

COURSE CODE:	ANP 502
COURSE TITLE:	Reproductive Physiology
NUMBER OF UNITS:	3 Units
COURSE DURATION:	Three hours per week

COURSE DETAILS:

Course Coordinator:	Dr. Abiona John Adesanya <i>B.Agric., M.Agric., PhD</i>
Email:	abionajohn@yahoo.co.uk ; abionajohn@gmail.com
Office Location:	Animal Physiology Staff Office, COLANIM
Other Lecturers:	Prof. Onogbesan, O. M., Prof. Osinowo, O. A., Dr. Ladokun, A. O. and Dr. James, I. J.

COURSE CONTENTS:

ANP502: REPRODUCTIVE PHYSIOLOGY (3Units)

Introduction to animal reproduction, reproductive efficiency and profitable livestock production; Biological basis of sex. Sex differentiation. Intersex, homologues of the male and female reproductive system.

Comparative anatomy of the male reproductive system. Comparative anatomy of the female reproductive system. Sexual development. Gametogenesis, spermatogenesis, sperm transport, sperm output, hormonal control of spermatogenesis; Oogenesis, ova physiology, follicular development, ovulation, hormonal control of oogenesis. Reproductive cycles. Pregnancy. Infertility in farm animals.

Practical: Gross anatomy of male and female reproductive tracts, histology of the testis, ductus deferens, ampulla, epididymis, vesicular glands, histology of the ovary, oviduct, uterus and cervix. Gross anatomy of the gravid uterus. Oestrus detection in cattle, pigs, sheep and goats. Pregnancy diagnosis in sheep and goats by ballottement

COURSE REQUIREMENTS:

This is a compulsory course for all students in the Department of Animal Physiology and as elective Course for other Departments that discovers its relevance in their programme. In view of this, students are expected to participate in all the course activities and have minimum of 75% attendance to be able to write the final examination.

READING LIST:

1. Austin, C. R. and Short, R. V. Reproduction in mammals. Cambridge University Press. 1990.
2. Lamming, G. E. Marshall's physiology of Reproduction. Churchill Livingstone, Edinburgh. 4th edition, Volumes 1&2. 1990.
3. O.A. Osinowo. Introduction to Animal Reproduction. Sophie Academic, Abeokuta, Nigeria. 2006
4. Hafez, E.S.E., Jainudeen, M.R. and Rosnina, Y. Hormones, growth factors, and reproduction. In: Reproduction in farm animals. Edited by E.S.E. Hafez and B. Hafez, Lippincott Williams and Wilkins Publishers, USA, 7th edition. Pp. 33-54. 2000.

LECTURE NOTES

Introduction to reproduction

Animal reproduction is the process through which offspring are produced by male and female parents. It normally involves heterosexual mating, conception, pregnancy, parturition and lactation. Conception occurs as a result of the fusion of the male and female gametes, namely spermatozoon and ovum respectively, in a process known as fertilization. Before animals can reproduce, they must first attain puberty or reproductive age, from when they become capable of gamete production. Reproduction in animals involves close coordination or synchronization of various physiological events and this is largely achieved through the actions of the reproductive hormones.

A proper understanding of animal reproduction would involve some knowledge of reproductive physiology, endocrinology, environmental physiology, cell biology, immunology, genetics, biochemistry, sociology, reproductive diseases, psychology, embryology, obstetrics and so on. In agriculture, animal production revolves around reproduction. Livestock products such as eggs and milk are direct outputs from reproductive

processes. Meat production depends primarily on production of offspring which are subsequently grown out or fattened for slaughter.

Role of reproduction in genetic improvement

Reproduction and animal breeding are inseparable in farm practice, though these subjects are often taught separately in most Colleges and Universities. Segregation and recombination of genes which is the sole basis for genetics occur as a central aspect of reproduction. In animal breeding, desired genotypes are obtained by allowing only selected individuals to reproduce. In any animal improvement scheme, the rate of genetic progress per year (ΔG_y) can be generally expressed as:

$$\Delta G_y = \frac{SD * h^2}{GI}$$

GI

where

SD = selection differential

h^2 = heritability estimate, and

GI = generation interval in years

In the above equation, both selection differential and generation interval are dependent on reproductive efficiency. A high reproductive rate will yield more animals for selection, resulting in higher selection differential. Also, the sooner both intended parents attain reproductive age the lower would be the generation interval. Faster rate of genetic progress would thereby ensue. Furthermore, selection differentials can be greatly increased through the use of artificial insemination (AI) and, to some extent, multiple ovulation and embryo transfer (MOET). Both of these techniques increase the selection differentials on the male and female sides respectively.

