attracts the male by calling, rolling and treading of the hind legs. In some cases (3/6 cats) in this study, behavioural changes were found to precede changes in vaginal cornification (Paper II), similar to previously reported by Shille *et al.* (1979). Thus, vaginal cytology is not a reliable tool for predicting the onset of oestrus in the cat but is advantageous for determining oestrus in timid cats that fail to exhibit obvious sexual behaviour (Shille *et al.*, 1979; Shille & Sojka, 1995).

At the onset of the follicular phase, a rapid rise of oestradiol-17β was observed from a base line of 13.2 pmol/L to a surge of 125.5 pmol/L on Day 2 of natural oestrus (Paper II), and of 119.2 pmol/L on Day 3 of induced oestrus (Paper IV). Thereafter, oestradiol-17β gradually decreased to approach baseline on approximately Day 8 of the natural oestrous cycle or on Day 7 of the induced oestrous cycle while the oestrous behaviour of most of the cats disappeared. The results were in accordance with those of Wildt *et al.* (1981), in that the mean concentration of oestradiol-17β is above base line on the first day of oestrus, remains elevated throughout oestrus and returns to baseline during the last 24 to 48 hrs of oestrus, or after the onset of ovulation.

Ovulation was successfully induced after four natural matings within one hour as confirmed by the presence of CH or CL in the ovaries (n=12) (Paper IV). The regimen of an eCG-induced oestrus followed by natural mating used in this study resulted in a high proportion of ovulations (87.9%) similar to the proportion of oocytes recovered after eCG-induced oestrus and hCG-induced ovulation (91.4%) as reported by Goodrowe *et al* (1988).

Transcervical catheterisation

The specially designed transcervical catheter developed in this study enabled the introduction of contrast medium into the uterus of the cats during all stages of the oestrous cycle (Papers I & III). Thus, positive contrast study of the uterine appearance in the cat is also possible when the cervix is closed. However, there was a risk of damage to the vagina and cervix when the catheter was introduced blindly into the vagina, which is narrow, and non-distensible (Swanson & Godke, 1994). Measurements with the tomcat catheter determined the length of the combined vestibule and vagina to be 45-60 mm, similar to the post-mortem findings of Watson and Glover (1993). A single-sized vaginal catheter might not fit properly into the ventral vaginal fornix of all cats since the anatomy of the posterior reproductive tract varies between individuals. It was found that the vaginal catheter could not always be properly placed in the cranial vagina because

the portio vaginalis uteri created a too shallow ventral vaginal fornix; consequently, the tomcat catheter did not attain the proper angle towards the cervical canal (K.Chatdarong, E. Axnér & C. Linde-Forsberg, unpublished). The overall success rate of introducing the inner catheter through the cervix (35.2%) (Papers I & III) was lower than the success rate reported in Paper I (76%), presumably as a result of more variation in anatomic structure when more cats were used. It was apparent that to perform transcervical catheterisation good knowledge of the anatomy of the reproductive tract and the individual variation between females as well as the skill of the veterinarian are required.

Cervical patency

Cervical patency could be demonstrated using vaginal deposition of a contrast medium, as well as a radiopharmaceutical, through a 3.5 French tomcat catheter in cats lying in dorsal recumbency and with the hindquarters elevated (Paper II) but not when in lateral recumbency (Paper I). It might be that cats lying in dorsal recumbency provided better pooling of the contrast medium around the opening of the cervical canal, compared to when the cats were positioned in lateral recumbency (Paper I). Similar observations have been reported in the bitch (Linde, 1978).

In most cases (13/16 observations), the cervix was found to be patent in relation to the presence of maximal cornification of the vaginal cells and following the peak of the serum concentrations of oestradiol-17β (Paper II). In bitches, the cervix is functionally open during declining serum concentrations of oestradiol-17β and prior to cytological metoestrus (Silva *et al.*, 1995). The cervical dynamics appeared to be a delayed effect of the high serum oestradiol-17β in the cat as well as in the bitch. The time when the cervix was open varied between cats in this study and was seen either only during late-oestrus, during midoestrus and late-oestrus, or throughout entire oestrus (Paper II). However, it was shown that the cervix in some of the cats did not permit entry of contrast medium even when full cornification of the vaginal cells was evident (Papers I & II).

