

Learning objectives for Female Reproductive Tract Lectures 1+2

- 1) Define the terms hermaphrodite, pseudohermaphrodites and freemartin and explain how these phenotypes develop
- 2) Outline the major congenital and acquired abnormalities of the ovaries
- 3) List the various types of ovarian neoplasia
- 4) Outline the major pathology of the uterine tubes and uterus including neoplasia
- 5) Understand and compare development of cystic endometrial hyperplasia and pyometra in cattle and dogs (especially know the pathogenesis of canine pyometra)
- 6) Know the clinical signs and extra-genital lesions of pyometra in dogs
- 7) Outline the major pathology of the vulva and vagina
- 8) Discuss the major non-infectious conditions that can affect the pregnant uterus
- 9) Describe the general responses of the uterus to disease during pregnancy
- 10) Outline the possible routes of infection of the uterus and be able to give some examples of causes of abortion

EMBRYOLOGICAL DEVELOPMENT OF THE GENITAL TRACT

Early embryo has small primitive structures which will become gonads and genital tract. There are paired mesonephric ducts (Wolffian) or paramesonephric (Müllerian) ducts which fuse with the urogenital sinus. **Chomosomal (genetic) sex** is determined at time of conception (XX or XY). **Gonadal sex** is determined by the sex chromosomes which facilitate differentiation of the bipotential gonad in the foetus e.g. presence of the SRY gene on the Y chromosome (sex-determining region of the Y chromosome) encodes “testicular determining factor” protein (TDF). Lack of SRY gene (i.e. XX females) leads to ovarian differentiation as other genes are expressed. The sex of the gonad then normally determines which reproductive duct system develops (**phenotypic sex**). Testes induce development of mesonephric (Wolffian) duct system into epididymis and vas deferens and cause regression of paramesonephric (Müllerian) duct by “Mullerian inhibitory substance” (MIS). Absence of testes and presence of ovaries allows paramesonephric ducts to develop under influence of oestrogen to oviduct, uterus, and cranial vagina. These will fuse caudally with the urogenital sinus (which will become the posterior part of the vagina and the vulva). All of the stages of development are controlled by genes and gene products.

DISORDERS OF SEXUAL DEVELOPMENT

Developmental abnormalities resulting in discordance between the genetic sex, gonadal sex and phenotypic sex. As there are many stages in sexual development, the exact mechanism can be difficult to determine in every case but may result in an “intersex” animal with “normal” or ambiguous external genitalia.

HERMAPHRODITISM

True hermaphrodite: An individual possessing gonadal tissue of both sexes. Rare and due to failure of embryological differentiation of gonads during foetal life which leads to the presence of both ovarian and testicular tissue (ovotestis or one gonad of each type). The tract

will contain tissues from both mesonephric and paramesonephric ducts (ducts may predominantly differentiate towards one sex but with remnants of the other).

Pseudohermaphrodite: Sex chromosomes and gonads are of one sex but the ducts and external genitalia are modified towards, or are of, the opposite sex. To class apseudohermaphrodite as male or female you go with the sex of the GONADAL TISSUE
Male pseudohermaphrodite (more common) = XY and testis present BUT predominantly female genitalia. May have ambiguous external genitalia e.g. small genitalia, hypospadias, variably fused vulvar lips, clitoromegaly etc. Testes may be in a scrotal structure or intra-abdominal (may even be attached to uterine horns). Female pseudohermaphrodite = XX and ovary +/- uterine horns and uterus present but male-like genitalia.

NOTE: Histologically some cases diagnosed grossly as pseudohermaphrodites may actually be hermaphrodites with ovotestes present. The gross appearance of the gonad is influenced by the amounts of ovarian and testicular tissue present.

Aetiology of intersex conditions: Genetic. XX Sex reversal (i.e. mutation allows 1 gene on X chromosome to act like Y chromosome) is reported in mice, dogs, pigs, and goats; XY sex reversal and/or androgen insensitivity (i.e. XY male with testes but mutation makes all cells insensitive to androgens). Administration of steroids to bitches during pregnancy may cause virilisation of female foetuses and pseudohermaphroditism etc.

Freemartinism (i.e. Chimeras): A freemartin is genetically female calf born co-twin with a male calf. During foetal development placental anastomosis allows embryonic blood to be shared by the twins (anastomosis occurs in 90% of bovine twins, <2% in other species). Each twin acquires genetically distinct population of hematopoietic cells from the other twin - **CHIMERISM**. Blood tests can be used to demonstrate different chromosome complement in nucleated WBC and presence of two blood groups in each twin. Most female calves born co-twin to a male will be freemartins and it is mainly recognised in cattle, but can occur in sheep, goats and pigs. As the testes develop earlier in embryonic life than ovaries, the TDF, MIS and testosterone enters the female twin's circulation via the placental anastomosis and impairs normal female gonadal development. In the female twin, paramesonephric (Müllerian) ducts do not form properly or may atrophy (remember these form the oviducts, uterus, cervix and cranial vagina) and mesonephric (Wolffian) ducts persist and may form rudimentary male tubular genitalia.

Morphological features in the affected female vary:

- a. External genitalia may appear relatively normal - condition only detected at puberty
- b. Enlarged clitoris, long tufts of hair round vulva, skin fold from groin to umbilicus.
- c. Vagina is hypoplastic and blind-ended (complete hymen). Vulva and vestibule hypoplastic. Patency can be checked using speculum or probe.
- d. Uterus variably under developed - fibrous cords or non-patent tubes. Vestigial vesicular glands present (consistent feature) at base of paramesonephric ducts.
- e. Small/underdeveloped mammary gland
- f. Ovaries are usually stunted and may contain seminiferous tubules (i.e. may be an ovotestis).

PATHOLOGY OF THE FEMALE GENITAL TRACT

Diseases of the female genital tract may be incidental or can interfere with fertility.