Biological basis of sex

What is sex?

Sex is the sum total of those differences in structure and function on the basis of which an organism is classified as male or female. Two main theories have been advanced to explain the mechanism of sex determination: These are the chromosomal theory and the genic balance theory.

The chromosomal theory of sex determination

According to this theory, sex is determined at fertilization by the so-called sex chromosomes. Each gamete contains the haploid number of chromosomes. In mammals and in most insects, all gametes produced by the female are similar, having one X chromosome. Males on the other hand produce two types of gametes in approximately equal numbers, one type bearing the X chromosome and the other type bearing the Y chromosome. Hence in mammals and most insects, the female is known as the homogametic sex while the male is heterogametic. At fertilization, the union of an egg with an X-carrying spermatozoon results in a zygote with 2 X chromosomes which develops into a female while the union of a Y-carrying spermatozoon with an egg results in an individual with one X and one Y chromosome, which develops into a male. In birds, the female is the heterogametic sex while the male is homogametic .

Chromosomal, genotypic or genetic sex is thus determined by the sex chromosomes received from the parents. Aberrations of genetic sex do occur, either as a result of non-disjunction of sex chromosomes, translocation, deletion or mutation. In maternal non-disjunction, fertilization of the ovum will lead to formation of either an XXX or an XXY zygote. In paternal non-disjunction, zygotes of XXY or XO genotypes result. Such cases of aneuploidy often result in gonadal or endocrine defects.

In man, the Klinefelter's syndrome in which the genotype is usually XXY instead of XY is

characterized by gonadal hypofunction. In women showing Turner's syndrome which is characterized by gonadal agenesis or aplasia, the genotype is frequently XO rather than XX.

Genic balance theory of sex determination

To some extent, genotypic sex may be considered an "all or none" or qualitative trait since usually male or female zygotes are formed at fertilization. However, sex is a phenotypic trait, determined by interaction between genotype and environment. Individuals vary in their degrees of maleness or femaleness.

C.B. Bridges in 1922 proposed the genic balance theory to explain apparent quantitative variability in sexual character. According to this theory, sex is determined by the autosomes as well as by the X chromosomes, the ratio of autosomes to X's being the significant relation. In *Drosophila* the X chromosome carries more genes for femaleness while the autosomes carry more genes for maleness. Which sex actually develops is decided by the balance between the two sets of genes.

Genetic dosage compensation / X-inactivation model of sex determination

The theory of mammalian X-chromosome inactivation proposed by Lyon in 1961 holds that almost all the genes on one of the two X-chromosomes in the somatic cells of females are suppressed as a dosage compensation mechanism. The choice of either the paternal or maternal X chromosome for inactivation is random. The inactivated X chromosome is identifiable as it is heterochromatic and usually has its DNA replicated later in mitosis than other chromosomes.

The ZFY gene, thought to constitute the primary sex-determining signal, was identified within a very small segment of the Y chromosome by Page and co-workers in 1987. However, the presence of a similar gene, ZFX, on the X chromosome prompted the propounding of the dosage compensation / X-inactivation theory of sex determination.

According to this theory, both ZFX and ZFY produced functionally interchangeable proteins.

It therefore follows that XY cells would have two active copies of the gene while XX cells would only have one active copy, due to X-inactivation. Embryos with two copies would thus develop into males while those with a single copy of the gene would develop into females.

In this way, the theory held that gene dosage determined sex. Though elegant, the theory became untenable when it later became evident that the ZFX gene escapes X-inactivation, thus contradicting the dosage compensation model.

SEXUAL DEVELOPMENT

The determination of sex and sexual differentiation are sequential processes that involve the establishment of the chromosomal (genetic) sex (xx or xy) in the zygote at the period of fertilization. In response to the genetic sex, the gonadal sex (ovaries and testes) is determined. In turn, the gonadal sex regulates the differentiation and development of the genital apparatus and the manifestation of phenotypic sex.

In placental mammals, maleness is associated with heterogametic sex (i.e xy). A gene on the y-chromosome, the testis-determining factor, determines that the gonadal anlage (the first accumulation of cells destined to develop into a distinct organ) is to differentiate into a testis. In birds, the sex determination is by the zz, zw chromosomes. The cock is homogametic (zz) whereas the hen is heterogametic (zw).