Transport of fluids, particles and spermatozoa through the cervix

The mechanism by which fluids, particles and spermatozoa are transported through the cervix is not clearly understood but is thought to involve muscular activity of the vagina, cervix and uterus (Harper, 1988). Rapid transport of the contrast fluid, the inert radiopharmaceutical particles and the spermatozoa from the vagina into the uterus of the cats, was observed by fluoroscopy, scintigraphy and flushing of the reproductive tract after natural mating (Papers II & IV). These findings demonstrated that the cervix of the cats allowed fluids, particulate matter and living cells to enter the uterus during a certain period of oestrus. In the rabbit, dead spermatozoa (Noyes *et al.*, 1958) as well as inert radioactive polystyrene microspheres (0.8 to 30 μm in diameter) (Glover & Patterson, 1963) placed in the vagina have also been shown to enter the uterus during oestrus. Fluoroscopy has

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also been used to determine that digital stimulation of the vulva induces vaginal contractions moving radiopaque solutions from the vagina into the uterus in oestrous rabbits (Akester & Inkster, 1961).

In this study, the Omnipaque was injected slowly and only a small volume of the radiopharmaceutical medium (0.02 ml) was used in order to avoid producing pressure towards the cervix. It has been reported in women that the function of the fallopian tubes was disturbed by injection of contrast medium and dye during hysterosalpingography, which leads to false negative tubular patency results compared with results obtained from hysterosalpingo-radionuclide scintigraphy (HERS) (Iturralde & Venter, 1981). The contrast fluid was observed in the uterine tubes in a few cases in this study (Paper III). However, the scintigraphic examination was considered as mimicking passive transport more naturally than fluoroscopy (Paper II) as the volume of ^{99m}Tc-HSA chosen was based on the normal ejaculate volume of the cat (Sojka, 1986).

Contractions of the female genital tract

Muscular activity during oestrus is considered induced by oxytocin. Mechanical manipulation by probing against the cervix, defined as vaginocervical mechanostimulation (VS), produces a release of oxytocin from the hypothalamus (Komisaruk & Whipple, 1995). Plasma concentrations of oxytocin are increased at coitus in women (Carmichael et al., 1994) and in the ewe (Garcia-Villar et al., 1985) causing vaginal and uterine contractility (Roberts & Share, 1969). However, distension of the vagina in non-pregnant and non-lactating bitches produced with a small rubber balloon and a pressure of 150 mmHg induces a myometrial electrical activity within 3.6±0.36 sec, implying a rapid reflex action rather than a hormonal involvement, which would require a latency period long enough (minutes) for a hormone release (Yacout et al., 1991).

Whether vaginocervical mechanostimulation has an effect on the contraction of the female reproductive tract via reflex action or release of oxytocin in the cat is unknown. However, this study provided clear evidence that both cervical opening and contractions of the female reproductive tract were important factors for the passage of contrast fluid, radiopharmaceutical particles or spermatozoa through the cervix into the uterus. When the cervix was closed during interoestrus, the fluid and the particles could not be seen to enter the uterus (Paper II), indicating that the cervix of the cat has an efficient valve-like action like previously shown in the rabbit (Blandau, 1973).

In cats in oestrus and when the cervix was open, both ascending and descending uterine contractions and an intrauterine movement of particles were observed by fluoroscopy and scintigraphy (Paper II). Peristaltic and antiperistaltic waves of contractions passing cranially and caudally have been demonstrated in the vagina of anaesthetised mated rabbits (Blandau, 1973). In the rabbit, uterine contractions initiated by stimulation of stretch receptors in the vagina have been reported (Fuchs et al., 1965). In women during midcycle, the directions of the uterine contractions are mainly from the cervical canal to the fundal part of the uterine cavity towards the cervix, whereas during menstruation they are directed from the

uterine cavity towards the cervix (Lyons *et al*., 1991). Using electromyography, the myometrial activity of mares is directed from the cervix to the oviducts and from the tips of the uterine horns to the cervix (Troedsson *et al*., 1998).