OVARIES: CONGENITAL ABNORMALITIES

- 1) **Ovarian agenesis or duplication.** Rare. **Ovarian remnant syndrome** is seen in female dogs and cats that have been neutered but still show signs of oestrus. May be due to anomalous duplication but you must remember it may also be due to inappropriate surgical technique.
- 2) **Ovarian hypoplasia.** Rare in Britain, recognised in Swedish Highland cattle (autosomal recessive gene). Usually bilateral with infantilism of the tract.
- 3) **Vascular Hamartoma** - Incidental finding in cow, pig, horse. Dark red mass on ovarian surface composed of connective tissue and vascular channels.
- 4) **Cysts** - Parovarian cysts arise from mesonephric or paramesonephric ducts, can be large. Mare dog, cat.

OVARIES: ACQUIRED PATHOLOGY

- 1) **CYSTS** – These are common and they may be physiological (Graafian/ovulatory follicles can be very large e.g. mare). There are many types e.g. follicular and luteinized cysts; epithelial inclusions cysts (which may cause infertility in mares); and bitches/queens may have cystic rete ovarii etc. In cattle and pigs the main types are:
 - a) Follicular cysts - Failure of follicle to ovulate due to failure of LH release. In cattle - >2.5cm and persist for >7days. These may secrete oestrogen and can affect cyclic activity e.g. anoestrus or nymphomania. They may also cause changes in the rest of reproductive tract, e.g. cystic endometrial hyperplasia and mammary hyperplasia.
 - b) Luteinised cysts - Failure to ovulate due to inadequate or delayed LH release. Cyst partially luteinised. Usually no oestrogen production but if multiple in sows may cause infertility. Important differential diagnosis is **cystic corpora lutea** which have a distorted outline due to the ovulation papilla.
- 2) **HAEMORRHAGE:** Ovarian haemorrhage may be intrafollicular and is often incidental or occurs at ovulation (corpus haemorrhagicum). However, it may occasionally be severe (even fatal) in mares OR after manual enucleation of corpora lutea in cattle.
- 3) **INFLAMMATION:** Oophoritis is rare but may be secondary to ascending infection. Repair is by granulation tissue formation with fibrous adhesions to bursa.

OVARIAN NEOPLASIA

- a. Germ cell origin – Teratoma or dysgerminoma.
- b. From gonadal stroma i.e. tumours of the granulosa / thecal cells and their luteinized forms. Granulosa-theca cell tumour - most common especially in mare and cow. Usually unilateral and benign. Encapsulated, nodular white grey surface. May be cystic ± haemorrhage. Often secrete oestrogen/progesterone/testosterone causing behavioural changes e.g. persistent oestrus or male behaviour; bitches may develop cystic endometrial hyperplasia or pyometra
- c. Tumours of surface epithelium i.e. Cystadenoma/adenocarcinoma - Elderly bitches, may be bilateral and may also have cystic endometrial hyperplasia. Pale nodular, cauliflower-like masses.

UTERINE (FALLOPIAN) TUBES: There are two main conditions

- 1) **Obstruction** results in hydrosalpinx i.e. uterine tubes distended with mucus secretions. May be congenital or acquired
- 2) **Inflammation** - Salpingitis/pyosalpinx, may affect fertility and is often only detectable microscopically. Secondary to ascending infection from uterus especially cows.

UTERUS CONGENITAL ABNORMALITIES

- 1) **Segmental aplasia of uterine horns and/or uterine body.** May vary from complete absence of one horn (uterus unicornis) to segmental aplasia. The tract proximal to the aplastic segment may become cystic/filled with necrotic debris and secretions.
- 2) **Failure of the caudal parts of the paramesonephric ducts (uterine body and cervix) to fuse.** The degree of fusion may vary e.g. failure of fusion of cervix and uterine body -> duplication of the cervix and uterine body **uterus didelphys** (normal in some marsupials, rodents and lagomorphs) OR failure of caudal portion of paramesonephric ducts results in a dorsoventral septum dividing the cranial vagina.
- 3) **Failure of fusion of the paramesonephric ducts (uterus) with the urogenital sinus (vagina)** i.e. imperforate hymen with remnant of tissue cranial to urethral opening. It may be a small piece of tissue but if complete, the uterine secretions may accumulate and distend the uterus (hydrometra/mucometra).

PATHOLOGY OF THE UTERUS

1) ABNORMAL POSITION/LOCATION

- a. **Torsion** – Enlarged uterus e.g. due to pregnancy or pyometra (generally uncommon but seen in cow, mare). Sequelae: congestion, oedema, foetal death, uterine rupture.
- b. **Prolapse** - Cow, ewe, sow. Post-partum, secondary to hypocalcaemia, prolonged dystocia. Sequelae congestion, oedema, necrosis, shock.
- c. **Rupture** - Secondary to torsion/prolapse, iatrogenic or due to untreated dystocia.

2) ENDOMETRIAL CHANGES

- a. **Atrophy** - due to loss of ovarian function. Senility, secondary to hypopituitarism, anoestrus (mare), following ovariectomy.
- b. **Hyperplasia** - Cystic endometrial hyperplasia, related to hormone influence e.g. granulosa cell tumours (see later for details in dogs). Non-cystic hyperplasia also occurs and in some species (mainly sheep) may follow ingestion of oestrogenic plants.
- c. **Endometrial polyps** – seen in bitch and queen, with focal enlargement of uterine horn. Pedunculated mass of stroma and dilated glands. May prolapse through cervix.

3) INFLAMMATION

Inflammation of the uterus may be infectious or non-infectious and is influenced by hormonal factors. The main routes of infection are either via cervix (at oestrus/ mating or parturition) or haematogenous. Any infection may result in reduced fertility.

Endometritis Inflammation of the endometrium

Metritis Inflammation of the whole uterine wall

Placentitis Inflammation of the foetal attachment point of the uterine wall

Endometrosis Chronic endometritis (mare)

Pyometra Chronic suppurative infection with accumulation of pus in the uterine lumen

a) Infection at mating - Mating causes a mild non-specific endometritis which is usually rapidly cleared by neutrophils. Infected semen may also transmit infection following AI or natural infection e.g. contagious equine metritis is a NOTIFIABLE condition in horses caused by *Taylorella equigenitalis* which leads to a purulent endometritis and cervicitis.

b) Post-partum - Infection of the non-pregnant uterus via the vagina after parturition or abortion. Increased risk in animals with dystocia, retained placenta or failure of involution (bitch). Possible aetiological agents: **Cow** - *T. pyogenes*, *Fusobacterium necrophorum*, *E. coli*; **Sheep** - Clostridia. **Bitch/queen** - *E. coli*, *Staphylococcus* spp., *Streptococcus* spp. **Mare** - *Strep. zooepidemicus*, *E. coli*, *Pseudomonas* spp, etc.

