University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Publications from USDA-ARS / UNL Faculty

U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska

1983

PHYSIOLOGY OF MALE REPRODUCTION

R. P. Amann
Colorado State University - Fort Collins

B. D. Schanbacher
USDA-ARS

Follow this and additional works at: https://digitalcommons.unl.edu/usdaarsfacpub

Part of the Agricultural Science Commons

Amann, R. P. and Schanbacher, B. D., "PHYSIOLOGY OF MALE REPRODUCTION" (1983). *Publications from USDA-ARS / UNL Faculty*. 765.
https://digitalcommons.unl.edu/usdaarsfacpub/765

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Publications from USDA-ARS / UNL Faculty by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

PHYSIOLOGY OF MALE REPRODUCTION

R. P. Amann[1] and B. D. Schanbacher[2]

Colorado State University, Fort Collins 80523
and
U.S. Department of Agriculture, Clay Center, NE 68933

Summary

The major advances over the past 25 yr in male reproductive physiology of cattle, sheep, swine and horses are reviewed. Those findings which have allowed efficient culling of subfertile males and exploitation of the reproductive capacity of genetically superior sires are emphasized.

Hormones secreted by the male gonad early in gestation induce regression of potentially female structures and development of the male reproductive tract. Compartmentalization of the testis and formation of the blood-testis barrier during prepubertal development permit the steroidogenic and gametogenic activities of the adult testes to proceed normally. The role of the central nervous system, hypothalamus and anterior pituitary in regulation of the endocrine and exocrine functions of the testes are discussed in view of the physiological constraints and reproductive requirements of the domestic male. The relationships among testes size, the efficiency of spermatogenesis and daily spermatozoal production are discussed. Ignorance about epididymal function and the physiology of spermatozoal maturation are mentioned. Successful techniques have been developed and applied to the extension and preservation of genetically superior bovine sperm, but similar success and exploitation has not been achieved in other species. A better understanding of the biochemical and biophysical differences among sperm from domesticated animals should enable development of procedures for successful extension and cryopreservation of sperm from the boar, ram and stallion. This would allow producers to make more efficient use of genetically superior sires.

Artificial insemination of beef cattle and sheep is likely to increase, but not to the extent achieved for dairy cattle or swine. Most sheep and beef cattle probably will continue to be bred by natural mating under conditions which maximize the utilization of forage but which make difficult, or preclude, application of high technology management practices. Sires used for natural mating routinely will undergo a breeding soundness examination. By the year 2,000, spermatozoal motility, velocity and concentration probably will be measured by dipping a fiber optic probe into a semen sample. However, visual examination of semen quality still will require a phase contrast microscope. Insemination of frozen semen enriched in sperm bearing either the X- or the Y-chromosome, with at least 80% offspring of the desired sex, is likely within the next 20 yr. Application of sexed semen in commercial dairy herds will be common, but its impact on other species of livestock may be marginal.

An expanded array of options for management of sires will be available to the livestock producer in the next decade. The greatest impact of those technologies will be on dairy cattle and swine. Future sires may be conceived as a result of planned matings accomplished by artificial insemination followed by sexing and splitting of embryos, transfer of desirable embryos to appropriate recipients, and possible manipulation of reproductive development before or after birth. A better understanding of factors controlling sexual behavior should enhance the usefulness of sires used for natural matings or in artificial insemination centers. Manipulation of the seasonal pattern in sperm production of rams and stallions also will be commonplace.

[1] Animal Reproduction Laboratory, Colorado State Univ.

[2] Roman L. Hruska U.S. Meat Anim. Res. Center, ARS, USDA.

This article is a U.S. government work, and is not subject to copyright in the United States.

(Key Words: Bulls, Rams, Boars, Stallions, Male Reproduction, Sperm Production, Semen Preservation, Male Fertility.)

Introduction

The past 35 to 40 yr have seen remarkable advances in our understanding of male reproductive physiology. These advances have led to techniques for exploitation of the reproductive capacity of bulls with semen from individual superior sires being used to inseminate up to 100,000 dairy cows annually. Such exploitation of genetically superior sires is a direct result of our increased understanding of testicular physiology and sexual behavior; improved procedures for extension, processing and cryopreservation of semen; the design of efficient packaging and storage containers; improved methods for evaluating spermatozoal quality; and establishment of the minimum number of motile spermatozoa required for maintenance of an adequate conception rate. The greatest impact of this knowledge has been through artificial insemination of dairy cattle. The same procedures are used for preservation of semen from beef bulls. However, our understanding of the physiology of beef bulls lags behind that for Holstein bulls and the commercial application of artificial insemination to beef cattle is limited by factors not related to male reproductive physiology. With horses, sheep and swine, artificial insemination of fresh semen is practical, but with frozen semen the conception rates and numbers of offspring born are less than those obtained with fresh semen or natural service.

Improved understanding of the neuroendocrine control of male reproductive function, sexual behavior, and approaches for evaluation of semen have led to widespread adoption of the breeding soundness evaluation for beef bulls used for natural mating and, to a lesser extent, evaluation of rams, boars and stallions for potential fertility. This application of basic research has increased pregnancy rates in herds and flocks through early culling of sterile or potentially subfertile males and males with a low reproductive capacity. It now is possible to shift the seasonal cycle of sperm production and fertility of rams. Manipulation of the seasonal cycle of stallions should be common within the next decade and manipulation of testicular development, puberty and body growth of future sires may be possible.

In this review, advances in our understanding of differentiation of the male reproductive system, the testis and spermatogenesis, neuroendocrine control of male reproduction, epididymal function, reproductive capacity of the male, spermatozoa and techniques for extension and preservation of semen will be considered. Physiological concepts have been illustrated using bulls and rams because a more complete picture is available for these species, but comparisons with boars and stallions are made when possible. Lack of space has necessitated the citation of many review articles rather than the primary references for all topics considered. We apologize to individuals who may feel slighted. We conclude with our speculations concerning advances within the next decade or two.

Differentiation and Development of the Mammalian Testis

The indifferent gonad in the developing fetus arises from a thickening of the coelomic epithelium on the medioventral aspect of the mesonephros. These thickenings, known as the genital ridges, are invaded by primordial germ cells which migrate from the mesenchyme (Gier and Marion, 1970). The chronology of development of the indifferent gonad is asymetric in that a male gonad develops days before the earliest changes in a female gonad (Jost et al., 1973). In normal males, expression of a gene on the Y-chromosome organizes the indifferent embryonic gonad to produce a male gonad from structures that otherwise would form an ovary at a later time. Thus, expression of male genetic sex is essential for testicular formation.

The early initiation of testicular function is essential for development of the male reproductive tract. The male embryo contains anlage of both the male and female excurrent ducts – the mesonephric tubules and duct and the Müllerian duct. The male genome contains a program for inhibition of female duct development. This sequence is activated by hormones secreted by the male gonad (Jost, 1970; Jost et al., 1972; Ford, 1982). The Leydig cells of the fetal male gonad produce testosterone (near day 35 in pigs or day 42 in cattle) which stimulates development of the mesonephric tubules and duct into the efferent ducts, ductus epididymidis, ductus

deferens and vesicular glands. The indifferent supporting cells (fetal Sertoli cells) produce a Müllerian duct inhibiting hormone which causes degeneration of potentially female structures (Josso et al., 1977, 1979). As differentiation of the mesonephric duct proceeds, induction of the enzyme 5α-reductase allows metabolism of testosterone to dihydrotestosterone which induces differentiation of the urogenital sinus into the prostate and bulbourethral glands and male type urethra and phallus (Wilson and Siiteri, 1973). The urogenital folds develop into a scrotum. Castration of a male bovine fetus on day 40 removes these influences of the testis and the animal develops as a phenotypic female (Jost et al., 1973). Final development of the mammalian testis involves testicular descent (Gier and Marion, 1970) which occurs long before birth in cattle, sheep and swine but within 2 wk before or after birth in horses.

The Adult Testis

Much of the early understanding of testicular function has resulted from the practice of castration. The exposed position of the testes and the observation that removal of the testes is not life-threatening provided the incentive for intentional orchidectomy by early civilizations. Hormonal contributions of the testes may have been deduced by ancient man, since castration was used before 7000 B.C. to produce tender meat for human consumption and Aristotle recognized the different consequences of prepubertal and postpubertal castration (Bremner, 1981). However, the contribution of the testes to fertility was not acknowledged until Leeuwenhoek observed spermatozoa in 1667 A.D. (Meyer, 1938) and de Graff (1668) described the seminiferous tubules. While the endocrine and exocrine functions of the testes long have been recognized, only in the past three decades has a fuller appreciation of the specialized functions of the testes been realized.

Compartmentalization and the Blood-Testis Barrier

The two basic functions of the testes are a) secretion of testosterone and other hormones through the process of steroidogenesis and b) production of spermatozoa through the process of spermatogenesis. These two functions occur, respectively, in the Leydig cells and seminiferous tubules (figure 1). Steroid secretion and spermatozoal production each are dependent on the separate actions of the two gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Nevertheless, both processes are intimately coupled in that adequate levels of testosterone are essential for the normal production and maturation of spermatozoa.

The testis can be considered to have three functional compartments. The interstitial tissue compartment, which includes the Leydig cells, surrounds the seminiferous tubules and bathes the seminiferous tubules with a fluid rich in testosterone. The other two compartments are within the seminiferous tubules which are divided functionally into a basal and an adluminal compartment (figure 1). The former contains spermatogonia which divide by mitosis whereas the latter contains a special isolated environment in which spermatocytes undergo meiosis and spermatids differentiate into spermatozoa. Although Sertoli cells extend from the basal compartment into the adluminal compartment, the two tubular compartments are separated by junctional complexes that function as the major component of the blood-testis barrier (Dym and Fawcett, 1971; Fawcett et al., 1970). The blood-testis barrier excludes, totally or partially, from the adluminal compartment many compounds found in blood or intersitital tissue fluid (Neaves, 1977; Setchell, 1980; Waites and Gladwell, 1982). Several substances believed to play key roles in the development and maintenance of testicular function are secreted by Sertoli cells and concentrated in the isolated adluminal compartment of the testis. Distention of seminiferous tubules and accumulation of fluid after ligation of the efferent ducts (Setchell, 1980) and the inability of large molecules such as lanthanum to penetrate the distended testis (Neaves, 1973), bear proof to the impermeable barrier which resides in the seminiferous tubules.

The Leydig Cell

Leydig cells are large, polyhedral cells occurring in clusters and are found in association with the lymphatics and blood capillaries in the interstitial compartment of the testis (Setchell, 1978). The cytoplasm of Leydig cells contains numerous lipid-filled vacuoles and an extensive smooth endoplasmic reticulum