Sperm transport during natural mating

This study demonstrated rapid passage of spermatozoa as illustrated by their presence in the ampulla in three cats and in the infundibulum in 4/6 cats at 30 min after mating. Sperm migration from the vagina to the uterus has been observed in the bitch as soon as 30 sec after natural mating (Tsutsui *et al*., 1989). In the rabbit, spermatozoa have been recovered from the upper ampulla and the fimbrial and ovarian surfaces 1 min after mating (Overstreet & Cooper, 1978a). This indicates rapid initial transport of spermatozoa in the female reproductive tract in these species. Using scintigraphy, radiolabelled rabbit spermatozoa inseminated vaginally were transported into the uterus within 5 min (Bockisch, 1993).

The rapid transport phase is a peri-coital event, characterised by the presence of sperm in the oviducts within minutes of mating or AI (Overstreet & Cooper, 1978a). This rate of transport is more rapid than sperm swimming speed and attributed to muscular contractility of the female tract (Harper, 1994). The rapid phase is followed by a sustained phase of sperm migration (Overstreet & Cooper, 1978b), during which the distribution of spermatozoa within the female tract continues and sperm reservoirs are established (Scott, 2000).

The differing numbers of spermatozoa recovered from the vagina and the reproductive segments above the cervix at 30 min and 3 hr after natural mating indicated that the cervix functioned as the primary physical sperm-barrier in the cat (Paper IV). The erect penis of the tomcat during intromission reaches to 15-20 mm from the cervix (Watson & Glover, 1993). Therefore, at natural mating cat spermatozoa have to pass through the cervix in order to reach the site of fertilization in the oviducts. The mating is thought to influence sperm transport as conception rates are higher in cats mated naturally than in those inseminated intravaginally (Sojka *et al*., 1970; Root *et al*., 1995; Tsutsui *et al*., 2002). Compared to intravaginal insemination, intrauterine insemination is an alternative to overcoming the cervical barrier as it increases conception rates (Platz *et al*., 1978; Tsutsui *et al*., 2001). The mechanical stimulus of mating enhances visceral contractions and sperm transport (Overstreet & Katz, 1990). Passage of spermatozoa through the cervix is stimulated by a second coital stimulus with a vasectomised male (Bedford, 1971), and it has been suggested (Anderson, 1991) that contractions of the vagina and uterus during intromission and orgasm in the female results in the suction of seminal fluids into the cervical canal and, thus, to sperm transport through the cervix.

Sperm barrier

Sharp gradients in the number of spermatozoa in the female reproductive tract of the cat after natural mating were illustrated in this study (Paper IV). The considerable variation in sperm numbers among the reproductive segments was

possibly because of the uterine activity as indicated by the movement of fluids and particles back and forth within and between the uterine horns (Paper II). The cervix and the UTJ were considered the major barriers for sperm transport in the cat due to the differences in sperm numbers in the vagina, in the uterine segments, including the UTJ, and in the uterine tubal segments (Paper IV).

Early sperm distribution within the female tract has been described as occurring in two phases, a rapid transport phase and a sustained transport phase (Overstreet & Cooper, 1978a; Overstreet & Cooper, 1978b). The rapid transport phase is attributed to muscular contractility of the female tract but not sperm motility (Harper, 1994) and is followed by a prolonged phase of sperm migration during which the competent spermatozoa arrive in the uterine tubes (Cooper *et al*., 1979; Scott, 2000). Whether sperm motility is necessary for transport through the cervix remains controversial. The recovery of non-motile spermatozoa from the uterus of the rabbit during the first 15 min after vaginal insemination indicates that sperm motility is not required for rapid transport (Overstreet & Tom, 1982). In contrast, Mattner and Braden (1969) reported that after intravaginal insemination of both dead and motile spermatozoa in the ewe, most of the motile spermatozoa and only small numbers of dead spermatozoa enter the cervix, whereas the majority of dead spermatozoa are blocked in the vagina by cervical mucus. Unlike in the rabbit, in ruminants, *e.g.* the ewe and cow, and in primates cervical mucus is considered to function as a barrier to the passage of spermatozoa and seminal plasma (Katz *et al.*, 1989) and to be a site for sperm storage (Mattner, 1973). Thus, sperm motility appears more essential for sperm migration through the cervix in ruminants than in the rabbit. However, cervical mucus and its function on sperm transport have not been investigated in the cat. In this study, a small amount of clear mucus was observed refluxing from the vulva of one cat during infusion of ^{99m}Tc-HSA into the vagina, thus, blocking the radiopharmaceutical medium from passing into the uterus (Paper II). Cervico-vaginal mucus has been identified in the bitch (England & Allen, 1989) but it is not present in sufficient large quantities (Lindsay, 1983) to represent a barrier to sperm transport (England & Pacey, 1998).

