LECTURE 1
Overview of veterinary reproduction. Anatomy and Physiology

In this course we will follow the events that relate to reproduction in a logical order; from sexual development of the juvenile, puberty, estrus cycles, conception through pregnancy and parturition. We will try to concentrate on aspects that relate to veterinary medicine and animal husbandry as much as possible. It is necessary though that we build a theoretical basis that includes developmental anatomy, adult anatomy, embryology, andrology and physiology in order to proceed with our discussions.

A. Overview of veterinary reproduction.

1. Scope of course

Basic Science
- Anatomy
- Physiology
- Endocrinology
- Gametes
- Fertilization
- Embryology
- Pregnancy
- Lactation
- Passive transfer of antibodies

Technologies
- Estrus control
- Artificial Insemination
- Embryo Transfer
- Advanced Techniques

Veterinary Medicine
- Obstetrics
- Pathology
- Therapy
- Surgery
- Neonatology

2. Reference materials

General Texts

1) Veterinary Reproduction & Obstetrics 7th Ed. (1996) Geoffrey H. Arthur, David E. Noakes, Harold Pearson, Timothy J. Parkinson, W. B. Saunders Company, ISBN 0-7020-1785-X
2) Reproduction in Mammals and Man (1993) Charles Thibault, Marie-Claire Levasseur, Ronald Henry Fraser Hunter, Ellipses, ISBN 2-7298-9354-7
3) Chordate Development (1987) H. Eugene Lehman, Hunter Textbooks Inc., ISBN 0-88725-083-1
4) Current Therapy in Theriogenology 2nd Ed. (1986) David A. Morrow, W. B. Saunders Co., ISBN 0-7216-6580-2

5) Current Therapy in Theriogenology (1980) David A. Morrow, W. B. Saunders Company, ISBN 0-7216-6564-0
6) Veterinary Obstetrics and Genital Diseases (Theriogenology) (1971) Stephen J. Robert, Published by Author

Individual Species Texts

7) Ratite Reproduction, Veterinary Clinics of North of America, Food Animal Practice 1998; 14:3, 437-453
8) Bull Infertility, Veterinary Clinics of North of America, Food Animal Practice 1997; 13:2, 195-366
9) Proceedings of Canine Male Reproduction Symposium (September, 1997) American College of Theriogenologists and the Society for Theriogenology, Box 2118, Hastings, NE 68902-2118, (402) 463-0392
10) Canine and Feline Endocrinology and Reproduction 2nd Ed. (1996) Edward C. Feldman, Richard W. Nelson, W. B. Saunders Company, ISBN 0-7216-3634-9
11) Proceedings of Mare Reproduction Symposium (August, 1996) American College of Theriogenologists and the Society for Theriogenology, Box 2118, Hastings, NE 68902-2118, (402) 463-0392
12) Proceedings of Swine Reproduction Symposium (August, 1996) American College of Theriogenologists and the Society for Theriogenology, Box 2118, Hastings, NE 68902-2118, (402) 463-0392
13) Perinatal Mortality in Beef Herds, Veterinary Clinics of North of America, Food Animal Practice 1994; 10:1, 1-185
14) Proceedings of Small Ruminant Short Course (August, 1994) American College of Theriogenologists and the Society for Theriogenology, Box 2118, Hastings, NE 68902-2118, (402) 463-0392
15) Female Bovine Infertility, Veterinary Clinics of North of America, Food Animal Practice 1993; 9:2, 223-420
16) Proceedings of Canine Theriogenology Short Course (August, 1993) American College of Theriogenologists and the Society for Theriogenology, Box 2118, Hastings, NE 68902-2118, (402) 463-0392
17) Swine Reproduction, Veterinary Clinics of North of America, Food Animal Practice 1992; 8:3, 435-760
18) Canine Reproduction, Veterinary Clinics of North of America, Small Animal Practice 1991; 21:3, 421-640
19) Equine Reproduction, A Review for Equine Practitioners (1983) Dean P. Neely, Irwin K. M. Liu, Robert B. Hillman, Veterinary Learning Systems Co., Inc.
20) Conception in the Human Female (1980) Robert G. Edwards, Academic Press, ISBN 0-12-232450-1