During early gestation, fetal gonad is indifferent since males and females have 2 sets of primitive genital tracts – the Wolffian and Mullerian ducts. Male sexual development is associated with the regression of the Mullerian ducts and requires the presence of testis. In contrast, female sexual development is associated with the regression of the Wolffian ducts. This occurs in the absence of masculinizing factors since the undifferentiated gonad has an inherent tendency to feminize and form a reproductive tract.

Interstitial cells of the testis secrete a hormone called Mullerian inhibiting substance (MIS) which causes the regression of the adjacent Mullerian ducts (see fig). MIS is a glycoprotein

hormone that also has a role in testicular descent to the scrotum. Low testicular level of MIS leads to CRYPTORCHIDISM – a state of incomplete descent of the tests or abdominal retention of one or both testes.

Testosterone secreted from the fetal Leydig's cells directly promotes the differentiation of the Wolffian ducts linking the mesonephric kidney to the urogenital sinus. The portions of the Wolffian ducts adjacent to the testes are transformed into the EPIDIDYMIS, the central portions become the vas deferens and the portions near the urogenital sinus develop into the seminal vesicles. Later testosterone induces (through DHT) to transform the early external genitalia. The pelvic portion of the urogenital sinus becomes the prostate, the bulbo-urethral (Cowper's Gland), and the membranous portion of the urethra. The genital tubercle elongates to form the glans penis.

Comparative Anatomy

All species present the basic male reproductive tract (i.e. 2 testis, 2 epididymides, 2 ductus deferens, and a prostate). However, considerable variation exists regarding the other accessory glands such as seminal vesicles, ampullary glands, bulbourethral glands (glands of Littre).

The ampulla: present in the bull, camel, rabbit, ram, stallion. It is a spiral-shaped, thickening of the terminal portion of the ductus deferens. In the male hamster, mouse and rat, it exists on the dorsal wall of the urethra. The secretion of the ampulla is rich in electrolytes (Na^+ , Ca^{++} , and Mg^{++}) and citric acid.

The Seminal Vesicle: This is a paired, bag-shaped glands in guinea pigs, rats and stallions. In bull, boar and rams, they consist of multiple lobes containing a system of ramified secretory ducts. It is absent in cats and dogs.

The Prostate: It is a tubule-alveolar gland that may be diffuse, it is confined around the urethra or discrete. It forms a definite body outside the urethral muscle. In rams the prostate

is of the diffuse type whereas both types exist in boars and bulls. It is the only sexual accessory gland in dogs.

Testes: Testes are paired encapsulated ovoid organs made up of seminiferous tubules separated by interstitial tissues. They are enclosed in a dense connective tissue capsule the tunica albuginea. In most mammals, the testes migrate from their origin in the abdomen to a subcutaneous invagination of the peritoneum, the scrotum. Testicular descent occurs during fetal life in large animal, (bull, ram) but only after birth in other animals (rats, dogs).

The seminiferous tubules have a lumen, a stratified epithelium composed of spermatogenic cells, Sertoli cells and a thin outer basement membrane. The loose connective tissue stroma between the tubules contain Leydig cells. The distal segment of the seminiferous tubules open and drain into the rete testis (a network of ducts). The rete testis carries spermatozoa and fluid in which they are suspended. A system of efferent ducts or ductus deferens links the rete testis to the epididymis. The ductus deferens is also an absorptive and secretory tissue.

Development of female Reproductive Tract

The ovary is seeded with thousands of primary oocytes, a minority of which will ultimately reach maturity after puberty. The Mullerian ducts develop, whereas the Wolffian ducts become vestigial. According to the species, the Mullerian ducts change to become the oviducts of a duplex uterus (rodents) or bicornuate uterus (pigs) or bipartite uterus (ruminants) or simple uterus (man, primates) (see fig). The genital tubercle develops into the clitoris, the genital fold into the urethral orifice and the genital swelling into the borders of the vulva.

In bird the female reproductive system is unique. Although paired Wolffian ducts appear, only the left genitalia primordia develop further to functional organs. Therefore, only the left ovary and oviduct are present.

In prepubertal female animals, the ovary is relatively static, apart from undergoing growth compared to the postpubertal animal. The ovary consists of an outer layer of cortex which contains thousands of follicles that carries a female gamete or primary oocyte. The inner layer of the ovary is the Medulla. Each oocyte is enveloped by a single layer of cells and it termed primordial follicle.

Puberty

Reproductive life characterized by gametogenesis and by a desire for sexual activity (libido), becomes possible at puberty and continues for most of the animal's life until senescence.

