Effect of selection for female reproductive rate on testicular physiology and histomorphology in Rambouillet rams

by Horacio C?ardenas Seijas

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science

Montana State University

Abstract:
Objectives of this study were to determine if scrotal circumference (SC), testicular weight (TW), seminal characteristics, histological components of the testicular parenchyma, capacity and affinity of the oLH/hCG receptor, ability and sensitivity of testicular parenchyma to secrete testosterone (T) in vitro after stimulation by oLH or hCG differed among Rambouillet rams from lines selected for low (LL) or high (HL) female reproductive rate and from a random-bred control line, (CL). Two trials were conducted, one during the winter of 1989 and the other during the winter of 1990. Lines had been selected for 19 and 20 years for each trial, respectively. Scrotal circumference (SC), testicular weight (TW) and in vitro T secretion after hCG were evaluated in Trial 1. These characteristics and in vitro secretion of T after oLH, receptor capacity and affinity, testicular histomorphology and seminal characteristics were evaluated in Trial 2. Rams among lines did not differ (P>.10) in SC and TW but LL rams had lower (P<. 05) percentage of progressively motile sperm than HL and CL rams. Volume percentage of Leydig cells were higher (P<.10) in HL rams than in LL and CL rams whereas volume percentage of vascular tissue was higher (P<.10) in LL rams than in HL and CL rams. Volume percentage of seminiferous tubules or seminiferous tubule diameter did not differ (P>.10) among rams of. these lines. Receptor capacity was higher (P<.10) but affinity was lower (P<.10) for oLH than for hCG in rams of these lines. Receptor capacity for hCG per g of testicular parenchyma did not differ (P>.10) among rams of these lines but capacity for oLH was higher (P<.10) in HL rams than in the other lines. Affinity for hCG was higher (P<.10) in HL and LL rams than in CL rams, however affinity for oLH did not differ (P<.10) among rams of these lines. There were line by time and dose by time interactions (P<.10) for in vitro T secretion by testicular parenchyma after oLH or hCG stimulation in rams of these lines. Selection applied upon female reproductive rate altered certain seminal, histomorphological and physiological male reproductive characteristics in Rambouillet sheep.

EFFECT OF SELECTION FOR FEMALE REPRODUCTIVE RATE ON TESTICULAR PHYSIOLOGY AND HISTOMORPHOLOGY IN RAMBOUILLET RAMS

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I would like to dedicate this work to my wife Luz and my parents Victor and Elena.

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ABSTRACT

Objectives of this study were to determine if scrotal circumference (SC), testicular weight (TW), seminal characteristics, histological components of the testicular parenchyma, capacity and affinity of the oLH/hCG receptor, ability and sensitivity of testicular parenchyma to secrete testosterone (T) in vitro after stimulation by oLH or hCG differed among Rambouillet rams from lines selected for low (LL) or high (HL) female reproductive rate and from a random-bred control line (CL). Two trials were conducted, one during the winter of 1989 and the other during the winter of 1990. Lines had been selected for 19 and 20 years for each trial, respectively. Scrotal circumference (SC), testicular weight (TW) and in vitro T secretion after hCG were evaluated in Trial 1. These characteristics and in vitro secretion of T after oLH, receptor capacity and affinity, testicular histomorphology and seminal characteristics were evaluated in Trial 2. Rams among lines did not differ ($P>.10$) in SC and TW but LL rams had lower ($P<.05$) percentage of progressively motile sperm than HL and CL rams. Volume percentage of Leydig cells were higher ($P<.10$) in HL rams than in LL and CL rams whereas volume percentage of vascular tissue was higher ($P<.10$) in LL rams than in HL and CL rams. Volume percentage of seminiferous tubules or seminiferous tubule diameter did not differ ($P>.10$) among rams of these lines. Receptor capacity was higher ($P<.10$) but affinity was lower ($P<.10$) for oLH than for hCG in rams of these lines. Receptor capacity for hCG per g of testicular parenchyma did not differ ($P>.10$) among rams of these lines but capacity for oLH was higher ($P<.10$) in HL rams than in the other lines. Affinity for hCG was higher ($P<.10$) in HL and LL rams than in CL rams, however affinity for oLH did not differ ($P<.10$) among rams of these lines. There were line by time and dose by time interactions ($P<.10$) for in vitro T secretion by testicular parenchyma after oLH or hCG stimulation in rams of these lines. Selection applied upon female reproductive rate altered certain seminal, histomorphological and physiological male reproductive characteristics in Rambouillet sheep.