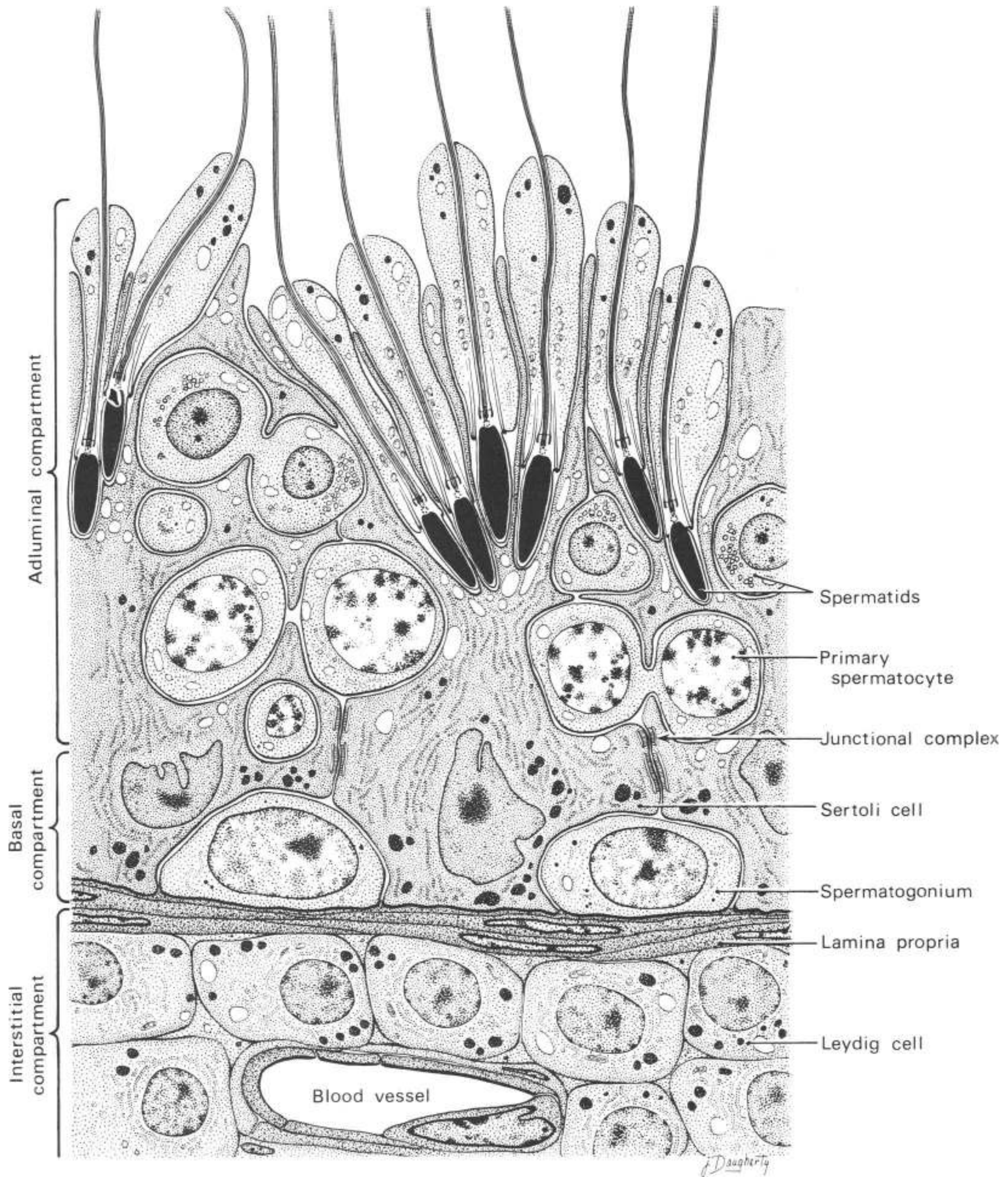

Figure 1. Drawing of part of a seminiferous tubule showing the relationship of the germ cells to the adjacent Sertoli cells. Formation of spermatozoa starts near the basement membrane when a spermatogonium divides to form other spermatogonia and ultimately primary spermatocytes. The primary spermatocytes are moved from the basal compartment, through the junctional complexes between adjacent Sertoli cells, into the adluminal compartment, where they eventually divide to form secondary spermatocytes (not shown) and spherical spermatids. The spermatogonia, primary spermatocytes, secondary spermatocytes, and spherical spermatids all develop in the space between two or more Sertoli cells and are in contact with them. During elongation of the spermatid nucleus, the spermatids are repositioned by the Sertoli cells to become embedded within long pockets in the cytoplasm of an individual Sertoli cell. When released as a spermatozoon, a major portion of the cytoplasm of each spermatid remains as a residual body within a pocket of the Sertoli cell cytoplasm. Note the intercellular bridges between adjacent germ cells in the same cohort or generation.

(Hooker, 1944, 1970; Zirkin et al., 1980). Leydig cells bind HCG (Schanbacher, 1979a) and in vitro the cells respond to LH by producing testosterone. Leydig cells are active in the early embryo, regress during later development, and reactivate during the onset of puberty (Hooker, 1970). In the boar there also is a neonatal period of Leydig cell differentiation (van Straaten and Wensing, 1978).

Leydig cells are the primary source of testicular steroids, including testosterone, progesterone and probably estrogen (Ewing and Brown, 1977; Zirkin et al., 1980). However, Sertoli cells also are a source of estradiol (Huggins and Moulder, 1945; Ritzén et al., 1981). Steroid production by a testis is correlated with the amount of smooth endoplasmic reticulum in the Leydig cells (Zirkin et al., 1980). Cholesterol is produced from acetate within the smooth endoplasmic reticulum of the Leydig cells and stored as the free or esterified compound. Formation of pregnenolone involves enzymatic cleavage of the side chain from cholesterol and occurs in the mitochondria. This step is rate limiting in the production of testosterone and formation of pregnenolone is stimulated by LH. Pregnenolone is rapidly metabolized to testosterone, via a number of Δ^4- and Δ^5-intermediates, in the smooth endoplasmic reticulum (Ewing and Brown, 1977; van der Molen and Rommerts, 1981). Although the testes of all species secrete a variety of steroids in addition to testosterone, the testes of boars and stallions produce relatively large quantities of estrogens (Bedrak and Samuels, 1969; Oh and Tamaoki, 1970). The boar also secretes a C_{16}-unsaturated steroid (Booth, 1982) that serves as a pheromone (Signoret, 1970).

The Sertoli Cell

Sertoli cells, which rest upon the lamina propria of the seminiferous tubule (figure 1), are the only somatic cells within the seminiferous tubules. Their cytoplasmic processes extend to the lumen of the seminiferous tubule and envelope the developing germ cells. Sertoli cells provide the only communication link across the blood-testis barrier. The precise function of Sertoli cells in spermatogenesis is not fully understood, but they provide the microenvironment required for germ cell development. The germ cells are anchored by desmosome-like, ectoplasmic specializations within the Sertoli cells. Organelles within the Sertoli cells, including the endoplasmic reticulum, have an active role in modifying the spermatid during nuclear elongation and acrosome formation.

The Sertoli cells have a pivotal role in the hormonal control of spermatogenesis. Biochemical and morphological changes in Sertoli cells are induced by FSH and Sertoli cells are the only testicular cell with specific binding sites for radiolabeled FSH (Schanbacher, 1979a). Thus, the action of FSH on spermatogenesis is indirect, via the Sertoli cells, rather than directly on the germ cells. Because of the blood-testis barrier, FSH must enter through the basal aspect of the Sertoli cell. In response to FSH stimulation, and with the availability of testosterone, Sertoli cells secrete fluid and specific products like androgen binding protein (ABP) and inhibin (Steinberger, 1981a,b). The functional role of ABP is believed to reside within the seminiferous tubules or epididymis where ABP presumably serves to attenuate changes in testosterone concentration or to aid in testosterone transport. Inhibin is thought to act on the anterior pituitary gland to suppress FSH secretion (Blanc et al., 1981; Ritzen et al., 1981). Both secretory proteins have been detected in peripheral plasma. It seems likely that ABP, inhibin and other secretory products of Sertoli cells enter the systemic circulation via two routes: a) the basal aspect of the Sertoli cells and b) absorption from the luminal fluid in the proximal epididymis (Setchell, 1978). Regardless of routes and mechanisms, continuous communication exists between the anterior pituitary, Sertoli cells and germ cells.

It currently is accepted that the number of Sertoli cells in the testis is established during puberty and that Sertoli cell number is constant in an adult male (Lino, 1971; Hochereau-de Reviers and Courot, 1978). However, the validity of this assumption has been challenged by Johnson and Thompson (1983) who found that the number of Sertoli cells within the stallion testis was 26% lower during the winter than in the summer. If this report is confirmed, consideration should be given to the possibility that slow replacement of Sertoli cells or seasonal changes in Sertoli cell number occur in other domesticated males. Additional studies of factors controlling the number of Sertoli cells at different times

of year or the number formed during the prepubertal period are needed.

Spermatogenesis

Spermatogenesis is the sum of the transformations that result in formation of spermatozoa from spermatogonia while maintaining spermatogonial numbers (Courot et al., 1970; Ortavant et al., 1977). This process involves mitotic division of A_1-spermatogonia to form more differentiated cells termed A_2-, A_3-, In-, B_1- and B_2-spermatogonia. The latter divide to form two primary spermatocytes that enter meiosis, form secondary spermatocytes, and finally spermatids. Spermatids undergo differentiation into spermatozoa. Early workers (von Ebner, 1888; Regaud, 1901; Schoenfeld, 1901) outlined spermatogenesis in domesticated animals from a morphological point of view. They observed that spermatogenesis could be classified into a series of cellular associations based on the general appearance of seminiferous tubule cross sections. Further advancement of knowledge concerning spermatogenesis was minimal until the 1950's.

In the mid-1950's, two techniques were developed which revolutionized the study of spermatogenesis. Clermont and Leblond (1955) noted that use of the periodic acid-Schiff stain allowed them to describe the morphologic changes of the acrosome in developing spermatids. By this approach, they were able to define a number of steps during spermiogenesis which later served as the basis for definition of 12 cellular associations within the bovine germinal epithelium (Berndtson and Desjardins, 1974). The second technique involved radiolabeling of DNA during synthesis by young primary spermatocytes. This enabled the time course of spermatogenesis and the pattern of spermatogonial divisions to be ascertained. Classic studies with rams (Ortavant, 1958) and bulls (Koefoed-Johnsen, 1958) utilized ^{32}P, but in subsequent studies (Hochereau et al., 1964; Swierstra, 1968a; Swierstra et al., 1974) ^{3}H-thymidine has been used because of increased specificity and resolution during radioautography.

A seminiferous tubule contains a number of reserve A_0-spermatogonia which are not part of the proliferating pool of germ cells (Hochereau-de Reviers, 1981) and are extremely resistant to radiation or toxic agents. In domesticated species, a number of stem A_1-spermatogonia in one area of a seminiferous tubule synchronously become committed to produce differentiated A_2-spermatogonia and the cohorts of germ cells resulting from these A_2-spermatogonia differentiate in unison. The progeny of a stem A_1-spermatogonium remain joined by intercellular bridges (Dym and Fawcett, 1971), except where cell death breaks the "chain" of interconnected cells. Because a synchronous population of developing germ cells occupies a considerable area of the basement membrane of a seminiferous tubule, the major part of most cross sections through a seminiferous tubule has the same appearance. However, adjacent cross sections frequently have a different appearance.

Eight to 12 different cellular associations have been discerned for the bull, boar, ram and stallion (Setchell, 1978). Each cellular association contains four or five types of germ cells organized in a specific, layered arrangement (figure 2). Each layer represents one cell generation with the most differentiated nearest the tubule lumen. The eight stage classification scheme (figure 2) is based on morphology of the spermatids and the relationships among all germ cell types. The cells that should be present in a given tubule cross section are listed in the eight columns. Modifications of this classification approach are useful with prepubertal animals (Courot, 1971; Curtis and Amann, 1981) or if the development of spermatids is impeded. An alternative approach for defining cellular associations is based on morphology of the acrosome in the developing spermatid – 12 cellular associations have been described for the boar, bull and ram (Setchell, 1978). With either approach, the complete series of cellular associations is termed the cycle of the seminiferous epithelium.

Collectively, the germ cells at a point within a seminiferous tubule sequentially acquire the appearance of each of the 8 to 12 cellular associations characteristic of that species. The interval required for one complete series of cellular associations to appear at a point within a tubule is termed the duration of the cycle of the seminiferous epithelium. This duration is uniform for a species (table 1) and is not influenced by any known factor.

Critical studies of the pattern of spermato-

gonial renewal have been published for the bull (Hochereau-de Reviers, 1981), boar (Frankenhuis et al., 1980) and ram (Ortavant, 1958), but alternative interpretations of certain aspects of spermatogonial development are possible. Even allowing for this uncertainty, the duration of spermatogenesis probably is between 4.3 and 4.7 cycles of the seminiferous epithelium or 3.9 and 61 d (table 1).

Any realistic appraisal of testicular function must include a quantitative analysis of testicular histology, because subtle and even substantial changes in germ cell production may not be detected by subjective evaluations (Berndtson, 1977; Amann, 1982). Excellent reviews of approaches to morphometric analyses (Berndtson, 1977; Bolender, 1982) should

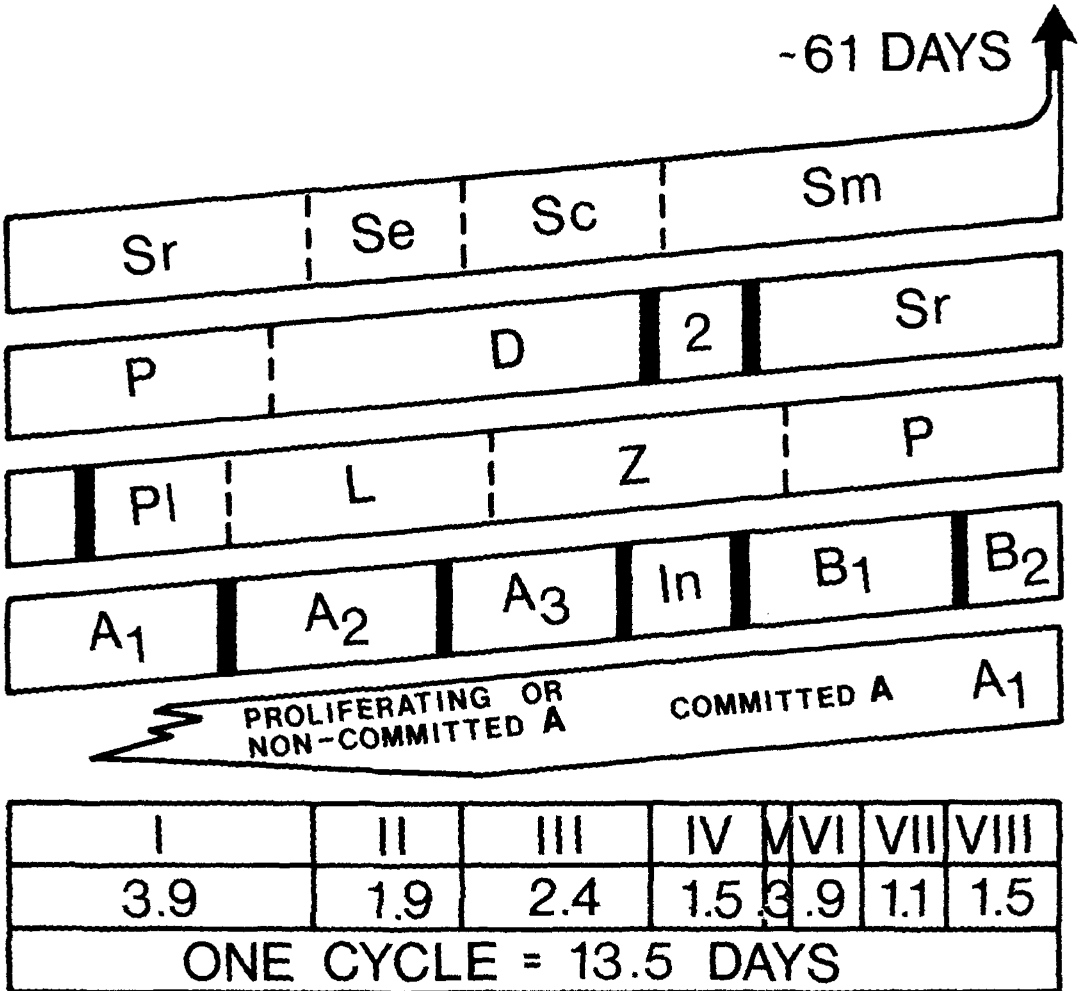

Figure 2. The cycle of the seminiferous epithelium in the bull. In this chart, the basement membrane is at the bottom and the lumen of the seminiferous tubule at the top. Each of the five upper rows represents one generation of germ cells that are increasingly (from bottom to top) more mature. The columns represent the eight cellular associations of the morphological classification scheme. The germ cells present in each cellular association can be discerned by reading up in each column. Germ cell types are A_1-, A_2-, A_3-, In-, B_1- and B_2-spermatogonia, preleptotene (Pl), leptotene (L), zygotene (Z), pachytene (P), diplotene (D) and secondary (2) spermatocytes and spermatids with a spherical (Sr), elongating (Se), condensing (Sc) or definitive (Sm) nuclear shape. The complete series of cellular associations is termed the cycle of the seminiferous epithelium. In the bull, the duration of one cycle of the seminiferous epithelium is 13.5 d. The duration of each cellular association also is shown. Since approximately 4.5 cycles of the seminiferous epithelium pass between commitment of an A-spermatogonium to differentiate and produce more advanced types of spermatogonia and release of the resulting spermatozoa from the germinal epithelium, the duration of spermatogenesis is 61 d in the bull.

