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The Effects of Testicular Nerve Transection and Epididymal White Adipose Tissue Lipectomy on Spermatogenesis in Syrian Hamster

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THE EFFECTS OF TESTICULAR NERVE TRANSECTION AND EPIDIDYMAL WHITE ADIPOSE TISSUE LIPECTOMY ON SPERMATOGENESIS IN SYRIAN HAMSTER

by

JEREMIAH E. SPENCE

Under the direction of Andrew N. Clancy, Ph.D.

ABSTRACT

Previous investigators demonstrated that epididymal white adipose tissue (EWAT) lipectomy suppressed spermatogenesis and caused atrophy of the seminiferous tubules. EWAT lipectomy, however, may disrupt testicular innervation, which reportedly compromises testicular function. To resolve this confound and better clarify the role of EWAT in spermatogenesis, three experimental groups of hamsters were created in which: i.) the superior and inferior spermatic nerves were transected (SSNx) at the testicular level, ii.) EWAT was extirpated (EWATx), and iii.) testicular nerves and EWAT were left intact (SHAM controls). It was hypothesized that transection of the superior and inferior spermatic nerves would disrupt normal spermatogenesis. The findings indicate a significant reduction in spermatogenic activity and marked seminal tubule atrophy within the EWATx testis, as compared to the SSNx and controls testes, which did not differ significantly from each other. From these data, it is concluded that EWAT, and not testicular innervation, is central to normal spermatogenesis.

INDEX WORDS: Epididymal white adipose tissue (EWAT); EWAT lipectomy; Gonadal fat pad; Male reproduction; Norepinephrine; Seminiferous tubule; Spermatogenesis; Superior and inferior spermatic nerve; Syrian hamster, Testes; Testicular nerve transection (denervation)

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DEDICATION

To my parents, for reasons beyond expression; Kesha, my closest friend, whose spirit and wisdom I envy; Alfie, a gifted listener and caring advisor; and lastly, Dr. Mary Sterner Lawson, my ol' Honors prof., whose positive leadership and lessons on logic, independent thought, humility and service to humanity I will forever embrace, and from whom I learned much about tolerance—that is, *different* and *wrong* are not synonyms. You are all invaluable. Thank You!

.

I shall be telling this with a sigh

Somewhere ages and ages hence:

Two roads diverged in a wood, and I—

I took the one less traveled by,

And that has made all the difference.

~Robert Frost

"The Road Not Taken"

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As we express our gratitude, we must never forget that the highest appreciation is not to utter words, but to live by them. ~John F. Kennedy

♦ ♦ ♦ ♦

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

25-HDC	25-hydroxycholesterol
2-DG	2-deoxyglucose
6-OHDA	6-hydroxydopamine
AA	ascorbic acid
ABC	avidin-biotinylated peroxidase complex
ABP	androgen-binding protein
Ach	acetylcholine
ACTH	adrenocorticotropic hormone
Ad	dark-staining Type A spermatogonia
ADR	adrenergic receptor
ANS	autonomic nervous system
Ap	pale-staining Type A spermatogonia
Ap1-Ap4	differentiating, pale-staining Type A (1-4) spermatogonia
AR	androgen receptor(s)
ARO	aromatase
Asn	asparagine
At	transitional/intermediate Type A spermatogonia
cAMP	cyclic AMP
CG	celiac ganglion
CGRP	calcitonin gene-related peptide
CNS	central nervous system
COMT	catechol-*O*-methyl transferase
CPC	committed progenitor cells (sing. CPC)
CPON	C flanking protein of NPY
CSF-1	colony-stimulating factor-1
CSF1-KO	colony-stimulating factor-1 knock-out mice
DAB	diaminobenzidine
DAG	diacylglycerol
DEAE	diethylaminoethyl
DHBA	dihydroxybenzylamine
DHEA	dehydroepiandrosterone
DHT	5α-dihydrotestosterone
DL	dorsolateral
DM	dorsomedial
DMSO	dimethyl sulfoxide

DMV	dorsal motor nucleus of the vagus nerve
E	epinephrine
E_1	estrone
E_2	estradiol
E_2R	estrogen receptor
E_2RKO	estrogen receptor gene knock-out mice
ECM	extracellular matrix
ECM	extracellular matrix
ENS	enteric nervous system
ER	endoplasmic reticulum
EST	Eastern Standard Time
EtOH	ethanol
EWAT	epididymal white adipose tissue
EWATx	EWAT lipectomy (bilateral)
FB	fat body
FB-A	aqueous fraction of FB homogenate
FB-L	lipid fraction of FB homogenate
FBWL	FB weight loss
FBX	fat body removal
FFAs	free fatty acids
FMF	fat-mobilizing factor
FSH	follicle-stimulating hormone
FSHR	FSH receptor
G2	second growth phase
GD	guanethidine
GH	growth hormone
GI	gastrointestinal
G_i	G protein inhibitory complex
GnRH	gonadotrophin-releasing hormone
GPCR	G protein-coupled receptor
G_q	G protein, heterotrimeric
G_s	G protein stimulating complex
GVA	general visceral afferent fibers
H & E	haematoxylin & eosin
h	hours
hCG	human chorionic gonadotrophin
HPLC	high-phase liquid chromatography
HSL	hormone-sensitive lipases

i.p.	intraperitoneal injection
ICC	immunocytochemistry
IFN-γ	interferon gamma
IGF-I	insulin-like growth factor-I
IL	inferior testicular ligament
IL-1α	interleukin-1, alpha
IL-1β	interleukin-1, beta
IL-6	interleukin-6
IMG	inferior mesenteric ganglia
IP_3	inositol triphosphate
ir	immunoreactive
ISN	inferior spermatic nerve
IWAT	inguinal white adipose tissue
IWATx	inguinal white adipose tissue lipectomy (bilateral)
IWATx	IWAT lipectomy (bilateral)
kDa	kiloDalton
LH	luteinizing hormone
LH-R	luteinizing hormone receptor
LIH	leukemia inhibitory factor
LMF	lipid-mobilizing factor
LPL	lipoproteinlipase
L-RPLND	laparoscopic retroperitoneal lymph node dissection
LSD	Fisher least significant differences test
LSN	lumber splanchnic nerve
MAO	monoamine oxidase
MAOIs	monoamine oxidase inhibitors
MAP	mean arterial pressure
MGC	multinucleated germ cell
MMPs	matrix malleoprotesases
MPG	major pelvic ganglia
MW	molecular weight
NDS	normal donkey serum
NE	norepinephrine
NO	nitric oxide
NOS	nitric oxide synthase
NPY	neuropeptide-Y
P450scc	side-chain cleave enzyme
PAG	pelvic accessory ganglia

PB	phosphate buffer
PCA	perchloric acid
PD	pars distalis of the pituitary gland
PDE	3’5’cyclic AMP phosphodiesterase
PDX	pars distalis removal
PG	pelvic ganglia
PG	prostaglandin(s)
PGE_2	prostaglandin E_2
$PGF_2\alpha$	prostaglandin $F_2\alpha$
PGJ_2	prostaglandin J_2
PI	phosphatidylinositol
PIP_2	phosphatidylinositol phosphate
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PMC	peritubular myeloid cells
POMC	proopiomelanocortin
PPARγ	peroxisome proliferator-activated receptor gamma
PR	progesterone receptor
PRL	prolactin
PRV	pseudorabies virus
PSN	pelvic splanchnic nerve
PSNS	parasympathetic nervous system
PVPI	povidone-iodine
PWAT	parametrial white adipose tissue
RER	rough endoplasmic reticulum
RIA	radioimmunoassay
RT	room temperature
RWAT	retroperitoneal white adipose tissue
S	DNA synthesis phase
SBF	spermatic blood flow
SCF	stem cell factor
Sch	sympathetic chain ganglia
SCI	spinal cord injury
SHAM	sham-operated males (controls)
SL	superior testicular ligament
SMG	superior mesenteric ganglion
SNS	sympathetic nervous system

SSN	superior spermatic nerve
SSNx	nerve-transected males
StAR	steroidogenic acute regulatory protein
T	testosterone
TAG	triglyceride(s)
TIMP-1	tissue inhibitor of metalloproteinases-1
TNF-α	tumor necrosis factor alpha
TSH	thyroid-stimulating hormone
UNI-EWATx	EWAT lipectomy (unilateral)
UNI-IWATx	IWAT lipectomy (unilateral)
VIP	vasoactive intestinal peptide
Vso	vasovastostomy (vasectomy reversal)
Vx	vasectomized; vasectomy
WAT	white adipose tissue
ZAG	zinc-α_2-glycoprotein
α_{1A}ADR	α_{1A}-adrenergic receptor
α_{1B} ADR	α_{1B}-adrenergic receptor
α_{1B}ADRKO	α_{1B} -adrenergic receptor gene knockout mice
α_{1D}ADR	α_{1D}-adrenergic receptor
α_{2A}ADR	α_{2A}-adrenergic receptor
α_{2B}ADR	α_{2B}-adrenergic receptor
α_{2C}ADR	α_{2C}-adrenergic receptor
α-LPH	α-LPH
α-MSH	α-melanocyte-stimulating hormone
β_1ADR	β_1-adrenergic receptor
β_2ADR	β_2-adrenergic receptor
β_3ADR	$\beta_3$adrenergic receptor
β-LPH	β-lipotropin
β-MSH	β-melanocyte-stimulating hormone
γ-LPH	γ-lipotropin

INTRODUCTION

I. Overview and Background

The kinetics of spermatogenesis. The cellular events which involve the mitotic differentiation of spermatogonia into spermatocytes, followed by the meiotic differentiation of these spermatocytes into the immature, non-motile spermatids is called spermatogenesis, whereas the transformation of the spermatids into mature, motile spermatozoa (=sperm) is called spermiogenesis (1-4). In mammals, spermiogenesis involves between 16-19 distinct, morphological stages, which occur in the epithelia of the seminiferous (seminal or germinal) tubules (2, 5, 6). In the present review, however, the two processes—spermatogenesis and spermiogenesis—are regarded as a singular, yet multistep biologic process, and the former term is adopted for ease of discussion. In studying the histopathological effects of a particular agent or a surgical treatment on spermatogenesis, Russell (2) suggests a thorough understanding of the kinetics of spermatogenesis under normal physiological conditions. Thus, the following discussion concerns the origins of the various types of spermatogonia and the development of these germ cells into primary spermatocytes.

Traditionally, two major groups of spermatogonia—Type A and B—have been classified on the basis of several morphological features, including organization of the rough endoplasmic reticulum (RER), stainability (e.g., dark vs. pale), chromatin grain (e.g., coarse vs. fine) and distribution within the nucleus (7, 8). The classification of spermatogonia by these criteria is quite often complex and difficult, while identification of the spermatogonia by the latter criterion is commonly obscured by poor fixation techniques. Technical advances in election microscopy have aided in the identification of three morphological subtypes of Type A spermatogonia, which have been characterized in higher mammals, including primates and rodents (9-12). The task of

identifying the Type A subtypes, however, is in itself complicated by the different spermatogonial naming systems, which vary somewhat from author to author (2, 13). The publication by Russell *et al.* (2) provides micrographs and detailed descriptions of each subtype, and consequently, the spermatogonial nomenclature used by Russell will be used for the remainder of this report.

The first spermatogonial subtype are the dark-staining Type A (Ad) spermatogonia, which are mitotically quiescent in the post-pubertal testis (14), yet proliferate in an gonadotrophin-independent fashion in the infant and juvenile testis of the Rhesus monkey (8). Thus, in the pre-pubertal testis, the Ad cells act as proliferative stem cells, which have the limitless ability to self-renew and produce committed progenitor cells (CPCs; 15, 16, 17) , whereas, in the normal adult, Ad cells serve as reserve (dormant) stem cells (7, 18). Despite divergence in the scientific community regarding the definition of the CPC, which has persisted over the past decade, Kalthoff (19) explains that the CPC is a undifferentiated cell type, which—restricted in its development—can only differentiate into a specific cell type.

Arising from the Ad cells, the progenitor Ap cells, the second subtype, undergoes several amplifying (mitotic) divisions to produce identical clones, which are linked by cytoplasmic bridges formed by incomplete cytokinesis (2). The cytoplasmic bridges represent intercellular connections which allow for the transfer of biomolecules between adjacent/attached germ cells, thereby mediating the synchronized development of the spermatogonial clones within the synctial chain (2, 19). The third subtype type —the transitional Type A (At) spermatogonia—which exhibit features intermediate to that of the Ad and Ap spermatogonia, are only visualized when Ad and Ap cells are present during the fetal period, 3-4 years preceding the appearance of

Type B spermatogonia (11). It is generally accepted the At subtype is not an intermediate form between Types A and B spermatogonia (12).

Once the Ap cells cease to divide, the Ap clones typically differentiate (through at least four cell types denoted Ap_1-Ap_4) into Type B spermatogonia (2, 14, 18, 20, 21). However, one study suggested that Ap spermatogonia may also generate Ad and At spermatogonia during each successive division (12). In this model, Ap spermatogonia behave as renewing stem cells to ensure continuation of spermatogenesis, while giving rise to Type B progenitor cells at puberty. To complete this first half of spermatogenesis, Type B cells differentiate into diploid primary spermatocytes, which subsequently enter meiosis to produce haploid secondary spermatocytes (21).

According to Russell *et al.* (2), fully differentiated spermatogonia are committed to becoming mature spermatozoa, unless germinal degeneration occurs during some part of their development. Degeneration, which is similar to program cell death (or apoptosis) in somatic cells, is a normal and constant phenomenon of the developing germ cells during spermatogenesis (22-24). Although the cause and purpose of degeneration are poorly understood, it has been suggested that degeneration maintains an optimal or ideal Ap- and/or germ-to-Sertoli cell ratio (25). In this way, when deviations from ideal conditions persist, the ratio may act as a critical limiting factor which triggers degeneration (25). According to estimates, only one-fourth of the theoretically possible number of primary (preleptotene) spermatocytes, which arise from fully differentiated Type B cells, are produced from the original population of Ap_1 spermatogonia (25). Consequently, the actual yield of sperm is consistently much lower than what can be predicted by the spermatogonial and spermatocytes division kinetics alone (2, 3).

The rate of germ cell degeneration is accelerated in pathogenic states. Germ cell degeneration is observed most often in the differentiating Ap_2 and Ap_3 cells, during meiosis (i.e., primary and secondary spermatocytes), and in stages 7 and 19 of spermiogenesis in normal adult male rats (24, 25) and stallion (26). Similarly, Huckins (25) reported that degeneration occurs less often in the Ap_4 cell type and rarely in the Ap_1 and Type B spermatogonia. Histological hallmarks of degeneration first appear once the Ap clones, which are linked cytoplasmic bridges, complete DNA synthesis (S phase) and enter the second growth (G2) phase of the cell cycle (25). Degeneration is characterized by an increase stainability of the nuclear envelope, karyorrhexis (literally, "nuclear fragmentation"), and pyknosis, which is shrinkage and clumping of the nuclear chromatin— the latter two phenomena mediated by an arsenal of apoptotic-promoting molecules (25, 27). The nuclear mass is eventually extruded from the cell, "leaving a cytoplasmic ghost" (25). The chronological steps in degeneration can be traced from one cell to its sister clones, suggesting that the apoptotic effectors synthesized within one cell may spread via cytoplasmic bridges to eventually affect all spermatogonia within the synctial chain (2, 25). Multinucleated germ cells (MGC), which are conspicuous markers of spermatogenic disorder and germ cell degeneration, are formed when the plasma membranes of adjacent spermatocytes (or round spermatids) coalesce during the latter stages of spermiogenesis (2, 28, 29). The subsequently widening of the cytoplasmic bridges leads to fusion of the cellular contents and formation of these unusually large cells, which are released into the seminal lumen where they will eventually degenerate (28, 29).

Numerous studies in laboratory rat demonstrated that degeneration can be initiated by environmental conditions, including: i.) deficiency of an essential nutrient, such as vitamin A, a retinoid critical for normal cell differentiation and stem cell biology (30-33) or ii.) exogenous

administration of pharmacologically-active agents, such as ethionine, a non-proteinogenic amino acid (34), nitrofurazone, an antibiotic (35), or methylmercury, a highly toxic bioaccumulent (36). Endogenous substances, such as nitric oxide (NO; 37), steroidal hormones (26, 38-40), and prolactin (PRL; MW 22 kDa; 41) have been linked to germ cell degeneration in higher vertebrates, especially when these substances are present at above normal physiological levels. Lastly, a wide range of specific and non-specific insults to the reproductive tract and testis—which include transection of the testicular nerve supply (42, 43), vasectomy (44-48), changes in testicular temperature (26, 38, 49, 50), prolonged lead or cadmium exposure (2, 26, 51), infectious disease (26, 51), and dietary deficiencies (52, 53)—increase the rate of degeneration of the developing germ cells, which dramatically reduce the spermatogenic yield.

*Stages of spermatogenesis and in the c*ycle. There are distinct morphological features of the spermatocytes and spermatids that distinguish the last ten spermatogenic stages of the rat from the first nine stages. These include the shape of the acrosome, level of nuclear condensation, flagellum status, status of the mitotic spindle, mitochondrial arrangement and the distribution and amount of cytoplasm (2, 5, 6, 54). Clermont *et al.* (55) described the cyclic transformations in the epithelial architecture, which represent characteristic spatial and temporal transformations in the organization and combination of the germ cells during spermatogenesis. The duration of this cycle of the seminiferous epithelium (or simply, the cycle), which consists of 6 to 14 stages—denoted by Roman numerals to prevent confusion with the stages of spermatogenesis—varies greatly between the mammalian species, and even among the various strains of animals of the same species (2, 20, 55, 56). Interestingly, a study involving the autoradiographic labeling of testicular tissue from incarnated, human volunteers demonstrated that the cycle in humans consists of 6 stages, which occurs over a 16-day period, while the entire

spermatogenic process spans 64 days (57). For these data, one can infer that spermatogenesis in man requires 4 cycles of the germinal epithelium (2). A quantitative assessment by Hess (56) revealed statistical differences in the spermatid (nuclear) shape and diameter, distance of the acrosomal tip from the basal lamina and frequency of mitosis between the various stages in the cycle over time. Additionally, Hess (56) includes a dichotomous key, which can be used to qualitatively classify the various stages of the cycle, while Hess *et al.* (58) proposed group size and sampling guidelines for evaluating alterations in the frequency of stages in the cycle as a indication of the effects of a particular [testicular] treatment on spermatogenesis.

Anatomy and physiology of the male reproductive tract. As previously mentioned, spermatogenesis is especially sensitive to changes in temperature (59, 60, reviewed in 61). Consequently, the testes are positioned outside the body in a sack of skin called the scrotum, which maintains the testes at an optimal temperature 2°C and 8°C below body temperature in man (52, 62, 63) and murine (64), respectively. Contraction of the cremaster and dartos smooth muscles of the scrotum affect the compensatory re-positioning of the testes in response to changes in environmental and body temperature (63). Testicular temperature is also maintained by modifying the rate of perfusion to the testis, which is mediated by autonomic neural control of the testicular vasculature (38, 59, 61, 65-67).