Macroscopically: Ranges from slight opacity of oestral mucus in mild cases to malodorous, dark brown to grey uterine contents +/- debris with an ulcerated, congested and haemorrhagic mucosa. In mares histopathological features of endometrial biopsies are carefully evaluated and graded for evidence and severity of chronic endometritis to help with fertility investigations.

Sequelae of endometritis may vary markedly:

- a) Ascending infection - salpingitis
- b) Descending infection - cystitis and pyelonephritis
- c) Septicaemia - fever, depression, death
- d) Embolic spread - endocarditis, renal infarcts
- e) Chronic endometritis – fibrosis and lymphoplasmacytic inflammation with glandular atrophy
- f) Pyometra – Suppurative infection of uterus following metritis or endometritis with pus accumulating in the lumen. In cattle may be associated with persistent corpus luteum due to failure of prostaglandin release (which may be secondary to endometritis/metritis) and the uterus is susceptible to infection but the cervix is closed. Pyometra may also occur as a result of dystocia/retained foetal membranes. In cats it is often seen post-partum and may be associated with retention of foetal membranes. **NOTE:** In the dog (and sometimes cats) pyometra is usually associated with cystic endometrial hyperplasia of the endometrium - see below.

4) CYSTIC ENDOMETRIAL HYPERPLASIA AND PYOMETRA IN THE DOG

Most bitches that develop pyometra are middle aged/old and have never been bred. Typically the animal will present 4-8 weeks after oestrus with a history of inappetance, lethargy, vomiting, polyuria and polydipsia and may or may not have a uterine discharge. Pyrexia, circulating neutrophilia and a painful/distended abdomen may also be noted. The animals typically have associated cystic endometrial hyperplasia which occurs due to oestrogen priming followed by elevated progesterone (i.e. normal cycle) which, along with local irritation/inflammation, allows the development of cystic endometrial hyperplasia, mucoid secretion and subsequent bacterial infection (*E. coli*, *Staph. aureus*, *Strep* sp.) with pus accumulation in the uterus results in **pyometra**. The cervix may be closed or open and there tends to be increased severity of signs if cervix closed.

Macroscopically – Distended uterine horns containing variable content (often purulent) within congested serosal surfaces. **Microscopically**- Marked endometrial hyperplasia with cystic glands, inflammatory cells (often neutrophils), congested blood vessels etc.

Extra-genital lesions: These include toxæmia/septicaemia, membranoproliferative glomerulonephritis (immune complex deposition), myeloid hyperplasia in the bone marrow and extra-medullary haematopoiesis (liver, spleen, etc).

5) MISCELLANEOUS UTERINE PATHOLOGY

Subinvolution of placental sites

Bitches post-partum. Failure of placental sites to involute. Bloody discharge ± necrotic placental tissue - cause unknown.

Hydrometra/mucometra – accumulation of fluid/mucus in the uterus due to endometrial hyperplasia or proximal to an obstruction in the uterus, cervix or vagina.

6) UTERINE NEOPLASIA

Usually primary tumours. Metastasis to the genital tract is rare

Uterine carcinoma - cow, incidental finding at slaughter. Metastases in lungs. Common in rabbits but rare in bitch

Leiomyoma (tumour of smooth muscle) - most common in the bitch. Leiomyosarcomas are also seen.

VAGINA AND VULVA PATHOLOGY

- 1) **Congenital defects:** Usually as part of intersex/failure of fusion of paramesonephric ducts with urogenital sinus (described above).
- 2) **Swelling:** May be normal (physiological change during oestrus) but abnormal oestrogen levels e.g. oestrogen producing tumours such as GCT or some mycotoxins may also cause swelling.
- 3) **Trauma** e.g. during parturition, following vaginal prolapse in late pregnancy ewes on oestrogenic pastures may result in inflammation, infection or even rupture.
- 4) **Inflammation i.e. Vulvo-vaginitis** may be due to local trauma (as above) or may be secondary to specific infectious agents e.g. CATTLE Infectious pustular vulvovaginitis due to *Bovine herpesvirus 1* (which also causes bovine rhinotracheitis) results in vaginal/vulvar hyperaemia and oedema, ulceration and lymphoid nodules,. HORSES – Contagious equine metritis (*Taylorella equigenitalis*) resides in the crypts/fossa around the clitoris.

VAGINA AND VULVA NEOPLASIA

Squamous cell carcinoma - Cow, ewe, mare, due to solar radiation.

Transmissible venereal tumour - Bitch, transmitted at coitus by transfer of intact cells. Not reported in UK. Macroscopically: Nodular, friable ulcerated lesion. Microscopically: sheets of round cells. Spontaneous regression may occur.

Melanotic tumours – Grey horses, may be seen on vulva in mares (also prepuce in male)

Vaginal leiomyoma – Bitch, single or multiple benign tumours. A vaginal polyp is an important differential for this lesion.

PATHOLOGY OF NON INFECTIOUS CONDITIONS OF THE PREGNANT UTERUS

- 1) **Rupture** - Usually during parturition as a result of untreated or mishandled dystocia.
- 2) **Ventral herniation** - Result of muscle weakness, old age, trauma. Heavily pregnant uterus can herniate through abdominal muscle wall lateral to mid-line. Cow and mare.
- 3) **Adventitial placentation** - Formation of additional (less efficient) sites of placentation between existing placentomes. May be congenital or due to loss of

caruncles due to previous endometritis. Cattle. Normally cows have 75-120 caruncles. If marked, the pregnancy is insecure and may abort mid-term.