Sperm motility is a prerequisite for the spermatozoa to be able to traverse the UTJ and to establish the sperm reservoirs (Scott, 2000). Despite the presence of smooth muscle contractions, only motile but not non-motile spermatozoa were found emerging from the cut oviductal end of the UTJ of excised tracts in the rat (Gaddum-Rosse, 1981); whereas, radiolabelled tracers of different molecular weights are rapidly transported across the UTJ in pigs (Einarsson *et al*., 1980; Viring *et al*., 1980).

The cervix and the UTJ, the sites of sperm restriction, contribute to the selection of a physiologically normal population of spermatozoa during transit (Scott, 2000). Exclusion of spermatozoa with cell surface abnormalities at the level of the cervix has been reported (Katz *et al*., 1989), whereas the UTJ appeared to block the passage of mouse spermatozoa with severe head abnormalities (Krzanowska, 1974). With scanning electron microscopy, more than 90% of spermatozoa visualised *in situ* at the UTJ in mares are morphologically normal, even when the inseminate contains high numbers of spermatozoa with major morphological

defects (Scott *et al.*, 2000). However, the morphology of the spermatozoa in the various reproductive segments of the cats was not examined in the present study.

Sperm reservoir

Ovulation in the cat takes place 26-36 hr after mating (Wildt *et al.*, 1980), indicating that specific regions, so-called sperm reservoirs, have to be established in the female reproductive tract in which populations of spermatozoa are accumulated to extend the viability of the spermatozoa over time. A high proportion of spermatozoa were found accumulated in the UTJ at 3 hr after natural mating whereas they remained mostly in the isthmus at 48 hr (Paper IV). Within 4 hr after preovulatory insemination, equine spermatozoa have been shown to accumulate in the UTJ (Scott *et al.*, 2000) and are still present 18 hr after insemination (Scott, 2000). From more than 30×10^9 spermatozoa in the ejaculate from the pig, only a maximum of 1.2×10^5 were found accumulated in the UTJ and oviductal lower isthmus after 5 to 60 min: this number remained for 24 hr following mating or until ovulation occurred (Rigby, 1966; Mburu *et al.*, 1997; Rodiguez-Martinez, 2003). Thus, the UTJ and lower isthmus are considered functional sperm reservoirs (Hunter, 1988). The roles of the sperm reservoirs are to maintain sperm viability and fertilizing capacity as well as to prevent attacks by the female immune system. Moreover, the sperm reservoirs help avoid polyspermy (Hunter & Leglise, 1971; Hunter & Nichol, 1988), a lethal condition that rarely occurs *in vivo*, by controlling the release of restricted numbers of spermatozoa in temporal relation to ovulation (Suarez, 1998; Töpfer-Petersen *et al.*, 2002). Fluorescent staining has shown that more than 40% of spermatozoa recovered by flushing the UTJ after ovulation had intact plasma membranes and these spermatozoa were presumably viable (Mburu *et al.*, 1996). In the dog, a greater flagellar activity has been shown in spermatozoa bound to the uterine tube epithelium *in vitro*, regardless of region, in cells cultured from bitches in oestrous than from bitches in luteal or anoestrous stages, suggesting that the entire uterine tube may form a functional sperm reservoir in the bitch (Pacey *et al.*, 2000). Due to the low number of spermatozoa recovered from each reproductive segment, determination of the viability of spermatozoa was not possible in the present study.