The glandular testis is encapsulated by the white, thick tunica albuginea (Fig. 1a-b), which is itself covered by the tunica vaginalis (not depicted in illustration), except at the junction of the epididymis and testis alongside the posterior border, and where the spermatic blood vessels enter the testis (63). The interior of the testis proper is subdivided into approximately 250 lobules, which each contain one to three highly-convoluted, seminiferous tubules that constitute the bulk of the testicular mass (68). Along with the Leydig cells, whose functions

will be discussed later, the testicular macrophages, fibroblasts, Sertoli cells, and peritubular myeloid cells (PMC) represent the somatic cell lines of the testis. The Leydig cells and testicular macrophages account for 25% of the cells in the interstitium of the testis (2, 69), the latter cell entering the germinal space only under severe pathological states to phagocytize nonviable cells and cellular debris (70). Although similar to the resident macrophage populations of other tissues, the testicular macrophages are not as immunologically potent as other macrophages, yet these cells secrete cytokines, lysozymes and present antigens (70, 71). The fibroblasts, also of the testicular interstitium, maintain the extracellular matrix (ECM) and provide attachment for the investing vasculature, which include blood vessels and the lymphatics (2).

The large, columnar Sertoli cells, which span from the basal lamina of the tubule wall to the adluminal surface, provide developmental support for the germ cells; the PMCs surround the entire seminiferous tubule (2, 19, 72). Tight junctions between adjacent Sertoli cells form the blood-testis barrier, creating two compartments: i.) an exterior compartment of premeiotic spermatogonia and ii.) an interior compartment of postmeiotic spermatocytes, spermatids, and spermatozoa within the seminal tubule (2, 19, 21). During the process of spermiation, cytoplasmic bridges between adjacent sperm are broken and sperm emerge tail first into the seminal lumen (or central cavity) of the seminal tubule (2, 73). Spermatozoa not released during spermiation, as well as apoptotic germ cells and discarded cytoplasmic contents (i.e., residual bodies) are phagocytized by the Sertoli cells (2, 74). Next, sperm are temporarily stored in the lumen before entering into a network of 30 short collecting tubules called the straight seminiferous tubules (2, 75). The straight tubules converge at the rete testis (Fig. 1c), which is a reticular space located in the medial to the testis proper, yet on the posterior pole of the testis (63, 76, 77).

The sperm exit the rete testis in the efferent ducts to enter the caput (head) epididymis (Fig 1), thence the corpus (body) epididymis, and lastly, the cells traverse into the cauda (tail) epididymis where the sperm will mature (i.e., become motile and acquire the ability to fertilize 78). The testis is bound tightly to the proximal end of the caput epididymis via the superior ligament of the testis (SL; Fig. 1a-b), where the tunica vaginalis is thickened into a dense network of connective fibers. The inferior ligament of the testis (IL), which is contiguous with a portion of the scrotal wall, connects the cauda epididymis to the inferior pole of the testis (Fig. 1a-b) and is characterized by a thick bundle of connective strands (79). During ejaculation, a two-stage event that begins with seminal emission, where the sperm are expelled from the cauda epididymis and enter the muscular vas deferens (Fig 1b), is followed by the ejaculation proper, where rhythmic contractions of the vas deferens propel the sperm through the remaining portions of the male reproductive tract, which include the ejaculatory duct, urethra, and the glans penis (62, 63, 77, 80, 81). Before exiting the body, the sperm are bathed in milky secretions from the seminal vesicles, bulbourethral and prostate glands (Fig. 2), which provide nourishment for the sperm, stimulate contraction of the female vagina and uterus, and lubricate the penis (63, 75).

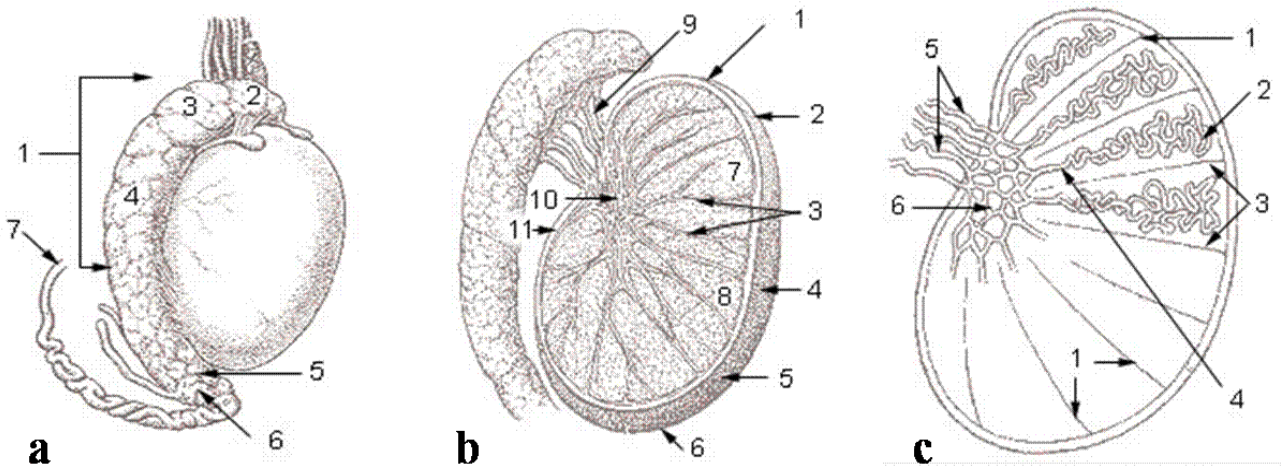

FIGURE 1: ULTRASTRUCTURE OF THE HUMAN TESTIS. Exterior surface of the testis (a) and in cross section (b and c).[1]

(a) Epididymis (**1**); Caput epididymis; region of superior ligament (**2**); Lobules of epididymis (**3**); Corpus epididymis (**4**); Cauda epididymis ; region of interior ligament (**5**); Duct of epididymis (**6**); Ductus deferens or vas deferens (**7**)

(**b)** Superior pole of testis (**1**); Tunica albuginea (**2**); Testicular septa (**3**); Anterior/ventral margin (**4**); Lateral surface (**5**); Inferior pole of testis (**6**); Testicular lobules (**7**); Parenchyma of testis (**8**); Efferent ducts (**9**); Mediastinum testis (**10**); Posterior/dorsal margin; region of testicular hilium (**11**)

(c) Testicular septa (**1**); Seminiferous tubules (**2**); Testicular lobules (**3**); Straight seminiferous tubules (**4**); Efferent ducts (**5**); Rete testis (**6**)

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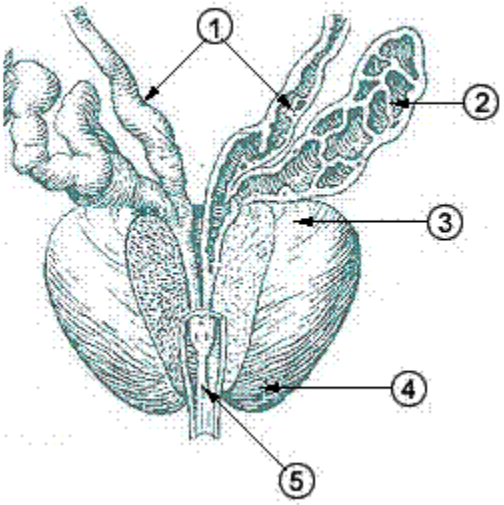

FIGURE 2: ACCESSORY ORGANS OF MALE REPRODUCTION. Schematic of the accessory sex organs of man, in cross section.[2] Vas deferens (**1**); Seminal vesicle (**2**); Base of the prostate (**3**); Apex of the prostate (**4**); and Prostatic urethra (**5**)

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Endocrine and local factors control testicular function and androgenesis. Endocrine control of the male gonads is accomplished primarily by two hormones— luteinizing hormone (LH) and follicle-stimulating hormone (FSH)—which are secreted by the gonadotrophs, a group of specialized cells in the pars distalis (PD) region of the adenohypophysis, called also the anterior pituitary gland (63, 82-85). In addition to thyroid-stimulating hormone (TSH), also of the adenohypophysis, and human chorionic gonadotrophin (hCG), which is secreted by the placental syncytiotrophoblasts (86), LH and FSH are members of a small, yet functionally diverse, family of proteins called the glycoproteins (83, 87, 88). The glycoproteins are composed of monomeric α and β subunits, which are linked by disulfide bridges at several amino acid positions (reviewed in 88). Oligosaccharide side chains are covalently bonded to the amide nitrogen of asparagine (Asn) residues on one or more of the subunits (83, 89, 90). In humans, the amino acid sequences of the α subunits of LH and FSH are identical— both hormones have a total of 204 amino acids and a MW of ~30kDa (83, 87, 91). According to a review by Ryan *et al.* (83), the ultrastructural characteristics of the glycoproteins are highly conserved across several vertebrate species, which is evident in the high sequence homology of each subunit. Sugar moieties at positions α-56 and α-82 are also conserved in the structures of LH and FSH throughout the mammalian taxon (83, 87, 89, 90, 92-94). Conversely, the variable nature of the β subunit confers distinct immunological responses, differential receptor binding capabilities, and unique pharmacological properties, which ultimately contribute to the highly-specific biological actions of the glycoprotein hormones (83, 87).

The gonadotrophins act in synergy to mediate androgen (male sex hormone) production/secretion and spermatogenesis, which are the two principle functions of the mammalian testis (85, 91). The synthesis and subsequent secretion of LH and FSH is governed

by gonadotrophin-releasing hormone (GnRH), a decapeptide neurohormone released from the anterior medial peptic area of the hypothalamus (91, 95, 96). In both males and females, secretion of GnRH is cyclical, occurring in episodic bursts every 90 minutes (91, 95). In males, the hypothalamopituitary-gonadal axis is regulated by a negative-feedback control mechanism with the actions of the sex hormones and inhibin, a testicular endocrine factor, serving to downregulate secretion of GnRH and the gonadotrophins (85, 91, 95).

LH and testicular steroidogenesis. Luteinizing hormone binds to the extracellular domains of the LH receptor (LH-R), a member of the G-protein coupled receptor (GPCR) family located on the surface of the interstitial Leydig cells (69, 97-99). Activation of the LH-R initiates an intracellular signaling transduction cascade mediated by the cyclic AMP (cAMP) second messenger system and the lipophilic prostaglandins (100). The phosphorylation of downstream signaling elements by cAMP activates transcriptional expression of the steroidogenic acute regulatory protein (StAR; MW 30 kDa), which transports cholesterol ($C_{27}H_{46}O$) to the P450 side-chain cleavage enzyme complex (P450scc) of the inner mitochondrial membrane (101-104). As the name implies, P450scc catalyzes the cleavage of the nonpolar side chain at the C-17 position of the cholesterol molecule which results in the production of pregnenolone (105). The side-chain cleavage reaction is the rate-limiting step in the *de novo* synthesis of the steroid hormones (98, 105, 106). Hydroxylation of pregnenolone produces 17-OH pregnenolone, which is subsequently converted to the prohormone dehydroepiandrosterone (DHEA), the primary precursor to the gonadal steroids in both sexes (91). Despite being a weak androgen, DHEA is a potent neurosteroid agonist of the sigma-1 receptor of the central nervous system (CNS; 91, 107, 108, 109). Reduction of DHEA by the enzyme 3β-hydroxysteroid dehydrogenase generates androstenedione, which is subsequently

reduced to testosterone (T) by the enzyme 3β-hydroxysteroid dehydrogenase (91, 110). It is worth noting here that the zona reticularis of the adrenal cortex is a secondary site for steroidogenesis, synthesizing <5% of the weak androgens in circulation (91).

There exist several reports of an intimate physiologic association between the testicular macrophages and male reproductive function, wherein the testicular macrophages influence steroidogenesis and modulate the responsiveness of the Leydig cell to LH (70, 111). Microvilli on the Leydig cell surface, which interdigitate with the coated pits or vesicles on the surface of the testicular macrophage, mediate the crosstalk between the two cells (111). In mice deficient for the gene encoding colony-stimulating factor-1 (CSF1-KO), the main growth factor for macrophage development within the reproductive tract, Cohen *et al.* (111) reported that mutants displayed low sperm number, decreased libido, and significantly reduced plasma T and LH concentrations as compared to wild-type males. Others investigators report that Leydig cells grown in media, which was previously exposed to testicular macrophages, secrete more T than control Leydig cells (70). The testicular macrophages secrete 25-hydroxycholesterol (25-HDC), an oxysterol (oxidized form of cholesterol) with myriad biologic functions. At micromolar concentrations, 25-HDC induces apoptosis of lymphocytes and is cytotoxic to tumor cells (112), however this oxysterol also mediates the differentiation of hepatocytes (113), keratinocytes (113), and promotes the development of Leydig cells *in utero* (70), and after birth (114). In the absence of cholesterol, 25-HDC is an excellent substrate for steroidogenesis by circumventing the StAR protein transport system of the Leydig cell (115). These independent observations suggest a central role that the testicular macrophages have in promoting the morphological development of the testis and in mediating Leydig cell physiology.

After secretion from the Leydig cell, DHEA, androstenedione, and T enter into systemic circulation where they are taken up by cellular targets in adipose tissue, CNS, bone, and the reproductive tract (91). Inside the target tissues, DHEA and androstenedione may bind to intercellular the androgen receptors (AR), members of the nuclear receptor superfamily which are similar in function to the progesterone and estrogen receptors (PR and E_2R, respectively; 116, 117-119). Activation of the AR leads to expression of various gene products that mediate lipid and carbohydrate metabolism, protein synthesis, muscle development, bone growth and maturation in nonreproductive tissue (120-122) as well as the promotion of spermatogenesis in the male reproductive tract (119, 123-126). Androgen receptors also have "nongenomic" functions, including the promotion of vascular function and induction of second messengers signaling systems (91, 116).

Alternatively, once inside of target tissues, DHEA and androstenedione can be converted into T, which, in turn, is reduced to 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase within the prostate, testis, and adrenal cortex (91, 110). During fetal development the *in utero* production of DHT, a powerful anabolic hormone which has an AR binding affinity 3 times that of T, drives the development of the internal and external genitalia, whereas in puberty, DHT is responsible for the onset of the male secondary sex characteristics (91, 110). Other successive metabolites of T include the testicular estrogens—specifically, estrone (E_1) and estradiol (E_2)—which are produced in a well-characterized biochemical reaction involving the enzyme aromatase (ARO; reviewed recently 127, 128-130).

The role of estrogen in reproductive tract function (128, 130-132) and mediating copulatory behavior (133-136) in males is multifaceted and the focus of ongoing research. Previous studies in rat demonstrate that administration of estrogen, in combination with T,

lowers testicular weights and leads to reversible azoospermia (137), but did not affect male mating behavior (138) , serum concentrations of T and E_2, and sex organ accessory weights (137). Simultaneous administration of both steroids led to antagonistic effects, i.e., initial decreases in testicular function and accessory organ weight caused by estrogen treatment were countered by sufficient doses of T (137). Robaire *et al.* (137) demonstrated that while low doses of estrogen, without added T, did not significantly affect serum E_2 levels, testicular weights and spermatid or spermatozoa counts, estrogen treatment resulted in significant reductions in serum LH and testosterone levels and in the weights of sex accessory organs; no significant changes in FSH were detected in all treatment groups. Higher doses of E_2, without administration of T, led to increased serum estradiol levels, and produced a sustained reduction in the testicular and sex accessory organ weights and testicular sperm count (137). Subsequent researchers, using mice homozygous for a mutation in the E_2R gene (E_2R knockout or E_2RKO), reported no visible anatomical aberrations in the prostate glands, epididymi, and seminal vesicles of E_2RKO mice (139). Mating studies demonstrated that the E_2RKO animals were infertile, as compared to the heterozygous E_2R mutants and wild-type animals that produced several offspring (139). Histological analysis of the seminiferous tubules of E_2RKO mice revealed enlarged luminal diameters, whereas the seminal epithelia of other animals were disorganized or mainly filled with Sertoli cells (139). In any case, spermatogenic cells were nearly absent in the seminal tubules of E_2RKO mice, suggesting a novel role of E_2R in mediating normal spermatogenesis (139).

The morphological integrity of the germinal epithelium is maintained by FSH. The formation of tight junctions (=zonula occludens) between adjacent Sertoli cells, which completely envelope the developing germ cells, is stimulated by FSH. The intimate association between the Sertoli and germ cells is the basis for the seminal epithelial architecture, which is a

characteristic feature of the healthy testis during the cycle (56). FSH and T also increase the synthesis of inhibin and androgen-binding protein (ABP) when they are bound to the extracellular domains of the Sertoli cell FSH receptor (FSHR), also a GPCR (91). Inhibin downregulates the secretion of GnRH and FSH, whereas ABP binds to T, DHT, and E_2 to decrease the hydrophobicity of these hormones, an effect which consequently increases the solubility and concentration of these hormones within the luminal fluid of the seminiferous tubules, epididymis, and systemically (91). In turn, the increased concentration of T and DHT promote spermatogenetic activity within the testis and the maturation of the spermatozoa within the cauda epididymis (76, 77, 81, 91).

According to the lumicrine hypothesis, factors that are secreted by upstream cells can regulate the activities of downstream cells via a luminal or ductal network (81). Support for this hypothesis is reviewed by Hinton *et al.*(81), who cite studies where ligation of the efferent ducts resulted in apoptosis and morphological changes in the epithelial cells of the proximal epididymis, whereas expression and the activity of the epididymal genes proteins, respectively, are affected by loss of testicular factors secreted by the upstream Leydig and/or Sertoli cells. ABP is one such testicular factor that is widely considered to exert its affects on the epididymis. Studies suggest that APB regulates expression of 5α-reductase within the epithelial cells of the caput epididymis, and thus the production and concentration of DHT within the caput lumen (81). As the initial point of sperm entry into the epididymis, the caput epididymis, under the influence of luminal factors, may play an important role in the maturation and development of the spermatozoa in more distal regions of the epididymis.

Gronning *et al.* performed *in vivo* experiments, which have further elucidated the actions of FSH on the Sertoli cell (140) . The investigators showed that the expression of tissue inhibitor

of metalloproteinases-1 (TIMP-1) in whole testes was significantly reduced in hypophysectomized male rats, along with serum levels of FSH (140). However, expression of TIMP-1 could be upregulated by exogenous administration of FSH in hypophysectomized males (140). Northern blot analysis confirmed the presence of TIMP-1 within the nuclei of peritubular and Sertoli cells, whereas the nuclei of Leydig cells, primary spermatocytes, and spermatids were not immunoreactive (ir) for TIMP-1 (140). TIMP-1 are the endogenous inhibitors to matrix metalloproteinases (MMPs), enzymes that degrade extracellular matrix (ECM) proteins, mediate cell proliferation, differentiation, and apoptosis—all integral functions of normal physiological tissue remodeling (141). These findings suggest that FSH, in regulating the expression of the endogenous inhibitors of MMPs, may also mediate tissue remodeling, which is critical for the germ cell rearrangements of testicular organogenesis and of the cycle (140).