INTRODUCTION

Reproductive rate is an important characteristic in sheep production. Improvement of reproductive rate by means of selection has been based primarily on characteristics that can be measured only in females such as ovulation rate or number of lambs born per lambing ewe. Because of the sex limited nature of these traits, selection based upon male characteristics has never been used to improve reproductive rate in domestic animals except for research purposes. A review of the literature of selection for reproductive rate in sheep, cattle and mice indicates that this type of selection induces direct responses in females under selection and, at the same time, indirect changes in reproductive characteristics of the male offspring. Recently, it was reported that changes in certain female characteristics related to reproductive rate occur when selection is applied to male reproductive traits.

It was hypothesized that genes that control physiological processes related to reproductive rate may be the same in males and females, therefore, selection to improve reproductive rate in domestic species could be based upon characteristics measured in males. This type of selection has the advantage of increased responses due to shorter generation intervals and higher selection intensities that can be applied in males relative to females.

The present review focuses on a) the physiology of reproduction of adult male sheep with emphasis on the physiology and histomorphology of the testis and the endocrinological control of testicular function, and b) the correlated responses in testicular characteristics of the male offspring when selection is applied to female reproductive rate.

REVIEW OF LITERATURE

Anatomy of the Adult Ram Testis

The testes are the primary organs of reproduction in male mammals. In sheep, they are paired ovoid organs located between the rear legs inside a sac-like structure called the scrotum.

Weight of the testis in sheep varies with age. Each testis weighs about 3 g by 1 month of age, 40 g by 4 months and 115 g by 6 months. The large change in testicular weight between 4 and 6 months of age, occurs soon after the interstitial cells (steroid producing cells) of the testis become fully functional (Gier and Marion, 1970). Further increase of testicular weight after 24 weeks of age, when rams reach full maturity (Ashdown, 1988), is considered insignificant and in general, testicular weight decreases as males get older. The size of each testis in relation to body weight of rams is about 0.5% (Setchell, 1984).

Blood concentrations of certain hormones and season of the year are known to influence testicular weight and size. A linear relationship was observed between testicular size and the concentrations of the pituitary hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH) and the testicular steroid hormone, testosterone (Courot and Ortavat, 1981). Seasonal variations in testicular diameter and weight

appear to be related to photoperiod since these characteristics decrease coincident with increasing photoperiod and increase as photoperiod decreases (Lincoln, 1981; Thimonier, 1981).

Anatomically, the testis is covered by a capsule which is formed by three layers of tissue, an outer layer of visceral peritoneum, the tunica vaginalis, a middle layer referred to as the tunica albuginea that consists of fibroblasts, collagen and "myofibroblasts" and, on the inside, the tunica vasculosa which is an extension of the testicular interstitium (Setchell and Brooks, 1988). The tunica albuginea is the most prominent layer of the testicular capsule. The testicular artery penetrates the tunica albuginea and runs around the inferior pole and up the anterior border of the testis, at which point it breaks into branches which enter the testicular parenchyma (Davis et al., 1970).

The testicular capsule surrounds the parenchyma, which is composed of two elements; highly coiled tubes known as the seminiferous tubules and the interstitium. The diameter of the seminiferous tubules of the ram varies between 200 to 260μm (Hochereau-de Reviers et al., 1979; Lunstra and Shanbacher, 1988). Both ends of each seminiferous tubule open into the rete testis, which is a complicated network of intercommunicated channels located longitudinally in the central part of the testis (mediastinum). Spermatozoa that are produced in the seminiferous tubules are transported,

suspended in a fluid, to the rete testis and then leave the testis through the efferent ducts.

A basement membrane forms the outer wall of the seminiferous tubules. This membrane is composed of a number of layers: several inner non-cellular layers followed by layers of myoid cells and collagen fibers and then, on the outside, a layer of endothelial cells (Bustos-Obregon and Courot, 1974).