Specific Subjects

21) Proceedings of Reproductive Pathology Symposium (September, 1997) American College of Theriogenologists and the Society for Theriogenology, Box 2118, Hastings, NE 68902-2118, (402) 463-0392
22) Diagnosis of Abortion, Veterinary Clinics of North of America, Food Animal Practice 1994; 10:3, 439-615
23) Congenital Abnormalities, Veterinary Clinics of North of America, Food Animal Practice 1993; 9:1, 1-222

Artificial Insemination

24) Techniques for Handling and Utilization of Transported Cooled and Frozen Equine Spermatozoa (1994 Proceedings), Equine Sciences Program, Colorado State University
25) AI Training Manual (1993) Select Sires Inc., P.O. Box 143, Plain City, OH 43064-0143, (614) 873-4683
26) Procedures for Collection, Evaluation and Utilization of Stallion Semen for Artificial Insemination (1987) B.W. Pickett, E.L. Squires, A.O. McKinnon, Animal Reproduction Laboratory, Colorado State University
27) Salmon's Artificial Insemination of Sheep and Goats (1987) Gareth Evans, WMC Maxwell, Butterworths, ISBN 0-409-49177-2

Embryo Transfer

28) Manual of the International Embryo Transfer Society, 3rd Ed., (1998) International Embryo Transfer Society, 309 W. Clark St., Champaign, IL 61820
29) Procedures for Recovery, Bisection, Freezing and Transfer of Bovine Embryos 6th Ed. (1995), R. P. Elsden, G. E. Seidel, Animal Reproduction and Biotechnology Laboratory, Colorado State University
30) Training Manual for Embryo Transfer in Cattle (1991) George E. Seidel, Sarah M. Seidel, FAO Animal Production and Health Paper 77, Food and Agriculture Organization of the United Nations, ISBN 92-5-102804-4
31) Bovine Embryo Transfer (1990 Short Course Proceedings), Animal Reproduction and Biotechnology Laboratory, Colorado State University
32) Techniques for Freezing Mammalian Embryos (1990 Short Course Proceedings), Animal Reproduction and Biotechnology Laboratory, Colorado State University
33) Equine Embryo Transfer (1984 Short Course Proceedings), Animal Reproduction Laboratory, Colorado State University
34) Mammalian Egg Transfer (1982) Cyril E. Adams, CRC Press, Inc. ISBN 0-8493-6140-0

3. Applying reproductive technologies in your career

In general practice
- Breeding soundness evaluation
- Semen collection for AI
- Embryo transfer

Artificial insemination technician
- Bovine
- Equine
- Canine

Laboratory technician
- Bull Stud
- Stallion Station
- Embryology laboratory
- In Vitro Fertilization laboratory
- Transgenic and Cloning laboratory (pharmaceuticals)

B. ***Male Anatomy of the Urogenital System***

1. **Testicle**: the site of **spermatogenesis**.
   A) Composed of **seminiferous tubules** and interstitial tissue. The seminiferous tubule is the basic structure of spermatogenesis.
      The main cell types found inside the basement membrane of the seminiferous tubules include:
      1) **Spermatogonia**: sperm cell precursors undergoing mitotic division
      2) **Spermatocytes**: sperm cell precursors undergoing meiotic division
      3) **Spermatids**: sperm cell precursors that have completed meiotic division and are completing the transition from dividing cuboidal cells to flagellated sperm cell. Spermatids have a 1N chromosome content.
      4) **Spermatozoa**: flagellated gametes that are ready to be released into the lumen of the seminiferous tubule.
      5) **Sertoli cells**: are large "nurse" cells that enclose the developing sperm cell precursors and the nearly mature spermatozoa prior to release into the tubule.