Sperm-epithelium contact

The endometrial crypts of the female reproductive tract hosted the majority of the spermatozoa after natural mating in the cat (Paper IV). The population of spermatozoa residing in the endometrial crypts cannot be recovered by the simple flushing technique tested. Moreover, close to the time of ovulation more spermatozoa were found trapped in the epithelial crypts than before ovulation (Paper IV). The heterotypic binding between cells involves carbohydrate recognition (Suarez, 1998). On the heads of uncapacitated hamster sperm, the lectin that binds an oligosaccharide moiety present on the oviductal epithelium has been postulated to be responsible for attachment of sperm to the epithelium (DeMott & Suarez, 1992). There is strong evidence from multiple species that

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sperm-epithelium contact is responsible for the formation of reservoirs (Racey *et al.*, 1987; Suarez, 1998), capacitation (Töpfer-Petersen *et al.*, 2002) and disposal of spermatozoa (Overstreet, 1983). Regardless of location or duration of storage, during storage spermatozoa are found displaying a typical linear, side-by-side arrangement, their heads closely associated with the luminal epithelium (Racey *et al.*, 1987). Binding of spermatozoa to oviductal epithelial cells extends the period of viability of spermatozoa in horses (Ellington *et al.*, 1993; Thomas *et al.*, 1996), pigs (Mburu *et al.*, 1997) and dogs (Pacey *et al.*, 2000) *in vitro*. Capacitation, characterised by an increase in intracellular Ca^{2+}, is regulated by the oviductal sperm reservoir, and the influx of free calcium ions is reduced by sperm interaction with the oviductal epithelium thus, increasing the life span of sperm (Dobrinski *et al.*, 1997; Petrunkina *et al.*, 2001). It has been reported that functional changes in sperm cell physiology, so called capacitation, induced by sperm interaction with the luminal fluids and epithelial surfaces during transit enable spermatozoa to acrosome react (Yanagimachi, 1994). Associated with sperm capacitation is hyperactivated motility which generates thrusting forces considered great enough to enable a sperm to be released from the storage site and to penetrate the ovum (Katz *et al.*, 1989).

Ovulation induces sperm redistribution

A release of spermatozoa from the reservoirs is induced by ovulation. At the time just after ovulation, the numbers of spermatozoa in the tissue sections of the ampulla and isthmus were found to be greater than the numbers before ovulation (Paper IV). Prior to ovulation, competent spermatozoa that pass through the UTJ accumulate in the lower isthmus until ovulation is imminent (Scott, 2000). To do the journey to the ampulla, the site where fertilisation takes place in the cat (Van Der Stricht, 1911), the spermatozoa detach from the sperm reservoir in the UTJ and progress to the ampulla and isthmus (Paper IV). In the rabbit, which is a reflex ovulator like the cat, the isthmic reservoir accumulates a number of spermatozoa following AI, but spermatozoa are not found in the upper uterine tube unless ovulation is induced (Overstreet & Cooper, 1979). Peri-ovulatory redistribution of spermatozoa has also been reported in the pig (Hunter, 1984; Mburu *et al.*, 1996) and the cow (Wilmut & Hunter, 1984). However, the signal that is transmitted by the egg or follicle near the time of ovulation to the sperm that are bound to the uterine tubal epithelial cells is unknown.

Elimination of spermatozoa from the female reproductive tract

Spermatozoa that are bound to the epithelium are removed from the female reproductive tract mainly by phagocytosis (Overstreet, 1983) and maybe by epithelial engulfment (Bedford *et al.*, 1997). In the pig, of 70-99% of spermatozoa transported into the uterus one-third is eliminated by vaginal efflux, whereas the rest are phagocytosed. Polymorphonuclear cells (PMNs) are found in the uterus of the pig at 30 min after ovulation, but not as early as after 10 min (Rodriguez-Martinez, 2003). Transient mating-induced phagocytic activity is observed within a few hours after natural mating and insemination in the mare (Troedsson *et al.*,

1998): spermatozoa alone (separated from semen plasma and bacterial contaminants) can cause an influx of PMNs into the equine uterus (Troedsson *et al*., 1998). In the ram, up to 60% of spermatozoa recovered from the uterus are phagocytosed (Phillips & Andrews, 1937). The non-significant difference between sperm numbers recovered at 30 min and 3 hr after mating between cats mated once and cats mated four times, indicated that elimination of spermatozoa from the female reproductive tract had occurred within 3 hr after mating (Paper IV). In addition, the decrease in sperm numbers over time after mating reflected continuous elimination of spermatozoa (Paper IV).