Inside the seminiferous tubules there are two types of cells; germ cells and somatic cells known as Sertoli cells. Just before puberty, germ cells begin their transformation into spermatozoa through the process of spermatogenesis. Sertoli cells are polymorphous cells, located at the periphery, against the internal side of the basement membrane. Adjacent Sertoli cells form junctional complexes that contribute to the formation of the blood-testis barrier (Fawcett, 1975; Waites and Gladwell, 1982). In the ram, proliferation of Sertoli cells occurs mainly in utero, but a relatively small increase in number of Sertoli cells occurs between birth and puberty. After puberty there is no further increase in number of Sertoli cells (Hochereau-de Reviers et al., 1987). Proliferation of Sertoli cells is markedly influenced by FSH (Orth, 1984). In sheep, the number of Sertoli cells per testis by 18 to 24 months of age varies with breed and ranges from 20×10^8 to 50×10^8 cells (Barenton et al., 1983; Hocherau-de Reviers et al., 1987).

The interstitium of the testicular parenchyma contains blood and lymphatic vessels, interstitial or Leydig cells, fibroblasts, nerves and collagen fibers. In the ram Leydig cells are found singly or in small clusters with no clear association with blood vessels. The primary function of these cells is steroid hormone synthesis and secretion. The connective tissue in the interstitium is abundant and includes many fibroblasts and much collagen. There are blood and lymphatic capillaries in almost all interstitial areas. This arrangement of elements in the interstitium of the ram is similar to that observed in cattle, elephants, monkeys and humans (Fawcett et al., 1973).

Other cell types are also present in the testicular interstitium. These include macrophages, lymphocytes, plasma cells and mast cells. The function of mast cells in the testicle is not well known (de Kretser and Kerr, 1988) and macrophages have been studied mainly in rat testis in relation to their localization and function. They appear to be endocytically active (Miller et al., 1983).

Testicular Physiology in Sheep

The testes produce male gametes, the spermatozoa, and perform critical endocrine functions, the synthesis and secretion of steroids (androgens) and inhibin, a protein hormone.

Endocrine Function of the Testis

Androgen Synthesis. Leydig cells synthesize and secrete androgens male mammals (Baillie et al., 1966; Christensen and Gillim, 1969; Hall et al., 1969; Christensen, 1975). Testosterone is the predominant androgen synthesized in the testis; other androgens are androstenedione and dehidroepiandrosterone which are less active than testosterone (Eik-Nes and Hall, 1965).

Development of Leydig cells in the testis exhibits a biphasic pattern in most mammals; the first phase occurs during gonadal differentiation (the fetal period of life) and the second around puberty (Gondos et al., 1976). In the pig an additional phase in Leydig cell development takes place in the perinatal period between 2.5 weeks before until 2.5 weeks after birth (Van Straaten and Wensing, 1978). A definitive population of Leydig cells is established in pigs (Van Straate and Wensing, 1978; Lunstra et al., 1986) and rats (Kaler and Neaves, 1980) just after puberty; however humans exhibit a decline in Leydig cell number (Kaler and Neaves, 1978) while horses experience an increase in Leydig cells with advancing age (Johnson and Neaves, 1981).

Ultrastructure of the ovine Leydig cell has been described only recently by Lunstra and Shanbacher (1988). A list of references on Leydig cells of other mammalian species can be found in the review by Christensen (1975). Leydig

cells of rams contain a large oval or elongated nucleus, numerous mitochondria, abundant densely packed smooth endoplasmic reticulum (SER), a characteristic of steroid secreting cells, and well developed Golgi apparati. The overall arrangement of organelles within Leydig cells of rams is similar to those found in other vertebrates including humans. Variation in some details, like number of lipid droplets or amount of SER among species has been reported (Christensen, 1975). Lunstra and Shanbacher (1988) reported that the percentage of testicular volume occupied by Leydig cells in rams was 2.7%, the volume of an individual Leydig cell was 416μm^3 and the total number of Leydig cells per paired testes was 26.4×10^9.

Synthesis of testosterone and other androgens by Leydig cells requires cholesterol as a precursor. Cholesterol can be either taken up from the circulation or synthesized inside Leydig cells from acetate. Most of the cholesterol (60 to 85%) required for steroidogenesis is synthesized in the SER of the Leydig cell and stored as lipid droplets (Morris and Chaicoff, 1959, Weibin and Chaikoff, 1961). Blood cholesterol to be used in steroid synthesis by Leydig cells is thought to be derived from low-density lipoproteins (Freeman and Ascoli, 1983).