TABLE 1. SPERMATOGENESIS IN ADULT DOMESTIC ANIMALS[a]

	Paired testes wt (g)	Duration of cycle (days)[b]	Duration spermatogenesis (days)[c]	Daily sperm production	
				Per gram parenchyma (10^6/g)	Per male (10^9)
Bull					
Hereford	650	13.5	61	10	5.9
Charolais	775	13.5	61	13	8.9
Holstein	725	13.5	61	12	7.5
Stallion	340	12.2	55	16	5.3
Ram					
Ile de France	500	10.4	47	21	9.5
Suffolk	475	10.4	47	25	12.5
Boar					
Yorkshire	939	8.6	39	20	16.8

[a]Compiled from the literature.

[b]Duration of one cycle of the seminiferous epithelium.

[c]Duration of spermatogenesis assuming it requires 4.5 cycles of the seminiferous epithelium.

be consulted before quantitatively evaluating testicular function. The method of tissue fixation is critical and use of buffered formaldehyde is unsatisfactory (Amann, 1982). Morphometric data should be used to calculate the total number of germ cells of each type within the testis. By this approach changes have been detected in the number of Sertoli cells and different classes of germ cells during puberty (Attal and Courot, 1963; Courot, 1971; Curtis and Amann, 1981), during different seasons of the year (Ortavant, 1958; Johnson and Thompson, 1983), or as a consequence of drug or hormone therapy (Berndtson et al., 1979).

Daily Sperm Production

When evaluating spermatogenesis, the ultimate end point is the number of potentially fertile sperm produced (table 1). Daily sperm production is the number of sperm produced per day by the testes (Amann, 1970, 1981). The efficiency of sperm production is the number of sperm produced per day per gram of testicular parenchyma. The efficiency of sperm production can be determined by three methods (Amann, 1970; Berndtson, 1977; Johnson et al., 1981a). In all species (table 1), the efficiency of sperm production is influenced by age, environmental factors, hormonal status and drugs. Normal, adult individuals of a given breed or species have a fairly uniform efficiency of sperm production at a given time of year (Swierstra, 1968b; Amann, 1981). Thus, daily sperm production is highly correlated with testicular weight. Sperm production of living animals can be estimated with an orchidometer (caliper) by measuring the length, breadth and width of each testis (Foote, 1969). For bulls and rams, the circumference of the scrotum around the widest point is highly correlated with testicular weight and sperm production (Willett and Ohms, 1957; Foote, 1978; Coulter, 1980). Measurements of testicular size should be an integral part of any andrologic examination or experiment on male reproductive function so that normalcy of testes size or experimentally induced changes in testicular size, and potential spermatozoal production, can be established.

The testes of rams (Ortavant, 1958, 1977; Lincoln and Short, 1980; Ravault et al., 1980; Lincoln, 1981) and stallions (Pickett et al., 1981; Johnson and Thompson, 1983) undergo a seasonal regression in function, but complete cessation of spermatogenesis does not occur. Similar but more subtle effects may exist in cattle (Amann, 1970) and high ambient temperature decreases sperm production by boars (Wettemann et al., 1978). An increased understanding of the mechanisms underlying the seasonal constraints to efficient lamb production has enabled animal scientists to overcome seasonal infertility in rams by use of modified light regimens or hormone

injections (Ortavant, 1977; Schanbacher, 1979c; Haynes and Schanbacher, 1983).

Endocrine Regulation of Testicular Function

The isolation and purification of testosterone from testicular tissue (David et al., 1935) and its subsequent assay (Lindner, 1959, 1961) were milestones in reproductive endocrinology. However, the development of sensitive radioimmunoassays for the rapid quantitation of protein (Yallow and Berson, 1960) and steroid (Midgley et al., 1969) hormones in blood and tissue revolutionized the field of endocrinology. For andrologists, the assays for LH (Niswender et al., 1969), FSH (McNeilly et al., 1976), testosterone (Flavo and Nalbandov, 1974; Schanbacher and D'Occhio, 1982), and GnRH (Nett and Adams, 1977) are especially important.

The Hypothalamus

The hypothalamus integrates inputs from the central nervous system and generally is considered to be the orchestrator of testicular activity (Schanbacher, 1982b). A massive and highly competitive research effort established how these signals are transmitted by the hypothalamus. Research teams headed by Guillemin and Schally extracted gonadotropin releasing factors from millions of sheep and pig hypothalami, respectively. Almost simultaneously, the two groups established the correct sequence for the decapeptide (Burgus et al., 1971; Matsuo et al., 1971) and showed that synthetic material caused the release of LH and FSH from the pituitary (Monahan et al., 1971). For both species the structure of gonadotropin releasing hormone (GnRH) is pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2). Many synthetic analogs now are available and certain of these have proven useful as profertility or antifertility agents.

GnRH is released from the hypothalamus into the portal system in discrete pulses (Levine et al., 1982) whereby it reaches the gonadotrophs of the anterior pituitary and causes the pulsatile discharge of LH and the discharge of FSH. Whereas LH secretion is immediate after GnRH stimulation, the discharge of FSH is slow and gradual (Lincoln, 1979). This fact, together with a slower clearance of FSH from blood, results in attenuation of changes in blood concentration of FSH. Evidence for hypothalamic control of the anterior pituitary has come primarily from studies employing hypothalamic lesions (Clegg et al., 1958) or immunoneutralization of GnRH activity (Schanbacher, 1982a). In both cases, hypogonadotropism and gonadal atrophy result. While these perturbations have severe consequences on gonadal function, less dramatic inputs from both external (e.g., photoperiod and physical stress) and internal (e.g., testicular steroid feedback) environments continually impinge on the hypothalamus (Schanbacher and Ford, 1979; Schanbacher, 1982b).

The Anterior Pituitary Gland

Regression of the rat testes following hypophysectomy provided evidence that the testes are controlled by the pituitary gland (Smith, 1927). This observation has been confirmed repeatedly in several species including sheep (Courot, 1967). It now is known that LH and FSH from the anterior pituitary are essential for normal function of Leydig cells and Sertoli cells, respectively. Injection of pituitary extracts containing LH and FSH prevent the testicular regression that follows hypophysectomy (Greep et al., 1936; Courot, 1967).

The Hypothalamic-Pituitary-Gonadal Axis

The hypothalamic-pituitary-gonadal axis (figure 3) is a self-regulating system. LH secretion is controlled by a complex interaction of the sex steroids and GnRH. In the mature male, a release of LH usually is followed by a rise in serum testosterone. Based on the castrate animal model, it appears that the peripheral concentration of testosterone largely dictates the pattern of LH secretion (Ford and Schanbacher, 1977; D'Occhio et al., 1982b). Regulation of LH secretion by feedback inhibition also can be induced by other androgens and estrogens (Parrott and Davies, 1979; D'Occhio et al., 1982a, 1983; Schanbacher et al., 1983).

The observations that both LH and FSH secretion increase following castration (Schanbacher and Ford, 1977; Amann and Walker, 1983) and cryptorchidism (Schanbacher, 1979b) are evidence that a hormonal factor(s) from the testes controls the secretion of both gonadotropins. Although testicular steroids inhibit FSH secretion in the castrate ram (D'Occhio et al., 1982c), a nonsteroidal factor,

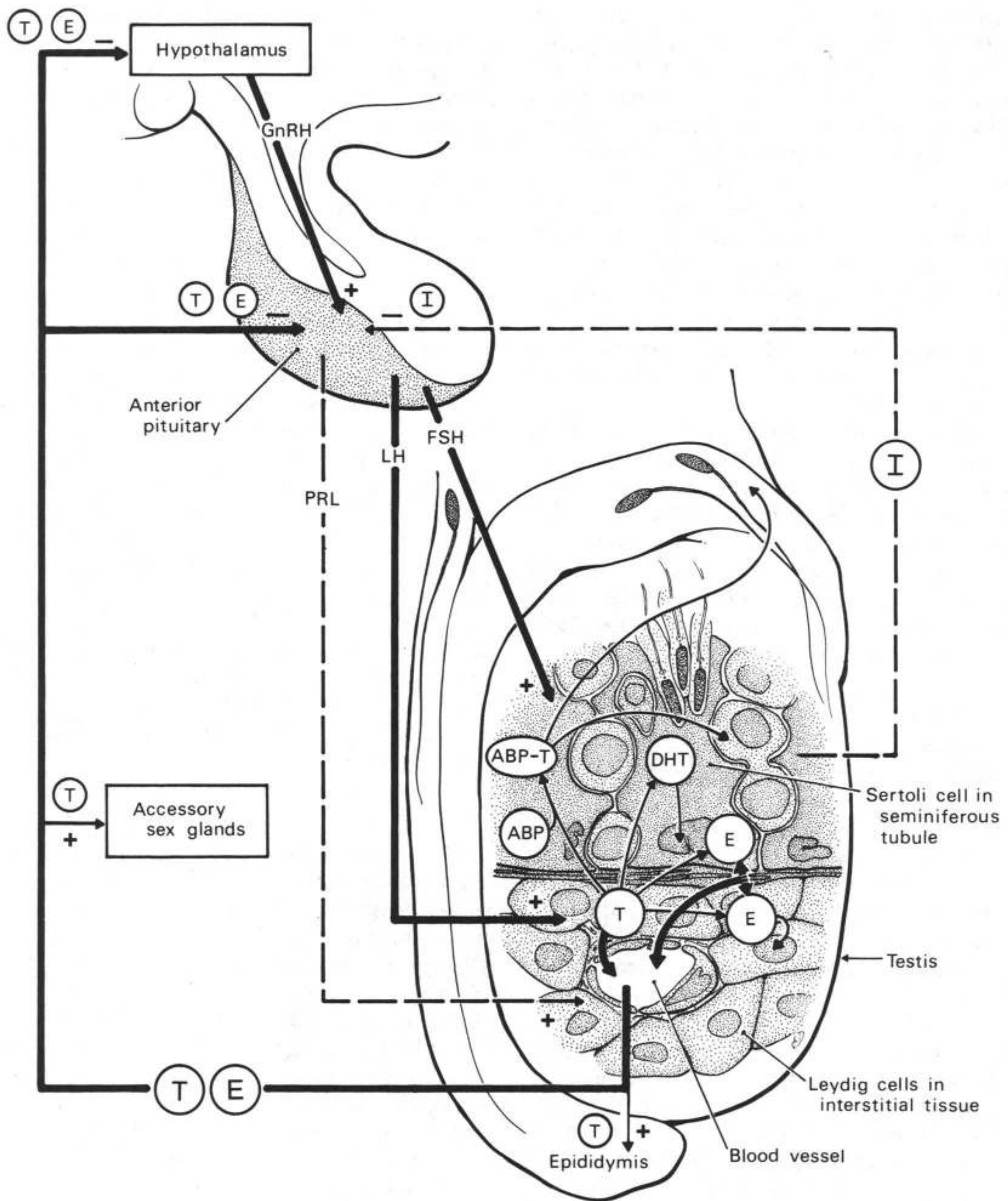

Figure 3. Diagram showing inter-relationships among hormone production in the Leydig cells and seminiferous tubules and the feedback control of gonadal hormones on the hypothalamus and anterior lobe of the pituitary gland. An increased level of testosterone (T) in peripheral blood, either as a result of increased production by the testes or following injection of exogenous hormone, provides negative feedback on the hypothalamus to suppress pulsatile discharge of GnRH and thus suppress discharge of luteinizing hormone (LH) from the anterior pituitary. Consequently, the Leydig cells receive less LH stimulation which results in less T being produced. Estrogens (E) are produced by the Leydig cells and to some extent by the Sertoli cells. The ratio of T to E reaching the anterior pituitary may affect the relative amounts of LH and FSH secreted by the gonadotrophs. Based on studies with rams, inhibin (I) suppresses discharge of FSH from the anterior pituitary gland. The physiological roles of E and prolactin (PRL) in adult domestic males remain conjuctural. Sertoli cells produce an androgen-binding protein (ABP) which serves as a carrier for testosterone and may aid in maintaining a high androgen concentration within the seminiferous tubules or in providing testosterone to the epithelium lining the proximal portion of the epididymis.

termed inhibin, originating from the seminiferous tubule may be the major regulator of FSH secretion (McCullagh, 1932). Hemicastration results in increased secretion of FSH and with prepubertal lambs is followed by compensatory growth of the remaining testis (Land and Carr, 1975; Walton et al., 1978, 1980). The feedback regulator of FSH secretion may be inhibin (Keogh et al., 1976; Blanc et al., 1981) and probably originates from the Sertoli cells. Although the physiological role of inhibin has been studied, isolation and characterization of inhibin has not been accomplished (Setchell et al., 1977; Franchimont et al., 1981).