- 4) **Hydramnios and hydroallantois** - Accumulation of excess fluid in amniotic and allantoic sacs. Mainly cattle and rare in other species. **Hydroamnios** is associated with foetal abnormalities (especially facial abnormalities) whereas **hydroallantois** is seen with adventitial placentation or twin pregnancy. **Anasarca** is generalised oedema and may occur in the foetus due to either of the conditions above.
- 5) **Prolonged gestation** - Due to foetal malformation, e.g. absence of pituitary gland (Holstein, Guernsey cattle), anencephaly. Disturbance of foetal pituitary adrenal axis leads to failure of release of foetal corticosteroids and failure of induction of parturition.
- 6) **Twinning** – In mares, abortion/mummification of twins may be due to insufficient placental space
- 7) **Post parturient haemorrhage** – May be secondary to trauma at time of parturition or due to retained foetal membranes.

GENERAL RESPONSES TO DISEASE DURING PREGNANCY

- 1) Early embryonic death and resorption with normal or delayed return to oestrus.
- 2) Mummification – When the foetus dies but the corpus luteum is retained and there is no bacterial infection. Foetal fluids are resorbed and the foetus is retained as black leathery mass. This may be a non-specific finding associated with many different causes e.g. genetic abnormalities, viral infection, placental insufficiency, etc.
- 3) Maceration and emphysema – Requires bacterial infection and results in maceration and resorption or expulsion. May cause endometritis or pyometra. Emphysema occurs when dead foetal tissue invaded by gas forming bacteria e.g. clostridia.
- 4) Abortion – Foetal death due to damage to uterus, placenta or foetus before the foetus is considered viable
- 5) Stillbirth - Foetal death due to damage to uterus, placenta or foetus after the foetus is considered viable
- 6) Live birth – With a clinically normal or abnormal neonate.
- 7) Congenital malformation of the foetus due to genetic factors (inherited or acquired) or environmental factors (physical, chemical, nutritional, hormonal, infectious agents).
- 8) Retained foetal membranes will cause metritis/endometritis.

INFECTIOUS DISEASES OF PREGNANT UTERUS

Pregnancy failure may occur as a result of:

- 1) Endotoxins or pyrexia, e.g. *Leptospira interrogans* (pigs, cattle), *Salmonella* (cattle and sheep) with no lesions in placenta or foetus although bacteria present.
- 2) Immediate placentitis, e.g. *Campylobacter fetus* ssp *fetus* (cows), *Listeria* (sheep). Lesions present in foetus and placenta (cotyledon) with abortion about 2 weeks after oral infection.
- 3) Delayed placentitis, e.g. *Brucella abortus* (cows), *Chlamydophila abortus* (sheep).
- 4) Direct damage to foetus due to viruses e.g. Bovine Viral Diarrhoea virus, parvovirus, - resulting in foetal death (also congenital abnormalities or persistent infection of neonate).

In many cases, the pathology relating to infertility/abortion is non-specific and a combination of a thorough clinical history, culture, serology and virus isolation are required to determine the cause. The principles of infertility/abortion investigation will be covered in farm animal medicine courses but essentially you should consider maternal, foetal and placental causes

and ensure your investigation has samples to cover all of these (e.g. take blood from the dam along with swabs from the uterus and submission of placental and foetal tissues to a lab). Autolysis may mask some changes in the foetus. Many infectious agents can affect the uterus during pregnancy and some of these are listed below. Infectious organisms often cause “abortion storms” i.e. >10% of animals abort but non-infectious organisms (e.g. fungal contamination of feed) may also cause similar problems. Infectious agents may gain access to the placenta via haematogenous, descending (i.e. from uterine horns) or ascending (transcervical) spread.

Some examples of specific diseases (remember that some are ZOONOTIC)

Viral

Herpes viruses - Species specific herpesvirus are seen in horses, cattle, pigs and dogs and may cause abortion. Foetus may be autolysed but the classic lesion is focal hepatic necrosis with intra-nuclear inclusions.

Bovine Viral Diarrhoea virus – Pestivirus, may result in abortion of fresh, autolysed or mummified foetuses. Calves may also be born alive with congenital defects e.g. cataracts, renal dysplasia, cerebellar hypoplasia etc. or they may appear clinically normal but be persistently infected.

Border disease virus – Pestivirus in sheep may cause abortion and stillbirths or birth of live small, weak, hairy lambs with CNS and skeletal abnormalities (“hairy shakers”).

Porcine parvovirus – A common cause of infertility with a range of clinical signs e.g. small litters, stillbirth, mummification, embryonic death or infertility.

Bacterial

Brucella abortus – Cattle, transmission by exposure to contaminated placental fluids resulting in placentitis and abortion typically occurs in last half of pregnancy. Placenta is covered by fibrinonecrotic tissue. Foetus often shows bronchopneumonia.

Listeria spp. - Abortions in cattle, sheep and goats, typically in last third of pregnancy and causes necrotizing and suppurative placentitis with retention of foetal membranes often leading to metritis. Focal pinpoint yellow lesions of hepatic necrosis in foetal liver.

Campylobacter fetus or C. jejuni – Sheep, often causes abortion storms. Oral transmission from faeces or aborted material. Late abortion, premature and weak lambs due to placentitis. Areas of focal hepatic necrosis in foetus.

Chlamydophila abortus - Enzootic abortion of ewes. Late abortion, premature lambing, retained foetal membranes. Infection by ingestion. Immunity develops following exposure therefore abortion only in ewes not previously exposed (i.e. ewe lambs or bought in animals). Aborted foetus may be oedematous with blood tinged fluid in body cavities. Cotyledons are necrotic, covered with exudate while foetal membranes may be thickened and oedematous.

Salmonella spp – The bacteraemic phase of infection may cause placentitis, foetal pneumonia, hepatitis etc.

Coxiella burnetii (rickettsial organism) infection by inhalation, ingestion or tick bites. Sheep and rarely cattle. Late abortion or birth of weak lambs/kids. Thickened leathery placenta covered in exudate. ZN stains can be used to demonstrate organisms

Protozoal

Toxoplasma gondii – Sheep. Abortion late in gestation following infection with oocysts from cat faeces. Ewes not clinically ill, foetus has no gross lesions. Necrotising placentitis, characteristically the cotyledons are bright red with multiple (1-3 mm) white/yellow foci (strawberry cotyledons).

Neospora caninum - Cattle. Late abortion of mummified or autolysed foetus. May also get foetal malformation, e.g. arthrogryposis.

Tritrichomonas foetus - Venereal infection of cattle which can cause early embryonic death and abortion.