The conversion of cholesterol to testosterone occurs by several enzymatic reactions that include three hydroxylations, two dehydrogenations, one isomerization and two C-C cleavages

(Hall, 1988). Cholesterol is transformed into pregnenolone in the mitochondria after two hydroxylations and one C-C cleavage between C 20-22. Hydroxylations are important reactions that take place in the mitochondrias of Leydig cells and are catalyzed by enzymes called monooxygenases. These reactions require NADPH and molecular O_2, the latter needs to be activated by cytochrome P_{450}. All reactions involved in the transformation of pregnenolone to testosterone take place in the SER. Pregnenolone is converted into progesterone by an oxidation and an isomerization. Pregnenolone and progesterone are C-21 steroids. The conversion of progesterone into androstenedione, a C-19 steroid, proceeds with a 17-α hydroxylation and a C 17-20 cleavage. Testosterone is formed by reduction of the 17-keto group of androstenedione in a reversible reaction catalyzed by the enzyme 17-ß hydroxysteroid dehydrogenase. The rate limiting step in the synthesis of testosterone is the formation of pregnenolone from cholesterol (Stryer, 1988).

Leydig cells of stallions and boars synthesize estrogens in addition to androgens (Bedrok and Samuels, 1969; Raeside and Renaud, 1982; Allrich et al., 1983). Furthermore, estrogens are probably synthesized in the seminiferous tubules because aromatization enzymes necessary to synthesize estrogens from testosterone are found in these structures in several species (Stainberger et al., 1979) including sheep (Setchell, 1978).

Secretion of Testosterone. Leydig cells, like other steroid secretory cells, store minimal amounts of their synthesized products (Eik-Nes, 1975). In the ram and in males of other species testosterone is secreted into the blood in a pulsatile manner (Sanford et al., 1974; Ortavat et al., 1982; Hall, 1988). The secretion of testosterone is regulated mainly by LH (Courot, 1967; Amman and Shanbacher, 1983). Testosterone and the other androgens diffuse directly into the blood, lymph and seminiferous tubules (Lindner, 1969).

Biological Functions of Androgens. Androgens exert important functions in male reproduction. These hormones control the development and maintenance of functionality of internal and external genitalia, appearance of secondary sexual characteristics, stimulate the process of spermatogenesis, interact with the hypothalamus-pituitary axis and stimulate the development of the musculo-skeletal system (Reeves, 1987; Hall, 1988).

Inhibin. The existence of the hormone called inhibin, a non-steroid substance that regulates pituitary function was first proposed by McCullag (1932), based on his studies on the endocrine activity of the testis. Currently, it is known that inhibin is a protein hormone, apparently produced in males only in the Sertoli cells of the seminiferous tubules, that exerts an inhibitory action on synthesis and secretion of FSH

from the hypophysis by mechanisms that remain to be elucidated (Steinberger and Ward, 1988).

Spermatogenesis

The process of formation of spermatozoa is called spermatogenesis. Spermatozoa have a precise function to carry genetic information of the male to the oocyte.

Spermatogenesis is usually divided into two continuous phases: spermatocytogenesis and spermiogenesis. The first phase includes the transformation by mitotic divisions of the stem cell A_0-spermatogonia (2n) into other types of spermatogonia (A_1, A_2, A_3, A_4, intermediate and B) which then divide to form primary spermatocytes (2n). In this series of divisions each new type of germ cell divides to produce the succeeding cell type. Primary spermatocytes undergo a reductional division (meiosis) to form secondary spermatocytes (result of first meiotic division) which then divide without further DNA synthesis to form the aploid cells known as spermatids (Garner and Hafez, 1987). In the ram, three type A, one intermediate and 2 type B spermatogonial generations have been identified (Hochereau-de Reviers, 1987).

During spermiogenesis spermatids undergo condensation of the nuclear chromatin, formation of the tail and development of the acrosome to become fully formed spermatozoa. These cells are then released into the lumen of the seminiferous

tubules by a process known as spermiation (Garner and Hafez, 1987). Additional morphological and physiological changes occur in spermatozoa during their passage through the epididymis. These changes are collectively called maturation (Voglmayr, 1975). The final result of spermatogenesis is a population of highly specialized cells with a very peculiar form consisting of a flattened head that contains the nucleus of the cell and a tail necessary for cell motility.

Within any cross section of a seminiferous tubule, different cell types of the spermatogenic process form defined associations that appear one after the other over a particular period of time. The appearance of series of associations repeated in a cyclic manner during the reproductive life of the male. The time elapsed between the disappearance of one association and the appearance of the same association is called the cycle of the seminiferous epithelium. In sheep, the duration of this cycle is 10.4 days (Hochereau-de Reviers et al., 1987) and the number of associations of germ cells, based on the morphology of the acrosome in the developing spermatid is 12 (Setchel, 1978). The duration of spermatogenesis in the ram is 47 days (Amman and Shanbacher, 1983).