      The main cell type found outside the basement membrane of the tubules is:
      6) **Leydig cell** lie adjacent to but outside the basement membrane of the tubule. The primary function of these cells is production of androgens.
   B) Seminiferous tubules empty into the **Rete testis** and then into several **efferent ducts**. The efferent ducts then empty into the **epididymis**.
   C) An adherent fibrous layer and visceral peritoneum surround the testicle. These layers are referred to as the **tunica albuginea**.
   D) The testicle and epididymis lie within a cavity surrounded by another fibrous layer and the parietal peritoneum. These layers are referred to as the **tunica vaginalis**.

2. **Epididymis, Vas Deferens (Ductus Deferens) and Ampulla**: The epididymis is a transport tube as well as an organ for storage and maturation of semen. The vas deferens is the duct system carrying spermatozoa from the body and tail of the epididymis to the urethra. The ampulla is part of the vas deferens and may provide short-term storage for fully mature sperm cells during ejaculation. The ampulla are vestigial or absent in small animals.

3. The **spermatic chord** is composed of several structures:
   A) The highly convoluted **testicular artery**, is closely associated with and encircles the venous drainage from the testicle,
   B) The **pampiniform plexus**, is the venous drainage of the testicle (the convoluted nature of the artery allows for heat exchange and possibly small molecule exchange between the artery and veins),
   C) The **vas deferens**,
   D) The **cremaster muscle** is a branch of the external oblique muscle that also assists in temperature control by raising and lowering the testicle inside the scrotum,
   E) The **vaginal tunic** (parietal peritoneum) and the visceral peritoneum are continuous with the peritoneal cavity,
   F) The **internal and external spermatic fascias** lie under the peritoneal layers and add support to the other layers.

4. **Scrotum**, the scrotum has the same fascia and peritoneal layers as the spermatic chord. The scrotum acts to lower the testicular and epididymal temperature by suspending the testicles outside of the body wall.

The **accessory genital glands**: are glands that secrete fluids into semen at the time of ejaculation. The fluids act to buffer pH, supply nutrients and add volume to the semen. Stallions, boars and canines secrete relatively large volumes of accessory genital gland fluid.

5. **Seminal Vesicles**: paired, loosely attached glands, and located lateral and parallel to the ampulla. Supplies a clear fluid to the ejaculate. The secretory ducts for these glands lie adjacent to the opening for the ampulla. Like the ampulla these gland are found in large domestic species. Not found in dogs or cats. They provide fluid, phosphate and carbonate buffers, fructose, sorbitol and other compounds. Add gel to the ejaculate of stallions.
6. **Prostate gland**: are very prominent in all domestic species except the ram. They consist of a bulbous portion and a diffuse portion both adjacent to the urethra just posterior to the neck of the bladder. The ram only has diffuse prostate tissue. The prostate provides fluid and inorganic ions, which provide osmotic regulation to semen. The secretion also contains various enzymes including a gel-forming enzyme that acts on seminal vesicle fluids.
7. **Bulbourethral glands**: are paired glands that lie further posterior in the pelvis than the other glands. They are paired and lie on either side of the caudal pelvic urethra. Dogs do not have bulbourethral glands. Also known as Cowper's glands. Primarily provides pre-ejaculate fluids. Flushes the urethra prior to ejaculate. Add gel to the ejaculate in some species (boars, man).

The Penis

8. The cloven-hoofed animal's penis includes a sigmoid flexure. The flexure in the bull, ram and buck penis lies caudal to the scrotum while the flexure in the boar lies anterior to the scrotum (or would it be more proper to say the scrotum lies caudal to the flexure). Stallions and carnivorous males all have a straight penis. The penis is composed of the following major structures:
   A) **Tunica albuginea**, surrounds the entire length of the penis,
      1) In animals with a sigmoid flexure this fibrous sheath is relatively thick and inelastic. These species attain erection through relaxation and straightening of the sigmoid flexure and engorgement of the relatively inelastic erectile structures.
      2) In animals with a straight penis the tunica albuginea is quite a bit thinner and more elastic. Erection is accomplished through vascular engorgement of the erectile structures and elastic extension of the penis.