The gonadotrophins also regulate the secretion of autocrine factors from the peritubular, Leydig, and Sertoli cells, which, in turn, govern the release of paracrine factors that mediate the development of undifferentiated spermatogonia, developing spermatocytes, and spermatozoa (142). In summary, a plethora of autocrine and paracrine factors of gonadal origin have been identified (reviewed extensively in 72). For example, the pro-inflammatory cytokines, which include: i.) interleukin-1 alpha, interleukin-1 beta, and interleukin-6 (IL-1α, IL-1β, and IL-6, respectively), tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ), along with several growth factors including: ii.) leukemia inhibitory factor (LIF) and stem cell factor (SCF), mediate crosstalk between adjacent Sertoli cells (142, 143) and promote germ cell proliferation of the spermatogonia and development of the spermatocytes (144). The Sertoli cell also secretes transferrin, ceruloplasmin, retinol-binding protein, which are likely to influence

germ cell development and the secretion of germ cell paracrine factors, such as nerve and fibroblast growth factors which influence Sertoli cell physiology (21).

Recent empirical evidence indicates that members of the IL-1 family: i.) increase Sertoli cell expression of testicular TIMP-1 (140), ii.) increase the proliferation of immature Sertoli cells, even more so than FSH (145), and iii.) increase the growth and differentiation of peritubular and Sertoli cells, while interacting with human growth hormone (GH; MW 22kDa) and insulin-like growth factor-I (IGF-I) to increase T secretion (146). Finally, other groups have demonstrated that abnormally high levels of pro-inflammatory cytokines, a characteristic feature of prolonged diseased states, negativity affects spermatogenesis and androgen production (142, 144).

II. Role of the autonomic nervous system in male reproductive function

Overview of the autonomic nervous system. The autonomic nervous system (ANS) is organized into ganglia (sing. ganglion), which are dense aggregations of neuronal cell bodies scattered throughout the periphery, and represents all the nerve pathways that form ganglion synapses outside of the CNS (75, 147). Neurons that synapse onto the peripheral ganglia are called preganglionic and have cell bodies in the brain or spinal cord (i.e., in the CNS), whereas neurons whose cell bodies are located within the peripheral ganglia are called postganglionic (75, 147). The ANS is divided into an efferent (motor) arm, which sends output various effectors, including glands and muscles of the vasculature, viscera, and genitourinary tract, via the cranial nerves (III, VII, IX, and X) and spinal preganglionics (75, 147). The afferent (sensory) arm receives information from the sense organs and viscera via the cranial nerves (VII, IX and X) and the general visceral afferent fibers (GVA; also called sympathetic afferent fibers; 75, 148). Traditionally, the motor arm of the ANS has been divided into three, distinct neuroanatomical divisions—the enteric, sympathetic and parasympathetic nervous systems (ENS, SNS and PSNS, respectively; 75). The interneurons, efferent and afferent neurons of the ENS are found within two types of ganglia—Auerbach's (myenteric) and Meissner's (submucosal) plexuses—situated in the mucosa of the gastrointestinal (GI) tract (147). Unlike the SNS and PSNS, which require input from the CNS to operate, the neurons of the ENS function autonomously, communicating with the CNS mainly by the parasympathetic vagus (X) nerve (75).

A general appreciation of the physiological features of the SNS and PSNS is essential to understanding the role of the ANS in mediating male reproductive function. Thus, the following discussion, albeit brief, is warranted and serves to provide a basic framework in which to view the more detailed discussions of this report.

Physiology of the autonomic nervous system. The cell bodies of the SNS are found in the intermediolateral gray matter of the thoracolumbar spinal cord and exit the CNS via the ventral roots located at T1-L2 (148). The preganglionic fibers of the SNS synapse onto postganglionics located in the 24 pairs of sympathetic (paravertebral) chain ganglia, which run on either side of the spinal cord (147). The SNS preganglionics may also synapse onto the prevertebral ganglia, which include the inferior and superior mesenteric ganglion (IMG and SMG, respectively), celiac ganglion (CG), and aortic-renal ganglia—unpaired, midline ganglia situated anterior to the aorta and vertebral column (75, 148). The preganglionic fibers of the PSNS (or the craniosacral outflow as it is more commonly called) originate from several brain stem nuclei, including the dorsal motor nucleus (DMV) of the vagus nerve, which provide extensive neural input to the viscera and urogenital tract (147), and from the sacral spinal cord. The sacral outflow exits via the lower three sacral roots (S2-S4) of the spinal cord to form the pelvic splanchnic nerve (PSN; known also as the nervi erigentes), which innervates the viscera and genitals (75, 149).

The actions of the SNS and PSNS, which are largely antagonistic to each other, are mediated by the release of the neurotransmitters acetylcholine (Ach) and norepinephrine (NE) from the pre- and postganglionic sympathetic neurons, respectively, and the release of Ach from all neurons of the PSNS (75). Neurons which release Ach are called cholinergic, whereas nerves which release NE are called adrenergic or catecholaminergic (75). It is also worth noting here that the adrenal medulla is widely considered a modified postganglionic sympathetic neuron, releasing the catecholamines NE and epinephrine (E) when stimulated by preganglionic neurons. The postganglionic neurons of the PSNS also release cotransmitters, such as vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY), neuronal nitric oxide synthase (NOS), in addition to Ach (148).

Not surprisingly, receptors which bind NE and E are called adrenergic receptors (ADR) and fall into two main groups—α and β—which are further subdivided into various morphophysiologically-distinct isoforms (reviewed in 150). The two subtypes of the α-ADR family, which include α_1ADR and α_2ADR, are generally associated with mediating effector excitation (reviewed in 151). Isoforms of α_1 are α_{1A}ADR, α_{1B}ADR, and α_{1D}ADR, whereas α_2 exists as α_{2A}ADR, α_{2B}ADR, and α_{2C}ADR (reviewed recently in 147). The intracellular domains of α_1ADR receptor are linked to the heterotrimeric G protein (G_q), a member of the GPCR superfamily (91). Activation of G_q via ligand binding to the extracellular domains of the α_1ADR mediates the catalytic activity of phospholipase C (PLC), which cleaves phosphatidylinositol phosphate (PIP_2), a membrane lipid, into inositol triphosphate (IP_3) and diacylglycerol (DAG). Diffusion of IP_3 into the cytoplasm mediates the release of calcium (Ca^{2+}) from the ER, whereas DAG activates protein kinase C (PKC), a downstream effector located in the plasma membrane (91). The liberation of Ca^{2+} triggers responses throughout the cell, including stimulation of PKC and the activation of Ca^{2+}-sensitive enzymes and of a Ca^{2+}-binding protein, calmodulin (91). The α_2ADR is linked to the G-protein inhibitory complex (G_i), which, when activated, downregulates activity of the adenylyl cyclase enzyme, and consequently, production of cAMP (91). In turn, reductions in cAMP levels inhibit cAMP-dependent protein kinase A (PKA) activity—a key enzyme responsible for the phosphorylation of downstream effectors (91).

The β-ADR exists in the β_1, β_2, and β_3 isoforms (reviewed recently 152 and 153), which are linked to the transmembrane G-protein stimulating complex (G_s), another GPCR. Activation of G_s increases activity of adenylyl cyclase, resulting in an increase in the intracellular levels of cAMP (91). The increased cAMP levels activate PKA, which phosphorylates downstream target proteins. Many enzymes (e.g., lipases and glycogen phosphorylase) are activated by

phosphorylation, whereas the catalytic ability of other enzymes (e.g., pyruvate dehydrogenase and glycogen synthase) are deactivated when phosphorylated by PKA (91). Termination of the activating signal (i.e., the neurotransmitter) results from the degradation of the neurotransmitter by monoamine oxidase (MAO) or catechol-*O*-methyl transferase (COMT), reuptake of the neurotransmitter by the presynaptic neuron and/or reuptake within the plasma membranes of non-neuronal cells in the vicinity (154). Inhibitors of COMT and MAO (MAOIs) prevent the breakdown of catecholamines, which increases their availability and greatly exaggerates the effects of the activated ADR (154). Due to potentially lethal drug and dietary contraindications, MAOIs are usually a last resort for the treatment of a wide range of psychiatric phenotypes including, depression, agoraphobia, social anxiety, and Parkinson's disease (155).

Epinephrine is considered to be the most potent, endogenous agonist of both groups of receptors, while the effects of NE are most pronounced on the α and β_1 receptors (91, 148). Thus, the adrenal medulla is central in the systemic activation of the ADR via release of E. Vasoconstriction of the blood vessels leading to the heart, skeletal muscles, genitalia and adipose tissue is mediated solely by NE, except in cases where more than one ADR type is represented (91, 148). In these cases, binding of E to the α- and β_2-ADRs results in vasoconstriction or vasodilation, respectively, depending on plasma concentrations of the ligand (91, 148). Although the α-ADR is less sensitive to E when activated, this receptor dominates the vasodilator effects of the β_2-ADR (91). As a result, high levels of circulating E cause vasoconstriction, while lower levels of circulating E enhance β-ADR stimulation, producing overall vasodilation. In this way, a single catecholamine can have differential effects, which are mediated by the binding affinity and serum concentration of the neurotransmitters as well as the makeup (or composition) of the ADR population on the surface of the effector (91, 148).

Aside from controlling vasomotor activity of the blood vessels, the adrenergic receptors mediate the contractile behavior of cardiac muscle, smooth muscle within the GI, respiratory and urogenital systems, endocrine function of the pancreatic (150). The ADR also mediates lipolysis, which is a multistep, catabolic process whereby triglycerides (TAG)—high-caloric molecules stored in white adipose tissue (WAT) pads located in the abdominal and thoracic cavities, subcutaneously, and within muscle and liver (156)— are hydrolyzed into free fatty acids (FFAs) and glycerol (reviewed previously, 157). Before entering into blood, the FFAs are conjugated to a water-soluble, plasma protein called albumin (157). Inside of target cells, FFAs are oxidized as fuels yielding acetyl-CoA, which powers the Krebs cycle, and ketone bodies, which act as a secondary fuel source for the brain (157). Various reproductive and metabolic functions that are mediated by the adrenergic receptor family are reviewed in the following sections within the context of primary research.

Neural control of male reproduction. As discussed previously, the development and proper functioning of the male reproductive tract are largely the result of hormonal influences, however, the successful completion of the principal reproductive event (i.e., insemination of the female) requires exact timing, and consequently, the involvement of the CNS (158) and ANS (66). For example, sexual arousal brought about by psychogenic stimuli and the physical stimulation of the male (and female) genitalia, followed by the events of the sexual act, is mediated by the somatic (voluntary motor) and autonomic nervous systems (75). Specifically, the medial preoptic area of the anterior hypothalamus and limbic areas of the CNS mediate sexual arousal (159), whereas autonomic neural control of the mammalian male reproductive organs by the SNS (42, 160-167) and the PSNS (162, 166, 168) ensure the proper functioning of the testes (i.e., androgen production and spermatogenesis) and successful fertilization, which

involves penile erection and glandular secretion. Thus, neural control of reproduction facilitates synchronized responses throughout the male reproductive tract and whole body, and most likely evolved to ensure the perpetuation of the species (75).

Innervation of the male reproductive organs is accomplished by three, major sets of nerve fibers—lumbosacral, sacral parasympathetic and the thoracolumbar sympathetic nerves—which converge at the pelvic plexus (159, 169). According to a review of the pelvic plexus by Dail (170), the interaction of the these nerve fibers at the pelvic plexus is not well understood. The lumbosacral outflow to the reproductive tract includes the genitofemoral (=inguinal), pudendal, and in certain species (e.g., man and equine), the ilioinguinal nerves (for review, see ref. 171). The genitofemoral nerve, which arises from the lumbar plexus at spinal roots L1 and L2, supplies the cremaster muscle and scrotal skin (171, 172). Originating in the sacral plexus, the pudendal nerve derives fibers from nuclei in the dorsomedial (DM) and dorsolateral (DL) cell columns near spinal segments L5-L6 and from the ventral branches of the second, third, and fourth sacral nerves (S2, S3, S4, respectively; 169, 173, 174). The pudendal, which at least in rat, branches into motor and sensory divisions at the level of the ischium nerve, supplies somatosensory innervation to the genitalia, specifically to the penis, scrotum, and perineum (169). The motor pudendal division carries somatic fibers to the striated penile muscles, whereas both divisions carry efferent sympathetics (involuntary motor) to the bulbospongiosus and ischiocavernosus muscles (63, 169). Stimulation of the bulbospongiosus and ischiocavernosus results in erection and the spasmodic contractions of sexual climax, which generally involve ejaculation and the euphoric sensation of orgasm (reviewed in 175). The ilioinguinal derives its fibers from L1 and innervates the skin over the root of the penis and the upper part of the scrotum (169).

The diffuse and extensive autonomic neural projections to the male genitalia have been described in cat (166), rat (176) , camel (161) and other animal species (79, 177-179). Accounting for less than 30% of the neuronal input to the sex accessory organs, the PSNS represents a minor contribution to the male reproductive genitalia (66). The primary source of parasympathetic input to the male reproductive organs is the PSN (149, 170, 180). The PSN receives virtually all of the parasympathetic neurons exiting the sacral spinal cord (149), supplying vasodilator (proerectile) control to the cavernous smooth muscles and vasculature of the penis (66, 170) and visceral afferent fibers to the pelvic plexus (171). Hill and Toivola (164) reported that sacral cordotomy, which is transection of the parasympathetic portion of the spinal cord at the lumbosacral articulation, significantly reduced the testicular weights and serum levels of testosterone of male guinea pigs. Transected pigs had severely atrophied seminiferous tubules, which showed little spermatogenic activity, whereas the epididymi of transected males lacked sperm (164). Considering the lack of empirical evidence demonstrating significant cholinergic innervation of the testis, outside of the testicular capsule and supporting vasculature (79), the findings of Hill are perplexing to say the least. Numerous groups have reported disruptions to spermatogenesis, the pituitary-gonadal axis, and testicular blood flow following spinal cord injury (SCI), which ultimately causes irreversible infertility in man (181-183) and the Sprague-Dawley rat (124, 184-194), whereas Campos reported that the testis is richly innervated by catecholaminergic (195) and serotonergic fibers (196). Thus, it is plausible that the surgical procedure performed by Hill and Toivola incidentally severed the sympathetic fibers to the testis (and possibly epididymis), which *has* been shown to cause spermatogenic defects and induce a pathophysiologic condition in testicular function (42, 43, 197, 198). For these reasons, the remainder of this discussion will focus on the role of the SNS in mediating male reproduction.

The testicular vasculature is innervated by adrenergic and peptidergic fibers. Using techniques common to classical immunocytochemistry (ICC), Rauchenwald *et al.* (163) reported that while VIP-ir neurons were not detected within the testis, NPY-ir neurons were dispersed throughout the tunica albuginea and superficial vasculature. The results of a subsequent study by Lakomy (165) corroborated the findings of the Rauchenwald group, demonstrating the presence of noradrenergic and peptidergic markers, which varied in degree between the cauda epididymis, testicular capsule and parenchyma of adult boars. Zhu *et al.* (79) reported that neurons immunoreactive for VIP, calcitonin gene-related peptide (CGRP) and C flanking protein of NPY (CPON; a marker for NPY) were present in the intracapsular interstitium and testicular blood vessels, although no neurons were found innervating the testicular parenchyma, seminiferous tubules, and the intratesticular area of the rete testis of the rat. The discovery of sparsely distributed catecholaminergic neurons throughout the vasculature and capsule provided new insight on the innervation of the testis (79, 163, 199).

Exiting from the thoracolumbar segments of the spinal cord, the lumber splanchnic (LSN) and hypogastric nerves mediate smooth muscle contraction of the testicular capsule (79, 163, 176), epididymis (200), and vas deferens (201-207), which propels the sperm out of the testis and into the epididymis and through the seminal tract during seminal emission (175, 208). The sympathetic nerve fibers also mediate glandular secretion of the seminal vesicles, bulbourethral and prostate glands during the ejaculatory event (66, 80, 170, 209, 210). Investigations involving the surgical treatment of testicular tumors in man have added to our understanding of the role of the LSN in the reproductive process. Ipsilateral or contralateral electrical stimulation of the LSN, during nerve-sparing, laparoscopic retroperitoneal lymph node dissection (L-RPLND), resulted in unilateral seminal emission on the stimulated side, which was

recorded by an endoscope inserted into the urethra (211). Although cross innervation of the vas deferens and ejaculatory ducts is widely accepted in other mammals (201, 202, 206), the results of this study ruled out cross innervation in humans and provided evidence for the ipsilateral, sympathetic efferent control of seminal emission (211).

Electrical stimulation of the LSN of canines, which arises from spinal segments L1-L2 and synapses onto the caudal mesenteric ganglion (which corresponds to the IMG and superior hypogastric ganglia in humans), resulted in seminal emission (203). Conversely, electrical stimulation of the thoracic splanchnic nerves, which originate from spinal segments T5 -T12 and synapse onto the CG and SMG, did not result in seminal emission (203). Similar investigations in canines demonstrated that transection of the spermatic nerves (to be discussed later), followed by electrical stimulation of the distal end of the severed nerve, did not cause seminal emission (200). However, electrical stimulation of the same portion of the severed spermatic nerve a month later, accompanied by transection of the hypogastric nerve and lumbosacral spinal cord, produced seminal emission (200). These findings indicate that the superior spermatic nerve, which arises, in part, from the upper lumbar sympathetic trunk, may provide a compensatory route for seminal emission if the hypogastric nerves or LSN are damaged.

The role of the spermatic nerves in testicular function. The contributions from the superior and inferior spermatic nerves (SSN and ISN, respectively) represent the principle supply of catecholaminergic input to the mammalian testis (66, 79, 195, 212), although an intratesticular source of catecholamines, which is not reviewed here, has been reported recently (213-216). The SSN carries output from the renal, spermatic, aortic, and mesenteric plexuses and descends bilaterally into the superior pole of the testis inside the spermatic cord (Fig. 1b). The SSN, which also receives afferent and possibly vagal parasympathetic fibers (171, 217), is carried into

the testis alongside the internal spermatic (=testicular) artery (61, 79). Originating primarily from branches of the pelvic and inferior mesenteric plexuses, the ISN, which carries mostly sympathetic fibers, accompanies the vas deferens to penetrate the cauda epididymis near the IL, where it penetrates the testis, although to a lesser extent than does the SSN (79, 180, 218).