Daily Sperm Production. Daily sperm production is the number of sperm produced per day by the testes (Amman, 1970) and represents one way by which efficiency of spermatogenesis

can be evaluated in a particular individual or species. Daily sperm production can be estimated by determining the cycle of the seminiferous epithelium by quantitative histological analysis of the testicular parenchyma, from counting number of spermatids in testicular homogenates and from daily sperm output measured in repeated ejaculates (Amman, 1970).

Estimation of daily sperm production was 4.81×10^9 in Ile-de-France and 3.54×10^9 in Romanov sheep during the breeding season (Hocherau-de Reviers et al., 1990). Based upon data from the literature, Amann et al. (1976) concluded that daily sperm production is highly variable between species, however when it was expressed as daily sperm production per g of testicular parenchyma it became relatively constant (approximately 21×10^6 to 25×10^6 cells per day) for most species, except dairy bulls and stallions (12×10^6 and 16×10^6 cells per day, respectively).

Epididymal Sperm Reserves. After being released into the lumen of the seminiferous tubules, sperm cells are transported to the epididymis where they undergo a process called maturation (Voglmayr, 1975; Holtz and Smidt, 1976) and are stored in the cauda epididymis and proximal deferent duct (Amman and Shanbacher, 1983). Exact changes that sperm undergo during maturation are not completely understood, however during this process sperm cells develop progressive motility, undergo morphological changes which include

reorganization of tail organelles, nuclear chromatin and plasma membrane, and changes in metabolism; all factors resulting in the acquisition of fertilizing capacity (Bedford, 1975; Orbegin-Crist and Olson, 1984). The average time required for sperm cells to transit through the epididymis of different mammals is approximately 10 days (Robaire and Hermo, 1988). Sperm transit through the epididymis and epididymal sperm reserves were estimated to be 16.4 days and >165 x 10^9 cells respectively for Ile de France rams and 10.4 days and >135 x 10^9 cells, respectively for Suffolk rams.

Spermatozoa are stored in the cauda epididymis until ejaculation or for more than 30 days if they are not ejaculated (Orbegin-Crist et al., 1975). Older sperm cells are selectively destroyed and reabsorbed after degradation (Bedford, 1975). In sexually "rested" rams a great number of sperm can be found in the urine (Lino et al., 1967).

Daily Sperm Output. The number of ejaculated sperm that can be obtained per day is called daily sperm output (Amann and Shanbacher, 1983). This is an important characteristic because fertility is correlated with the number of sperm deposited by the male or by artificial insemination in the female reproductive tract. If an adequate number of ejaculations are collected at short and regular intervals for several weeks, daily sperm output becomes highly correlated with and approaches daily sperm production (Amann, 1970).

Daily sperm production in mammals are markedly different and Chang (1945) reported that daily sperm output in rams was approximately 8.6×10^9 cells.

Sperm cells are ejaculated as part of the semen. The other component of the semen is the seminal plasma, a fluid formed by the secretions of the accessory organs of the male reproductive tract. Seminal characteristics differ among domestic animals. In rams, the volume of the ejaculate is 0.8 to 1.2 ml; the concentration of sperm cells is 2×10^9 to 3×10^9 per ml; the percentage of motile sperm is 60-80% and the percentage of morphologically normal sperm is 80 to 95% (Garner and Hafez, 1987).

Regulation of Testicular Function

Steroidogenesis

Luteinizing hormone, sometimes called Interstitial Cell Stimulating Hormone (ICSH), stimulates synthesis and secretion of testosterone by Leydig cells (Brady, 1951; Hall and Eik-Nes, 1962; Connell and Eik-Nes, 1968; Eik-Nes, 1975). Luteinizing hormone promotes de novo synthesis of testicular steroids and has almost no effect on secretion of preformed steroids from the Leydig cells (Eik-Nes, 1975). In rats, Hall et al. (1979) demonstrated that LH stimulated the transport of cholesterol to the inner mitochondrial membrane where side-

chain cleavage of this molecule takes place.