Secretion of testosterone by the Leydig cells provides a high concentration of testosterone around the seminiferous tubules which is essential for spermatogenesis (Albert, 1961). The tissue concentration of testosterone probably approaches that in testicular vein blood which is 20 to 100 times higher than in peripheral blood (Lindner, 1959, 1961; Amann and Ganjam, 1976, 1981).

Regulation of Puberty

Puberty is when a male first produces sufficient sperm to impregnate a female. For practical reasons, puberty in bulls has been defined as the age when an ejaculum is obtained containing 50×10^6 sperm of which $\geq$10% are motile (Wolf et al., 1965). This definition also is suitable for boars, rams and stallions. For boars, bulls and rams, the pubertal period is associated with rapid testicular growth, changes in LH secretory pattern, a gradual increase in blood testosterone, and the initiation of spermatogenesis. Puberty is not synonymous with sexual maturity or adult status which occurs months or years later (Amann, 1970, 1981).

The hypothalamus is believed to play a key role in initiating puberty because the pituitary gland, gonads and steroid-dependent target tissues each are competent and ready to respond to their respective tropic hormones prior to puberty (Davidson, 1974). In the boar, bull and ram, puberty appears to be the culmination of a continuous and lengthy process of endocrine changes which begin shortly after birth (Levasseur, 1977; FlorCruz and Lapwood, 1978; Foster et al., 1978; Hafs and McCarthy, 1979; Lacroix and Pelletier, 1979; Amann, 1983). The most conspicious early change is an increase in the frequency of pulsatile discharge of LH. This is followed by altered testicular steroidogenesis, increased circulating levels of testosterone, differentiation of Sertoli cells and the onset of spermatogenesis (figure 4). Ramirez and McCann (1963) proposed that puberty occurs when an animal becomes desensitized to the feedback inhibition imposed on the hypothalamic-pituitary complex by gonadal steroids. Presumably, this allows an increase in GnRH discharge and a greater response by the pituitary gland to GnRH. The repeatedly observed increased frequency and amplitude of LH discharges could reflect such a shift. This hypothesis also is supported by the observation that testicular growth, testosterone secretion and spermatogenesis were delayed in pubertal-age bull calves administered low doses of estradiol (Schanbacher, 1982b). Numerous environmental factors (both internal and external) influence the central nervous system to modulate the endocrine system and, thereby, alter the chronological age at which a given animal reaches puberty. Energy intake, breed and season of birth are major factors affecting age at puberty.

Old Age

With few exceptions, the testes of a mature male continue to secrete testosterone and produce spermatozoa at a rate adequate to meet the breeding requirements of the species. Old age impinges on reproductive performance in man and certain laboratory species, but detailed longitudinal studies of changes in the pituitary-gonadal axis or of spermatogenesis with advancing age have not been made for domestic animals. Certain disorders (e.g., genetic diseases, autoimmune disease, orchitis and tumors) can contribute to testicular failure. The best data are for Holstein bulls in artificial insemination organizations (Kratz et al., 1983). About 46% of the genetically desirable bulls eventually are removed for reproductive reasons although many are removed for muscular-skeletal problems.

Epididymal Function

In the mid-1950's, virtually nothing was known about the function of the epididymis. Early morphologists (Benoit, 1926) described

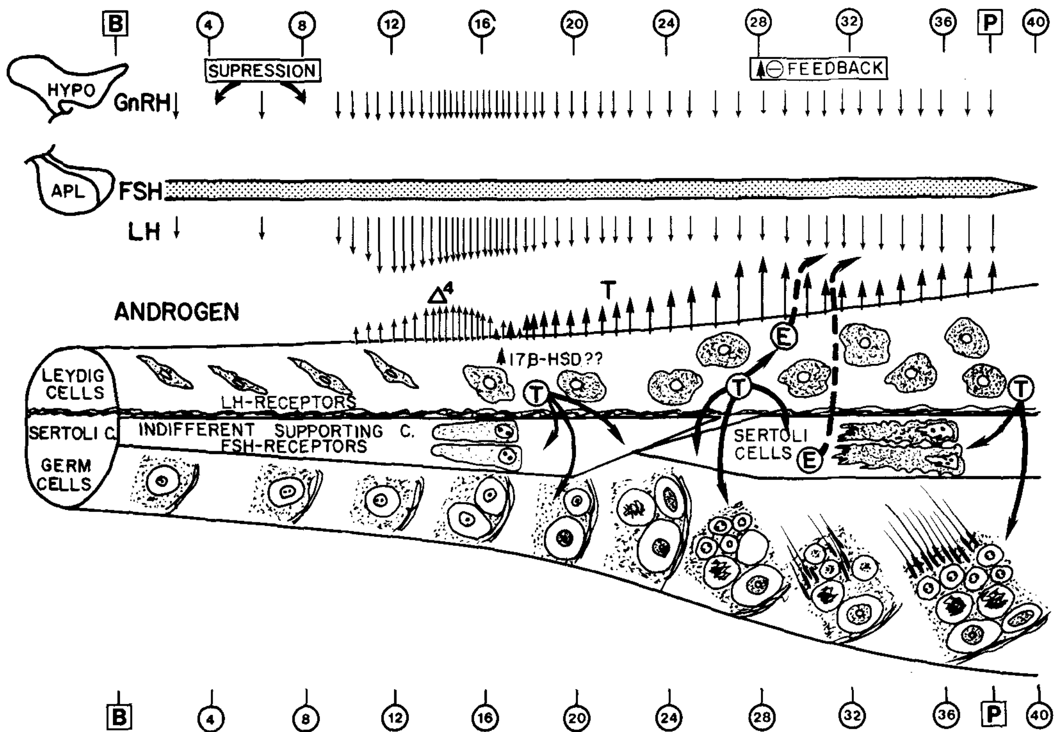

Figure 4. A postulated sequence of events in neuroendocrine development, differentiation of the testes, and the initiation of spermatogenesis between birth (B) and puberty (P) in well-fed Holstein bulls. Age is shown in weeks. Starting at about 12 wk, a sequence of events induces a) initiation of pulsatile discharge of LH, possibly resulting from a diminished suppression of GnRH discharge from the hypothalamus or from an increased capacity of the anterior pituitary to respond to GnRH; b) LH-induced differentiation of Leydig cells with increased secretion of androstenedione in response to LH stimulation; c) further differentiation of Leydig cells resulting in LH-stimulated secretion of testosterone; d) testosterone-induced differentiation of indifferent supporting cells to Sertoli cells concomitant with testosterone induced differentiation of gonocytes to prespermatogonia and A-spermatogonia; e) an increased sensitivity of the hypothalamic-pituitary complex to the negative feedback action of gonadal steroids; f) a somewhat diminished frequency and amplitude of LH discharges; g) cessation of Sertoli cell formation followed by formation of junctional complexes between adjacent Sertoli cells and establishment of the blood-testis barrier; h) sequential formation of primary spermatocytes, spermatids and spermatozoa; and i) continued increases in the efficiency of spermatogenesis until sufficient sperm are produced to provide the first ejaculum around 37 wk.

regional differences in epithelial morphology and the concentration of sperm within the epididymal duct. The notion that sperm from proximal part of the epididymis were infertile, whereas those from the distal cauda epididymidis were fertile, was accepted based on classic studies with guinea pigs (Young, 1931). It also was recognized that the cauda epididymidis served as a reservoir for sperm.

It now is recognized that from a functional point of view the epididymis has three segments, although the duct has many more cytologically distinct regions (Nicander, 1958; Glover and Nicander, 1971). The efferent ducts plus initial segment of the caput are involved in the resorption of fluid and solutes from the ductal lumen and may secrete other compounds. Sperm entering the epididymis are infertile, but acquire fertilizing capacity during a maturational process. The middle segment of the epididymis, which is comprised of the major portions of the caput and corpus, is the site of sperm maturation (Orgebin-Crist et al., 1975; Holtz and Smidt, 1976; Fournier-Delpech et al., 1979; Johnson et al., 1980). The terminal segment, essentially the cauda epididymidis, together with the proximal deferent duct, is involved in the storage of

TABLE 2. SPERM RESERVES OF SEXUALLY RESTED, ADULT DOMESTIC ANIMALS (10^9 /MALE)[a]

	Epididymis				
	Caput	Corpus	Cauda	Ductus and ampulla	Total
Bull					
Hereford	11	1	21	6	40
Charolais	18	4	35	7	64
Holstein	20	5	39	8	72
Stallion	12	17	54	7	90
Ram					
Ile de France	23	11	126	?	>165
Suffolk	17	9	104	?	>135
Boar					
Yorkshire	76	51	234	?	>361

[a]Compiled from the literature.

fertile sperm (table 2). Movement of sperm through the caput and corpus epididymidis primarily is by continuous peristaltic contractions of the smooth muscle surrounding the ductus epididymidis. In the cauda, contractions are much less frequent and the duct is inactive except when the smooth muscle is stimulated to contract. Consequently, the time required for movement of sperm through the caput and corpus epididymidis is not altered by ejaculation and is similar for common mammals (table 3).

The biology of sperm maturation is not understood. Evidence for sperm maturation includes the acquisition of fertilizing capacity, acquisition of progressive motility, changes in spermatozoal morphology, changes in characteristics of the spermatozoal plasma membrane and changes in sperm metabolism (Bedford, 1975; Orgebin-Crist et al., 1981; Hammerstedt et al., 1982). Simple retention of sperm within a given segment of the epididymal duct is insufficient to induce sperm maturation. Secretions by the epididymal epithelium must modify the spermatozoa. Sperm leaving the corpus epididymidis are fertile. However, studies with rams have lead to the concept that survival of embryos resulting from sperm leaving the distal corpus may be lower than for embryos resulting from sperm taken from the cauda epididymidis (Fournier-Delpech et al., 1979). Based on research with ram sperm (Hammerstedt et al., 1982), it is likely that changes in the plasma membrane are central to the process of sperm maturation. These changes certainly include alteration of exposed glycoproteins, membrane fluidity and transfer of substrates across the plasma membrane. Sperm maturation is dependent on secretions of the epididymal epithelium as well as the transport of sodium, potassium and other compounds. These processes are dependent upon dihydrotestosterone, aldosterone and possibly other hormones (Orgebin-Crist and Jahad, 1978; Turner and Cesarini, 1981).

The conditions and processes by which fertile sperm remain viable for several weeks within the cauda epididymidis are of great biologic importance, but virtually unexplored. The temperature of the cauda is lower than that of the caput or corpus epididymidis. Indeed, it has been suggested that the scrotum evolved as a device to cool the cauda epi-

TABLE 3. TRANSIT TIME OF SPERMATOZOA THROUGH THE EPIDIDYMIS (DAYS)[a]

	Caput and corpus	Cauda
Bull		
Hereford	2.0	3.6
Charolais	2.5	3.9
Holstein	3.1	5.2
Stallion	4.0	9.8
Ram		
Ile de France	3.6	12.8
Suffolk	2.1	8.3
Boar	5.4	6.4

[a]Compiled from the literature. Transit time through the caput-corpus epididymidis is unaffected by ejaculation, but transit time through the cauda is reduced two to four days by daily ejaculations.

didymidis rather than the testis (Bedford, 1978; Foldesy and Bedford, 1982). Although not universally accepted, it generally is assumed that sperm are immotile within the confines of the ductus epididymidis and become motile only by removal of contact inhibition (Cascieri et al., 1976) or dilution of suppressor molecules (Turner et al., 1978). The substrates utilized for energy production by sperm within the cauda epididymidis and the problem of moving substrates or metabolites across the considerable distance from the ductal epithelium to the center of the duct have not been addressed. Molecular transport to and from sperm within the ductal lumen may be facilitated by the mixing of sperm that probably occurs through ductal contractions and physical manipulations.