The role of the SNS in testicular function, specifically, spermatogenesis, androgen production, testicular blood flow, and seminal emission was elucidated by selective nerve transection and stimulation (electrical) experiments involving the SSN, ISN, and lumbosacral spinal cord. Previous investigators observed alternations to spermatogenesis and/or testicular endocrinology following testicular denervation by surgical (42, 43, 197-199, 212, 219-222) and chemical means (35, 223-225) and after SCI (42, 43, 124, 181-191, 193, 194, 197-199, 212, 221, 222), which suggest that nonendocrine mechanisms (i.e., neural input) mediate normal testicular function—specifically, germ cell differentiation, development, androgenesis, and maintenance of the germinal structural integrity. Chow *et al.* (42) surgically transected the SSN and ISN of adult rats and observed a significant regression in spermatogenesis, increased phagocytosis of mature sperm, and delayed spermiation, whereas other groups observed atrophy of the testes, degeneration of the seminal tubular epithelia, and hypertrophy of interstitial secretory tissue following interruption of testicular innervation in dogs (226), guinea pigs (227) and rabbits (219). In stark contrast, Frankel and Ryan (228) reported that bilateral denervation of the spermatic plexus, which gives rise to the SSN, produced no effect on testicular weights, serum testosterone levels, spermatogenesis, or mating behavior. The authors reported that the pelvic nerve plexus (which supplies the ISN) was left intact, which may explain these conflicting findings.

As the principle neurotransmitter substance of postganglionic sympathetic neurons, much study has been devoted to the role of NE in mediating spermatogenesis and androgen production. Recall that binding of NE to the $\alpha_{1\beta}$ADR activates hydrolysis of phosphatidylinositol (PI), which upregulates mitosis, thereby enhancing cell proliferation (229-231). Allen *et al.* (229) showed that transfection of rat fibroblasts with a plasmid containing a constitutive-activating mutation in the α_{1B}ADR gene: i.) increased expression of the wild-type α_{1B}ADR within the mutant fibroblasts, ii.) enhanced the mitogenic effects of NE on mutant fibroblasts, and iii.) promoted tumor development in nude mice injected with mutant fibroblasts. Mhaouty-Kodja *et al.* (230) described novel effects of NE on male gonadal function, presumably through its activation of the α_{1B}ADR in male mice. Adult mice, which were homozygous for mutations in the α_{1B}-adrenergic receptor gene (α_{1B}ADRKO), showed decreased testicular weights, whereas the undifferentiated germ cells of α_{1B}ADRKO mice entered into an apoptotic pathway at meiosis (230). Additionally, α_{1B}ADRKO mice exhibited severely reduced concentration of serum T, resulting in significantly elevated serum LH levels when compared to the wild-type controls (230). Consequently, approximately 30% of the mutant mice were infertile due to premature apoptosis and abnormal serum T (230). Intratesticular production of inhibin was increased in the α_{1B}ADRKO mice, which was correlated with increased expression of the inhibin gene (230). Finally, *in situ* hybridization demonstrated that α_{1B}ADR messenger RNA (mRNA) was present in the early spermatocytes of the wild-type testis (230). Publications by Mayerhofer (223) and Campos *et al.*(212) may serve to explain the elevated levels of LH reported by Mhaouty-Kodja. Mayerhofer reported that injection of 6-hydroxydopamine (6-OHDA), a neurotoxin which selectively targets the noradrenergic neurons of the SNS, significantly reduced the concentration of LH-R and testicular T in the hamster testis after 24 hours (h), as compared to vehicle-treated

control, contralateral testis (223). Injection of NE into previously untreated hamsters reduced the concentration of the LH-R and testicular T after 24h, which suggests that NE may downregulate gonadal function during stress (223). At 72h, the LH-R and testicular T levels were significantly increased, whereas LH-R and T levels of animals sacrificed at 10h, 48h, 144h (6 days), or 168h (7 days) were not significantly different as compared to controls (223). At144h and 168h, the testicular weights of animals treated with 6-OHDA or NE was significantly reduced, an effect which was secondary to the degenerative morphological changes in the seminal epithelium, including the invasion of neutrophils into the lumina of centrally-located tubules (223). Bergh (232) demonstrated 6-OHDA had no effect on germinal morphology or spermatogenic ability in rats after a 49-day treatment period, which suggests that 6-OHDA treatment may be ineffective in suppressing testicular function following prolonged exposure to the neurotoxin. Subsequent investigators replicated these findings, demonstrating that bilateral transection of the SSN nerve in rats decreased the number of LH receptors and significantly reduced the *in vitro* production of androgen in response to hCG stimulation (212). In theory, the reduction in LH-R and T caused by 6-OHDA treatment and SSN denervation may have led to over secretion of LH in the α_{1B}ADRKO mice. Together, the findings of Mhaouty-Kodja and Campos suggest a central role of NE (and thus, the ANS) in gonadal function in demonstrating that disruption of adrenergic receptors indirectly impairs germ cell development by compromising Leydig-Sertoli cell communication and androgenesis (230). Moreover, these studies suggest that NE, through its activation of the α_{1B}ADR, might be critical for promoting the synchronized mitogenic activity of spermatogenesis.

Lamono-Carvalho *et al.* (167, 233) reported that selective guanethidine-induced sympathectomy of the epididymis and vas deferens of prepubertal male rats increased

intratesticular progesterone levels and reduced the intratesticular androstenedione and T levels, yet had no effect on spermatogenic ability of the testis. In late-pubertal and adult rats, guanethidine therapy increased the concentration of spermatozoa in the caudal epididymis, indicating the inability of sympathectomized males to emit semen, control fluid resorption, and clear unejaculated spermatozoa (233). Moreover, pre-pubertal rats subjected to subcutaneous injections (10 mg/kg/day) of guanethidine (GD), an inhibitor of NE release from sympathetic preganglionics, show significantly elevated intratesticular progesterone levels, reduced T levels, which were purportedly caused by the "specific blockade" of 17-α-hydroxylase affected by GD therapy (234). Catecholamine content assays confirmed denervation of the testicular tissue, whereas histological analysis confirmed that spermatogenesis was not affected by the GD therapy (234). These findings are largely in concert with published studies in adult rats, which report that spermatogenic ability of the testes, serum levels of T and LH, fertility of epididymal sperm are unaffected by guanethidine-induced sympathectomy of the epididymis (191, 235).

The role of the SSN and ISN in T secretion and spermatic blood flow (SBF) was elucidated in classical electrical stimulation experiments. These studies demonstrated that moderate (25-35V) to high-intensity (36-70V) electrical stimulation applied to the SSN in cats increased plasma concentration of T within the spermatic vein, but decreased the overall SBF though this vessel; NE concentrations in arterial blood and the spermatic vein also increased from basal values after electrical stimulation (67). Conversely, moderate stimulation of the ISN had no effect on plasma concentrations of T, NE output, or SBF, whereas high-intensity stimulation increased SBF only (67). It is important to note, however, that heart rate and mean arterial pressure (MAP) did not change with the fluctuations in SBF, suggesting that the modifications in vascular diameter were elicited by neuromediators (67). The differential effects

of electrical stimulation of the SSN and ISN on T concentration and testicular blood flow: i.) provide evidence for the neural control of T secretion and ii.) demonstrate that vascular tone of the spermatic artery is mediated by adrenergic input from the SSN and ISN that control arterial vasoconstriction and vasodilation, respectively (67).

III. Energy availability, reproduction, and white adipose tissue

Warm-blooded animals require a constant supply of energy to power the life-sustaining metabolic activities of the cell that maintain body temperature and power biosynthetic pathways. Thus, in an energy deficit, organisms suspend energetically-expensive functions, such as gametogenesis, gonadal steroid production, and lactation, which are not critical for the immediate survival in favor of function that sustain life (reviewed in 236 , 237). The evolution of neural, metabolic, and endocrine signals, which mediate the crosstalk between critical metabolic pathways and non-critical reproductive functions, ensured that life-sustaining processes, such as cell growth/development, aerobic respiration, and thermoregulation, continued during prolonged metabolic challenges—e.g., when the levels of circulating (e.g., carbohydrates) or stored fuels (e.g., glycogen and triglycerides) dropped below a critical value (236, 238).

Carbohydrate oxidation, a stepwise process and ubiquitous hallmark of heterotrophic organisms, is the preferred method of energy transfer in warm-blooded animals. During metabolic challenges circulating fuels and glycogen stores are generally depleted first, followed by release of triglycerides from WAT (i.e., lipolysis), which ensures that energy needs are met and life-sustaining process are not compromised (156, 239-243). However, when WAT stores are depleted, non-critical processes, such as those that support reproduction, are severely compromised (237). The direct relation between availability of energy and reproductive fitness is especially true in females, whose reproductive costs some believe to be greater than that of males (237, 239, 244-248). Frisch (249) reviewed a large body of evidence which provides strong evidence that excessive leanness caused by malnutrition, rapid weight loss or heavy physical exercise disrupts normal energy metabolism and reduces gonadotrophin and estrogen secretion, thereby preventing menarche in prepubertal girls, while lessening the reproductive

ability and fertility of athletic women. Food-restriction studies done in prepubertal female rats demonstrate that when energy levels drop below certain critical values, secretion of GnRH is inhibited which, in turn, inhibits secretion of pituitary LH, decelerates follicular development and disrupts ovulation (237, 240, 241, 244, 250, 251). Additionally, partial excision of the parametrial white adipose tissue (PWAT) depots surrounding the ovaries of pregnant, Djungarian hamsters produced no effect on the litter or pup size, but inhibited the investment in a second reproductive attempt, while decreasing the size of second litters as compared to the size of second litters in control females (252). Thus, internal control mechanisms—mediated by the neural and humoral signals mentioned previously—monitor the availability of reserve and dietary energy, which allow animals to prioritize their reproductive functions and behavioral options, especially when nutritional needs are unmet (236, 237, 240, 241, 253).

IV. Role of white adipose tissue in male reproductive function

The role of the gonadal WAT in mammalian reproduction. Although well documented in human females (236, 237, 244, 246, 254, 255), rodent (244, 250, 252, 253, 256), Rhesus monkey (257) and dairy cow (245, 247, 248), the exact role that energy availability or reserves plays on reproductive function in males, specifically gonadotrophin secretion, androgenesis, spermatogenesis and mating behavior, is less understood and begs to be explored. Previous investigators demonstrated that bilateral removal of the gonadal epididymal white adipose tissue (EWATx) decreased spermatogenic activity in albino rats (258) and Syrian hamsters (*Mesocricetus auratus*; unpublished data), whereas unilateral or bilateral removal of an equal amount of the non-gonadal inguinal white adipose tissue (UNI-IWATx and IWATx, respectively)—with or without transplantation of the excised IWAT pads under the dorsum of the donor hamster—had no effect on spermatogenesis as measured by ejaculatory sperm counts (unpublished data). Moreover, male mating behavior—indicated by mount, intromission, and ejaculation frequencies (see 259 for descriptions of rat copulatory behavior)—did not differ significantly between any of the experimental group, however the ejaculate recovered from the vaginas of females mated with EWATx males contained virtually no sperm, even when the excised EWAT pads were transplanted dorsally (unpublished data). Conversely, the ejaculate recovered from females mated with IWATx (with or without transplantation) and UNI-IWAT males was abundant in sperm (unpublished data).

Microscopic examination revealed that the seminiferous tubules of EWATx rats were markedly atrophied (258), whereas quantitative measurements revealed that (on average) the testes of EWATx hamsters had significantly thinner seminal tubule epithelial widths and fewer sperm than the hamsters which received unilateral EWAT removal (UNI-EWATx) or sham

surgery (unpublished data). Compared to the sham controls, relatively normal spermatogenesis and normal seminal tubule widths were observed in the EWAT-intact testes of UNI-EWATx males, however, the contralateral, EWAT-lipectomized testis within the same hamster had significantly fewer sperm-positive tubules, reduced sperm counts, and thinner epithelial widths (unpublished data).

Hormone radioimmunoassay (RIA) revealed that plasma levels of FSH were significantly higher in EWATx hamsters than in the UNI-EWATx or shams, suggesting loss of testicular inhibitory signals which downregulate the hypothalamo-hypophyseal axis (unpublished data). For instance, the marked seminal tubule atrophy and germ cell degeneration of the EWATx testis might indicate a regression in Sertoli cell function, especially in the maintenance of tight junctions and in the secretion of ABP and inhibin—a key inhibitory signal of GnRH and FSH secretion. Circulating levels of LH and T did not differ significantly between the experimental groups, indicating that testicular (and adrenal) androgenesis was normal in all groups (unpublished data). The value of the summed intratesticular NE levels from both testes of the EWATx males was significantly depressed when compared to that of the sham controls, whereas the NE content of the prostate gland did not differ significantly between these two groups (unpublished data). While tentative, the reduced NE levels suggests that the EWATx testes were incidentally denervated by the EWAT lipectomy surgery, which implies that the EWAT and testis share a common neural supply via an *en passant* neural connection which might course first through the EWAT en route to testis. Moreover, the relatively unremarkable gonadotrophin and T serum profile of the EWATx group raises an important question: Considering that secretion of FSH, LH, and T in EWATx males was not severely impaired, why were the tubular lesions and anti-spermatogenic defects of the EWATx procedure not ameliorated or altogether

prevented? A highly probable answer to this question might be found in studies done in Amphibia, which are discussed later in this report.

The weights of the emptied or fluid-filled sex accessory organs (seminal vesicles and prostate glands) of the EWATx, UNI-EWATx, and SHAM males did not differ significantly from each other, whereas the average weight of both testes of EWATx males was significantly lower that of the latter two groups, which did not differ significantly from each other (unpublished data). While the epididymal sperm counts of the latter two groups did not differ significantly from each other, epididymal sperm count measures could not be performed for the EWATx animals, owing to epididymal atrophy in some males and the total absence of the epididymis in others (unpublished data). Srinivasan *et al.* (258) noted that EWATx rats sacrificed 15 days after the lipectomy procedure had significantly lower testicular and accessory organ weights (prostate, epididymis, seminal vesicle) than that of the sham controls, while spermatogenetic activity and accessory organ weights of animals sacrificed 30 days post-lipectomy did not differ significantly from control values. The latter finding suggests that the health of the internal genitalia and spermatogenesis might be restored in a time-dependent fashion following EWAT removal, which the authors contribute to the regrowth of EWAT in only the rats sacrificed 30 days post-lipectomy (258). Cohen *et al.* (260) reported that excision of an EWAT fat pad in rat resulted in a significant increase in the weight and fat content of the contralateral pad within 3 days of the lipectomy procedure, despite a decrease in body weight. No significant differences in size were detected in the intact fat pad size when longer periods were tested (260), a finding which was replicated in experiments by Mauer and Bartness (261) wherein UNI-EWATx Siberian hamsters (*Phodopus sungorus*) were sacrificed 12 weeks post-lipectomy, and by other investigators (unpublished data).

The subsequent regrowth of EWAT pads following bilateral excision (258), as well as the accelerated growth of the EWAT after excision of its contralateral mate (260), may have been caused by a blood-borne (humoral) factor of EWAT origin that became less active with time, a theory which was first proposed by Cohen *et al.* (260). Mauer and Bartness (262) extended the ideas of Cohen, proposing that that total body fat might be regulated by humoral and/or afferent (sensory) signals released from WAT pads throughout the organism in response to changes in total body fat. These signals would target energy-monitoring centres within the CNS (possibly the hypothalamus and brainstem), which, via sympathetic efferent projections to WAT, mediate various compensatory responses, including increasing white adipocyte proliferation and decreasing the lipolytic activity of intact WAT pads (262).

The role of the gonadal WAT in amphibian reproduction. Studies on the fat body (FB) stores of several amphibian species, including the Green frog (*R. esculenta*) and *Rana hexadactyla* Lesson (no common name), may provide further insight into the role of the gonadal fat stores in mammalian reproduction and furnish an answer to the question posed in the previous section. Sharing a common mesodermal origin with the gonads, the FBs are finger-like structures bilaterally situated at the cephalic end of the gonads of both sexes (263, 264). These fat stores represent the largest lipid (triglyceride and cholesterol) deposits in the amphibian body (263-265), comprising nearly 7% of total body weight (266). Zancanaro (263) noted that the FBs of frogs caught after winter hibernation contained lipoproteinlipase (LPL) and S-100 protein, markers generally associated with mammalian adipocytes (157). Conversely, the adipocytes of the amphibian fat body lacked a definite external lamina, but instead possess cellular prolongations and spike-like projections ("spicula") produced by extrusion of the cell membrane, features not observed in mammalian adipocytes (263).

Dubois (267) reported that partial orchidectomy resulted in the enlargement of the FBs, a discovery which was the basis of an early theory concerning the secretion of a nutritive or hormonal factor from the amphibian FB which may sustain gonadal function. Evidence in support of Dubois's theory was presented by Chieffi (268), who isolated steroid hormones from the FB of male Northern crested newts (*Triturus cristatus carnifex*), whereas other workers (cited in 264) reported that the FB of this same species contained steroidogenic enzymes, which could use cholesterol as the precursor molecule. Moreover, bilateral extirpation of the fat body (FBX) resulted in ovarian atrophy in females and degeneration of the "sexual products," i.e., spermatids and spermatozoa of male newts (*Notophthalmus uiridescens*; 53). Fat body removal also inhibited protein synthesis, uptake of lipids, and attenuated the ovarian growth of *R. esculenta* during recrudescence (263, 269). Das (266) reported that the masses of the FBs and livers of adult male *R. hexadactyla* caught during the peak mating months were smaller than in frogs caught in other times of the year. The size and masses of these organs in male frogs fluctuated inversely with spawning peaks, suggesting that FB contents may support courtship behavior and/or spermatogenesis (266). However, Das reported that only the FB, and not the liver masses, of adult female frogs showed seasonal variation. Lastly, the size of the abdominal (visceral) depots showed seasonal peaks in both male and females, which were correlated to fluctuations in feeding peaks. Das concluded that TAG stored in the gonadal FBs are most likely used for reproduction and not for maintenance during periods of increased nutritional stress (i.e., the dry season), since the FBs and livers of males during the dry season are larger than in other times of the year (266).

Zancanaro (263) replicated the findings of Das (266), reporting that the FBs of frogs caught in the fall month of August were consistently larger than the FBs of frogs caught during

the peak mating season in May, however, the FBs of females were consistently larger than that of males during all seasons (263). The reduced fat body (lipid) content of the May frog provides evidence that the lipid stores were consumed to support the increased gonadal activity during the mating months (263). Microscopic analysis revealed that intercellular contact between adjacent adipocytes in the May frog is maintained by interdigitations of the plasma membrane, whereas the presence of prominent Golgi complexes and pinocytotic vesicles supports the theory that the active cellular secretion of lipids increased during the mating months (263).

To date, Chieffi *et al.* (265, 270) provides the strongest evidence in support of Dubois's theory (267) in the amphibian model. Chieffi *et al.* (265) reported that FBX in adult frogs resulted in rapid germ cell degeneration and testicular lesions, noting that the general pathologic pattern began in the central "testicular elements" and later spread to the peripheral seminal tubules. Atrophy of the testes following FB removal led to compensatory gonadotrophin hypersecretion as measured by changes in the cytology of the PD (265). Unilateral FBX experiments caused histopathological damage to the ipsilateral testis only (265, 271), whereas India ink tracing studies confirmed that the testicular vasculature had not being damaged during the FBX procedure (265). Interestingly, the onset of testicular atrophy and germinal degeneration in fall-caught frogs was delayed following FBX, whereas the histopathological effect of FBX on the testes of spring-caught frogs was rapidly manifested (265). Thus, when demand for the putative FB nutrient(s) is high, which is secondary to the increased gonadal activity of the spring season, the FBX procedure resulted in rapid testicular atrophy and germ cell depletion, whereas the inverse is true following FBX during the low-demand (autumn) season. To study the ability of extracts of the FB to restore testicular function, the researchers performed replacement experiments where FB homogenates were separated into aqueous (FB-A)

and lipid (FB-L) fractions using a multistep, solvent extraction procedure (265). Compared to the controls, intraperitoneal (i.p.) injection of the FB-L fraction was highly significant in reversing the effects of FBX, even more so than the FB-A fraction or total FB homogenate (265).