Luteinizing hormone is a 28.3 kD glycoprotein (Sairam, 1983) synthesized and secreted by cells (gonadotropes) of the hypophysis (Pearce and Parsons,1981; Pierce, 1988). This hormone consists of two polypeptide chains or subunits, α and ß, that are glycosylated at specific residues and are internally cross-linked by disulfide bonds. The hormone is biologically active only when both subunits are present and actively interacting (Pierce and Parsons, 1981).

In the male, LH is secreted in pulses which induce a pulsatile secretory response in testosterone (Sanford et al., 1974; Ellis and Desjarnis, 1982; Ortavat et al., 1986; Hall, 1988). The biological significance of pulsatile secretion of testosterone is not understood (Ellis and Desjarnis, 1982).

The action of LH in Leydig cell function, is mediated by a specific receptor located in the plasma membrane to which the LH molecule binds with high affinity (Catt and Dufau, 1977). The LH receptor is a dimeric transmembrane glycoprotein with a molecular weight of approximately 90 kD (Rajaniemi et al., 1989; McFarland et al., 1989; Loosfelt et al.,1989).

Using cDNA techniques for sequencing proteins, McFarland et al. (1989) predicted that the LH receptor should consist of a 341-residue hydrophilic extracellular domain and a 333-residue region containing seven hydrophobic transmembrane segments; a total of 674 amino acid residues with a molecular

weight of 75 kD. Because of glycosylation of the extracellular domain of the natural receptor, its molecular weight was calculated to be 93 kD. Similarly, Loosfelt et al.(1989), by means of cDNA clones encoding porcine LH receptor, predicted a 333-residue extracellular domain, which precedes a 266-residue domain that displays seven possible transmembrane segments. However, they found three other types of clones corresponding to small proteins in which the transmembrane domain was absent. Furthermore, and demonstrating that there is not complete agreement about the structure of the LH receptor, Rajaniemi et al. (1989) predicted only one transmembrane segment.

The LH molecule probably binds to the extracellular domain of its receptor (McFarland et al., 1989) by mechanisms that are not completely understood. Rajaniemi et al.(1989), hypothesized that the LH receptor binds one hormone molecule and that the most important interaction of the gonadotropin with the receptor occurs through its α-subunit, after the ß-subunit recognizes the receptor. Katikineni et al. (1980) found that binding of Human chorionic gonadotropin (hCG), a hormone with LH-like activity, to rat testicular membranes progresses from a loose to a tight binding; they hypothesized that this change may represent the beginning of the degradation of the hormone-receptor complex.

Recently, it was reported that the LH molecule exhibited disulfide isomerase activity (Boniface and Reichert, 1990).

This activity is similar to the function of thioredoxin an enzyme that catalyzes the formation of disulfide bonds. This common function is probably due to the amino acid sequence, Cys-Gly-Pro-Lys, found in the ß-subunit of LH and the active site of thioredoxin. It was suggested that this catalytic activity may be involved in the generation of hormone-receptor complexes, and may be an explanation of irreversible hormone binding to receptors found by Katikineni et al. (1980).

Binding of LH to its receptor stimulates binding of GTP to the membrane. This results in the activation of specific membrane proteins that participate in the activation of adenylate cyclase, which in turn stimulates cAMP synthesis from ATP. Cyclic AMP, acting as a second messenger, stimulates the phosphorylation of Leydig cell proteins through the action of cAMP-dependent protein kinases. The phosphorylated proteins presumably stimulate synthesis and secretion of steroids (Catt and Dufau, 1977; Pierce and Parsons, 1981; Hunzicker-Dunn and Birnbauner, 1985).

McFarland (1989) found structural similarities of the LH receptor with the G Protein-couplet receptor family. In this group of receptors, the formation of the hormone-receptor complex activates many molecules of G-protein located in the plasma membrane which then activate adenylate cyclase (Gilman, 1987; Stryer, 1988). Other authors suggest that Ca^{++}/phospholipid-dependent protein kinase may be involved in the process of LH stimulation in addition to cAMP for protein

phosphorylation (Tähkä, 1986; Hall, 1988).