Fungal - Abortions due to mycotic agents affect individual animals rather than cause abortion storms. In cattle may be associated with intestinal ulcers infected with fungus resulting in haematogenous spread to uterus. Lead to abortion late in pregnancy, placenta often retained. Leathery thickening may affect whole placenta. Raised grey/white plaques may be present on skin of foetus. Organisms involved include *Aspergillus* spp, *Mucor/Rhizopus/Absidia* spp. Cattle and horses (also sheep and goats).

Mammary Lecture Learning Objectives

- 1) Describe the congenital/non-infectious conditions of the mammary gland
- 2) Be aware of the infectious skin conditions of the mammary gland
- 3) Know the basic model of mastitis and be able to outline the pathophysiology and sequelae of the disease
- 4) Know examples of mastitis in cattle
- 5) Know examples of mastitis in other species
- 6) Know about mammary neoplasia in the dog and cat and any species differences

PATHOLOGY OF MAMMARY DISEASE

SELF STUDY: Review lectures on mammary gland anatomy

Mammary glands develop from ventrolateral ectoderm in the wall of the embryo. These modified apocrine sweat glands are composed of lobules of glandular tissue formed by tubuloacinar secretory units (alveoli) and ducts in fibrofatty connective tissue. Alveoli are lined by a single layer of cuboidal to columnar epithelium. Ducts are lined by a bilayer of cuboidal epithelium. Myoepithelial cells around the alveoli and ducts and control milk flow. The amount of secretory tissue varies depending on the age of the animal (pre-pubertal vs post-pubertal), pregnancy and lactational status.

Ruminants have a single teat canal whereas other species have 2 or more. The teat canal lining is stratified squamous epithelium with an innermost layer of smegma (waxy material composed of epithelial debris and milk solids). The basic function of the gland is for neonatal nutrition and provision of passive immunity (immunoglobulin transfer in colostrum). Normal milk contains many factors (both humoral and cellular) that inhibit bacterial growth.

Remember that males also develop mammary glands and may or may not have nipples (e.g. male rats, mice and horses do not have nipples).

CONGENITAL/NON INFECTIOUS CONDITIONS

Rarely neonatal animals may secrete fluid/milk from their mammary glands (galactorrhea; witches milk) due to the effects of maternal hormones on the foetal pituitary. This should resolve spontaneously.

Mammary gland/nipple aplasia, hypoplasia, inversion, non-patency may all occur and their significance depends on whether the animal will be used for breeding/milk production.

Supernumerary teats are relatively common especially in cattle (where the incidence may be up to 30%). These may interfere with attachment of the neonate or milking machines.

Abnormal melanin pigmentation (melanosis) of the mammary glands (and ventral abdominal fat) is seen in gilts (aka “seedy cut”) and usually disappears following first lactation.

Note: Galactorrhea (also known as a “false pregnancy” in adult bitches) may be seen in the normal dioestrus period and may become permanent if bitches undergo ovariectomy during dioestrus.

INFECTIOUS CONDITIONS OF THE SKIN OF THE MAMMARY GLAND

- 1) **Bovine herpes mammillitis:** Localised Bovine herpesvirus 2 infections in lactating dairy cows -> **ulcerative mammillitis** (inflammation of skin of the teat and udder) with teat swelling, ulcers, crusting etc. Usually sporadic and secondary to teat trauma. The pain may cause reluctance to be milked and can contribute to secondary mastitis. On histology there are epithelial syncytia with intra-nuclear eosinophilic inclusion bodies.
- 2) Bovine herpesvirus 4 also causes **mammary pustular dermatitis** on the udder but does not involve the teat.

MASTITIS

This is **inflammation of the mammary gland**, usually due to bacterial infection via one of the following routes: haematogenous, percutaneous or invasion of the teat canal. Most commonly seen in production species (but can occur in other species) and can be peracute, acute, subacute or chronic. The clinical presentation may range from subclinical to severe or even fatal. A wide variety of microbes may be involved. The udder has a normal bacterial flora (similar to skin) and disease typically occurs when there is disruption of the microenvironment. Several factors contribute to the development of disease in the gland and these include innate and acquired resistance, teat damage, milking machines etc. Trauma and cell damage allows bacterial entry and proliferation; there is inflammation and epithelial cell death which lead to exudation and desquamation causing changes in the milk and/or gland. The infection may be fatal, may resolve, or with continued cell damage, progress to replacement of mammary tissue by fibrosis and/or abscessation (summary below).

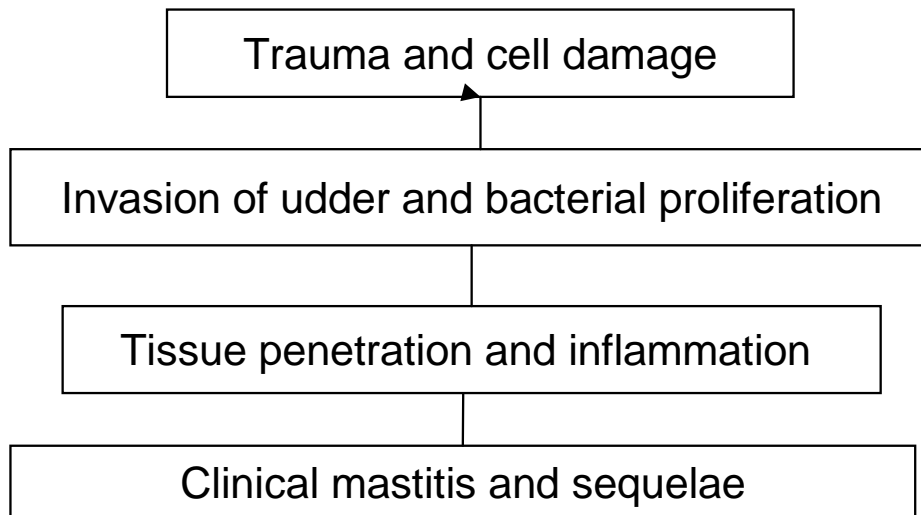


Figure 1: Basic model of the pathogenesis of mastitis

BOVINE MASTITIS

Typically this is divided into two categories:

a) Contagious mastitis (Cow-Cow transmission): due to bacteria on the skin of the teat which may be passed from one cow to another e.g. *Streptococcus agalactiae*, *Staphylococcus aureus*, etc.

b) Environmental mastitis: Due to organisms which don't normally live on the udder but are present in the environment, especially if it is heavily contaminated e.g. bedding contaminated with faecal material. The bacteria generally enter the teat canal following contamination of the end of the teat e.g. *E. coli*.