In the mare, the efflux of luminal spermatozoa through the cervix is accomplished by uterine contractility, whereas the phagocytic activity of the PMNs eliminates the spermatozoa residing in the epithelial crypts (Troedsson *et al*., 1995). Using electromyography, the myoelectrical activity in the uterus of mares following insemination with fresh semen was seen to immediately increase and last for 30 min. The second phase of myoelectrical activity was observed from 4 hr up to at least 12 hr after insemination, which was similar to the activity seen following bacterial inoculation in normal mares (Troedsson *et al*., 1993). Therefore, uterine contractility may be the result of an inflammatory reaction to semen, and possibly is more important for sperm removal from the reproductive tract to provide a proper environment for an embryo rather than for transport of a fertile population of spermatozoa to the fertilization site (Troedsson *et al*., 1998).

Techniques for studying sperm distribution in the domestic cats

The absence of significant differences in sperm numbers recovered from tissue sections between the flushed and the non-flushed reproductive tract segments in this study suggested that most spermatozoa had been firmly attached to the epithelium since 30 min after mating (Paper IV). Simple flushing was not an efficient method for recovering all the spermatozoa; however, histological examination is considerably more time consuming than evaluation of flushings (Larsson & Larsson, 1985). This observation supports the statement of Smith and Yanagimachi (1990) that sperm attachment to the epithelium can lead to a gross underestimation of sperm numbers in the reproductive tract when flushing is used to evaluate the sperm numbers.

Hysterography in relation to histology of the uterus

During the oestrous cycle, the uterus undergoes morphological changes under the influence of ovarian hormones. Using a positive contrast technique and histology, differences in uterine morphology in the inactive, follicular, luteal and postpartum stages of the oestrous cycle and due to pathological conditions in the cat were demonstrated in the present study (Paper III). Although laparoscopy was introduced to study the uterine morphology during the various stages of the oestrous cycle in the cat (Wildt & Seager, 1980), diagnostic imaging is an alternative, non-invasive technique; however, most radiographic and

ultrasonographic studies of the feline uterus are conducted to determine pregnancy or to predict parturition (Schmidt *et al.*, 1986; Beck *et al.*, 1991; Miles, 1995).

The criteria and nomenclatures introduced in this study for describing the hysterographic and histological characteristics were found practical for the cats in various stages of the oestrous cycle or under MPA-treatment as well as the cats having developed uterine pathological conditions. The hysterographic appearance was related to the histological characteristic of the uterus (Paper III). A straight-shaped uterine horn with a straight luminal cavity and a smooth inner contour was found to be characteristic for cats in the inactive stage of the oestrous cycle. Uterine horns in cats in the inactive stage have been described as being straight, smooth and 3-5 mm in diameter (Shille & Sojka, 1995), which is in agreement with the shape and the size of 3.9±0.11 mm found in this study (Paper III). Straight-shaped uterine horns with a wavy luminal cavity and a smooth inner contour were characteristic for cats in the follicular stage of the oestrous cycle and the width of the uterine horns was 5.6±0.11 mm (Paper III). The uterine horns have been reported as being enlarged and reaching up to 7 mm in diameter due to oedema of the endometrium and myometrium during follicular stage (Shille & Sojka, 1995). This corresponded to the current observations, where thicker endometrium and myometrium were recorded in the follicular stage, compared to the inactive stage of the oestrous cycle (Paper III). In cats in the luteal stage of the oestrous cycle, MPA-treated cats and cats that had developed uterine pathology, the hysterograms revealed coil-shaped uterine horns with overall widths of the uterine horns greater than in the inactive group (Paper III). The uterus examined in gross specimens is reported as being large, turgid and twisted inside the serosa, forming irregular corkscrew bulges in the luteal stage of the oestrous cycle (Shille & Sojka, 1995). Waviness and coiling of the uterine lumen was indicative of progestagenic effect as observed in MPA-treated cats, whereas irregular filling defects in the contrast medium were indicative of endometrial cystic changes (Papers II & III). The coiled-shape uterine cavity described in this study was similar to the typical spiral-shaped uterine cavity observed as the characteristic uterine appearance in metoestrus in bitches (Funkquist *et al.*, 1985; Nomura *et al.*, 1987).