Besides the endocrine regulation of Leydig cell function by LH, other studies have demonstrated that paracrine and autocrine mechanisms may be involved in the control of Leydig cell function. There are data to support interactions of Leydig cells with seminiferous tubules (Bergh, 1982; Wilton and Kretser, 1984), macrophages (Bergh,1985) and lymphocytes (Guo et al., 1990). Interleukin-1, a protein secreted by macrophages, decreased hCG binding to Leydig cells, blocked the hCG-stimulated secretion of testosterone and synthesis of cAMP in rat testicular tissue in vitro (Calkis et al., 1988). Similarly, Interleukin-2, a protein secreted by T lymphocytes, has a potent inhibitory effect on Leydig cell steroidogenesis through the inhibition of cAMP formation and the conversion of several androgen precursors to testosterone (Guo et al., 1990). It was also found that interferon gamma inhibits steroidogenesis in cultured porcine Leydig cells (Orava et al., 1989).

Regulation of the number of LH receptors is exerted mainly by LH itself and probably by other hormones (prolactin, FSH, estrogen) and other unknown factors of Sertoli cell origin (Tähkä, 1986). Increasing doses of LH, above biological concentrations, first induced an increase in number of receptors (up-regulation) and then a reduction (down-regulation) in LH receptors number. Down-regulation is usually related to an impairment of the Leydig cell to secrete

testosterone with additional hormone stimulation, a phenomenon also referred to as desentization (Dufau et al., 1979; Dufau et al., 1984). The block in steroidogenesis in desentisized Leydig cells is produced beyond adenylate cyclase activation and cAMP and pregnenolone formation. Presumably this block occurs in the conversion of 17α-hydroxyprogesterone to androgens precursors (Cigorraga et al., 1978; Dufau et al., 1979).

In human females, LH receptors also interact with hCG. It is now recognized that LH and hCG are hormones with similar chemical structure (Pierce and Parsons, 1981). They bind to the same receptor by similar mechanisms in both males and females and elicit qualitatively equivalent biological responses (Eik-Nes, 1975; Pierce and Parsons, 1981; Strickland et al., 1985; McFarland et al., 1988; Rajaniemi et al., 1988). However, studies performed in male rats and rams showed that even when the biological responses induced by LH and hCG are comparable the behavior of each hormone during its biochemical interaction with the receptor appears to differ. Hutaniemi and Catt (1981) found a higher association constant (K_a) of rat testicular receptors for hCG than for hLH or oLH, although the quantitative binding for the three hormones were similar. Contrary to these results, Sairam et al. (1988) reported similar affinities but markedly different capacities for oLH and hCG in testicular membrane preparations of rams.

Spermatogenesis and Sertoli Cell Function

Spermatogenesis is controlled primarily by the endocrine system. Two hormones are necessary for spermatogenesis; FSH stimulates and maintains the functions of Sertoli cells and testosterone, which is necessary for spermatid maturation and Sertoli cell function (Garner and Hafez, 1987; Bardin et al., 1988; Sun et al., 1990). Luteinizing hormone is required indirectly because it stimulates Leydig cells to synthesize and secrete testosterone that diffuses into the seminiferous tubules to exert its actions on spermatogenesis. Sun et al. (1990) found that the process of transformation of round to elongated spermatids was mainly testosterone dependent with limited effects of pituitary factors. The levels of testosterone necessary for this purpose in the rat was only 10-15% of those observed in normal rats.

A high correlation was found between total number of A1 spermatogonia and Sertoli cells, which was interpreted as the existence of important influences of Sertoli cell population on sperm production (Hochereau-de Reviers et al., 1987). Therefore, multiplication of Sertoli cells and establishment of an adequate population was considered important for sperm production.

The arrangement of Sertoli cells in the seminiferous tubules where they make contact to each other and to germ cells contributes to the organization and cohesion of the

seminiferous epithelium and the coordination of spermatogenesis. Some metabolites reach and leave the sperm cell by passing through the Sertoli cells which is facilitated by the closeness between both types of cells. Furthermore, Sertoli cells can reabsorb the residual bodies of spermatids and germ cells undergoing degeneration by phagocytosis (Courot et al., 1970).

The junctional contacts between Sertoli cells assist in the formation of the blood-testis barrier which makes the fluid of the seminiferous tubules and rete testis different from lymph and blood (Fawcett, 1975). The blood-testis barrier acts excluding molecules in such a manner so as to protect and isolate developing germ and sperm cells from mutagenic agents and the immune system (Bardin et al., 1988). Most proteins present in the fluid of the seminiferous tubules are secreted by Sertoli cells. These proteins and other compounds secreted by Sertoli cells provide the proper environment for the development of germ cells (Mather et al., 1983).