Since the rate of sperm movement through the caput and corpus epididymidis, those areas where sperm maturation occurs, is not influenced by ejaculation (Amann and Almquist, 1962; Swierstra, 1971), the fertility of sperm should not be depressed even in males ejaculating frequently. This is true for daily or thrice weekly ejaculation (Hafs et al., 1959; Almquist, 1982) and for 5 or 11 ejaculates collected in succession (Salamon, 1962; Martig et al., 1970). Although the transit time for sperm through the caput and corpus epididymidis is not altered by ejaculation, the interval that sperm spend in the cauda is influenced profoundly by ejaculation frequency (Amann and Almquist, 1976; Amann, 1981). The number of sperm in the cauda is maximal in males that have not ejaculated for at least 7 to 10 d, but is reduced by at least 25% in males ejaculating daily or every other day (Ortavant, 1958; Amann and Almquist, 1962; Swierstra, 1971; Pickett et al., 1981).

Sperm are produced continuously regardless of ejaculation frequency. Since sperm enter the epididymis at a constant rate, they also must leave the excurrent duct at a relatively constant rate, although this rate is influenced by ejaculation. Based on studies with bulls and rams (Koefoed-Johnsen, 1964; Lino and Braden, 1972), it is evident that sperm that are not ejaculated at copulation or voided by masturbation are eliminated periodically during urination. Resorption within the excurrent duct system of significant numbers of sperm does not occur (Paufler and Foote, 1969; Lino, 1972; Amann et al., 1976).

Reproductive Capacity

Semen Collection

The general approaches for semen collection have not changed greatly during the past three decades. These approaches are use of an artificial vagina with the bull, ram or stallion; electroejaculation with the bull and ram; and digital manipulation for the boar. The most notable advancement has been the improvement of electrostimulators and in the design of rectal probes used for collection of bull and ram semen (Ball, 1974). Use of a rectal probe with two or three parallel electrodes on the ventral surface, rather than circumferential electrodes or longitudinal electrodes equally spaced on the surface of the electrode, has led to a great reduction in stress of the animal and an increased success in seminal collection.

Although the equipment used for semen collection has not changed, the procedures used during semen collection and the general management of bulls have changed dramatically. In the early 1950's, it was believed that collection of semen more than once weekly would damage testicular function and might be detrimental to the health of a bull. These notions were dispelled by a series of studies (Bratton and Foote, 1954; Baker et al., 1955; Almquist and Hale, 1956; VanDemark, 1956; Hafs et al., 1959; Dukelow et al., 1960; Hale and Almquist, 1960) that convincingly demonstrated that bulls could be collected routinely 2, 3 or 7 d per week, week after week.

A genetically superior sire is in essence a sperm factory producing packets (spermatozoa) of genetically superior DNA. Rejection of the notion that frequent collection of a breeding male was harmful was essential for exploitation of a superior sire. Education of lay and professional personnel was required to gain acceptance that the important criterion of semen quality was the number of sperm harvested per day or per week, rather than the volume or concentration of spermatozoa in a given ejaculate (Almquist and Hale, 1956; VanDemark, 1956; Hafs et al., 1959). Collection of semen from boars, rams and stallions

daily, or even several times daily, is without deleterious effect on fertility.

Sexual Behavior

A full understanding of sexual behavior is critical to successful collection of four to seven ejaculates weekly from a male. Classic studies outlined procedures to maximize the number of sperm obtained in a given ejaculate and minimize the time expended in collecting the ejaculate (Collins et al., 1951; Branton et al., 1952; Hale and Almquist, 1960; Hafs et al., 1962; Hale, 1966). Bulls differ greatly in their inherent level of sexual activity (Hale and Almquist, 1960; Chenoweth, 1983).

Sexual stimulation is presentation of a stimulus situation which will ellicit mounting in the shortest possible time. Attainment of this objective is dependent upon novelty (Hale and Almquist, 1960). Novelty can be obtained by a) introducing a new stimulus animal or combination of stimulus animals in a given location, b) presenting familiar stimulus animals in a new location, or c) changing both the stimulus animal and the location simultaneously.

The goal of sexual preparation is to collect the greatest possible number of high quality sperm in an ejaculate. This is achieved by prolongation of the period of sexual stimulation beyond that needed for mounting and ejaculation (Hale and Almquist, 1960). Approaches for sexually preparing a male are a) the use of false mounts in which a male is allowed to mount the stimulus animal but not ejaculate, b) restraint of a male a few feet away from the stimulus animal, or c) a combination of false mounting and restraint (Branton et al., 1952; Hafs et al., 1962; Hale, 1966; Almquist, 1973). The concept of sexual preparation is applicable to sheep and boars (Hemsworth and Galloway, 1979), but not stallions (Pickett and Voss, 1973).

Daily Sperm Output

If routine semen collections every one to three days are initiated with a previously sexually rested male, the first few ejaculates will contain more sperm than subsequent ejaculates. This is because the number of sperm within the cauda epididymidis is reduced by ejaculation until it stabilizes at about 25% lower than that characteristic of a sexually rested male (Amann, 1970). Once such stabilization has occurred, the number of sperm obtained in subsequent ejaculates should oscillate around a mean value characteristic of that male at that time of year. When expressed as the mean number of sperm obtained per day, this value is termed daily sperm output.

If one ejaculate is collected each day, or if several ejaculates are collected every 2 or 3 d, daily sperm output will be maximal provided appropriate sexual preparation is used prior to collection of each ejaculate. If sufficient samples are collected to obtain a valid estimate, daily sperm output is highly correlated with and approaches daily sperm production (Amann, 1970, 1981; Amann et al., 1976). Daily sperm output increases for some time following puberty, may vary with season and differs greatly among males of a given breed or species because of differences in their testicular size.

The need for collection of multiple samples to study testicular function on the basis of semen characteristics often is overlooked. Evaluation of a single ejaculate provides no valid information on the number of sperm being produced, other than if the male had been producing spermatozoa in the last few weeks, but can provide limited information on the morphology and motility of sperm produced (Amann, 1981, 1982). Before undertaking an experiment involving semen collection from boars, bulls, rams, or stallions, appropriate publications (Almquist, 1973; Amann and Almquist, 1976; Foote, 1978; Pickett et al., 1981; Amann, 1982) should be consulted.

Spermatozoa, Semen Extension and Preservation

Spermatozoa

Enormous strides have been made within the past 25 yr in our understanding of the biology of spermatozoa. This topic is an excellent example of how improvements in techniques for analysis of cellular ultrastructure, metabolic processes, membrane structure and function, immunology and molecular biology can be focused on a single complex cell to begin to unravel its life story. Because of the impetus of artificial insemination of dairy cattle, by the early 1960's the fine structure of the bovine sperm (Saacke and Almquist, 1964) and the dependence of sperm on glucose or fructose as major energy sub-

strates (Mann, 1954) had been established. The biology of spermatozoal development and function is not considered in this review because excellent, recent reviews are available (Bellvé, 1979, 1982; Hammerstedt, 1981; Mann and Litwak-Mann, 1981; Zaneveld and Chatterton, 1982).

In the past decade, studies of spermatozoal biology have solidified the notion (Mann, 1954) that one should not consider as separate, discrete events the processes of spermatogenesis, epididymal maturation of sperm, storage of sperm outside of the animal body, transit of sperm into the female reproductive tract, capacitation and activation of sperm, and fertilization. Rather, following completion of the second division of meiosis, the newly formed spermatid undergoes a series of morphologic, biochemical, and membrane changes which continue after release of a spermatozoon from the germinal epithelium, during its transit through the epididymis and the female reproductive tract, and culminate in the process of fertilization. Thus, observed spermatozoal characteristics may have been induced by preceding events. Similarly, modifications of the normal process, as induced by freezing and thawing spermatozoa or by artificial insemination of fresh or frozen semen, almost certainly change the nature and timing of subsequent events of the normal process. Such changes, even if undetected, may be responsible for the observed variation among males in fertility of frozen semen.

Liquid Semen

By the mid-1950's, artificial insemination of cattle using bull semen extended in either egg yolk citrate or heated cows' milk was routine (Salisbury and VanDemark, 1961). Inclusion of the antibiotics, penicillin and streptomycin, was an accepted practice. For semen stored at 5° C, fertility was satisfactory for two days following collection and extension. Procedures also were available for short-term preservation of semen from boars, rams and stallions (Maule, 1962), although insemination of fresh semen was more conventional.

In the late 1950's, two approaches were developed to obtain satisfactory conception rates for 3 to 5 d following extension of bull semen. One utilized egg yolk extenders with a complex buffer that was gassed with CO_2 (VanDemark and Sharma, 1957) or generated CO_2 from bicarbonate and citric acid incorporated in the buffer (Foote et al., 1960). The second was based upon the addition at 5° C of 10% glycerol to semen extended in heated milk (Almquist, 1962). Both the CO_2-containing and glycerol-containing extenders gave marked improvements in conception rate for semen used on the third and fourth day after collection. A variety of other buffers have been evaluated (Salisbury et al., 1978). Inclusion of caproic acid in a citrate-buffered egg yolk extender gassed with nitrogen (Shannon, 1972) has been used routinely to the present time in New Zealand.

The economics of artificial insemination of sheep and especially horses and swine is very different from the situation with cattle because of the number of females that can be inseminated with one ejaculate. With cattle, up to 1,000 cows can be inseminated with one ejaculate. With boar, ram or stallion semen, however, insemination of only 5 to 50 females per ejaculate is possible. This limitation has reduced the potential genetic and economic impacts of artificial insemination for those species. Procedures for storage of boar, ram and stallion semen have been reviewed (Graham et al., 1978).

Frozen Semen

The advent of frozen semen (Polge et al., 1949), and especially its wide-scale adoption for artificial insemination of cattle, revolutionized cattle breeding. Initially, semen was packaged in 1-ml glass ampules and frozen and stored in alcohol cooled with solid carbon dioxide (Salisbury and VanDemark, 1961). This system was used commercially with frozen semen in the U.S. However, the widespread adoption and international use of frozen semen came only after the development of superinsulated, cryogenic field storage units in 1959 and reports that use of liquid nitrogen, to provide storage at −196° C, resulted in higher conception rates than storage at −79° C and essentially indefinite storage (Salisbury et al., 1978; Herman, 1981). For about 15 yr after glycerol was shown to be a suitable cryoprotective agent for freezing bull spermatozoa, an extraordinary effort was aimed at development of improved extenders and procedures for packaging and freezing semen, and on the interaction between

freezing rate and thawing rate (Pickett et al., 1978; Salisbury et al., 1978).

The introduction of plastic straws in 1964 resulted in more efficient storage of semen, reduced losses of sperm during insemination, and gave a slight improvement in conception rate (Pickett and Berndtson, 1974). In most parts of the world, semen now is packaged in 0.25-ml straws, although in the U.S. the 0.5-ml straw is standard. Freezing semen in the form of a small pellet has been tested. This approach never received widespread acceptance with bull semen, but has been used with boar (Johnson et al., 1981b) and ram (Salamon and Lightfoot, 1970) semen. Even with the best procedures for freezing and thawing bull sperm, at least 20% of the sperm are rendered immotile. Damage probably is caused by the interactions of two physical factors (Watson, 1979). These are a) formation of ice crystals that damage the spermatozoa and b) increases in solute concentration as pure water is withdrawn by crystallization of the extracellular solvent.

Conception rates for cattle inseminated with frozen and thawed semen potentially are equivalent to those obtained by insemination of unfrozen semen or by natural service. In some situations, this potential is not achieved because of inaccurate detection of estrus or faulty insemination technique. The success of procedures used to freeze bovine spermatozoa is exemplified best by the drastic reduction in the number of sperm used for each insemination dose. For many bulls of known fertility, it is possible to reduce the number of motile sperm per insemination dose to 4×10^6 without reducing conception rate (Pace et al., 1981). Consequently, as many as 100,000 insemination doses can be processed annually from a superior sire and numerous Holstein bulls have been mated to more than 50,000 cows annually.

Although the application of frozen semen technology to cattle breeding progressed rapidly during the early 1960's, application with other species has been limited. Conception rates and litter size achieved with frozen boar semen are lower than with unfrozen semen (Johnson et al., 1981b). Similarly, results with frozen ram (Graham et al., 1978) and stallion (Klug et al., 1980; Loomis et al., 1983) semen have been disappointing. There are distinct species' differences in the extender required for optimum survival of sperm during the freezing-thawing process (Watson, 1979). With some species, it is essential to change the composition of the extender after thawing the spermatozoa to maximize conception rate (Larsson and Einarsson, 1976). However, with all three species, the results are adequate to allow preservation and use with valuable germ plasm when breed regulations permit this.

The Future

By the year 2,000, animal scientists will have expanded our basic knowledge of male reproductive physiology to the point that farmers and ranchers will have many options for manipulation of reproductive processes. The extent to which available processes are applied will be determined by the economic tradeoffs associated with producing food and fiber. It is reasonable to expect that efficient exploitation of genetically superior sires of all classes of livestock will have increased. However, the greatest impact of this research will be on dairy cattle and swine.

Many future sires will be conceived as a result of planned matings accomplished by artificial insemination followed by sexing and splitting of embryos, transfer of desirable embryos to appropriate recipients, and possibly manipulation of reproductive development before or after birth. The objective of these manipulations will be to produce individuals genetically superior for economically important traits which also have an enhanced reproductive capacity. Manipulations of the neuroendocrine system should allow reduction of the age at puberty and development of males producing more sperm than today's sires. Although it is unlikely that the number of sperm produced per gram of testis can be increased greatly above current values, the normal attrition of potential germ cells may be reduced. The sexual behavior of sires used in artificial insemination may be modified by hormones, pheromones or drugs acting on the central nervous system to minimize the time required for semen collection. Manipulation of the seasonal pattern in sperm production of rams and stallions will be commonplace.