Some organisms can persist in both of the above locations (i.e. on skin and in environment) e.g. *Streptococcus uberis* and *Strep. dysgalactiae*

Clinically: Varies from no macroscopic abnormalities (subclinical mastitis) to marked abnormalities of the udder and milk (clinical mastitis) e.g. swelling, heat, redness and pain of the mammary gland; thin, watery to bloody milk secretion with flakes, clots or purulent material; decreased milk production.

PATTERNS OF MASTITIS

- 1) Acute catarrhal – with hyperaemia, interstitial oedema, lymphatic dilation and migration of neutrophils into interlobular tissue and alveoli e.g. Streptococci. May be subclinical
- 2) Acute suppurative - the organism survives in the ducts and penetrates the connective tissue with production of purulent material. May become chronic e.g. Staphylococci, *Trueperella (Arcanobacterium) pyogenes*
- 3) Necrotising – the organism or toxin causes acute necrosis; may lead to gangrene of the gland and death e.g. *T. pyogenes*, *S. aureus*.
- 4) Endotoxic shock syndrome – Fever, anorexia, hypocalcaemia with oedema and/or haemorrhage of mammary glands e.g. *E. coli*. Often peracute
- 5) Chronic suppurative OR Granulomatous – *Staphylococci*, *Trueperella* and *Actinobacillus* may all invade deeply into the tissue and cause chronic or

granulomatous infection. Other organisms that may cause granulomatous inflammation include *Nocardia asteroides* and atypical mycobacteria.

OTHER TYPES OF BOVINE MASTITIS

Summer mastitis - *Trueperella pyogenes* causes sporadic cases of mastitis usually following penetrating injuries and results in necrotizing and suppurative inflammation targeting the ducts (galactophoritis). May be seen in dry animals.

Mycoplasma mastitis – Often causes sudden agalactia with a firm, swollen and painless gland (there may be concurrent arthritis in some animals). There is abundant emigration of neutrophils initially, with alveolar and ductal hyperplasia in more chronic cases.

Tuberculous mastitis – due to *Mycobacterium bovis* and usually due to haematogenous infection with spread along the ducts.

Other bacteria, fungi, yeasts and algae may all be involved in cases of mastitis.

MASTITIS IN SHEEP AND GOATS

Teat injuries, udder chilling and areas with high reservoirs of bacteria (e.g indoor flocks/lowland farms with high stocking densities) all contribute to the development of mastitis. *Staphylococcus aureus* (producing alpha-haemolysin) and *Mannheimia haemolytica* are common isolates which cause acute necrotising or gangrenous disease that may result in sudden/unexpected death.

Typically only one gland is affected (enlarged, tense gland which may be bluish and watery milk is expressed). In per-acute cases animals may die within 12-48hours due to toxemia. The outcome for acute to chronic cases varies and depends on maintenance of vascular supply. If the infection is mild, healing with complete resolution may be possible. However, often (provided the vascular supply is maintained or re-established) and the tissue heals with fibrosis and/or chronic abscesses may develop. If there is marked vascular thrombosis and tissue necrosis the affected mammary tissue may slough over a 3-4week period.

Contagious agalactia due to *Mycoplasma agalactiae* is primarily seen in goats and occasionally sheep. Initially the animal is septicaemic and if the animal survives this stage (which is often fatal) there is keratoconjunctivitis, arthritis and mastitis. Pregnant females may abort or deliver live, infected foetuses.

Maedi-visna is a lentivirus which typically affects the lungs (ovine progressive pneumonia) and may also cause encephalitis (visna) and bilateral, lymphoplasmacytic mastitis primarily affecting the interstitial and periductal connective tissue. Goats develop a similar mastitis due to **Caprine arthritis-encephalitis virus** (another lentivirus).

MASTITIS IN SOWS

Generally seen sporadically in intensive breeding units; contributory factors include hygiene/husbandry levels and teat injuries. Coliforms cause acute infection in the early post partum period (similar to cattle).

Mastitis-metritis-agalactia syndrome – 12-48 hours post-partum. Lethargy, pyrexia, swollen, firm mammary glands with agalactia. Several contributing causes (coliforms, management changes etc) and usually seen in intensive units.

MASTITIS IN HORSES

Not common but may be associated with *Streptococcus zooepidemicus*.

MASTITIS IN DOGS AND CATS

Often non-specific, acute disease in lactating animals (may be seen in pseudopregnant animals) and galactostasis may contribute to the development of disease. *Streptococcus* and *Staphylococcus* spp. are usually involved.

MAMMARY NEOPLASIA

The first two conditions described are NON-NEOPLASTIC but will present as mammary masses and so are included here.

Mammary ductal and lobular hyperplasia: Seen in bitches and occasionally in queens; may be pre-neoplastic.

Mammary fibroepithelial hyperplasia (also known as feline mammary hypertrophy, feline mammary fibroadenomatosis, feline fibroadenoma etc.). Typically seen in young (<2years old) cycling or pregnant queens but may be induced in older entire females or males by treatment with synthetic progestagens. Can spontaneously regress or require ovariohysterectomy. There is marked swelling of mammary tissue with erythema +/- ulceration and necrosis. May cause oedema of the hind limbs due to compression/obstruction of lymphatics if severe. Microscopically there is a proliferation of well differentiated ductular epithelium and stroma with marked stromal oedema. Non-neoplastic condition.

Mammary neoplasia is common in dogs and cats but rare in other species.

Mammary gland neoplasia in the dog is common and generally affects older bitches (usually 8-11years) with younger dogs having an increased tendency for benign tumours. It is rare in animals less than 5 years and ovariohysterectomy prior to first oestrus decreases the risk of development of mammary tumours. Miniature and toy breeds often over-represented in studies. Approximately 50% of tumours are malignant and can metastasize via lymphatics to the lungs and regional lymph nodes. It is important to note that many tumours may appear histologically malignant but have a benign course of biological behaviour. Clinical signs which may be suggestive of malignancy include rapid growth, ulceration, local lymphadenopathy and/or dyspnoea (latter may suggest pulmonary metastasis has occurred).