Removal of the pars distalis (PDX) produced defects in the testicular histology, which were similar to those caused by FBX, however, the degree and rapidity of pathology following PDX was less severe than those caused by FBX (265). Moreover, whereas transection of the hypothalamo-hypophyseal tracts or PDX did not significantly affect FB weight loss (FBWL), administration of a PD homogenate to PDX animals caused a highly significant increase in FBWL, while administration of a hypothalamic homogenate alone did not affect FBWL (265). Conversely, i.p. injection of hypothalamic homogenates into PD-intact animals led to a rapid, highly significant increase in FBWL (265). Chieffi *et al*. (265) and other researchers (264) reported that castration resulted in rapid FBWL, a finding which, at first, is largely inconsistent with the findings of Dubois, although the former group of researchers did not address this inconsistency in their report. It is highly probable that partial castration lessened the testicular demand on the FB (267), while the continued production of inhibitory signals (e.g., inhibin & T) by the intact remaining testicular tissue suppressed the hypothalamo-hypophyseal axis and secretion of the fat-mobilizing factor (FMF)—two effects which could have led to the hypertrophic FB growth (267). Total castration, however, completely removed these inhibitory signals, which, in turn, resulted in FMF hypersecretion, and consequently, an increase in FBWL (264, 265).

Lipid mobilization, gonadal function and identification of novel lipolytic agents. In a fascinating model, Chieffi *et al.* (265) theorized that a novel FMF secreted from the PD mediates the mobilization of FB contents into the amphibian testis. According to Chieffi's model, the

subsequent production of testicular steroids and other inhibitor products might regulate the secretion of a hypothalamic-releasing factor, which, in turn, controls production/secretion of the FMF (265). Anatomists have long since established that the vascular supply of the amphibian testis and FB is independent , while lanthanum tracing experiments confirmed the presence of a vesicular-tubular system within the fat body, which presumably connects the adipocyte cytoplasm with the interstitial spaces of the fat body (263). Chieffi *et al.* (265) used India ink to trace a portal system between the gonads and FBs, which, along with capillary intervening network of the two structures, may serve as a conduit for the direct transport of the liberated fat body contents into the testis. In the testis, the FB contents might have several actions, including supporting steroidogenesis and germ cell development, mediating Sertoli cell function, or supplying mitogenic factors. The authors proposed that LH and FSH are incapable of stimulating the testes in the absence of the FB (or its contents), which explains why the pathogenic effects of FBX (and possibly EWATx) were not corrected by hypersecretion of the gonadotrophins from the basophilic (A1 and B2) cells of the PD (265).

Chieffi *et al.* (265) demonstrated that the hypothalamo-hypophyseal axis controlled the mobilization of stored body fat through release of a FMF, a hypothesis first proposed and tested by Best and Campbell (272, 273), which sought to explain the mobilization of fat into rat liver following administration of an anterior pituitary extract. Seifter and Baeder (274) reported that injection of cortisone increased the concentration of a lipid-mobilizing factor (LMF) in the plasma of horse and man, whereas repeated intravenous or intramuscular injection of the LMF into rat resulted in hyperlipemia and loss of body weight, despite inducing hyperphagia. The LMF was not detected in the plasma of hypophysectomized rats (274), yet intravenous injection of a dialysate containing the LMF caused hyperlipemia within this group (274) doubled the

serum concentrations of cholesterol, FFAs, and phospholipids of human subjects. Conversely, the dialysate had no effect on serum glucose levels (275). Considering that the LMF was undetected in rat plasma following removal of the hypothalamus, it is highly likely that the LMF of Seifter and Baeder (274, 275) is secreted primarily from the hypothalamus. This finding effectively rules out leptin (MW 16 kDa), an endocrine hormone secreted principally from white fat whose function it is to inform the CNS on the size of peripheral fat stores (for reviews, see refs. 254, 276, 277). Exogenous administration of leptin results in loss of body weight and *decreased appetite* (277), thus it is unlikely the LMF was leptin, as multiple injections of the LMF resulted in hyperphagia (274). Since multiple injections of the LMF resulted in severe hyperlipemia in fasted (275) and fed subjects (274), the researchers concluded that hyperlipemia was not induced postprandially.

Seifter (278) isolated a LMF from the pituitary gland of hog, whereas other teams isolated LMF/FMFs from the pituitary glands of various higher vertebrates, including man (279-282), rat and human urine (283, 284), rat hypothalamus (279, 285) and sheep midbrain (286). Larsen (287) described a FMF secreted from the PD of the common toad (*Bufo bufo*), whereas Trygstad (288) isolated a 2.1 kDa FMF from crude pituitary extracts of human. Subsequent studies revealed that the adipokinetic effects of the FMF could be prevented by a proteolytic factor isolated from human plasma (289-291). Li (292) and Seidah *et al.* (293) characterized the chemical and lipolytic properties of γ-lipotropin (γ -LPH; MW 6.9 kDa) isolated from the rat PD, whereas Spiess *et al.*(294) sequenced the amino acid profile of β-LPH (MW 11.2 kDa) isolated from the human pituitary gland. Lastly, Kastin (279) presents a thorough review of several lipid-mobilizing substances which had been characterized at the time of the report's publication.

Later studies revealed that the lipotropins are produced from proopiomelanocortin (POMC; MW 28-31 kDa[3]), a high-molecular weight precursor polypeptide of 131 amino acids, which is cleaved into adrenocorticotropic hormone (ACTH; MW 4.5 kDa[1]) and β-LPH by prohormone convertases within the corticotrophs of the anterior pituitary (85, 295-297). The successive cleavage of ACTH generates melanocyte-stimulating hormone (α-MSH; MW 1.7 kDa), whereas β-LPH is cleaved into γ-LPH and β-endorphin (MW 3.5-4 kDa[1]), thence β-MSH (MW 2.6 kDa[1]) and met-enkephalin (MW 573.7 Da) respectively, which occurs within the cells of the pars intermedia (295-297). To distinguish the newly-discovered LMF/FMFs from previously characterized lipolytic agents (e.g., ACTH, GH, α-MSH, E and NE), early investigators employed various biochemical techniques including RIA (298), gel electrophoresis, Sephadex gel filtration, ultraviolet spectroscopy, and diethylaminoethyl (DEAE) cellulose chromatography (288, 289, 299).

Receptors for the POMC-derived peptides (or simply, melanocortins) are ubiquitous components of many tissue types, thus the functions of the melanocortins are extensive (reviewed in 300). Previous researchers reported that β-LPH modulated adrenal steroidogenesis (301, 302), whereas others groups demonstrated that POMC-derived peptides—specifically, β-endorphin, β-LPH, ACTH, and α-MSH —may mediate Leydig and Sertoli cell development (303-307) and testicular androgenesis (303, 304). Boston (308) presents several lines of evidence regarding the lipolytic effects of the melanocortins on white fat.

Zinc-α_2-glycoprotein (ZAG; MW 41-43 kDa), a water-soluble protein first isolated from human plasma (309), is also found in human and mice adipocytes (310-314), human urine (315, 316) and from the secretory epithelia of the GI tract, breasts, liver, and eccrine sweat glands (317). Binding of ZAG and tumor-derived LMFs to the β_3ADR activates the G_s complex, which

[3] In human

increases the activity of adenylyl cyclase (312, 318, 319). As discussed previously, stimulation of adenylyl cyclase increases intracellular cAMP levels, promoting the activation of PKA, which, in turn, phosphorylates hormone-sensitive lipases (HSL)—hydrolytic enzymes which degrade TAG into DAG and monoacylglycerols during the first steps of lipolysis. The latter two compounds are eventually converted into FFAs and glycerol by monoacylglycerol lipase (157). Thus, overexpression of ZAG in malignant tumor cells is a marker of cancer and results in the subsequent mobilization of lipid stores and cachexia in humans and *ob/ob* mice (310, 311, 313, 315, 320, 321). Bao *et al.* (310) reported that ZAG gene expression within adipocytes is downregulated by exogenous TNF-α, yet administration of a selective agonist of the peroxisome proliferator-activated receptor gamma (PPARγ; which is a hormone nuclear receptor, which is activated by PGJ_2) increased ZAG mRNA levels (310). These findings demonstrate the local (paracrine and autocrine) regulation of ZAG synthesis, while endocrine mechanisms mediate the actions of ZAG at more distal targets.

The Chieffi group replicated the findings of Kasinathan *et al.* (271, 322), confirming that bi- or unilateral removal of the FB of *R. hexadactyla* impaired spermatogenesis, whereas Kasinathan *et al.* (323) implicated the B2 and B3 cells of the PD as the primary sites of gonadotrophin production. Kasinathan *et al.* (323) reported pronounced changes in the number and histocytology of the latter cells following castration (bi- or unilateral; with or without steroid hormone replacement) and FBX (bi- or unilateral; with or without FB extract therapy). Together, these findings led to extensive studies by the Kasinathan group that sought to characterize: i.) the steroidogenic and spermatogenic effects of various compounds, including cAMP, cholesterol, and the prostaglandins $F_2\alpha$ and E_2 ($PGF_2\alpha$ and PGE_2, respectively) and ii) the role of the FB in regulation of the hypothalamo-hypophyseal-gonadal axis and in testicular

function/maintenance. A brief review of the literature revealed several roles that the cAMP second messenger system and prostaglandins (PGs) in: i.) regulating spermatogenesis (324-329), ii.) mediating the plasma concentrations and actions of FSH and LH, and the production of T (325, 329-332), iii.) regulating cholesterol and lipid metabolism (330, 331), and iv.) influencing seminal accessory organ development (333). Kasinathan *et al.* (264) demonstrated that injection of either prostaglandin was successful in reviving all spermatogenic stages following FBX, even more so than administration of cAMP alone. As previously reported by the Chieffi group, Kasinathan *et al.* (264) confirmed that injection of a FB extract into FBX male also restored spermatogenesis. Kasinathan *et al.* (264) proposed from these findings that the prostaglandins represent the primary spermatogenic factors of FB origin, while cAMP acts as a secondary factor, promoting the, "completion of the [latter stages of the spermatogenic] process."

Injection of PGE_2 and $PGF_2\alpha$ into the dorsal lymph sac reduced the intratesticular level of cholesterol, while exerting the opposite effect on the FB cholesterol content within the same animal (264). Of particular note, the reduction of cholesterol following PGE_2 injection was highly ($p < 0.001$) significant; however, both PGs increased testicular weight and the seminal tubule diameter (264). These findings suggest several (putative) roles for the PGs, including increasing testicular steroidogenesis, while inhibiting FB steroidogenesis. Alternatively, these PGs might prevent the mobilization of cholesterol from the FB into the testis, which would effectively reduce the cholesterol-derived synthesis of hormones within the testis (264). Results of parallel experiments performed in rat have a definite bearing on elucidating the role of the PGs in gonadal function. Didolkar *et al.* (332) reported that serum gonadotrophin levels were not affected following injection of these PGs. However, injection of PGE_2 (but not $PGF_2\alpha$) significantly reduced testicular cholesterol levels, whereas PGE_2 was shown to increase testicular

weight (327, 332). Blood plasma levels of testosterone were significantly reduced following injection of $PGF_2\alpha$ and PGE_2 (332). Considering that both teams observed a reduction in testicular cholesterol following administration of PGE_2, the findings of the Didolkar team substantiate the latter theory regarding PG function (that is, the PGs may prevent the mobilization of cholesterol from the FB, and consequently, testicular androgenesis is compromised). In any case, the studies described here provide strong evidence of a cholesterol equilibrium between the testis and FB, which might be maintained by a novel, prostaglandin-mediated mechanism (264, 332).

Administration of cAMP, with or without prostaglandins, reduced cholesterol content of the FB and testis, suggesting that cAMP and the PGs might differentially affect gonadal function (264). The reduction of FB cholesterol content was not caused by the mobilization of cholesterol from the FB to the testis, considering that administration of cAMP, with or without the PGs, led to the concomitant and significant decrease in testicular cholesterol content (264). Instead, these findings suggest that cAMP, more so than the PGs, increased the steroidogenic utilization of cholesterol within the testes and fat body (264). Conventional wisdom dictates that if the FB provided only PG and cAMP, FBX would result in reduced intratesticular levels of both compounds (264), which, in turn, would decrease the steroidogenic activity of the testis, while increasing testicular cholesterol. Kasinathan *et al.* (264), however, reported that FBX decreased intratesticular content of PG and cAMP, while dramatically reducing cholesterol levels to nearly a fourth of control values, which provides strong evidence that the gonadal FB might supply *all* three compounds to the testis. To this end, Veeraragavan *et al.*(334) isolated hydroxylmethyglutaryl-CoA (HMG-CoA) reductase—the enzyme which catalyzes the rate-limiting step in cholesterol biosynthesis—from the FBs of *R. hexadactyla*. This group also

reported that the radioactive signal of ^{14}C-acetate and ^{14}C-glucose was incorporated into cholesterol molecules produced in the FB, indicating that acetate and glucose could serve as precursor molecules for the synthesis of cholesterol (334). The authors noted that glucose resulted in nearly 9.5 times more ^{14}C-cholesterol synthesis than acetate, presumably because glucose provides an abundant supply of protons for cholesterol biosynthesis unlike the highly-oxidized acetate.

When coupled, the results of these studies suggest that the testis requires cholesterol from the FB *and* from its own stores to synthesize the gonadal steroids (264, 334), while lipid mobilization is mediated by the release of a LMF/FMF from the hypothalamus (279, 335), pituitary (292, 299, 336, 337) and/or adipose organ (310-312). Although it appeared that testicular steroidogenesis increased following FBX, spermatogenic activity remained severely disrupted (264). The increased utilization of cholesterol, presumably caused by a compensatory increase in steroid hormone production within the testis, could not revive spermatogenesis without the aid of additional factors from the FB (264)—a highly plausible conclusion regarding the role of the FB in spermatogenesis analogous to Chieffi's (265) conclusions.

The thesis set forth by Kasinathan *et al.* (264), which stated that cAMP and PGs mediated the actions of the gonadotrophins and sex hormones, presupposes that these compounds are endogenous to the testis and FB under normal physiological conditions. To indirectly confirm the presence of cAMP within the gonads and FB, Kasinathan *et al.* (338) reported that 3'5' cyclic AMP phosphodiesterase (PDE)—an enzyme which converts cAMP to 5'AMP— was active in both gonadal tissues, although the activity of PDE was 1.5 times higher in the FB than in the testis. Kasinathan *et al.* (264) reported that FBX increased the concentration of several enzymes, including lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase

and acid phosphatase within the testis, indicating an increase in the metabolic activity of the testis, whereas Kasinathan *et al.* (338) reported that the concentration of PDE within the testis decreased by 20% following FBX, which suggests a biochemical link between the testis and FB where the FB directly supplies the testis with PDE.

The 20% reduction in the testicular concentration of PDE should effectively increase the testicular cAMP concentration, which, in turn, would upregulate expression of the StAR protein, ultimately leading to increased steroidogenesis (338). As previously discussed, Kasinathan *et al.* (264) reported that FBX caused a highly significant reduction in testicular cholesterol levels (i.e., steroidogenic activity increased), which Kasinathan *et al.* (338) suggests is likely secondary to the reduction in PDE levels following FBX. These findings provide strong evidence that the amphibian gonadal FB plays an important role in the local regulation of testicular function by a rapid, direct method and possibly via an indirect, slow (systemic) process (271, 338).

Past evidence suggests that gonadal WAT mediates reproductive function. These findings to date raise the possibility that the gonadal WAT uniquely support spermatogenesis, possibly through the release of: i.) PGs, cAMP, growth factors or cytokines such as IL-1α and IL-1β, which influence Sertoli cell differentiation and steroidogenesis (10, 143-145, 146, 339-341); or IL-6, which influences germ cell proliferation and testicular maturation (144, 342); ii.) high-energy storage molecules, such as low-density lipoproteins, cholesterol, or FFAs in response to a FMF/LMF of PD or hypothalamic origin (156, 343); or iii.) some other previously unidentified adipokine (276, 277, 344, 345). Alternatively, EWAT removal may incidentally damage the nerve supply of the testis or induce an autoimmune response within the epididymis, the latter effect accompanied by an increase in the concentration of pro-inflammatory cytokines within the epididymis followed by the recruitment and migration of immunologically competent

cells into the lumen of the epididymis. Phagocytosis of the autoantigenic spermatozoa stored in the epididymis results in the subsequent production of anti-sperm antibodies by the naïve immune cells (346-349), an autoimmune condition, which has been previously linked to infertility in men (350-352).

Considering the anatomical relationship of the epididymis to the testis, an inflammatory response is free to spread to the testis via the contiguous network of vessels (Fig . 1), which are unobstructed in the healthy testis (63). The subsequent destruction of the Sertoli cell blood-testis barrier by lymphocytes and the invasion of the germinal and epididymal lumina with antisperm antibodies produced in the epididymis and testicular (interstitial) macrophages has been shown to severely disrupt spermatogenesis following vasectomy (Vx) and/or vasectomy reversal by vasovasostomy (Vso) in rodents (46, 47, 346, 347, 349, 353-363), rabbit (45, 364), canine (363) and man (349, 365-370). EWAT lipectomy, like vasectomy, might also impair testicular endocrinology and trigger apoptosis in the developing germ cells, which significantly reduces spermatogenic yield, leading to a pathophysiologic state of irreversible infertility (371, 372). Lastly, edema associated with EWATx-induced inflammation might increase intratesticular pressure and inhibit capsular contraction necessary to expel spermatozoa out of the testis (364, 370, 373), which results in testicular atrophy (374-377), and consequently, reduces spermatogenic yield (363, 378, 379).