Artificial insemination of beef cattle and sheep will increase, but will remain at a far lower percentage saturation than for dairy cattle or swine. Even with superior methods

for control of estrus and ovulation, it probably will not be economical to use artificial insemination with herds and flocks maintained on the range. It seems inevitable that the majority of the sheep and beef cattle will continue to be bred by natural mating under conditions which maximize the utilization of forage but make difficult, or preclude, application of high technology management practices. However, sires used for natural mating will have undergone a breeding soundness examination because farmers and ranchers will have recognized that failure to select sires for reproductive performance is a false economy.

Techniques for artificial insemination of cattle with frozen semen probably will not change dramatically. However, the artificial insemination of swine using non-frozen semen may become a widely accepted practice. This will occur if our understanding of sperm membrane physiology allows development of techniques that enable normal conception rates and litter size following insemination of far fewer sperm than currently are necessary. Improved procedures for freezing sperm from the boar, ram and stallion may provide increased survival of these sperm within the female reproductive tract. Conception rates using frozen semen with these three species should approach that attainable with unfrozen semen, as has been true with cattle for the past decade. The greatest impact of this line of research will be a great reduction in the number of sperm required for successful insemination of swine. For all species the accuracy and ease of detecting estrus may be improved.

Insemination of frozen semen enriched for sperm bearing either the X- or the Y-chromosome, with at least 80% offspring of the desired sex, seems likely. Attainment of this goal is reasonable because of the recent development of procedures for monitoring the effectiveness, or lack thereof, of a given separation technique. Although application of sexed semen in commercial dairy herds seems likely, its impact on other species of livestock may be marginal. The utilization of freeze-dried spermatozoa for routine artificial insemination is not envisioned. Under special circumstances, however, lyophilized spermatozoa may be directly injected into an oocyte with resulting embryonic development.

By the year 2,000, a modern semen laboratory still will have a phase-contrast microscope to allow visual examination of semen quality. However, spermatozoal motility, velocity and concentration probably will be measured by dipping a fiber optic probe into a semen sample. Sperm morphology may be evaluated by computer. In the research laboratory, the quality of frozen and thawed spermatozoa will be evaluated by measuring selected attributes of the spermatozoal plasma membrane in addition to an automated assessment of spermatozoal motility. The role of the spermatozoon as a cause of embryo death may be clarified.

As farmers and ranchers enter the twenty-first century, they will have an unprecedented array of procedures to modify reproductive function in the male. Benefits derived from these procedures certainly will contribute to humanity through enhanced efficiency of food and fiber production.

Literature Cited

Albert, A. 1961. The mammalian testis. In: W. C. Young and G. W. Corner (Ed.) Sex and Internal Secretions (3rd Ed.). Vol. 1. p 305. The Williams & Wilkins Co., Baltimore, MD.

Almquist, J. O. 1962. Diluents for bovine semen. XI. Effect of glycerol on fertility and motility of spermatozoa in homogenized and skim milk. J. Dairy Sci. 45:911.

Almquist, J. O. 1973. Effects of sexual preparation on sperm output, semen characteristics and sexual activity of beef bulls with a comparison to dairy bulls. J. Anim. Sci. 36:331.

Almquist, J. O. 1982. Effect of long term ejaculation at high frequency on output of sperm, sexual behavior, and fertility of Holstein bulls; relation of reproductive capacity to high nutrient allowance. J. Dairy Sci. 65:814.

Almquist, J. O. and E. B. Hale. 1956. An approach to the measurement of sexual behavior and semen production of dairy bulls. Proc. 3rd Inter. Congr. Anim. Reprod. and A.I., Plenary Papers: 50.

Amann, R. P. 1970. Sperm production rates. In: A. D. Johnson, W. R. Gomes, and N. L. VanDemark (Ed.). The Testis. Vol 1. p 433. Academic Press, NY.

Amann, R. P. 1981. A critical review of methods for evaluation of spermatogenesis from seminal characteristics. J. Androl. 2:37.

Amann, R. P. 1982. Use of animal models for detecting specific alterations in reproduction. Fund Appl. Toxicol. 2:13.

Amann, R. P. 1983. Endocrine changes associated with onset of spermatogenesis in Holstein bulls. J. Dairy Sci. 66:(In press).

Amann, R. P. and J. O. Almquist. 1962. Reproductive capacity of dairy bulls. VI. Effect of unilateral

vasectomy and ejaculation frequency on sperm reserves; aspects of epididymal physiology. J. Reprod. Fertil. 3:260.

Amann, R. P. and J. O. Almquist. 1976. Bull management to maximize sperm output. Proc. 6th Tech. Conf. Artif. Insem. Reprod. p 1. Nat. Assoc. Anim. Breeders, Columbia.

Amann, R. P. and V. K. Ganjam. 1976. Steroid production by the bovine testis and steroid transfer across the pampiniform plexus. Biol. Reprod. 15:695.

Amann, R. P. and V. K. Ganjam. 1981. Effects of hemicastration or hCG-treatment on steroids in testicular vein and jugular vein blood of stallions. J. Androl. 3:132.

Amann, R. P. and O. J. Walker. 1983. Changes in the pituitary-gonadal axis associated with puberty in Holstein bulls. J. Anim. Sci. 57:(In press.)

Amann, R. P., J. F. Kavanaugh, L. C. Griel, Jr. and J. K. Voglmayr. 1976. Sperm production of Holstein bulls determined from testicular spermatid reserves, after cannulation of rete testis or vas deferens, and by daily ejaculation. J. Dairy Sci. 57:93.

Attal, J. and M. Courot. 1963. Développement testiculaire et établissement de la spermatogenèse chez le tauraeau. Ann. Biol. Anim. Bioch. Biophys. 3:219.

Baker, F. M., N. L. VanDemark and G. W. Salisbury. 1955. The effect of frequency of ejaculation on the semen production, seminal characteristics and libido of bulls during the first post-puberal year. J. Dairy Sci. 38:1000.

Ball, L. 1974. Electroejaculation of bulls. Proc. 5th Tech. Conf. A.I. and Reprod. p 101. Nat. Assoc. Anim. Breeders, Columbia.

Bedford, J. M. 1975. Maturation, transport and fate of spermatozoa in the epididymis. In: D. W. Hamilton and R. O. Greep (Ed.) Handbook of Physiology Sect. 7. Vol. 5. Male Reproductive System. p 303. Amer. Physiol. Soc., Washington, DC.

Bedford, J. M. 1978. Anatomical evidence for the epididymis as the prime mover in the evolution of the scrotum. Amer. J. Anat. 152:483.

Bedrak, E. and L. T. Samuels. 1969. Steroid biosynthesis by the equine testis. Endocrinology 85:1186.

Bellvé, A. R. 1979. The molecular biology of mammalian spermatogenesis. Oxford Rev. Biol. 1:159.

Bellvé, A. R. 1982. Biogenesis of the mammalian spermatozoon. In: R. P. Amann and G. E. Seidel, Jr., (Ed.). Prospects for Sexing Mammalian Sperm. p 69. Colorado Assoc. Univ. Press, Boulder.

Benoit, M. J. 1926. Recherches anatomiques, cytologiques et histophysiologiques sur les voies excretrice du testicle, chez les mammifères. Arch. Anat. 5:173.

Berndtson, W. E. 1977. Methods for quantifying mammalian spermatogenesis: A review. J. Anim. Sci. 44:818.

Berndtson, W. E. and C. Desjardins. 1974. The cycle of the seminiferous epithelium and spermatogenesis in the bovine testis. Amer. J. Anat. 140:167.

Berndtson, W. E., J. H. Hoyer, E. L. Squires and B. W. Pickett. 1979. Influence of exogenous testosterone on sperm production, seminal quality and libido of stallions. J. Reprod. Fertil. (Suppl. 27):19.

Blanc, M. R., M.-T. Hochereau-de Reviers, C. Cahoreau, M. Courot and J. C. Dacheux. 1981. Inhibin: effects on gonadotropin secretion and testis function in ram and rat. In: P. Franchimont and C. P. Channing (Ed.). Intragonadal Regulation of Reproduction. p 299. Academic Press, NY.

Bolender, R. P. 1982. Sterology and its uses in cell biology. An. NY Acad. Sci. 383:1.

Booth, W. D. 1982. Testicular steroids and boar taint. In: D.J.A. Cole and G. R. Foxchroft (Ed.). Control of Pig Reproduction. p 25. Butterworth, London.

Branton, C., G. D'Arensborourgh and J. E. Johnston. 1952. Semen production, fructose content of semen and fertility of dairy bulls as related to sexual excitement. J. Dairy Sci. 35: 801.

Bratton, R. W. and R. H. Foote. 1954. Semen production and fertility of dairy bulls ejaculated either once or twice at intervals of either four or eight days. J. Dairy Sci. 37:1439.

Bremner, W. J. 1981. Historical aspects of the study of the testis. In: H. Burger and D. de Kretser (Ed.). The Testis. p 1. Raven Press, NY.

Burgus, R., M. Butcher, M. Amoss, N. Ling, M. Monahan, J. Rivier, R. Fellows, R. Blackwell, W. Vale and R. Guillemin. 1971. Structure moleculaire du facteur hypothalamique (LRF) d'origine ovine controlant la secretion de l'hormone gonadotrope hypophysaire de luteinisation (LH). C. R. Acad. Sci. (Paris) 273: 1611.

Cascieri, M., R. P. Amann and R. H. Hammerstedt. 1976. Adenine nucleotide changes at initiation of bull sperm motility. J. Biol. Chem. 251:787.

Chenoweth, P. J. 1983. Sexual behavior in the bull: a review. J. Dairy Sci. 66:173.

Clegg, M. T., J. A. Santolucito, J. D. Smith and W. F. Ganong. 1958. The effect of hypothalamic lesions on sexual behavior and estrous cycles in the ewe. Endocrinology 62:790.

Clermont, Y. and C. P. Leblond. 1955. Spermiogenesis of man, monkey, ram and other mammals as shown by the "periodic acid-Schiff" technique. Amer. J. Anat. 96:229.

Collins, W. J., R. W. Bratton and C. R. Henderson. 1951. The relationship of semen production to sexual excitement of dairy bulls. J. Dairy Sci. 34:224.

Coulter, G. H. 1980. Testicular development: its management and significance in young beef bulls. Proc. 8th Tech. Conf. p 106. Nat. Assoc. Anim. Breeders, Columbia.

Courot, M. 1967. Endocrine control of the supporting and germ cells of the impuberal testis. J. Reprod. Fertil. 2:89.

Courot, M. 1971. Établissement de la spermatogénèse chez l'agneau (Ovis aries) Étude expérimentale de son contrôle gonadotrope; impor-