In previous studies on the bitch, hysterographic appearance with cystic endometrial hyperplasia has been described as having distinct filling defects (Cobb & Archibald, 1959; Funkquist *et al.*, 1985). The hysterograms of bitches have been categorised into seven groups according to the appearance of the uterine lumen: a normal straight shape; a tight spiral shape; having irregular filling defects; a string of pearls shape; a cotton-reel-like appearance; a corkscrew shape; and uterine lumen dilated uniformly or in a saccular manner (Lagerstedt, 1993). Histopathological examination indicated that the straight-shaped uterine lumen was characteristic of the normal inactive uterus; the tight spiral-shaped uterine lumen was the normal appearance in metoestrus; and the corkscrew shape with filling defects were caused by cystic formations in the endometrium (Lagerstedt, 1993), which is in accordance with our findings in the cat (Paper III). However, the string of pearls and the cotton-reel-like appearances of a postpartum uterus and the saccular dilatation of the uterine lumen which was indicative of purulent

endometritis in the bitch (Lagerstedt, 1993) were not observed in the cats in this study (Paper III).

Mitosis of endometrial epithelial cells

Although a relationship between the PCNA index and the stages of the oestrous cycle and with pathological conditions of the endometrium could not be observed in this study, the expression of PCNA in the luminal and glandular epithelial cells of the cat uterus was established. PCNA has been extensively used as a marker for determining the proliferation of endometrial cells in women with endometrial hyperplasia and adenocarcinoma (Ito *et al*., 1993) during the normal menstrual cycle and in the post-menopausal period (Li *et al*., 1993), suggesting that the PCNA is a reliable marker for endometrial cell proliferation. It is possible that proliferation of cells varies with time and within the stages defined in this study. Thus, further studies on mitotic activity should be performed on cats where the exact days of their oestrous stage are known.

Future prospects

Although this study has confirmed that transcervical catheterisation using a specially designed catheter was possible on cats, further data on the variation of cervical anatomy among individuals is needed. Different sizes of catheter may need to be developed for different sizes of cats since a non-fit placement of the vaginal catheter in the vaginal fornix seems to deviate the cervical catheter from the cervical canal, which may result in vaginal perforation. The specially designed transcervical catheter should be further applied for intrauterine insemination as a non-invasive technique of artificial insemination to obtain a higher conception rate compared to the commonly used intravaginal insemination, particularly in countries where surgical insemination is not permitted.

Since it is controversial whether mating and ovulation influence oestrous length (Verstegen, 2000), the dynamics of cervical patency in the ovulatory cycle should be further investigated. Paape *et al*. (1975) reported that the duration of oestrus was shortened by the occurrence of ovulation whereas Shille *et al*. (1979) reported that coital contact lengthened the duration of oestrus. On the other hand, no difference in oestrus length between ovulating and non-ovulating cats, and no effect of coitus or ovulation on the length of follicular function, have been reported in other studies (Wildt *et al*., 1981; Root *et al*., 1995; Feldman & Nelson, 1996). Thus, cervical patency in the ovulatory cycle could provide important information for the optimal period for semen deposition both by natural mating and by intravaginal artificial insemination.

Although intrauterine insemination appears to be a useful tool for overcoming the cervical barrier, the conception rates are still higher for natural mating (Sojka *et al*., 1970; Tsutsui *et al*., 2002), despite the fact that semen deposition during natural mating is vaginal (Blandau, 1973; Watson & Glover, 1993). Mating is thought to influence sperm transport in cats, but this should be determined by a comparative study of sperm distribution between natural mating, intravaginal insemination and intrauterine insemination. Moreover, the function of sperm reservoirs in cats would also be a further area for study. Electron microscopy is the preferred tool for studying sperm-epithelium contact and sperm viability in the sperm reservoirs, as has been demonstrated *in vitro* in dogs (Pacey *et al*., 2000), horses (Ellington *et al*., 1993; Thomas *et al*., 1996) and pigs (Mburu *et al*., 1997).

Cats that ovulate without fertilization undergo pseudopregnancy or luteal stage and produce progesterone for a period of about 25-45 days (Verstegen, 2000). Differences in the hysterograms of cats in the luteal stage were observed in this study. Using cats subjected to routine spaying limited the demonstration of changes in uterine appearance in this study because the exact day of oestrous cycle was unknown and because each cat could only be examined once. Definition of changes of uterine appearance with time during the luteal stage would be of use for a more detailed determination of progestagenic effects on the uterus, as well as for comparing the effects of exogenous progestagens, which are commonly used for oestrus prevention in cats. The use of contrast fluoroscopy and radionuclide

hysteroscintigraphy could be further developed for studies of uterine contractions and physiology of sperm transport in the female reproductive tract. In addition, in timed uterine specimens, mitotic activity of endometrial epithelium can also be undertaken to determine any relationship between PCNA index and stages of oestrous cycle.