Puberty arrives before growth is completed when animal, have reached a state of somatic development (mass or body wt) for each species or for each breed. Females reach this threshold body mass sooner than their male counterparts and show puberty at a younger age.

Approx Age (months) at Puberty

Cow 6 – 18

Goat 4 – 8

Guine pig 4

Horse 12 – 20

Mouse 2

Pig 5 – 10

Rabbit 6

Rat 2 – 3

Sheep 6 – 12

Chicken 4 – 6

A critical sign of the onset of puberty is the involution of the thymus.

Breed size, quality and quantity of feeding and season of birth regulate the onset of puberty. Factors that delay the appearance of puberty include large body size in some breeds (such as dog, beef cattle), malnutrition, and birth at the end of the breeding season. In sheep, the body size required is 40kg. For all species a high intake of energy allows puberty to occur early.

In seasonal breeders such as cat, goat, horse, sheep, birth near the beginning of the coming breeding season prevents the onset of puberty inside that season. These animals mature sexually at the following breeding season and the beginning of their reproduction cycle occurs more than 6 months later.

Until the young male or female animal reaches, puberty, it does not show cyclical interest in sexual activity. Puberty signals the onset of periodic hypothalamo-adenohypophysio-gonad hormonal processes that cause interest in sexual activity. In females, puberty indicates that the progressively developing hypothalamic gonadostat has reached an adequate and sufficiently mature state (re: no more highly sensitive to inhibiting E2 feedback effect). At puberty, the system for secreting gonadotrophin has a decreased sensitivity and allows gonadotrophin secretion to increase and to generate the requisite cyclical endocrine pulses and surges of GnRH, LH, FSH and prolactin.

In nonbred females, emergence into postpubertal life is manifested by the onset of estrus-cycles during which spontaneous ovulations recur periodically throughout the year in polyestrous species (mare, cow, goat, sheep, pig). It is defined as the first appearance of menstruation or 'menarche'.

In the young male, puberty is signaled by the production and the ejaculation of fertile spermatozoa and also by sexual desire. The secretory function of accessory reproduction glands appears earlier than spermatozoa. Enlargement of the testes marks an important sign of puberty since it represents an increase in the volume of the seminiferous tubules. The functioning of the seminiferous tubules (spermatogenesis) follows a gradual maturation of the

hypothalamic gonadostat, which leads eventually to pulsatile (basal) synthesis of GnRH, LH, FSH and testosterone.

Endocrinology of reproduction

In animal species, the hypothalamus and the pituitary gland form a physiological unit that is most important in the synthesis of peptide hormones. An important function of the hypothalamic-pituitary complex is the control of gonads for reproductive activities. The two organs are located close to each other in the lower part of the brain.

The hypothalamus acts as a neuroendocrine transducer and it translates neural activity into hormonal output. The specific hormones produced by the pituitary are regulated by the peptide hormones produced by the hypothalamus. The regulatory hormones produced by the hypothalamus reach the pituitary via the portal veins which convey blood from the hypothalamus to the pituitary.

Hormones of the Hypothalamus

GnRH (LHRH, FSH-RH)

GnIH

Prolactin-releasing factor (PRF)

Prolactin-Inhibiting factor (PIF)

GnRH: GnRH is produced in hypothalamus and transported by axonal flow and stored in small granules in the terminals of the nerves on median eminence (lower hypothalamus).

GnRH is released in pulses (every 2 hours) and causes the liberation of LH and FSH from the pituitary gland. Those two hormones participate in the control of reproduction in male and female animals. GnRH increases the pituitary secretion of LH & FSH. The secretion of GnRH is increased during coitus in the cat followed by LH surge. The secretion of GnRH is

controlled by feedback effects of steroid hormones produced in the ovary or testis. In small animals, secretion of GnRH is also stimulated by administration of synthetic, non-steroidal estrogen analog (clomiphene citrate) or an anti-estrogen (clomiphene citrate) or an anti-estrogen analog (tamoxifen). An example of neural inputs that influence hypothalamic output of GnRH are pheromones, that stimulate the olfactory bulb (rams).

The induction of puberty is marked by increase in the production of GnRH. Changes in photoperiod also control GnRH production (sheep). In sheep and goats of the temperate countries, shortening of day length increases melatonin production, initiate GnRH production. Lambs and kids that have grown sufficiently reach puberty and then sexually mature. In adults animal breeding season starts when day length shortens. In wild animals and horses, GnRH increase coincides with the breeding season when day lengths increase.