tance des cellules de la lignée sertolienne. D. Sc. Thesis, Univ. Paris, Paris.
Courot, M., M.-T. Hochereau-de Reviers and R. Ortavant. 1970. Spermatogenesis. In: A. D. Johnson, W. R. Gomes, and N. L. VanDemark (Ed.). The Testis. Vol. 1. p 339. Academic Press, New York.
Curtis, S. K. and R. P. Amann. 1981. Testicular development and establishment of spermatogenesis in Holstein bulls. J. Anim. Sci. 53:1645.
David, K., E. Dingemanse, J. Freud and E. Laquer. 1935. Uber krystallinisches mannliches Hormon aus Hoden (Testosteron) wirksamer als aus Harn oder aus Cholesterin bereitetes Androsteron. Z. Physiol. Chem. 233:281.
Davidson, J. M. 1974. Hypothalamic-pituitary regulation of puberty, evidence from animal experimentation. In: The Control of Onset of Puberty. p 79.
de Graaf, R. 1668. A treatise concerning the generative organs of men. Translated by H. D. Jocelyn and B. P. Setchell. J. Reprod. Fertil. (Suppl. 17):1.
D'Occhio, M. J., J. E. Kinder and B. D. Schanbacher. 1982a. Patterns of LH secretion in castrated bulls (steers) during intravenous infusion of androgenic and estrogenic steroids: pituitary response to exogenous luteinizing hormone releasing hormone. Biol. Reprod. 26:249.
D'Occhio, M. J., B. D. Schanbacher and J. E. Kinder. 1982b. Relationship between serum testosterone concentration and patterns of luteinizing hormone secretion in male sheep. Endocrinology 110:1547.
D'Occhio, M. J., B. D. Schanbacher and J. E. Kinder. 1982c. Testosterone feedback on FSH secretion in male sheep. J. Reprod. Fertil. 66:699.
Dukelow, W. R., E. C. Frederick and E. F. Graham. 1960. Frequency of ejaculation in the bovine. J. Dairy Sci. 43:1335.
Dym, M. and D. W. Fawcett. 1971. Further observations on the numbers of spermatogonia, spermatocytes and spermatids connected by intercellular bridges in the mammalian testis. Biol. Reprod. 4:195.
Ewing, L. L. and B. L. Brown. 1977. Testicular steroidogenesis. In: A. D. Johnson and W. R. Gomes (Ed.). The Testis. Vol. 4. p 239. Academic Press, New York.
Fawcett, D. W., L. V. Leak and P. M. Heidger, Jr. 1970. Electron microscopic observations on the structural components of the blood-testis barrier. J. Reprod. Fertil. (Suppl. 10):105.
Flavo, R. E. and A. V. Nalbandov. 1974. Radioimmunoassay of peripheral plasma testosterone in males from eight species using specific antibody without chromotography. Endocrinology 95:1466.
FlorCruz, S. V. and K. R. Lapwood. 1978. A longitudinal study of pubertal development in boars: Investigation of the relationship between gonadal and epididymal development and plasma luteinizing hormone and testosterone profiles. Int. J. Androl. 1:317.
Foldesy, R. G. and J. M. Bedford. 1982. Biology of the scrotum. I. Temperature and androgen as determinants of the sperm storage capacity of the rat cauda epididymidis. Biol. Reprod. 26:673.
Foote, R. H. 1969. Research techniques to study reproductive physiology in the male. In: Techniques and Procedures in Animal Science Research. p 80. Amer. Soc. Anim. Sci., Champaign, IL.
Foote, R. H. 1978. Factors influencing the quantity and quality of semen harvested from bulls, rams, boars and stallions. J. Anim. Sci. 47(Suppl. 2):1.
Foote, R. H., L. C. Gray, D. C. Young and H. O. Dunn. 1960. Fertility of bull semen stored up to four days at 5°C in 20% egg yolk extenders. J. Dairy Sci. 43:1330.
Ford, J. J. 1982. Testicular control of defeminization in male pigs. Biol. Reprod. 27:425.
Ford, J. J. and B. D. Schanbacher. 1977. Luteinizing hormone secretion and female lordosis behavior in male pigs. Endocrinology 100:1033.
Foster, D. L., I. H. Mickelson, K. D. Ryan, G. A. Coon, R. A. Drongowski and J. A. Holt. 1978. Ontogeny of pulsatile luteinizing hormone and testosterone secretion in male lambs. Endocrinology 102:1137.
Fournier-Delpech, S., G. Colas, M. Courot, R. Ortavant and G. Brice. 1979. Epididymal sperm maturation in the ram: motility, fertilizing ability and embryonic survival after uterine artificial insemination in the ewe. Ann. Biol. Anim. Bioch. Biophys. 19:597.
Franchimont, P., K. Henderson, G. Verhoeven, M. T. Hazee-Hagelstein, C. Charlet-Renard, A. Demoulin, J. P. Bourguignon and M. J. Lecomte-Yerna. 1981. Inhibin: mechanisms of action and secretion. In: P. Franchimont and C. P. Channing (Ed.). Intragonadal Regulation of Reproduction. p 167. Academic Press, New York.
Frankenhuis, M. T., D. G. de Rooij and M. F. Kramer. 1980. Spermatogenesis in the pig. Proc. 9th Inter. Congr. Anim. Reprod. and A.I. 5:17.
Gier, H. T. and G. B. Marion. 1970. Development of the mammalian testis. In: A. D. Johnson, W. R. Gomes and N. L. Vandemark, (Ed.). The Testis. Vol. 1. p 1. Academic Press, New York.
Glover, T. and Nicander, L. 1971. Some aspects of structure and function in the mammalian epididymis. J. Reprod. Fertil. (Suppl. 13):39.
Graham, E. F., B. G. Crabo and M. M. Pace. 1978. Current status of semen preservation in the ram, boar and stallion. J. Anim. Sci. 47(Suppl. 2):80.
Greep, R. O., H. L. Fevold and F. L. Hisaw. 1936. Effect of two hypophyseal gonadotropic hormones on the reproductive system of the male rat. Anat. Rec. 65:261.
Hafs, H. D. and M. S. McCarthy. 1979. Endocrine control of testicular function. In: H. W. Hawk, C. A. Kiddy and H. C. Cecil (Ed.). Animal Reproduction. p 345. John Wiley & Sons, New York.
Hafs, H. D., R. S. Hoyt and R. W. Bratton. 1959. Libido, sperm characteristics, sperm output, and fertility of mature dairy bulls ejaculated daily or weekly for thirty-two weeks. J. Dairy Sci. 42:626.
Hafs, H. D., R. C. Knisely and C. Desjardins. 1962.

Sperm output of dairy bulls with varying degrees of sexual preparation. J. Dairy Sci. 45:788.
Hale, E. B. 1966. Visual stimuli and reproductive behavior in bulls. J. Anim. Sci. 25(Suppl.):36.
Hale, E. B. and J. O. Almquist. 1960. Relation of sexual behavior to germ cell output in farm animals. J. Dairy Sci. 43(Suppl.):145.
Hammerstedt, R. H. 1981. Monitoring the metabolic rate of germ cells and sperm. In: K. W. McKerns (Ed.). Reproductive Processes and Contraception. p 353. Plenum Press, New York.
Hammerstedt, R. H., S. R. Hay and R. P. Amann. 1982. Modification of ram sperm membranes during epididymal transit. Biol. Reprod. 27:745.
Haynes, N. B. and B. D. Schanbacher. 1983. The control of reproductive activity in the ram. In: W. Haresign (Ed.). Sheep Production. p 441. Butterworths, London.
Hemsworth, P. H. and D. B. Galloway. 1979. The effect of sexual stimulation on the sperm output of the domestic boar. Anim. Reprod. Sci. 2:387.
Herman, H. A. 1981. Improving Cattle by the Millions. Univ. of Missouri Press, Columbia.
Hochereau, M.-T., M. Courot and R. Ortavant. 1964. Marquage des cellules germinales du bélier et du taureau par injection de thymidine tritiée dans l'artère spermatique. Ann. Biol. Anim. Bioch. Biophys. 4:157.
Hochereau-de Reviers, M.-T. 1981. Control of spermatogonial multiplication. In: K. W. McKerns (Ed.). Reproductive Processes and Contraception. p 307. Plenum Press, New York.
Hochereau-de Reviers, M.-T. and M. Courot. 1978. Sertoli cells and development of seminiferous epithelium. Ann. Biol. Anim. Biochem. Biophys. 18:573.
Holtz, W. and D. Smidt. 1976. The fertilizing capacity of epididymal spermatozoa in the pig. J. Reprod. Fertil. 46:227.
Hooker, C. W. 1944. The postnatal history and function of the interstitial cells of the testis of the bull. Amer. J. Anat. 74:1.
Hooker, C. W. 1970. The intertubular tissue of the testis. In: A. D. Johnson, W. R. Gomes and N. L. VanDemark (Ed.). The Testis. Vol. 1. p 483. Academic Press, New York.
Huggins, C. and P. V. Moulder. 1945. Estrogen production by Sertoli cell tumors of the testes. Cancer Res. 5:510.
Johnson, L. and D. L. Thompson, Jr. 1983. Age related and seasonal variation in the Sertoli cell population, daily sperm production, and serum concentrations of follicle stimulating hormone, lutenizing hormone and testosterone in stallions. Biol. Reprod. (In press).
Johnson, L., R. P. Amann and B. W. Pickett. 1980. Maturation of equine spermatozoa. Amer. J. Vet. Res. 41:1190.
Johnson, L., C. S. Petty and W. B. Neaves. 1981a. A new approach to quantification of spermatogenesis and its applications to germinal cell attrition during human spermiogenesis. Biol. Reprod. 25:217.
Johnson, L. A., J. G. Aalbers, J. T. Willems and W. Sybesma. 1981b. Use of boar spermatozoa for artificial insemination. I. Fertilizing capacity of fresh and frozen spermatozoa in sows on 36 farms. J. Anim. Sci. 52:1130.
Josso, N., J.-Y. Picard and D. Tran. 1977. The anti-Müllerian hormone. Rec. Prog. Horm. Res. 33:117.
Josso, N., J.-Y. Picard, J. L. Dacheux and M. Courot. 1979. Detection of anti-Müllerian activity in boar rete testis fluid. J. Reprod. Fertil. 57:397.
Jost, A. 1970. Hormonal factors in the sex differentiation of the mammalian fetus. Phil. Trans. Roy. Soc. (London) B.259:119.
Jost, A. B. Vigier and J. Prepin. 1972. Freemartins in cattle: the first steps of sexual organogenesis. J. Reprod. Fertil. 29:349.
Jost, A., B. Vigier, J. Prepin and J. P. Perchellet. 1973. Studies on sex differentiation in mammals. Rec. Prog. Horm. Res. 29:1.
Keogh, E. J., V.W.K. Lee, G. C. Rennie, H. G. Burger, B. Hudson and D. M. de Kretser. 1976. Selective suppression of FSH by testicular extracts. Endocrinology 98:997.
Klug, E., J. C. Martin and G. Kluge. 1980. Besamungsstation im Landgestüt Celle – Ergebnisbericht aus der Saison 1979. Hannoversches Pferd 54:63.
Koefoed-Johnsen, H. H. 1958. Undersøgelser over saeddannelsen. p 18. Aarsberetning, Kgl. Veter-Landbohøjskole, Inst. Sterilitetsforskning, Copenhagen.
Koefoed-Johnsen, H. H. 1964. Spermieproduktion hos tyre. Udskillelsen af spermier med urinen ved forskellige saedopsamlingshyppigheder. p 23. Aarsberetning. Kgl. Veter-Landbohøjskole, Inst. Sterilitetsforskning, Copenhagen.
Kratz, J. L., C. J. Wilcox, F. G. Martin and R. B. Becker. 1983. Vital statistics and reasons for disposal of United States and Canadian artificial insemination dairy sires. J. Dairy Sci. 66:642.
Lacroix, A. and J. Pelletier. 1979. LH and testosterone release in developing bulls following LH-RH treatment. Effect of gonadectomy and chronic testosterone propionate pre-treatment. Acta Endocrinol. 91:719.
Land, R. B. and W. R. Carr. 1975. Testis growth and plasma LH concentration following hemicastration and its relation with female prolificacy in sheep. J. Reprod. Fertil. 45:495.
Larsson, K. and S. Einarsson. 1976. Fertility of deep frozen boar spermatozoa. Influence of thawing diluents and of boars. Acta Vet. Scand. 17:43.
Levasseur, M.-C. 1977. Thoughts on puberty. Initiation of the gonadotropic function. Ann. Biol. Anim. Biochem. Biophys. 17:345.
Levine, J. E., K.-Y. F. Pau, V. D. Ramirez and G. L. Jackson. 1982. Simultaneous measurement of luteinizing hormone release in unanesthetized, ovariectomized sheep. Endocrinology 111:1449.
Lincoln, G. A. 1979. Differential control of luteinizing hormone and follicle-stimulating hormone by luteinizing hormones releasing hormone in the ram. J. Endocrinol. 80:133.
Lincoln, G. A. 1981. Seasonal aspects of testicular function. In: H. Burger and D. de Kretser (Ed.). The Testis. p 255. Raven Press, New York.
Lincoln, G. A. and R. V. Short. 1980. Seasonal breeding: nature's contraceptive. Rec. Prog. Horm. Res. 36:1.

Lindner, H. R. 1959. Androgens in the bovine testis and spermatic vein blood. Nature (Lond) 183: 1605.

Lindner, H. R. 1961. Androgens and related compounds in the spermatic vein blood of domestic animals. IV. Testicular androgens in the ram, boar and stallion. J. Endocrinol. 23:171.

Lino, B. F. 1971. Cell count correction factors for the quantitative histological analysis of the germinal epithelium of the ram. Anat. Rec. 170:413.

Lino, B. F. 1972. The output of spermatozoa in rams. II. Relationship to scrotal circumference, testis weight, and the number of spermatozoa in different parts of the urogenital tract. Australian J. Biol. Sci. 25:359.

Lino, B. F. and A.W.H. Braden. 1972. The output of spermatozoa in rams. I. Relationship with testicular output of spermatozoa and the effect of ejaculations. Australian J. Biol. Sci. 25: 351.

Loomis, P. R., R. P. Amann, E. L. Squires and B. W. Pickett. 1983. Fertility of unfrozen and frozen stallion spermatozoa extended in EDTA-lactose-egg yolk and packaged in straws. J. Anim. Sci. 56:687.

Mann, T. 1954. The Biochemistry of Semen. Methuen & Co., Ltd. London.

Mann, T. and C. Lutwak-Mann. 1981. Male Reproductive Function and Semen. Springer-Verlag, Berlin.

McCullagh, D. R. 1932. Dual endocrine activity of testes. Science 76:19.

McNeilly, Judith R., A. S. McNeilly, J. S. Walton and F. J. Cunningham. 1976. Development and application of a heterologous radioimmunoassay for ovine follicle-stimulating hormone. J. Endocrinol. 70:69.

Martig, R. C., J. O. Almquist and J. Foster. 1970. Reproductive capacity of beef bulls. V. Fertility and freezability of successive ejaculates collected by different methods. J. Anim. Sci. 30:60.

Matsuo, H., R.M.G. Nair, A. Arimura and A. V. Schally. 1971. Structure of the porcine LH- and FSH-releasing hormone: the proposed amino acid sequence. Biochem. Biophys. Res. Comm. 43:1334.

Maule, J. P. 1962. The Semen of Animals and Artificial Insemination. Commonwealth Agric. Bureaux, Farnham Royal.

Meyer, A. W. 1938. The discovery and the earliest representation of spermatozoa. Bull. Inst. Hist. Med. 6:89.

Midgley, A. R., Jr., G. D. Niswender and J. Sri Ram. 1969. Hapten-radioimmunoassay: a general procedure for the estimation of steroidal and other haptenic substances. Steroids 13:731.