V. Overview of neutral control of white adipose tissue

The SNS controls the mobilization of lipids from WAT. The earliest reports which describe the lipolytic influences of the nervous system on white fat date back nearly 100 years (380). Mansfeld (380) reported that unilateral spinal cordotomy increased the accumulation of white fat in the paralyzed leg of canine ipsilateral to the cordotomy. Unilateral denervation of the LSN, a sympathetic fiber, of several mammalian species increased the size of the ipsilateral fat pad, as compared to the LSN-intact contralateral (control) pad (for reviews see 381, 382-384). Weiss and Maickel (385) defined the physiological role of the SNS in adipose function in eloquent electrical nerve stimulation experiments. The researchers reported that electrical stimulation of fibers innervating the IWAT and EWAT of rat enhanced lipolysis, whereas the lipolytic effects of electrical stimulation was significantly reduced in rats pretreated with compounds which prevent the presynaptic packaging or release of NE and E and with other compounds that antagonize the α-ADR. Treatment with 2,2'-dichlorobiphenyl (DCB), a selective β-ADR antagonist, completely blocked lipolysis induced by electrical nerve stimulation, while treatment with pargyline, a MAOI, and theophylline, which inhibits PDE, enhanced the lipolytic effects of electrical stimulation (385). In summary, these studies demonstrate the central role of the SNS in mediating lipid mobilization (384), specifically through the α_2-ADR, which when activated by the binding of NE, inhibits lipid mobilization, and the $\beta_{1\text{-}3}$-ADRs, which when activated, promotes lipolysis.

Transynaptic retrograde axonal tracing using the pseudorabies virus (PRV) demonstrated the extensive SNS outflow from brain to WAT (386-388). Youngstrom and Bartness (389) characterized the postganglionic sympathetic innervation of WAT using fluorescent retrograde and anterograde tract tracers. Occasionally contacting individual adipocytes (2-3%), the

postganglionic, sympathetic fibers associate with the parenchyma, vasculature and innervate the major fat depots of most mammals, including humans (243) and rat (390). Slavin indicated that the blood vessels through EWAT (except for veins and venules) are extensively innervated with adrenergic fibers down to the capillary bed of EWAT, more so than the vasculature of IWAT. Fredholm (391) observed gap-like junctions between adipocytes which could provide communication routes between innervated and non-innervated adipocytes.

CNS modulates lipolysis via anterior hypothalamic inputs. Our understanding of the central neural control adipocyte function was enhanced by novel studies involving 2-deoxyglucose (2-DG), a competitive inhibitor to glucose. Following 2-DG administration, an experimental animal experiences a compensatory increase in FFA mobilization and plasma glucose concentration (392-394). Teixeira *et al.* (395) observed that administration of 2-DG reduced FFA serum concentration to subnormal levels in hypothalamic deafferentated rats, owning to a significant reduction in the mobilization of FFA from fat stores. However, 2-DG elicited the normal/expected increase in plasma glucose levels in deafferentated animals (395). Conversely, the researchers observed a significant reduction in the 2-DG-induced increase in plasma glucose and FFAs levels in deafferentated and adrenodemedullated animals, whereas FFAs concentration was not significantly different in adrenodemedullated animals than in intact animals after 2-DG administration. These results rule out significant adrenal medullary control of lipolysis (i.e., through its release of NE and E), and suggest that CNS, via hypothalamic input to the SNS outflow to WAT, mediates lipid mobilization (384, 395).

Sensory innervation of WAT may serve to monitor energy reserves. Evidence for the sensory innervation of WAT has been reviewed previously (see refs. 381, 383, 384). Sensory innervation of white fat was first demonstrated by the injection of True Blue, an anterograde tract

tracer, into the IWAT and dorsal subcutaneous pads of rat, resulted in labeling of the neuronal cells in the dorsal root ganglion, whose afferents relay sensory information from the periphery into the CNS (381, 384). Subsequent researchers reported that the IWAT and EWAT pads of Siberian hamster and laboratory rat were immunoreactive to substance P and CGRP, both neuropeptide neurotransmitters produced and secreted primarily from sensory fibers (381, 383).

Although the exact function of sensory innervation of WAT is unknown, it is speculated that the sensory innervation informs the CNS of the size of lipid stores, a function which is homologous to that of leptin and insulin (384). Alternatively, the chemoreceptors of sensory neurons might also relay information regarding the lipolytic rate by measuring the primary products of lipolysis—FFAs and glycerol—the latter serving as a better lipolytic indicator since newly-liberated FFAs are unreliable indicators because these compounds are oftentimes returned immediately to adipocytes to be reesterified to TAG (384). Sensory innervation might also mediate the perfusion rate to WAT via a feedback loop that regulates the vasomotor activity of the WAT vascular bed or the WAT afferents could synapse onto SNS centres within the CNS, thereby indirectly controlling the rate of lipolysis by innervating the sympathetic output to WAT (262, 384).

Parasympathetic drive to white fat. The exact nature of the parasympathetic drive to white fat is controversial (243, 396, 397). Kreier *et al.* (398) reported that injection of PRV into the sympathectomized retroperitoneal white adipose tissue (RWAT) of rats resulted in bilateral infection of the DMV, the chief nucleus of the PSNS. Conversely, Giordano reported "sparse" labeling of the DMV after bilateral injection of PRV into hamster IWAT (399). Critics of parasympathetic innervation of white fat argue that there is a total absence of immunoreactivity to PSNS-specific markers (e.g., VIP, NOS, and vesicular acetylcholine transporter protein) in the

epididymal, inguinal, or retroperitoneal fat depots of rats after ICC assay (396, 399). Moreover, Ballantyne (400) reported that acetylcholinesterase—an enzyme responsible for the breakdown of Ach— could not be detected in WAT, whereas Rosell and Belfrage (401) found little evidence of cholinergic innervation of WAT. The injection of PRV into the IWAT of Siberian hamsters, previously treated with the catecholaminergic toxin 6-OHDA—which, in effect, spared the putative parasympathetics—did not result in viral infection of the sympathetic chain or CNS, whereas vehicle-injected controls showed classic sympathetic chain and CNS viral infection patterns—findings which provide strong evidence against significant parasympathetic innervation of WAT (396, 399).

VI. Specific aims of the present study

While beyond the scope of the present study, it is apparent that further investigation is warranted to effectively elucidate the functions of the WAT afferents as well as the exact nature of the autonomic innervation of WAT, which, concerning the latter problem, would definitively resolve the discrepant findings of the investigative groups led by Kreier and Giordano. Despite the vagaries regarding neural control of WAT, the evidence presented in the preceding discussion raised several questions, most notably, does testicular innervation uniquely contribute to the maintenance of normal spermatogenesis, or alternatively, does EWAT alone play a role in this process? The findings discussed here provide considerable evidence that the spermatogenic process, which has been shown to be partly under hormonal and autonomic neural control, could be disrupted by transection of the testicular nerves, namely the SSN and ISN, at the immediate surface of the testicular capsule, a hypothesis tested in the present study.

To dissociate the effects of nerve transection and EWAT lipectomy on spermatogenesis, three experimental groups (n = 6 males/group) of Syrian hamsters (*Mesocricetus auratus*) were created which received one of the following surgical treatments: i.) transection of the testicular nerves, most notably the SSN and ISN, which may course through EWAT en route to testes, ii.) surgical removal of EWAT, which may also denervate the testes, or iii.) sham testicular nerve transection and sham EWAT lipectomy. In this report, the abbreviations SSNx, EWATx, and SHAM, respectively, will be used to refer to these treatment groups.

Previous experiments suggest that EWAT lipectomy significantly reduced intratesticular NE content (unpublished data). To ensure the success of the testicular denervation procedure and to characterize the anatomical arrangement of the nerves to the testis and EWAT, animals were assayed for NE content (testicular and EWAT) at autopsy using high-phase liquid

chromatography (HPLC). Moreover, to assess the effects of our surgical treatments on spermatid development (i.e., spermiogenesis in this case) and seminal tubular morphology among the three experimental groups, a spermatozoa maturity index was created, which was based on the observations of Russell *et al.* (2) and a modification of the techniques of Hess (56, 58), to classify stages 10-19 of spermatogenesis. Lastly, to test the hypothesis that EWAT lipectomy results in an inflammatory response (specifically, antisperm autoimmunity), which has been theorized to compromise testicular histology and spermatogenesis (353, 361, 362), testicular sections from each treatment group were subjected to ICC using the anti-mouse P84 antibody (402, 403). The P84 antibody is immunoreactive against the CD172a cell adhesion marker found on the surfaces of myeloid cells (i.e., all white blood cells, except lymphocytes) and macrophages, which are situated in the testicular interstitium under normal physiological conditions (70, 71, 404). In accordance with the autoimmunity hypothesis, it was predicted that immune cell labeling should be distributed to the interstitial *and* luminal regions of the EWATx testis, but only to the interstitial regions of the SSNx or SHAM testes.

MATERIALS AND METHODS

I. General experimental procedures

Animals. Eighteen Syrian hamsters (2.5-3 months old), obtained from Harlan Sprague Dawley, were divided into three experimental groups (*n*= 6 hamsters/group), matched for body mass and percent body mass change, after a 1-2 week single-housing adaptation period. The animals were weighed once per week and were housed at 20°C with lights on 14h per day (off from 12:30 pm to 10:30 pm EST). All animals were supplied with ad libitum chow and tap water. The Georgia State University Institutional Animal Care and Use Committee approved all experimental procedures in accordance with the Public Health Service and United States Department of Agriculture guidelines.

General surgical treatments. Each experimental group received one of the following surgical treatments: i.) bilateral surgical transection of the testicular nerves, namely the SSN and ISN (SSNx, *n* = 6), ii.) bilateral removal of EWAT (EWATx, *n* = 6) or iii.) bilateral sham lipectomy and sham testicular nerve transection (SHAM, *n* = 6). Hamsters were anesthetized with isoflurane (2.0-4.0% isoflurane delivered in 100% oxygen) for all surgeries. After initial anesthesia, continuous anesthesia was maintained using 1.0-3.0% isoflurane in 100% oxygen delivered by nosecone. Hair was removed from the incision site and swabbed with a povidone-iodine (PVPI) topical solution.

Following surgery, body mass and food intake was measured weekly to the nearest 0.01 g for 12 weeks, a time when compensation for lipectomy-induced lipid deficits is complete (405). At the end of 12 weeks, the animals were sacrificed by rapid decapitation, the fat pads and testes harvested, weighed and processed for histological verification.

Testicular nerve transection and sham surgery. A single median incision was made in the ventral abdomen, through which the testes and both EWAT pads were withdrawn from the peritoneal cavity, with blood vessels and nerves intact. The testes and EWAT pads were kept moist by laying them on 0.15M NaCl-soaked gauze during the denervation procedure according to the method of Shi and Bartness (405). The SSN was localized with the dissecting microscope, gently separated from the spermatic artery near the SL, and a section of approximately 1cm was carefully excised, according to a modification of the techniques of Kullendorff (406). The ISN was separated from the vas deferens near the IL using the dissecting microscope and a ~1cm section was excised (222, 406). To ensure complete testicular denervation, nerves entering into the hilium at the posterior pole of the testis, as well as nerves embedded in the peritoneum (dorsal to the caput epididymis), were also transected (171, 222). Sham surgery for each type of nerve transection was done exactly as described above, however, no testicular nerves were removed.

EWAT lipectomy and sham lipectomy. In the EWATx group, the testes and EWAT pads were removed from the peritoneal cavity and kept moist by laying them on a NaCl-soaked gauze as described above for the denervation procedure. The EWAT pads were dissected by severing them from the surrounding tissue and blood supply or were subjected to sham surgery, which involved all steps except the severing of the EWAT pads from the animal. The EWAT pads were excised with care taken to avoid damage to the testicular blood supply, nerves or musculature, as previously described (407, 408). The abdominal peritoneum was closed with sterile absorbable gut sutures and sterile wound clips were used to close the skin. Nitrofurozone powder was applied to the incision site surface to decrease the incidence of infection (407, 408).

Tissue harvesting, processing, and microscopic analysis. Twelve weeks post-surgery, the animals were sacrificed by rapid decapitation. The EWAT pads and testes from the SSNx and SHAM males were extirpated, along with the testes of the EWATx males. The excised EWAT pads and one testis (randomly selected) from each animal were then weighed and frozen in liquid nitrogen at -80°C. To verify successful nerve transection, the fat pads and testis were assayed (separately) for NE content using HPLC with electrochemical detection, which is described in Section IV.

The remaining testis from each male was weighed and submerged at room temperature (RT) into Bouin's fixative mixed with 1% (vol/vol) dimethyl sulfoxide (DMSO). The testes remained submerged for 24h and dehydrated in an ascending ethanol (EtOH) series to 100% EtOH with several changes of each concentration over a 10-day period until most of the fixative was removed. Each testis was then cross sectioned into two parts and subjected to several washes in xylene solution over a 10-day period. The testes was then immersed in several changes of liquid paraffin in a 64°C oven, embedded in paraffin blocks, cut into 5-8 µm thick sections, and mounted on slides. The slides were stained with Harris haematoxylin and eosin (H & E), and cover-slipped. In each animal, 30-40 seminal tubules were chosen at random and examined at a power of 800X. Seminal tubules, which best characterized the experimental group, were photographed, and a histopathological (qualitative) assessment was completed for each experimental group.

II. Quantification of testicular histology and spermatogenesis

Seminal tubule thickness and sperm count measurements. Randomly selected, non-overlapping microscopic fields (*n* = 50 fields, each measuring 243 µm x 178µm) were viewed at a power of 800X by an observer blind to the experimental treatments, and in each field, sperm were counted and epithelium thickness was measured in one seminiferous tubule. Microscope field measurements were then averaged to yield the following dependent variables: i.) seminiferous tubule epithelial width (in microns) and ii.) number of spermatozoa per seminiferous tubule up to *n* = 50 sperm heads observed.

Proportion of sperm-positive tubules. At a power of 800X, an observer blind to the experimental treatments examined adjacent, non-overlapping microscopic fields (*n* = 25 fields, each measuring 59 µm x 43µm), and in each field, the observer examined randomly-selected seminiferous tubules (*n* = 25) for the presence of spermatozoa. Microscopic field measurements from each testis were then averaged to yield a third dependent variable for each experimental group: (iii.) Proportion of sperm-positive seminiferous tubules observed per randomly-selected seminiferous tubules (*n* = 25).

Sperm maturation index. Concurrently, with the preceding measurement, randomly-chosen, sperm-positive seminiferous tubules (*n* = 25) were assigned a numerical variable for the highest stage of spermiogenesis observed. The aim of this measurement was to identify spermatids (and spermatozoa) in stages 10-19 (the upper spermatogenic stages), which were distinguished from stages 1-9 (the lower spermatogenic stages) by various morphological differences in the acrosomal (nuclear) head, flagellar status, and distribution of cytoplasm (2, 56, 58, 68). The acrosomal tip, which according to Russell (2), first appears during stage 10, was used to delimitate the upper stages from the lower stages. Sperm classified into stages 10-11 had compressed nuclear heads with poorly-staining flagella, which, concerning the latter feature, was

considered a distinct feature of stages 10-11 of spermatogenesis (2). Sperm classified into stages 12-14 were characterized by the polarization of the cytoplasm towards the base of the flagella (i.e., distal to the acrosome), whereas the curved acrosome, which was the most distinguishing feature of sperm in stages 15-17, was fashioned into a "pronounced hook" and the space immediately below the acrosome protruded into a conspicuous "ventral fin" (2). Finally, the appearance of sickled-shaped acrosomal heads and loss of cytoplasmic volume indicated fully mature sperm, which were classified into stages 18-19. Microscopic field measurements from each testis were averaged to yield a fourth dependent variable for each experimental group: (iv.) average number of sperm-positive seminiferous tubules where stage 18 or higher was observed.

III. ICC labeling of the CD172a cell adhesion molecule

Because the testicular sections were previously mounted and coverslipped, the ICC assay was performed directly on the glass slides, according to a technical modification of the method of Huddleston *et al.* (136). The slides were first soaked in xylene for 48h to remove the coverslips, thence rehydrated in 50% EtOH for 1h, followed by subsequent washes (5×15 min) in 0.1M phosphate buffer (PB). A ring of high vacuum [silicone] grease (Dow Corning Corp., Midland, MI) was applied to Field A, the section nearest the frosted side of each slide, to which was pipetted100 µl of the primary antibody—purified rat anti-mouse P84 (=CD172a) monoclonal antibody (diluted 1:1000; catalog no. 552371, BD Biosciences, San Jose, CA; generously provided by Dr. Yuan Liu, Georgia State University)—which was solubilized in 0.1M PB and 3% normal donkey serum (NDS; Jackson Laboratories, Bar Harbor, ME). Field A was incubated at RT for 2h, after which the slides were transferred to 0.1M PB for 30 min., followed by addition of 100 µl of the secondary antibody— donkey anti-rat biotinylated antibody (diluted 1:200; catalog no. 712-065-150, lot 28689, Jackson Laboratories)—solubilized in 0.1M PB and 3% NDS for 2h at RT. Slides were subsequently washed in 0.1M PB for 30 min and 100 µl of 1/10 strength avidin-biotinylated peroxidase complex (ABC; Vectastain Elite standard ABC peroxidase kit, Vector Laboratories, Burlingame, CA) solubilized in 0.1M PB, which was pipetted onto Field A. Slides were rinsed in 0.1M PB for 20 min, followed by addition of 100 µl of 0.2M mg/ml diaminobenzidine (DAB; Sigma, St. Louis, MO) solubilized in 0.1 µl/ml of H_2O_2 and 0.1M PB. The sections were incubated at RT for ~10 minutes before being transferred to 0.1M PB to end the DAB reaction, following by dehydration in 90% EtOH for 30 minutes (136). Finally, the slides were cleared in fresh xylene (which also removed the

silicone grease), re-coverslipped with a DPX mounting medium and allowed 48h to cure before the sections were viewed under lower power (250X) with the light microscope.

IV. Norepinephrine content assays and statistical analysis

HPLC norepinephrine (NE) content measures. The norepinephrine tissue content was measured using reverse-phase, HPLC with electrochemical detection, following Youngstrom and Bartness's (389) modification of the method of Mefford *et al.* (409) as described recently (410). Briefly, tissue was thawed and homogenized in a solution containing dihydroxybenzylamine (DHBA, internal standard) in 0.2M perchloric acid (PCA) with 1mg/ml ascorbic acid (AA). The amount of tissue processed and DHBA added was variable to obtain NE values within the range of the standards. Following centrifugation for 15 min (7500x g at 5°C), catecholamines were extracted from the homogenate with alumina and eluted into the PCA/AA. The catecholamines were assayed using an ESA Biosciences (Bedford, MA) HPLC system with electrochemical detection (Coulochem II). The mobile phase was Cat-A-Phase II, while the column was a HR-80 reverse phase column. Testicular NE content was expressed as pictogram (pg)/testis, whereas NE content for both EWAT pads was averaged and reported in nanograms (ng).

Statistical analysis. Comparisons between groups were analyzed by 1-way analysis of variance (ANOVA), followed by post hoc, pair-wise comparisons among means using the Fisher least significant differences (LSD) tests (411, 412). In situations where ANOVA could not be employed, the Student's *t* test for independent samples was performed for between group comparisons. Two-tailed probabilities are reported in all cases.