   Erectile structures include:

   B) **Corpus spongiosum penis** (CSP), surrounds the urethra and originates at the base of the penis just posterior to the, the **glans penis** is a distal enlargement of the CSP, the arterial supply to the CSP is through the bulb of the penis while venous drainage is through the glans penis,

C) The **corpus cavernosum penis** (CCP), are paired cavernous erectile structures on the dorsum of the penis, both the arterial supply and the venous drainage of the CCP are through the crura or base of the CCP penis. This anatomic detail is responsible for engorgement of the CCP during erection when the venous drainage is occluded by contraction of the ischiocavernosus muscle.

D) **Urethra**, during ejaculation semen components enter the urethra in an area called the **colliculus**, in this area the paired vas deferens or ampulla open separately into the urethra, the prostate and seminal vesicles also empty into the urethra in the colliculus, other accessory gland open into the urethra more distally.

Accessory structures of the penis include:

E) **Retractor penis muscle**; attached to the ventral surface of the penis on the tunica albuginea, relaxation allows extension of the penis and straightening of the sigmoid flexure in animals that have this structure,

F) The **Ischiocavernosus muscle** surrounds the CCP. The origin of the muscle is on the ischiatic arch and inserts around the crus (base) of the penis. Contraction of this muscle compresses the base of the CCP against the ischiatic arch, occluding venous drainage from the CCP, like a loose tourniquet, causing engorgement of the CCP erectile tissue,

G) **Bulbocavernosus muscle** surrounds the corpus spongiosum penis at the base of the penis, during ejaculation, rhythmic contractions of this muscle causes waves of increased blood pressure to propagate down the CSP from base to tip; these waves simulate "peristaltic like" waves which propel the ejaculate through the urethra.

9. **Erection, emission and ejaculation**

A) **Parasympathetic nerve impulses**, from the sacral parasympathetic nerves that innervate the penis, **control erection** and cause:

1) Vasodilation in the arteries that supply the erectile tissue of the corpus cavernosum and the corpus spongiosum,
2) Inhibition of sympathetic tone to the smooth muscles of the fibro-elastic sheaths of the corpus cavernosum and corpus spongiosum also allow engorgement and extension of the penis,
3) Relaxation of the retractor penis muscle, in animals that have this muscle allows extension of the penis,
4) Secretion of mucous and gel by urethral glands and the bulbourethral glands is stimulated by parasympathetic nerves.

B) **Sympathetic impulses** from the hypogastric nerve plexus **control the emission processes**. These nerve impulses cause the expulsion of semen and it's mixing with glandular secretions in the urethral colliculus. This process is referred to as emission:

1) Sympathetic nerve impulses cause muscular contractions and peristalsis in the ampulla and vas deferens causing the expulsion of semen into the urethral colliculus,

2) Muscle contractions in the sheaths of the prostate gland and the seminal vesicles cause their secretions to empty into the colliculus and be mixed with the semen,
3) Filling and stretching of the urethral colliculus with semen and glandular secretion may be the initiator of the final sympathetic ejaculatory reflex.

C) **Sympathetic nerves** also **control ejaculation**
1) Muscle contraction of the ischiocavernosus muscle occludes venous drainage from the base of the corpus cavernosum, increasing erection
2) Contraction of the muscular prostatic sheath and urethral muscles surrounding the colliculus force semen down the urethra,
3) Rhythmic contractions of the bulbocavernosus muscle result in pressure waves in the engorged corpus spongiosum that mimic peristaltic waves in the urethra, propelling the semen down the urethra.

C. *Female Anatomy of the Urogenital System*

1. **Ovary**: the site of **oogenesis**. The center or medulla of the ovary contains the vascular supply, lymphatic, nerve and connective tissues while the outer layer or cortex contains oocytes, follicular cells, connective tissues and the other structures related to follicular turnover. The follicles are present in groups referred to as germinal chords that are embryologically analogous to the seminiferous tubules in the male. The ovary is surrounded by fibrous connective tissue and an epithelial layer that are referred to as the **tunica albuginea ovarii**. The surface epithelium has been incorrectly referred to as the germinal epithelium. The surface epithelium is actually composed of cuboidal epithelial cells, which are not precursors to germinal cells.