Benign tumours include simple/complex adenomas, fibroadenomas, benign mixed tumours etc. (NOTE: complex tumours include the acinar epithelial component and spindle shaped myoepithelial component).

Malignant tumours include ductular carcinoma, carcinoma in situ, simple/complex carcinoma etc. Mammary sarcomas e.g. osteosarcoma, fibrosarcoma, chondrosarcoma are all reported.

Mammary gland neoplasia in the cat, is less commonly seen than in dogs but it is frequently malignant (80- 90% of cases), and metastasizes to regional lymph nodes, lungs, liver etc.

Mammary neoplasia is reported in mares and rarely in cattle and goats.

Male Reproductive Pathology Learning Objectives

1. Outline the major abnormalities of the scrotum
2. Describe the developmental, degenerative, circulatory and inflammatory disorders of the testis
3. Know the details of canine testicular neoplasia
4. Describe the disorders of the epididymis
5. Be aware of the disorders of the accessory genital glands
6. Describe the major developmental, non-inflammatory, inflammatory and neoplastic disorders of the penis and prepuce

MALE REPRODUCTIVE TRACT PATHOLOGY

SCROTUM + TUNICA VAGINALIS

The scrotum is a pouch of perineal skin, lined by an evagination of peritoneum (tunica vaginalis).

Developmental/congenital anomalies: agenesis (in cryptorchid animals) or failure of fusion resulting in scrotal clefts or bifurcation.

Scrotal skin lesions: frostbite; infectious/autoimmune/parasitic dermatitis; neoplasia (e.g. mast cell neoplasia or haemangiomas in dogs).

Scrotal (or inguinal) hernia is rare except in the horse which has both congenital and acquired forms. It may occur following open castration in horses or guinea pigs.

Fluid may accumulate between the layers of the tunica vaginalis e.g. **hydrocoele** in animals with ascites or anasarca; **haematocoele** is usually due to trauma. Neoplasia of the tunica vaginalis is rare but may include primary mesothelioma.

TESTES

Developmental/congenital anomalies: Anorchia (complete failure of testicular development) or testicular agenesis (failure of development of one testis) are rare. Disorders of sexual development are seen and mainly cause female phenotype (see female lectures).

Cryptorchidism - Incomplete descent of the testes which may be found anywhere from the posterior pole of the kidney to the external inguinal ring. One of the most common abnormalities of male genitalia (especially in cat and horse). Usually unilateral but may be bilateral. The cryptorchid testis has reduced fertility and is at increased risk of developing neoplasia or testicular torsion. The aetiology is unknown but may be hereditary. The testis is small and firm (hypoplastic) and histologically contains hypoplastic tubules (which often only contain Sertoli cells), increased fibrous tissue and thickened basement membranes.

Testicular hypoplasia - Testes fail to grow to normal size; spontaneous or associated with cryptorchidism/intersex conditions. Unilateral or bilateral, and the affected testis can be smaller or of similar size compared to the normal testis. Multifactorial aetiology and the small size is due to reduced numbers, length, and/or diameter of tubules. Germ cells may be

present or absent, and produce fewer/no spermatozoa. Basement membranes are usually smooth (compare with testicular atrophy/degeneration) and there is no inflammation.

Testicular degeneration/atrophy: Most common cause of reduced fertility. Unilateral or bilateral depending on whether the cause is local or systemic. Regeneration can occur if the aetiological agent is removed before the spermatogonia are completely destroyed. Numerous potential aetiologies including infection (localised or systemic); nutritional disorders; chemicals/hormones; hyperthermia, hypoxia etc. Affected testis will be small and soft with loss of turgor (cut surface does not bulge). With chronicity the testis may become firm with a wrinkled surface and areas of mineralization. Epididymis is not affected and may appear disproportionately large. On histology there is failure of maturation, formation of multinucleated spermatids and loss of spermatogonia. Sertoli cells develop cytoplasmic vacuolation and eventually are lost. Mineralization and fibrosis develop and there may be variable inflammation. The basement membrane of the tubules is thickened and wavy due to tubular collapse.

Circulatory Disturbances: Testicular blood supply is vulnerable to trauma as the arterial supply and venous return are restricted within the spermatic cord. Although the organ has high metabolic demands, the blood flow is relatively low. Examples include occlusion of testicular artery; torsion of spermatic cord (especially with cryptorchid testis); or thrombosis (idiopathic or secondary to trauma) etc. Arteritis and vasculitis may be seen with infections e.g. equine arteritis virus in horses and malignant catarrhal fever in cattle respectively. Disruption to the vascular supply may result in degeneration, atrophy or necrosis depending on the duration and severity of the insult. **Variocoele** – dilated/tortuous veins within the pampiniform plexus; common in old rams and often incidental but can affect thermoregulation causing reduced fertility.

Inflammation: Orchitis is inflammation of the testes and is generally rare/sporadic and must be differentiated from epididymitis which is more common (see later). Release of sperm into interstitial tissue due to trauma/infection/tubular damage will cause a granulomatous response (sperm granuloma). Inflammation may be interstitial, intratubular or necrotizing and caused by haematogenous spread (e.g. *Brucella* spp; Feline Infectious Peritonitis; *Corynebacterium pseudotuberculosis* etc.); ascending infection (usually causes intratubular orchitis); or secondary infection (localised trauma). The testicle will swell and become hot and painful with variable exudate. As the tunica albuginea cannot expand much, any severe swelling may progress to necrosis. Wide variety of histological changes ranging from necrosis of seminiferous epithelium ± haemorrhagic or fibrinopurulent exudate (acute form) to abscessation, granulomatous inflammation, fibrosis and mineralization (chronic form).