RESULTS

I. Microscopic analysis

Histopathologic findings. All stages of spermatogenesis and in the cycle were observed in the seminiferous tubules of the SSNx and SHAM testes, which were abundant in sperm, whereas a majority of the seminal tubules in the EWATx testes were azoospermic, indicating that spermatogenic activity was severely disrupted within this group (Fig. 3). The tubular epithelia of the latter two groups contained the full complement of germ cells (i.e., Type A and B spermatogonia, spermatocytes and spermatids), whereas these cells were either absent or degenerate (i.e., the nuclei were pyknotic and karyorrhexic) in the majority of epithelia in the EWATx testes. Qualitative estimates suggested that the seminal lumina of the EWATx testes were greatly dilated, a histopathological feature not observed in the SSNx and SHAM testes. Moreover, the distribution of apoptotic cells and atrophied tubules in the EWATx testes was not uniform, but was spatially restricted to tubules in the center of the tissue section where it was apparent that contact between the Sertoli and germ cells had been lost resulting in disorganized cell associations and disruption in the stages of the cycle. Seminal tubules along the periphery of the EWATx tissue section appeared healthy, had plentiful sperm and were indistinguishable from the seminal tubules of the SSNx and control testes.

In stark contrast to the SSNx and SHAM testes, the lumina of the EWATx testes were remarkable for irregular masses of seminal epithelium, which contained sloughed, elongated spermatids. The spermatids were easily identified by their flagella which protruded from the center of the mass. Numerous MGCs were observed within the seminal lumina of the EWATx testes, but not in the SSNx and SHAM testes.

II. Quantification of testicular histology and spermatogenesis

Seminal tubule thickness measurements. Relative to hamsters in the SSNx (n = 4) and SHAM (n = 6) groups, in which the seminiferous tubular epithelium was thick and robust, the seminal epithelium in the EWATx (n = 5) group showed significant regression, specifically in seminiferous tubular epithelial thickness, yielding a significant difference among groups ($F_{2,14}$=11.16, $p < 0.002$). Follow-up, post hoc statistical comparisons revealed that the EWAT group had significantly reduced seminiferous tubule thickness compared to both the SSNx and the SHAM animals ($p < 0.01$), respectively (Fig. 4). The seminiferous tubule epithelial thickness in the EWAT-intact testes of the SSNx and SHAM groups did not differ significantly from each other.

Sperm count measurements. Sperm counts in the testes of the EWATx group were significantly lower compared with counts from the testes of the SSNx and SHAM animals ($F_{2,14}$=14.52, $p < 0.001$). The post-hoc group comparisons (Fig. 5) revealed that the sperm count in the EWAT-intact testes in the SSNx and SHAM groups did not differ significantly from each other, whereas EWATx hamsters had significantly lower sperm counts than either SSNx or SHAM groups ($p < 0.01$), respectively.

Proportion of sperm-positive tubules. One-way ANOVA revealed that the proportions of sperm-positive seminiferous tubules (mean ± error) differed significantly between the three groups ($F_{2,14}$ = 4.04, $p < 0.046$). Follow-up, pair wise comparisons using Fisher LSD test (Fig. 6) revealed that the EWATx testes had significantly fewer sperm-positive seminiferous tubules than the SSNx and SHAM testes ($p < 0.05$, respectively), whereas the latter two groups did not differ significantly from each other (p = n.s., respectively).

Sperm maturation index. Group comparisons of sperm maturity revealed no significant difference in the level of sperm maturity among the three groups [Mean ± SEM: SSNx; 23.5 ± 1.19, EWATx; 20.80 ±1.66 and SHAM; 22.33 ±1.4], $F_{2,14} = 0.76$, p = n.s.

ICC labeling for the CD127a cell adhesion molecule. Under low magnification (250X), the CD127a-ir pattern within the tissue sections of the SSNx group was somewhat varied. While Cd127a-ir was distributed extensively to the interstitial, epithelial, and intraluminal regions of some SSNx sections, CD127a-ir labeling of the interstitial, epithelial and intraluminal regions of other SSNx sections was sparse and punctate at best. The distribution of interstitial CD127a labeling on the EWATx sections was relatively sparse, while the pattern and distribution of epithelial and intraluminal CD127a labeling of EWATx sections closely resembled that of the SSNx sections. CD127a-ir labeling on all regions on the SHAM sections was sparse and nearly absent in some cases. In all cases—irrespective of the treatment group or intratesticular region—CD127a-ir labeling did not involve readily identifiable immune cells, but instead involved the labeling of non-descript particulate matter, a preliminary observation confirmed under high power (1000X).

HPLC norepinephrine content measures. Group comparisons of testicular NE content indicated a significant difference among groups ($F_{2,13} = 10.71$, $p < 0.003$). Follow-up, pair-wise group comparisons using the Fisher LSD test (Fig. 7) revealed that the testicular NE content of SSNx ($n = 4$) males was significantly lower ($p < 0.05$) than that of the EWATx ($n = 5$) and SHAM ($n = 5$) males, which did not differ significantly from each other ($p < 0.05$, respectively). Conversely, *t* test analysis revealed that EWAT NE content in the SSNx males did not differ significantly from that of the SHAM controls (Fig. 8) [Mean ± SEM: SSNx; 73.54 ± 18.26 and SHAM; 52.04 ±3.41], $t(8) = -1.43$, p = n.s.

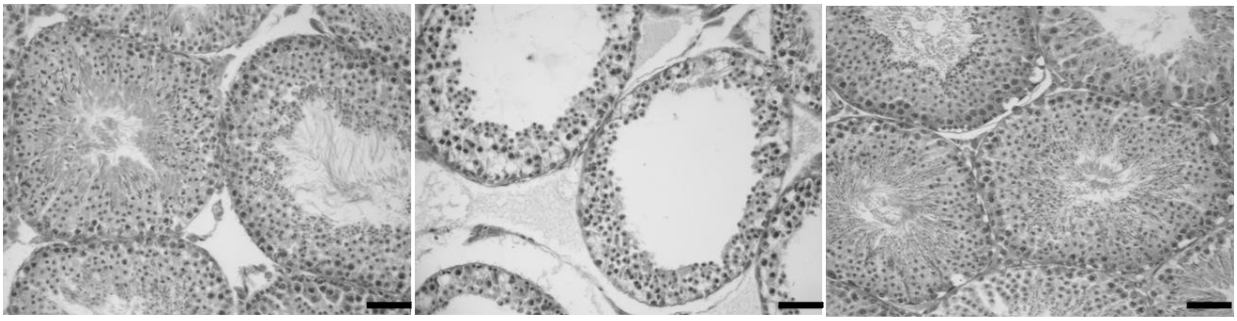

FIGURE 3: PHOTOMICROGRAPHS OF TESTICULAR SECTIONS (800X). The various stages of spermatogenesis are visible in the healthy seminal tubule epithelia of the SSNx and SHAM testes, whereas very little spermatogenic activity and severely atrophied seminiferous tubules were observed in the tubules of EWATx males. Bar = 50 µm.

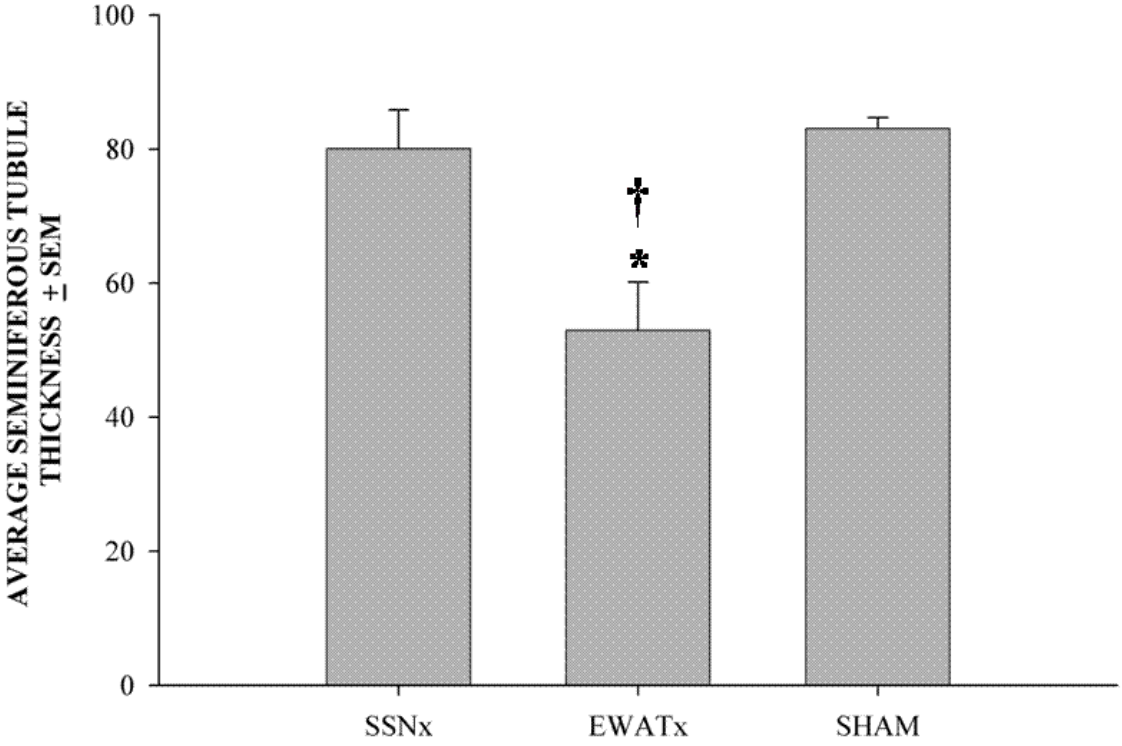

FIGURE 4: SEMINIFEROUS TUBULE THICKNESS. Seminiferous tubule thickness (μm, mean ± error) in males that received EWAT lipectomy was significant smaller than that of males in the SSNx and SHAM groups, which did not different significantly from each other. Asterisk (*****) denotes significantly different from SHAM ($p < 0.01$). Dagger (†) denotes significantly different from SSNx ($p < 0.01$).

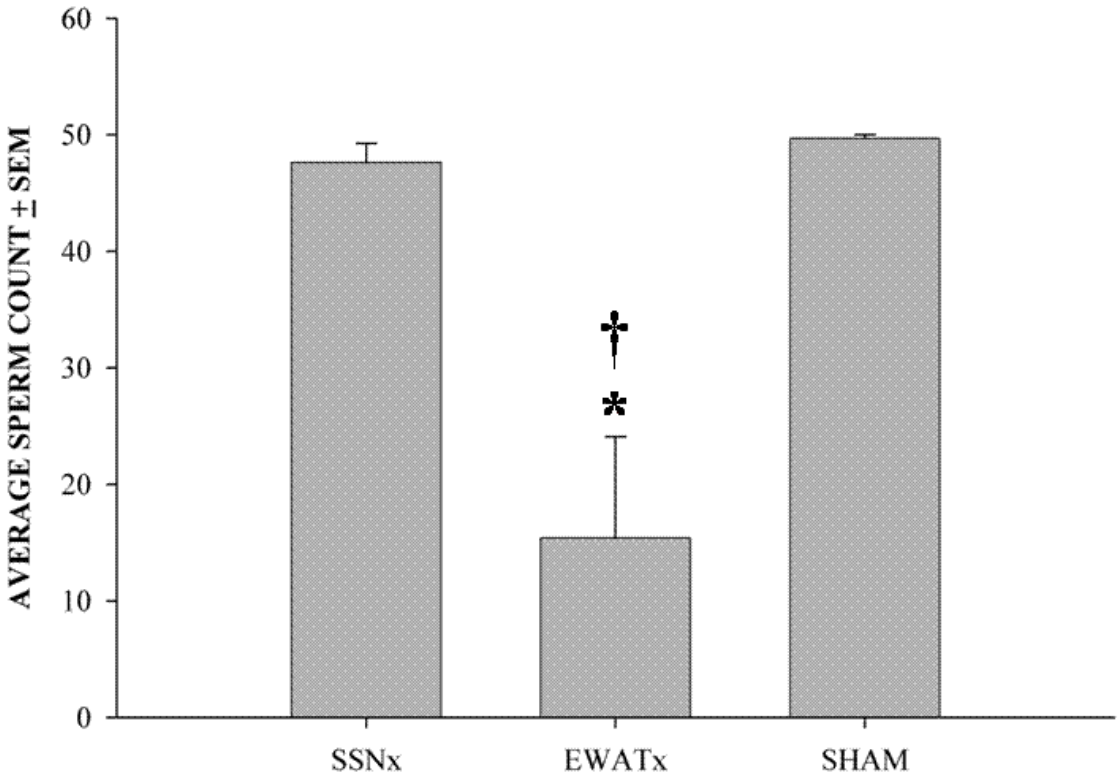

FIGURE 5: SPERM COUNT. Sperm counts (mean ± error) in males that received EWAT lipectomy was significantly lower than that of males in the SSNx and SHAM groups, which did not differ significantly from each other. Asterisk (*****) denotes significantly different from SSNx ($p < 0.01$). Dagger (†) denotes significantly different from SHAM ($p < 0.01$).

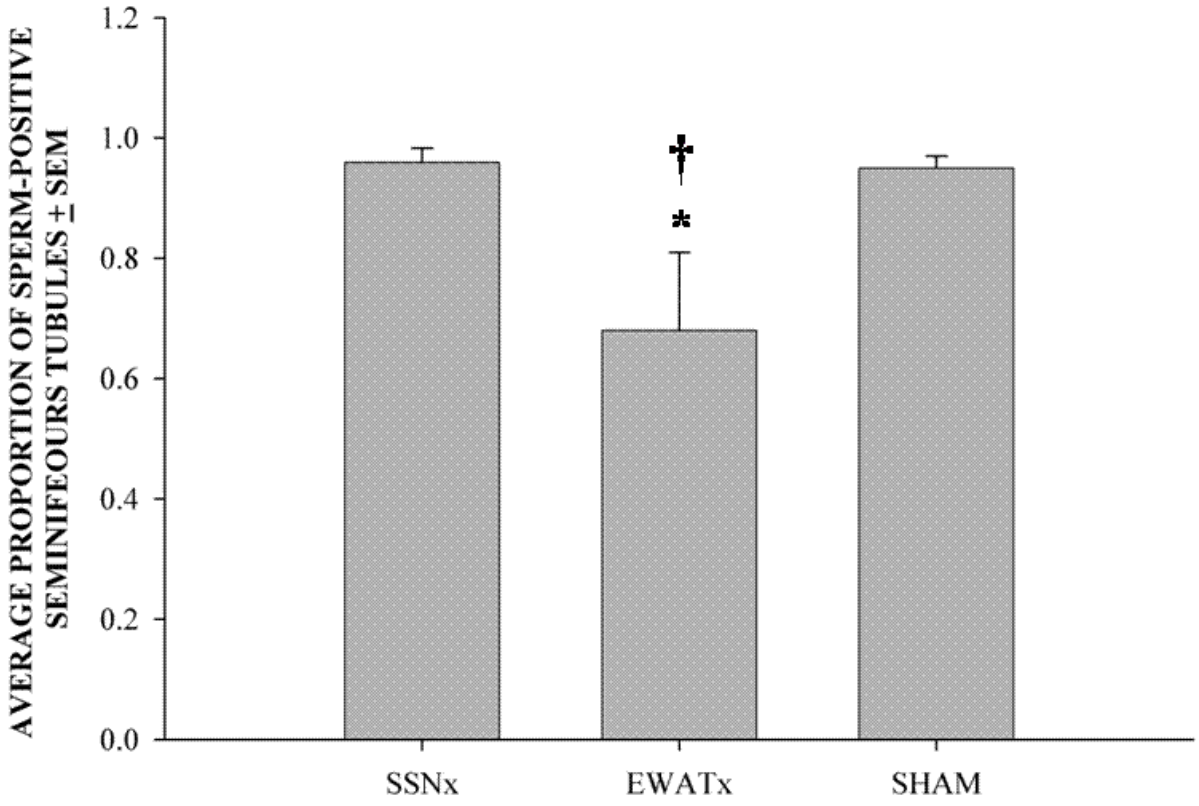

FIGURE 6: PROPORTION OF SPERM-POSITIVE SEMINIFEROUS TUBULES. Proportion of sperm-containing seminiferous tubules (mean ± error) in males that received EWAT lipectomy was significantly lower than that of the SSNx and SHAM groups, which did not differ significantly from each other. Asterisk (*****) denotes significantly different from SSNx ($p < 0.05$). Dagger (†) denotes significantly different from SHAM ($p < 0.05$).

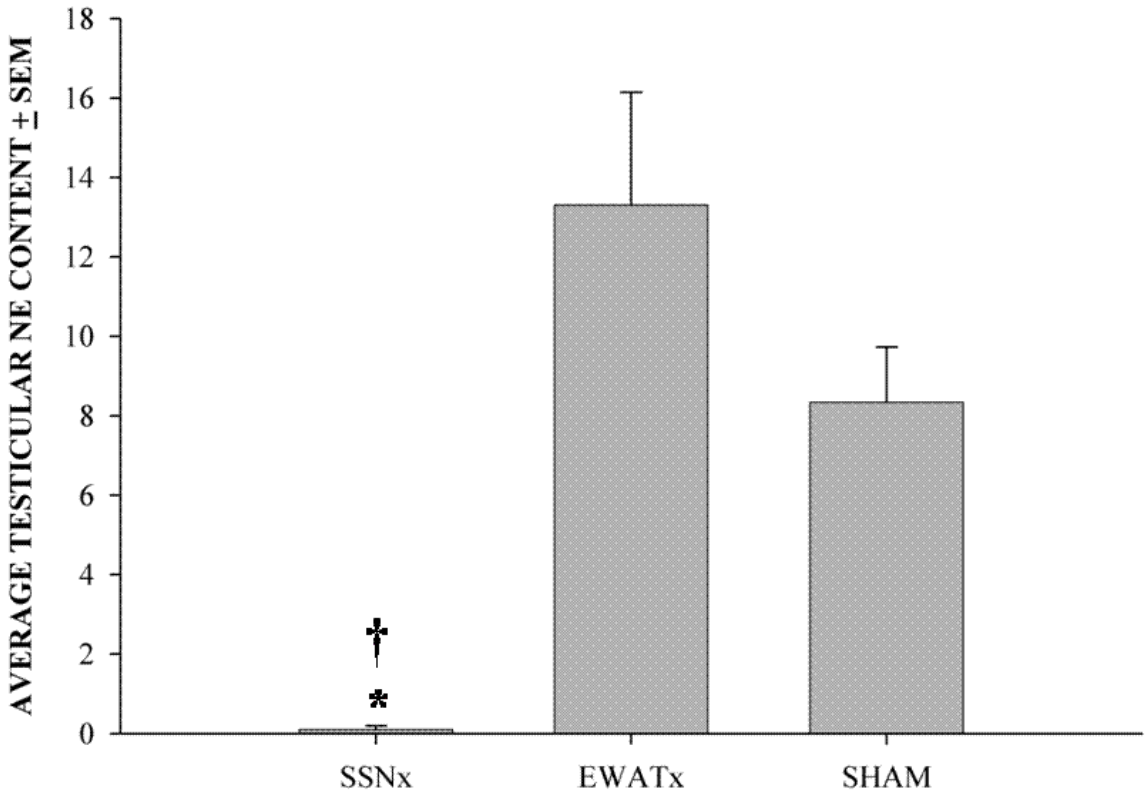

FIGURE 7: TESTICULAR NE CONTENT. Testicular NE content (pg/testis, mean ± error) in males in the SSNx group was significantly lower than that of the EWATx and SHAM groups, which did not differ significantly from each other. Asterisk (*****) denotes significantly different from EWATx ($p < 0.05$). Dagger (†) denotes significantly different from SHAM ($p < 0.05$).