Mitosis of the **oogonia** cells (the precursors of oocytes) occurs during fetal development. By the time of birth, proliferation of oogonia ceases and the resulting oocytes enter meiosis. The numbers of oocytes, which are arrested in an early stage of prophase of first meiotic division, are fixed at birth. The ovaries of the larger domestic species and man contain approximately 100,000 oocytes (or less) at the time of birth. The oocyte will remain in meiotic arrest (for years) until the time of ovulation when the oocyte will resume meiosis and complete the first meiotic division.

A) **Follicle**: Mature (and smaller) follicles are divided into several layers:
1) **Oocyte**: the germ cell, which is surrounded by a glycoprotein coat called the **zona pellucida**
2) **Cumulus oophorus**: a layer of specialized granulosa cells that envelop the oocyte during maturation and ovulation, function to nourish the oocyte and provide paracrine (hormonal) support to oocyte maturation. Cumulus cells remain in contact and invest the oocyte until after ovulation and fertilization in the oviduct. The oocyte and it's investment is called the **Cumulus Oocyte Complex** (COC).
3) **Granulosa layer**: consists of several layers of cells that line the antral cavity of the follicle. Granulosa cells secrete antral fluid and produce

steroidal hormones, primarily estrogen, during the follicular phase of the estrus cycle. FSH receptors are present on these cells early in their pre-antral development. LH receptors are expressed during the follicular phase of the estrus cycle and are necessary for ovulation.

4) **Basement membrane (basal lamina)**: separates the granulosa layer and the thecal layers of the follicle, provides a fluid and vascular barrier for the interior follicle.
5) **Theca interna**: The layer of epithelial cells just outside of the basement membrane, produces androstenedione (an androgen similar to testosterone) which is the precursor to and utilized by granulosa cells to manufacture estrogen.
6) **Theca externa**: A layer of cells external to the theca interna. These cells are probably the precursors of the theca interna cells early in the primary follicle development. They provide connective tissue like support to the follicle. Hormonal activity?

B) **Corpus hemorrhagicum**: The CH is the structure that results from ovulation of the follicle. The CH is a transitional early stage in the formation of the corpus luteum.
C) **Corpus luteum**: The CL is the structure that forms from the ovulated follicle and is responsible for secretion of progesterone and the maintenance of pregnancy. Both granulosa cell and theca cells are transformed and contribute to the formation of the CL.
D) **Corpus albicans**: The CA is the scar that remains from the involution of previous Cls.

2. **Oviduct**: transport tube for the ovulated oocyte and the site of fertilization. There are four sections of the oviduct:
   A) **Fimbria:** The erectile, non-muscular sheet of tissue that surrounds the ovary and is responsible for picking up the ovulated oocyte and directing it into the infundibulum of the oviduct. It is also referred to as the ovarian bursa.
   B) **Infundibulum**; the funnel shaped entrance to the oviduct.
   C) **Ampulla**: the lightly muscled first half of the oviduct. This section has a highly folded epithelial lining and a relatively large potential lumen. The ampulla is the region of the oviduct where fertilization occurs and where the zygote and embryo spends the first several days (2 to 6 days depending on species) after ovulation. The fertilized egg lies in the ampulla at the **ampullary-isthmic junction**.
   D) **Isthmus**; this is the region of the oviduct that connects the ampulla to the uterus. The isthmus has a heavily muscled wall and few epithelial folds. Muscle contractions in the isthmus during estrus move from the uterus towards the ovary. This assists in moving sperm cells to the ampulla for fertilization. During metestrus the muscle contractions are greatly reduced and unorganized. After the start of diestrus the muscular contractions throughout the length of the ampulla and the isthmus begin to move from the ovarian end of the ampulla towards the uterus. This results in the fertilized embryo being transported rapidly though the length of the isthmus, through the **utero-tubal junction** and into the uterus.