Testicular Neoplasia: Most common in the dog. May be multiple, bilateral and/or more than one tumour type within the testes. Increased incidence in cryptorchid testes. Most primary tumours are benign but malignancy is reported. Tumour may arise from:

- | | | |
|------------------|------|--|
| Germinal cells: | i) | Teratoma - young stallion (especially if cryptorchid) |
| | ii) | Seminoma - dogs and stallions. |
| Sex cord-stroma: | iii) | Sertoli cell tumours - old dogs (more common than seminoma). |
| | iv) | Leydig (Interstitial) cell tumours - old dogs (common), bulls. |

See Table 1 at end for details of gross pathology and histological features of these tumours.

Feminisation syndrome: associated with Sertoli cell tumours due to oestrogen production. Clinical signs: Attractive to male dogs, decreased libido, symmetrical alopecia, mammary hyperplasia, preputial oedema, hyperplasia/squamous metaplasia of prostate, contralateral testicular atrophy. May also have bone marrow suppression due to the presence of high levels of circulating oestrogen which is myelotoxic.

EPIDIDYMIS

Sperm granuloma: As above, sperm are released into surrounding tissue and cause a foreign body (i.e. granulomatous) response. Can be caused by blind-ending efferent tubules and may lead to pressure degeneration of testis.

Spermatocoele - Cystic dilation of epididymis causes accumulation of semen with rupture and formation of sperm granulomas. Due to congenital or acquired occlusions of the ducts.

Epididymitis: Inflammation of the epididymis and may occur along with inflammation of the accessory sex glands. Epididymitis may be confused with orchitis and is often due to ascending bacterial infection but systemic spread, direct penetrating injury and viral infection also occur. Grossly there will be increased size of epididymis with a variable consistency which depends on duration of the infection/inflammation (increased fibrous tissue in chronic cases) with concurrent testicular atrophy. Histologically ducts contain fibrin, inflammatory cells, degenerate spermatozoa and epithelial cells. Sperm granulomas often form.

NOTE: Funiculitis - Inflammation of the spermatic cord especially following castration. Horses, dogs and pigs. Chronic infection (e.g. *Staph. aureus*, *A. pyogenes*) causes granulation tissue formation with multiple small abscesses (also called “scirrhous cord”).

ACCESSORY GENITAL GLANDS

Ampullitis and Vesicular adenitis: Latter is most common in young bulls (<2years). Rectal palpation reveals changes in the gland (enlarged and painful if acute, shrunken and firm if chronic). Reduced fertility due to exudate in the ejaculate.

PENIS+PREPUCE

Developmental/congenital anomalies: Absence, hypoplasia, duplication may be associated with disorders of sexual development. Absence of sigmoid flexure is seen in some rams and bulls. Persistent penile frenulum - bulls and dogs. Associated with cork-screw or deviation of penis. Phimosis is due to small preputial orifice and the penis cannot be extruded from prepuce whereas with paraphimosis the penis cannot retract into the prepuce (may be congenital or acquired).

Hypospadias/epispadias – urethral canal malformations due to failure of the fusion of urogenital folds that create abnormal urethral openings on the ventral (hypospadia) and dorsal (epispadia) surface of the penis respectively. Animals may have concurrent abnormalities e.g. deformed/deviated penis, incomplete preputial closure etc. Boston Terriers are considered to be predisposed.

Non-inflammatory lesions: Priapism (persistent erection) and penile paralysis are reported e.g. in horses following acepromazine administration. Trauma will result in severe (possibly fatal) haemorrhage and in dogs the os penis may fracture.

Inflammation: Posthitis is inflammation of prepuce, balanitis is inflammation of glans penis but usually both are involved (balanoposthitis). There are numerous possible causes:

Dogs – bacterial infection (purulent discharge) e.g. *E.coli*, *Staphs*, *Streps* etc; *Canid herpesvirus 1* causes hyperemia, petechiation and lymphoid nodules; leishmaniasis may cause proliferative lesions.

Bulls - IBR virus (Bovine herpesvirus 1) causes mucosal necrosis and ulceration which may become secondarily infected. Respiratory and genital disease rarely occur simultaneously.

Rams and wethers – Ulcerative posthitis is important condition which occurs due to *Corynebacterium renale* (possible spread by contaminated bedding and flies) and urea rich urine (especially animals on high protein/leguminous diets). More common in wethers possibly because of under-developed genitalia with increased tendency to urinate within the sheath. Starts as small area of epidermal necrosis at the tip of the prepuce and progresses.

Horses- Equine coital exanthema (equine herpesvirus 3) -> large penile and preputial ulcers

Penile/preputial neoplasia:

- 1) Transmissible fibropapilloma - seen in bulls, bovine papillomavirus type 1+2. Multiple luxuriant growths, painful, easily traumatised. Transmissible to females..
- 2) Squamous cell carcinoma (SCC) – especially horses. Less common in bulls and dogs, low-grade malignancy may ulcerate and haemorrhage.
- 3) Melanoma - occur on prepuce and scrotum of grey horses. Often benign.
- 4) Transmissible venereal tumour - seen in dogs and bitches, may regress or generally responds well to chemotherapy. Grossly there is a proliferative, nodular or papilliform mass, with a friable/ulcerated surface. Histologically there are sheets of round to oval or polyhedral cells

Table 1: Classification of testicular tumours

	Teratoma	Seminoma	Sertoli Cell tumour	Interstitial (Leydig) cell tumour
Gross appearance	Irregular shape Solid ± cysts Variable colour	Spherical and lobulated/irregular Large Soft friable White / grey and bulges on cut surface	Spherical and large often nodular/lobulated Irregular bands of dense connective tissue (firm texture) ± Haemorrhage or cysts White/ cream	Encapsulated Small to medium size Soft ± cysts Tan / orange ± Haemorrhage
Histological appearance	Chaotic ± hair, cartilage, bone, glandular	Homogenous sheet Round/polygonal cells with some multinucleated forms Scant cytoplasm and no fat in cells Small amount of stroma Mitotic figs +++ Lymphocyte foci	Tubular or diffuse pattern Tall columnar cells with indistinct cell borders +/- lipid Dense fibrous stroma Mitotic figs ±	Solid sheets Polyhedral/rounded cells Large amount of cytoplasm, often with prominent lipid vacuoles Small amount of stroma Mitotic figures rare
Metastases	Rare	Rare	Rare	Very rare
Functional	No	No	Yes Oestrogen may cause atrophy of contralateral testicle	Sometimes Oestrogen Androgen?