Monahan, M., J. Rivier, R. Burgus, M. Amoss, R. Blackwell, W. Vale and R. Guillemin. 1971. Synthèse totale par phase solide d'un décapeptide qui stimule la sécrétion des gonadotropines hypophysaires LH et FSH. C.R. Acad. Sci. (Paris) 273:508.

Neaves, W. B. 1973. Permeability of Sertoli cell tight junctions to lanthanum after ligation of ductus deferens and ductus efferentes. J. Cell Biol. 59:559.

Neaves, W. B. 1977. The blood-testis barrier. In: A. D. Johnson and W. R. Gomes (Ed.). The Testis. Vol. 4. p 126. Academic Press, New York.

Nett, T. M. and T. E. Adams. 1977. Further studies on the radioimmunoassay of gonadotropin-releasing hormone: effect of immunoreactivity in serum. Endocrinology 101:1135.

Nicander, L. 1958. Studies on the regional histology and cytochemistry of the ductus epididymidis in stallions, rams and bulls. Acta Morphol. Neerlando. Scand. 1:337.

Niswender, G. D., L. E. Reichert, A. R. Midgley and A. V. Nalbandov. 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84:1166.

Oh, R. and B. Tamaoki. 1970. Steroidogenesis in equine testis. Acta Endocrinol. 64:1.

Orgebin-Crist, M.-C. and N. Jahad. 1978. The maturation of rabbit epididymal spermatozoa in organ culture: inhibition by antiandrogens and inhibitors of ribonuncleic acid and protein synthesis. Endocrinology 103:46.

Orgebin-Crist, M.-C., B. J. Danzo and J. Davies. 1975. Endocrine control of the development and maintenance of sperm fertilizing ability in the epididymis. In: D. W. Hamilton and R. O. Greep (Ed.). Handbook of Physiology, Sect. 7, Vol. V. Male Reproductive System. p 319. Amer. Physiol. Soc., Washington, DC.

Orgebin-Crist, M.-C., G. E. Olson and B. J. Danzo. 1981. Factors influencing maturation of spermatozoa in the epididymis. In: P. Franchimont and C. P. Channing (Ed.). Intragonadol Regulation of Reproduction. p 393. Academic Press, New York.

Ortavant, R. 1958. Le cycle spermatogénétique chez le bélier. D. Sci. Thesis. Univ. Paris, Paris.

Ortavant, R. 1977. Photoperiodic regulation of reproduction in sheep. In: Management of Reproduction in Sheep and Goats. p 58. Amer. Soc. Anim. Sci., Champaign, IL.

Ortavant, R., M. Courot, and M.-T. Hochereau-de Reviers. 1977. Spermatogenesis in domestic animals. In: H. H. Cole and P. T. Cupps (Ed.). Reproduction in Domestic Animals, 3rd Ed. p 203. Academic Press, New York.

Pace, M. M., J. J. Sullivan, F. I. Elliott, E. F. Graham and G. H. Coulter. 1981. Effects of thawing temperature, number of spermatozoa and spermatozoal quality on fertility of bovine spermatozoa packaged in .5-ml French straws. J. Anim. Sci. 53:693.

Parrott, R. F. and R. V. Davies. 1979. Serum gonadotrophin levels in prepubertally castrated male sheep treated for long periods with propionated testosterone, dihydrotestosterone, 19-hydroxytestosterone or oestradiol. J. Reprod. Fertil. 56:543.

Paufler, S. K. and R. H. Foote. 1969. Sperm retention and resorption in sexually active rabbits after epididymal ligatures. Proc. Soc. Exp. Biol. Med. 131:1179.

Pickett, B. W. and W. E. Berndtson. 1974. Preservation of bovine spermatozoa by freezing in straws: a review. J. Dairy Sci. 57:1287.

Pickett, B. W. and J. L. Voss. 1973. Reproductive management of the stallion. Colo. State Univ. Gen. Series Bull. no. 934.

Pickett, B. W., W. E. Berndtson and J. J. Sullivan. 1978. Influence of seminal additives and packaging systems on fertility of frozen bovine spermatozoa. J. Anim. Sci. 47(Suppl. 2):12.

Pickett, B. W., J. L. Voss, E. L. Squires and R. P. Amann. 1981. Management of the stallion for maximum reproductive efficiency. Colo. State Univ. Gen. Series Bull. no. 1005.

Polge, C., A. Smith and A. S. Parkes. 1949. Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature (London) 164:666.

Ramirez, V. D. and S. M. McCann. 1963. A comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. Endocrinology 72:452.

Ravault, J. P., M. Blanc, R. Ortavant, J. Pelletier and M. M. de Reviers. 1980. Variations circadiennes et circannuelles de la sécrétion de prolactine, LH et FSH chez les animaux domestiques males. In: R. Ortavant and A. Reinberg. Rythmes et Reproduction. p 115. Masson, Paris.

Regaud, C. 1901. Étude sur la structure des tubes séminifères et sur la spermatogénèse chez les mammiferes. Arch. Anat. Micr. 4:101.

Ritzén, E. M., V. Hansson and F. S. French. 1981. The Sertoli cell. In: H. Burger and D. de Kretser (Ed.). The Testis. p 171. Raven Press, New York.

Saacke, R. G. and J. O. Almquist. 1964. Ultrastructure of bovine spermatozoa. I. The head of normal, ejaculated sperm. Amer. J. Anat. 115: 143.

Salamon, S. 1962. Studies on the artificial insemination of Merino sheep. III. The effect of frequent ejaculation on semen characteristics and fertilizing capacity. Australian J. Agr. Res. 13: 1137.

Salamon, S. and R. J. Lightfoot. 1970. Fertility of ram spermatozoa frozen by the pellet method. III. The effects of insemination technique, oxytocin and relaxin on lambing. J. Reprod. Fertil. 22:409.

Salisbury, G. W. and N. L. VanDemark. 1961. Physiology of Reproduction and Artificial Insemination of Cattle. W. H. Freeman and Co., San Francisco.

Salisbury, G. W., N. L. VanDemark and J. R. Lodge. 1978. Physiology of Reproduction and Artificial Insemination of Cattle (2nd Ed.). W. H. Freeman and Co., San Francisco.

Schanbacher, B. D. 1979a. Relationship of in vitro gonadotropin binding to bovine testes and the onset of spermatogenesis. J. Anim. Sci. 48:591.

Schanbacher, B. D. 1979b. Testosterone secretion in cryptorchid and intact bulls injected with gonadotropin-releasing hormone and luteinizing hormone. Endocrinology 104:360.

Schanbacher, B. D. 1979c. Increased lamb production with rams exposed to short day lengths during the nonbreeding season. J. Anim. Sci. 49:927.

Schanbacher, B. D. 1982a. Responses of ram lambs to active immunization against testosterone and luteinizing hormone-releasing hormone. Amer. J. Physiol. 242:201.

Schanbacher, B. D. 1982b. Hormonal interrelationships between hypothalamus, pituitary and testis of rams and bulls. J. Anim. Sci. 55 (Suppl. 2):56.

Schanbacher, B. D. and M. J. D'Occhio. 1982. Validation of a direct radioimmunoassay for testosterone in unextracted serum from five species: application to study of the hypothalamic-pituitary-gonadal axis in males. J. Androl. 3:45.

Schanbacher, B. D. and J. J. Ford. 1977. Gonadotropin secretion in cryptorchid and castrate rams and the acute effects of exogenous steroid treatment. Endocrinology 100:387.

Schanbacher, B. D. and J. J. Ford. 1979. Photoperiodic regulation of ovine spermatogenesis: relationship to serum hormones. Biol. Reprod. 20:719.

Schanbacher, B. D., M. J. D'Occhio and T. W. Gettys. 1983. Pulsatile luteinizing hormone secretion in the castrate male bovine: effects of testosterone or estradiol replacement therapy. J. Anim. Sci. 56:132.

Schoenfeld, H. 1901. La Spermatogénèse chez le tareau et chez les mammiféres en général. Arch. de Biol. (Liege), 18:1.

Setchell, B. P. 1978. The Mammalian Testis. Cornell Univ. Press, Ithaca.

Setchell, B. P. 1980. The functional significance of the blood-testis barrier. J. Androl. 1:3.

Setchell, B. P., R. V. Davies and S. J. Main. 1977. Inhibin. In: A. D. Johnson and W. R. Gomes (Ed.). The Testis. Vol. 4. p 189. Academic Press, New York.

Shannon, P. 1972. The effect of egg yolk level and dose rate on conception rate of semen diluted in caprogen. Proc. 7th Inter. Congr. Anim. Reprod. and A.I. 2:1441.

Signoret, J. P. 1970. Reproductive behavior of pigs. J. Reprod. Fertil. (Suppl. 11):105.

Smith, P. E. 1927. The disabilities caused by hypophysectomy and their repair. J. Amer. Med. Assoc. 88:158.

Steinberger, A. 1981a. The Sertoli cell and its role in spermatogenesis. In: G. Frajese, E.S.E. Hafez, C. Conti and A. Fabbrini (Ed.). Oligozoospermia: Recent Progress in Andrology. p 35. Raven Press, New York.

Steinberger, A. 1981b. Regulation of inhibin secretion in the testis. In: P. Franchimont and C. P. Channing (Ed). Intragonadal Regulation of Reproduction. p 283. Academic Press, New York.

Swierstra, E. E. 1968a. Cytology and duration of the cycle of the seminiferous epithelium of the boar; duration of spermatozoan transit through the epididymis. Anat. Rec. 161:171.

Swierstra, E. E. 1968b. A comparison of spermatozoa production and spermatozoa output of Yorkshire and Lacombe boars. J. Reprod. Fertil. 17:459.

Swierstra, E. E. 1971. Sperm production of boars as measured from epididymal sperm reserves and quantitative testicular histology. J. Reprod. Fertil. 27:91.

Swierstra, E. E., M. R. Gebauer and B. W. Pickett. 1974. Reproductive physiology of the stallion.

I. Spermatogenesis and testis composition. J. Reprod. Fertil. 40:113.

Turner, T. T. and D. M. Cesarini. 1981. The effect of an aldosterone antagonist on the sperm concentrating ability of the rat epididymis. Biol. Reprod. 24(Suppl. 1):45A.

Turner, T. T., D.D'Addario and S. S. Howards. 1978. Further observations on the initiation of sperm motility. Biol. Reprod. 19:1095.

VanDemark, N. L. 1956. Quantitative aspects of semen production in bulls. Proc. 3rd Inter. Congr. Anim. Reprod. and A.I., Plenary Papers: 80.

VanDemark, N. L. and U. D. Sharma. 1957. Preliminary fertility results from the preservation of bovine semen at room temperatures. J. Dairy Sci. 40:438.

van der Molen, H. J. and F.F.G. Rommerts. 1981. Testicular steroidogenesis. In: H. Burger and D. de Kretser (Ed.). The Testis. p 213. Raven Press, New York.

van Straaten, H.W.M. and C.J.G. Wensing. 1978. Leydig cell development in the testis of the pig. Biol. Reprod. 18:86.

von Ebner, V. 1888. Zur Spermatogenese bie den Säugethieren. Arch. Mikr. Anat., 31:236.

Waites, G.M.H. and R. T. Gladwell. 1982. Physiological significance of fluid secretion in the testis and blood-testis barrier. Physiol. Rev. 62:624.

Walton, J. S., J. D. Evins and G.M.H. Waites. 1978. Feedback control of follicle-stimulating hormone in pre- and post-pubertal rams as revealed by hemicastration. J. Endocrinol. 77:75.

Walton, J. S., J. D. Evins, M. A. Hillard and G.M.H. Waites. 1980. Follicle-stimulating hormone release in hemicastrated prepubertal rams and its relationship to testicular development. J. Endocrinol. 84:141.

Watson, P. F. 1979. The preservation of semen in mammals. Oxford Rev. Biol. 1:283.

Wettemann, R. P., M. E. Wells, I. T. Omtvedt, C. E. Pope and E. J. Turman. 1978. Influence of elevated ambient temperature on reproductive performance of boars. J. Anim. Sci. 42:664.

Willett, E. L. and J. I. Ohms. 1957. Measurement of testicular size and its relation to production of spermatozoa by bulls. J. Dairy Sci. 40: 1559.

Wilson, J. D. and P. K. Siiteri. 1973. Developmental pattern of testosterone synthesis in the fetal gonad of the rabbit. Endocrinology 92:1182.

Wolf, F R., J. O. Almquist and E. B. Hale. 1965. Prepubertal behavior and puberal characteristics of beef bulls on high nutrient allowance. J. Anim. Sci. 24:761.

Yallow, R. S. and S. A. Berson. 1960. Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. 39:1157.

Young, W. C. 1931. A study of the function of the epididymis. III. Functional changes undergone by spermatozoa during their passage through the epididymis and vas deferens in the guinea pig. J. Exp. Biol. 8:151.

Zaneveld, L. J. and R. Chatterton. 1982. Biochemistry of Mammalian Reproduction. John Wiley & Sons, New York.

Zirkin, B. R., L. L. Ewing, N. Kromann and R. C. Cochran. 1980. Testosterone secretion by rat, rabbit, guinea pig, dog, and hamster testes perfused in vitro: correlation with Leydig cell ultrastructure. Endocrinology 106:1867.