The suppression of GnRH release by the administration of high levels of steroids (testosterone, estrogen, progesterone) form the basis for oral contraception in humans. Inhibin, a glycoprotein hormone, produced in the follicles of the ovary or in the seminiferous tubules of the testis, reduces GnRH secretion. Prolactin and LH from short-loop negative feedback can also inhibit GnRH.

Anterior Pituitary Hormones (Adenohypophysis)

LH,

FSH

In females and males, FSH and LH are synthesized and released from the anterior pituitary (adenohypophysis) upon stimulation by GnRH. The basal or tonic output of FSH and LH is pulsatile (with modulation of frequency and amplitude of the pulses) during most of the reproductive life of females and males. In females, a massive surge of LH during the oestrus cycle is associated with ovulation.

The basal pulsatile secretion of FSH regulates follicular growth in females and controls spermatogenesis in males. The synthesis and release of FSH is under the negative feedback of inhibin and of sustained high plasma levels of estradiol.

The basal pulsatile output of LH functions mainly for the maintenance of follicular maturation in the ovulated female. In males, tonic LH stimulates the interstitial (Leydig cells) cells of the testes to produce testosterone. The basal pulsatile secretion of LH is regulated by GnRH and gonadal steroid feedback (estradiol mostly).

In mammals, LH surge near ovulation is associated with the positive feedback of high estradiol levels whereas in the birds it is associated with the positive feedback of progesterone.

Posterior Pituitary Hormones (Neurohypophysis)

Prolactin (PRL)

Growth hormone (GH)

The posterior pituitary secretes prolactin (a single chain folded polypeptide). The secretion of prolactin is under tonic inhibitory control of dopamine and prolactin-inhibiting hormone.

In all animals, lactation and suckling are associated with high plasma levels of prolactin.

In females, the most obvious roles of PRL (prolactin) are to start the differentiation of mammary gland cells into producers of milk proteins and constituents and to initiate and maintain lactation. The function of PRL in male animals is unknown but may consist of an action on the testis to enhance the activity of LH and to stimulate testosterone production.

During pregnancy, in conjunction with estradiol, progesterone and placental lactogen, PRL levels rise constantly stimulating the growth of the mammary gland elements. Following parturition, the abrupt decrease in estradiol and progesterone and the maximal high levels of

PRL permit the initiation of maximal PRL secretion which gradually declines as lactation progresses.

In primates and rodents, hyper prolactinemia of lactation prevents the liberation of GnRH, together with gonadotropins, and the return of the reproductive cycles.

Testis

The major hormones produced by the testes are testosterone by Leydigis cells and inhibin by sertoli's cells. Their synthesis is regulated by the gonadotrophs (LH and FSH).

Inhibin induces the maturation and growth of the seminiferous tubule cells and the aromatization of androgens to estradiol. FSH supports sertoli cells for the morphological maturation of spermatids into spermatozoa.

The hypothalamic-pituitary-testicular axis is a closed-loop system, that is, a system in which the control action depends on the output. It has a negative feed-back system in which testicular hormones (testosterone and inhibin) depress the pituitary secretion of LH and FSH. Castration results in elevated levels of LH and FSH in circulation. The negative feedback of testosterone on hypothalamic GnRH makes it the primary regulator of gonadotropin secretion whereas inhibin would suppress mostly FSH release. Testosterone induces androgenic (male characteristics) and anabolic (growth-promoting) effects.

Besides stimulating the differentiation of the wolffian ducts during embryogenesis, testosterone is directly responsible for growth of the penis and of accessory glands and for the initiation and maintenance of spermatogenesis. Other actions include aggressiveness, sexual drive (libido), erection, growth of the male (horses) and growth and shedding of male anthers in deer.

The production of male pheromones (i.e substances that modify the reproductive behaviour of females) is stimulated by testosterone. In sows in estrus, the smell of boar's urine or saliva produces a typical immobility. The growth and maturation of large antral follicles-entering their final stage of development.

Ovarian Hormones

These include Progesterone, pregnenolone, androstenedione, testosterone, estrone, estradiol and estrin.

Pregnenolone is the key precursor of all steroids. It is readily converted to progesterone by follicles at all growing stages of development. In all animals, the greatest stimulation of progesterone biosynthesis follows the LH surge and ovulation. The major source of progesterone is the granulosa cells of the follicle and lutein cells of the corpus luteum.

Androstenedione and testosterone are androgens secreted by theca cells of the follicle. They are produced by hydroxylation of pregnenolone or progesterone. LH increases androgen secretion by theca cells. These androgens also act as substrate for conversion into estrogens.