3. **Uterus**: The uterus is divided into two sections (really three) the **right and left horns** (cornua) and the **body of the uterus**.

A) The wall of the uterus, from the interior out, is composed of:

1) Endometrium; the epithelial and glandular layer of the uterus,
2) Internal circular musculature,
3) External longitudinal musculature,
4) Serosa
5) In some species, notably the bovine, there are oblique muscle fibers also.

B) There are four different types of uterus

1) **Double uterus**: with separate horns and each horn has it's own separate cervix. This type of uterus is typical of rodents and lagamorphs. The paired cervices may open independently into the vagina as in the rabbit and rat or they may fuse into a single opening as in the mouse. This type uterus is usually relatively long and adapted to carrying a litter. The body of the uterus does not really exist in these species.
2) **Bicornuate uterus**; has two long convoluted uterine horns that fuse for 5% or less of it's length into a short body and have a single cervix connecting the body of the uterus to the vagina. This type uterus occurs in animals that have large litters.
3) **Bipartite uterus**; has two relatively short uterine horns that are fused for up to 50% of their length yet divided by a septum. The lumens of the horns join to form a body and have a single cervix connecting the body of the uterus to the vagina. Animals that can easily carry twins have longer curled uterine horns (cow and ewe) while animals that can only successfully carry a singlet have straight horns (mare).
4) **Simplex uterus**; is typical of primates, having very short or non-existent uterine horns the uterus is comprised almost entirely of uterine body attached to a single cervix.

4. **Cervix:** The cervix is composed of an epithelial layer and a strong musculo-fibrous layer. The cervix exhibits a typical cyclic motility with a relaxation phase that lasts 30 to 90 minutes and a short 5 to 10 minute contraction phase. During estrus the relaxation phase lasts about 30 minutes and the contraction phase for about 3 to 5 minutes. During contraction the lumen is functionally obliterated while during relaxation the lumen dilates. This probably has some function in sperm transport. During the luteal phase of the cycle the muscle activity persists but the relaxation phase is replaced by a period of muscular quiescence and dilation of the lumen does not occur. The contraction phase only occurs every 60 to 90 minutes.

During estrus the periodic contractions of the cervix have implication for practitioners performing intrauterine artificial insemination. In cattle an occasional animal will have a very tight and difficult to penetrate cervix. If the inseminator will wait for 3 to 5 minutes the cervix may relax and the AI instrument may then be passed through the cervix with

very little trauma. In mares it is also advantageous to be patient if one finds the cervix closed during AI.

At parturition relaxation dominates the cervical cycle. In addition to relaxation there is an estrogen induced increase in collagenase activity, which breaks down the fibrous collagen support present in the cervix of pregnancy. Relaxation and the biochemical changes in the connective tissues allow for dilation of the cervix to occur.

5. **Vagina:** The vagina is composed of a variable epithelial layer that is renewed with each estrus cycle and a muscular, fibro-elastic layer. Under the influence of estrogen at estrus and at parturition the cells of the vaginal mucosa proliferate and the surface cells change from cuboidal to keratinized squamous cells. During the luteal or anestrus phases of the cycle the vaginal cell type reverts to cuboidal. These changes in the vaginal cytology are obvious enough to allow for diagnosis of impending ovulation in the bitch, rodents and perhaps other species. During pregnancy, the luteal phase of the estrus cycle and at the onset of proestrus only cuboidal cells are found. During estrus keratinized cells increase to a maximum at ovulation. After ovulation leukocytes are also found among the keratinized cells. At metestrus, keratinized cells and leukocytes decrease and by the start of diestrus only cuboidal cells are found.

   At parturition, in addition to the estrogenic changes to the mucosa, there is also relaxation of the muscular layers and an estrogen induced increase in collagenase activity, allowing for softening and dilation of the fibro-elastic layers of the vagina, similar to the situation in the cervix.