Sertoli cells are thought to secrete up to 100 proteins (Bardin et al., 1988) including testes specific proteins and some proteins that are normally found in serum (e.g., transferrin, Wright et al., 1981). Androgen binding protein (ABP) and inhibin are the best known proteins secreted by Sertoli cells. The function of inhibin was reviewed previously. Androgen Binding Protein has a molecular weight

of 85 kD and exhibits androgen-binding activity. It has been postulated to act in the following manner: a) as a carrier of testosterone within the Sertoli cell and from the testis into the epididymis, and b) to maintain high concentrations of androgens in the seminiferous tubules and epididymis necessary for sperm development (Bardin et al., 1988).

Other compounds secreted by Sertoli cells are growth factors (Feig et al, 1980), plasminogen activator (Lacroix et al., 1977), steroids (Welsh and Wiebe, 1978) and several others that may play a role in paracrine and autocrine regulation of testicular function (Tähkä, 1986).

Follicle stimulating hormone, like LH, is a glycoprotein consisting of an α and ß subunits with a molecular weight of 29 kD (Pierce, 1988). This hormone binds to its receptor located in the membrane of the Sertoli cell and stimulates the adenylate cyclase cascade which results in phosphorylation of proteins involved in the activation of a particular Sertoli cell functions (Bardin et al., 1988). The participation of Ca^{++} as a second messenger working through calmodulin for phosphorylation, has also been proposed as another mechanism of action of FSH on Sertoli cells (Means, 1981).

Factors that control Sertoli cell funtions are numerous and include FSH, insulin, insulin-like growth factors, glucagon, calcitonin, androgens, estrogens, progesterone, several vitamins and pro-opiomelanocortin-derived peptides (Bardin et al., 1988). These numerous factors, besides the

paracrine effects from other components of the testicle, make the mechanisms of control of Sertoli cell functions highly complex and not well understood. Recent findings on paracrine regulation of Sertoli cell function indicate that mRNA for the ß-subunit of inhibin changes according to the state of the cycle of the seminiferous epithelium, which suggested that certain germ cell factors may alter gene expression in Sertoli cells (Bhasin et al., 1989). Luteinizing hormone may also influence expression of the Inhibin ß-subunit gene (Krummen et al., 1990).

Hypothalamic-Hypophyseal-Testicular Axis

The two gonadotropins, LH and FSH, that play major roles in control of testicular function are synthesized and secreted by the adenohypophysis. The hypothesis that the pituitary controls testicular function was proposed about 50 years ago and it was confirmed in sheep by Courot (1967) who prevented testicular regression by injecting pituitary extracts that contain LH and FSH into hypophysectomized rams.

Gonadotropin secretion is controlled by the central nervous system through the hypothalamus and by feedback mechanisms from the testicles. Environmental factors also influence gonadotropin secretion by way of the central nervous system (Fink, 1988).

Neurons from different parts of the hypothalamus, secrete

a number of peptides that control pituitary function (Guillemin, 1978). Among them is a decapeptide called Gonadotropin-releasing hormone (GnRH, Matsuo et al., 1971). Gonadotropin-releasing hormone is released in a pulsatile manner into the hypophyseal portal vessels (Fink and Janieson, 1976; Levine et al., 1982; Carty and Locatelli, 1988) and is transported to the adenohypophysis where it stimulates the synthesis of LH and FSH and the release of pulses of LH and the slow and gradual release of FSH by the same pituitary cells (Fink, 1988). Gonadotropin releasing hormone appears to act on gonadotropes by altering intracellular Ca^{++} and the phosphatidylinositol cascade (Mason and Waring, 1985).

Androgens exert a negative feedback on secretion of LH and FSH (D'Occhio et al., 1982) and inhibin inhibits secretion of only FSH (Stainberger and Ward, 1988). The negative feedback of testosterone apparently works by inhibiting binding of GnRH to its receptors on the gonadotropes (Clayton and Catt, 1981; Kitahara et al., 1990) and by inhibiting release of GnRH from the hypothalamus (Levine and Duffy, 1988; Caraty and Locatelli, 1988). Endogenous opiods modulate the secretion of LH probably by acting at the level of the hypothalamus. It was found that naloxone, an opiod antagonist, produces an increase in serum LH in males and females (Cicero et al., 1979; Ebling and Lincoln, 1985). Other studies have demonstrated that alpha-adrenergic receptor stimulation can induce LH release and that there is a