Conclusions

- Using a specially designed catheter it was possible to catheterise the cervix during interoestrus, oestrus, metoestrus and postpartum and to introduce contrast fluids into the uterus to study uterine appearance using hysterography also when the cervix was closed. Cervical dynamics could be observed by the passage of contrast or radiopharmaceutical medium from the vagina through the cervix into the uterus when cats lay in dorsal recumbency with the hindquarters elevated. With these methods, hysterography proved to be a useful technique for studying the uterine appearance of the domestic cat. Cannulation of the cervix with the specially designed catheter was performed to introduce the contrast medium into the uterus when the cervix was closed. Transcervical catheterisation is likely to enhance the success rate of feline assisted reproduction, and is a non-surgical technique that is potentially useful for other diagnostic and therapeutic purposes. Using a contrast medium, the transport of fluid through the cervix, and the uterine contractility in the cat could be observed with fluoroscopy. The dynamic images of the hysteroscintigraphy provided valuable information on the transport of inert particles in the uterine horns created by the uterine contractions.

- Vaginally deposited contrast fluid, inert radiopharmaceutical particles and spermatozoa were transported rapidly through the cervix of the cats in oestrus. The patency of the cervix was related to the degree of vaginal cornification, and, thus, presumably, with the serum concentration of oestradiol-17β.

- Spermatozoa are distributed in the female reproductive tract of cats with sharp gradients in sperm numbers from the vagina to the uterus and from the uterus to the oviducts. The cervix and the UTJ, therefore, seem to act as barriers for the spermatozoa. The uterine crypts and the UTJ are initial sperm reservoirs before ovulation, whereas the isthmus seems to function as a sperm reservoir around the time of ovulation in the cat. Both the flushing technique and histological sectioning can be used to study sperm distribution in the cat, although the latter method is more exact but it is also more elaborate and time consuming.

- Differences in the hysterographic appearance and the histological characteristics of the endometrium during the various stages of the oestrous cycle in the cat, and differences in the hysterographic features due to some pathological uterine conditions, were observed. Straight and wavy luminal cavities were characteristic for the uterus in the inactive and follicular stages of the oestrous cycle. Straightness, irregular waviness, and coiling of the luminal shape can be seen in cats in the luteal stage of the oestrous cycle. A coiled luminal shape was suggestive of endometrial hyperplasia, whereas irregular filling defects were indicative of generalised cystic changes in the endometrium.

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Acknowledgements

I would like to express my sincere gratitude to all those who have directly and indirectly contributed to this work, and especially to the following:

Professor Stig Einarsson, former head of the Department of Obstetrics and Gynaecology, and Professor Hans Kindahl, present head of the Department of Obstetrics and Gynaecology, for a welcome to study in Sweden and for placing the facilities of the Department at my disposal.

My scientific supervisor Professor Catharina Linde-Forsberg, for believing in me, I am grateful for her everlasting encouragement, interest and fruitful guidance both on academic and personal life over the years. Thanks for intensively criticising my manuscripts and thesis, particularly, during the wonderful Swedish summer time. Her dedication is appreciated. Thanks also for completing my research competence. Last, but not least, special thanks for always being in my electronic mailbox as soon as I needed support. The size of the World is nothing for us!

Eva Axnér, my scientific co-supervisor, for her friendship, generous advice, productive discussion and expertise in feline reproduction. She is always positive and always has time for me.

Associate Professor Chainarong Lohachit, my scientific co-supervisor at Chulalongkorn University, for his endless encouragement and support. Thanks for always understanding and being positive.

Associate Professor Karin Östensson, former director of SIPAR, for providing valuable opportunity to participate in the International Master of Science programme which initiated my post-graduate studies and second life in Sweden.

Professor Annop Kunavongkrit, head of the Department of Obstetrics, Gynaecology and Reproduction, Chulalongkorn University, for his never-ending support throughout my study leave.

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