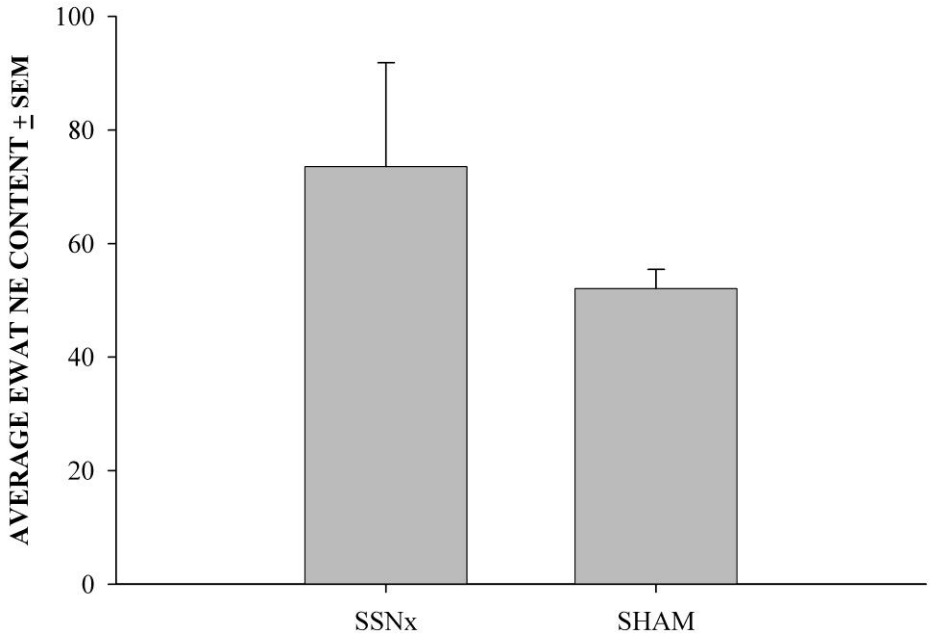

FIGURE 8: EWAT NE CONTENT. The EWAT NE content (ng, mean ± error) of the SSNx animals did not differ significantly from that of the SHAM controls.

DISCUSSION

I. Major findings

EWAT removal results in morphological changes to the testis. The presence of MGCs and the sloughing of epithelia and germ cells into the seminal lumen indicate widespread germ cell degeneration in the EWATx testis. While it is not known what factors cause the spermatids to form MGCs instead of immediately degenerating (2), there are numerous reports of testicular MGC formation after vasectomy (45, 361), epididymal obstruction (413), and treatment with $PGF_2\alpha$ (333) or with 6-OHDA (223). Moreover, MGC formation increases with age and prolonged disease (29). Although early studies provided strong evidence that vasectomy promoted the production of anti-spermatogenic antibodies within the epididymis, which purportedly impair the spermatogenic ability of the testis, the absence of intact immune cells in the seminal lumina of the EWATx testis and the lack of an observable difference in the CD127a-ir labeling distribution between the treatment groups effectively rules out the hypothesis that EWAT lipectomy induces an inflammatory (autoimmune) response. Alternatively, it is probable that lumicrine factors, which are secreted by the epididymal epithelium (or possibly the EWAT), may mediate germ cell development, a hypothesis which has not been tested. In this way, considering that T, LH, and FSH secretion was relatively normal in the EWAT lipectomized, azoospermic males of an earlier study (unpublished data), the atrophy or total absence of the epididymis in the EWATx group might have impaired the secretion of epididymal factors, therefore compromising germ cell development.

Although Sertoli cells were present and still attached to the basal lamina, the intercellular associations between the Sertoli and developing germ cells were absent, in addition to the compartmentalized microenvironments created by these associations which are critical for proper

germ cell development and viability (2). Consequently, apoptotic pathways are triggered, followed by massive germ cell sloughing and loss of the morphological integrity of the germinal epithelium (27). Russell warns that germ cell sloughing could also result from the mechanical stress of histological processing (2). Considering the excellent epithelial health and integrity of the SSNx and SHAM control testes as well as the absence of germ cell sloughing in these groups, the likelihood that mechanical stress caused the histopathologic effects observed in the EWATx testis is highly improbable.

Neural supply to testis and EWAT are independent. The results of the NE content assays provide new insight on the neuroanatomical organization of EWAT and the testis. First, the significantly reduced NE content of the SSNx testis confirms the success of the testicular denervation procedure. Despite the SSNx testis having significantly reduced intratesticular NE content, as compared to SHAM and EWATx males, spermatogenesis was not impaired, suggesting that NE is not central in promoting spermatogenesis. However, Mhauoty-Kodja demonstrated that NE (through its activation of the α_{1B}ADR) mediates Leydig and Sertoli cell function and spermatogenesis, making the role of NE in spermatogenic function somewhat more difficult to characterize (230). Second, the intratesticular NE values of the EWATx testis, when compared to the SHAM control values, demonstrates that EWAT lipectomy does not disrupt autonomic input to the testis. This finding, although inconsistent with the unpublished data of a previous study, demonstrates that the nerve supply to the testis and EWAT are independent (Fig. 9). An explanation for these discrepant findings is at hand. In the present study, special care was taken in the removal of the EWAT, unlike in an earlier study (unpublished data), in which EWAT removal might have damaged the testicular nerves. Thus, regarding the separate innervation of the testis and EWAT, we consider the present finding to be conclusive. Whether

neural input to the EWAT is collateralized with that of the testis, completely separate, or a combination of these two possibilities cannot be definitively determined from the results of the NE content assays. Third, the lack of a significant difference between the NE content of the EWAT pads of the SSNx and SHAM animals demonstrates that testicular nerve cuts do not compromise the sympathetic nerve supply to EWAT (Figures 8-9). Lastly, in addition to the spermatic nerves, which innervate portions of the rat epididymis, it is likely that EWAT is also innervated by the mixed (sympathetic and afferent) hypogastric nerve, which, according to Hodson (171), arrives at the rat epididymis via the vas deferens and its artery.

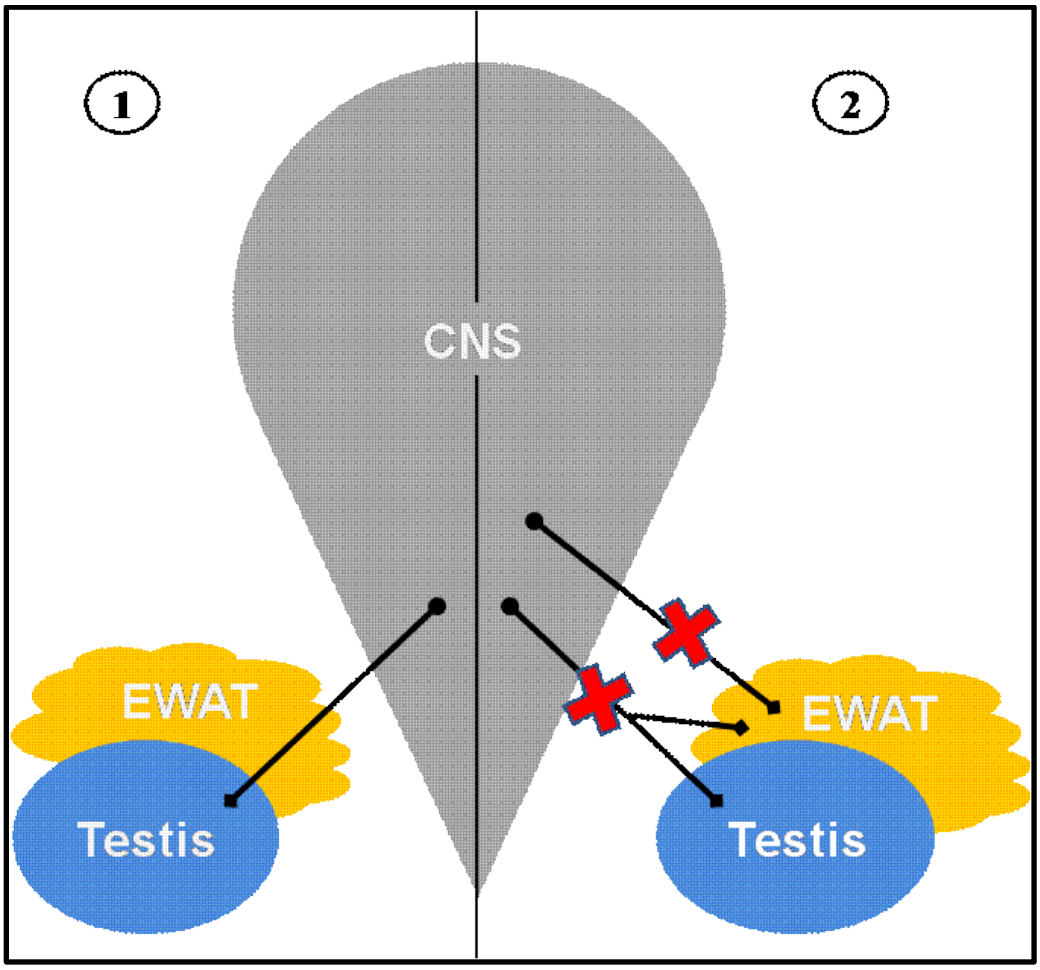

FIGURE 9: NEUROANATOMICAL ORGANIZATION OF THE EWAT AND TESTIS. Neural supply to EWAT and testis are independent. In Scenario ①, *en passant* innervation of EWAT and testis was hypothesized. Thence, it was predicted, that EWAT lipectomy would effectively denervate the testis (as measured by testicular NE content), a finding which was not supported by the testicular NE content data of the EWATx testis. Thus, Scenario ① was ruled out. In Scenario ②, innervation of EWAT and the testis are independent (i.e., collateralized or completely separate). In this scenario, EWAT lipectomy would not disrupt testicular innervation, a prediction which was supported by the results of the NE assay. Please see text for a complete explanation of the red *X*'s in Scenario ②.

II. Conclusions

EWAT, and not autonomic innervation, sustains spermatogenesis. The lack of testicular pathology and the presence of sperm in the SSNx males effectively rule out a central role for testicular innervation in spermatogenesis, which is in stark contrast to earlier studies that reported significantly reduced spermatogenic ability and severe regression of the seminal tubule following surgical denervation of the testis (42, 43). The results of this study are consistent with earlier experiments performed in our lab (unpublished data) and the observations of Srinivasan *et al.* (258), which demonstrate that EWAT lipectomy disrupts spermatogenesis and contributes to atrophy of the seminiferous tubules and epididymis. Although hormonal RIA was not conducted in the present study, gonadotrophin and sex hormone levels in EWATx males of an earlier study were relatively normal, despite severe impairment to spermatogenesis and to the testicular histology (unpublished data). Hence, it is concluded that the gonadal EWAT, alone, sustains normal spermatogenesis, and thus, the present data do not support the hypothesis that sympathetic innervation of the testis supports normal spermatogenesis.

Study Limitations. Although outside the aims of the present study, the mechanism by which EWAT promotes spermatogenesis is perplexing to say the least. The present study and earlier work in hamster (unpublished data), Albino rat (258) and frog (271) provide strong evidence that the gonadal white fat is central to normal spermatogenesis and androgen production. The studies in amphibians cited previously raise the possibility of novel a LMF/FMF secreted from the pituitary gland and/or hypothalamus (279), which, in addition to the sympathetic nervous system, mediates the mobilization of lipids and other nutritive factors from the EWAT. Mobilization of the EWAT pad contents promotes the proliferation and development germ cells and Sertoli-Leydig cell function. Moreover, the production and the

subsequent secretion of epididymal proteins into the testis could also mediate testicular function. In light of the themes presented by Kasinathan, Chieffi, and Hinton this explanation is highly plausible.

Alternatively, the seminal epithelial pathology and germ cell aberrations, which were observed in the EWATx testis only, could be the result of an inflammatory immune caused by the lipectomy procedure. In the context of the literature reviewed here, the effects of inflammatory/immune response on testicular pathology is regarded as secondary to EWAT removal (i.e., the histopathologic features observed in the EWATx testis were caused primarily by removal of EWAT and/or its nutritive factors, which may have been exaggerated by an immune response). To elucidate how EWAT lipectomy contributes to testicular histopathogenesis, which may also involve temporally- and spatially-regulated events such as inflammation, longitudinal studies might be valuable, where the animals are sacrificed at consecutive, randomly-chosen time intervals, according to a modified method of Srinivasan (258). Pro-inflammatory cytokines, such as IL-1α, which are expressed constitutively in the Sertoli cells of rat testis, mediate germ cell development and act as autocrine or paracrine factors to stimulate Sertoli cells growth and physiology to a greater degree than FSH, however, the cytokines may exert negative effects on testicular function in prolonged diseased states (10, 143). Disruptions to physiological tissue remolding caused by increased production of IL-1 (and thus, increased expression of TIMP-1) in prolonged pathologic states such as inflammation (141), might have severely compromised the maintenance of the seminal epithelium during stages in the cycle and reduced the spermatogenetic yield. It is noted, however, that TIMP-1 levels were not measured in any of the three groups, so this explanation is merely speculative.

Despite the EWATx testis having significantly reduced sperm counts and 30% less sperm-positive tubules than the SSNx and SHAM groups, sperm maturity measurements revealed no significant differences in the level of sperm maturity between the three groups. Thus, sperm, when present, were able to reach full maturity in the EWATx testis. In light of the profound pathology observed in the EWATx testis, this finding is considered to be tentative and most likely the result of the unevenly distributed pathology in the EWATx testis, a phenomenon which was observed in the amphibian testis following removal of the gonadal fat bodies (265). A power analysis performed by Hess *et al.* (58) advises that a minimal of: i.) $n = 12$ animals per experimental group and ii.) $n = 200$ seminiferous tubule cross sections should be observed to detect a difference of two standard deviations from the mean with a 2% error rate when classifying the stages in the cycle of the seminiferous tubules. Owing to loss of sample, the average group size for the sperm maturity measurement was $n = 5$, whereas $n = 25$ seminiferous tubules were observed. Thus, due to the pathologic changes in the EWATx testis, which were restricted to the center of the tissue section, and the group size number, the sperm maturity tool was underpowered and insensitive, failing to effectively reveal statistical variations in sperm maturity among the treatment groups.

The sequelae of the EWAT lipectomy procedure, which is remarkable for testicular lesions and disruptions to the spermatogenetic ability of the EWATx animal, may have far-reaching clinical significance for the treatment of male infertility. The present findings are consistent with a tentative interpretation that the EWAT supplies a trophic factor which sustains normal testicular physiology. Thus, isolation of the putative trophic factor might yield an effective pharmacologic therapy for the treatment of male infertility, whereas selective agonists against the receptor for this trophic factor might also provide an effective target for enhancing

male productive capacity. Conversely, antagonists directed against the same receptor might possibly provide an effective male contraceptive, while gene expression studies on EWATx, SSNx, and SHAMx animals may lead to advances in the treatment of male infertility via novel molecular/genetic approaches. Although surgical vasectomy has traditionally been the widely preferred method of sterilization, blockade of the putative EWAT trophic factors might provide an alternative route for reversible sterilization.

In light of the recent reports regarding neural control of white fat, it is likely that neural input to EWAT promotes lipid mobilization and/or the secretion of the trophic factors, which in turn, support spermatogenesis and testicular function. Whereas earlier workers reported spermatogenic defects, severe regression of the seminiferous tubules following SCI (182, 185, 194), chemical (35, 223-225), surgical denervation at more distal locations [e.g., at the spinal autonomic outflow (164) and near the pampiniform plexus of the spermatic cord (42, 43, 197, 198)], it is emphasized here that transection of the nerves in the present study was performed at the immediate testicular surface. In this way, all nerves entering the testis were denervated, while the nerves serving the epididymis, EWAT, and vas deferens were spared. Considering the neuroanatomical organization of the EWAT and testis, which is theorized here, it is hypothesized that the nerve cuts performed by earlier workers incidentally denervated the EWAT, which, in effect, disabled the sympathetic drive necessary for lipid mobilization. Therefore, it is predicted that axotomy of the nerves leading into the EWAT (see red *X*'s in Fig. 9) would likely impair the supportive function of the EWAT thereby compromising the spermatogenic ability of the testis.

REFERENCES

1. Fawcett, D.W., *The mammalian spermatozoon.* Dev Biol, 1975. **44**(2): p. 394-436.
2. Russell, L.D., R.A. Ettlin, A.P. Sinha Hikim, and E.D. Clegg, *Histological and histopathological evaluation of the testis*. 1st ed. 1990, Clearwater, Fl: Cache River Press. xiv, 286 p.
3. Roosen-Runge, E.C., *The process of spermatogenesis in mammals.* Biol Rev Camb Philos Soc, 1962. **37**: p. 343-77.
4. Eddy, E.M. and D.A. O'Brien, *The spermatozoon*, in *The physiology of reproduction*, E. Knovil and J.D. Neill, Editors. 1994, Raven Press: New York. p. 29-77.
5. Clermont, Y., *[Kinetics of spermatogenesis in mammals].* Arch Anat Microsc Morphol Exp, 1967. **56**(3): p. 7-60.
6. Clermont, Y., *Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal.* Physiol Rev, 1972. **52**(1): p. 198-236.
7. Clermont, Y. and C.P. Leblond, *Differentiation and renewal of spermatogonia in the monkey, Macacus rhesus.* Am J Anat, 1959. **104**: p. 237-73.
8. Simorangkir, D.R., G.R. Marshall, J. Ehmcke, S. Schlatt, and T.M. Plant, *Prepubertal expansion of dark and pale Type A spermatogonia in the rhesus monkey (Macaca mulatta) results from proliferation during infantile and juvenile development in a relatively gonadotropin independent manner.* Biol Reprod, 2005. **73**(6): p. 1109-1115.
9. Rowley, M.J., J.D. Berlin, and C.G. Heller, *The ultrastructure of four types of human spermatogonia.* Cell and Tissue Research, 1971. **112**(2): p. 139-157.
10. Amjad, A.I., O. Soder, and T. Sultana, *Role of testicular interleukin-1alpha tIL-1alpha in testicular physiology and disease.* J Coll Physicians Surg Pak, 2006. **16**(1): p. 55-60.
11. Fouquet, J.P., *[Ultrastructural evolution of fetal germinal cells to adult spermatogonia in Macaca fascicularis monkeys].* Reprod Nutr Dev, 1982. **22**(5): p. 767-83.
12. Fouquet, J.P. and J.P. Dadoune, *Renewal of spermatogonia in the monkey (Macaca fascicularis).* Biol Reprod, 1986. **35**(1): p. 199-207.
13. Rooij, D.G.D., B.H.G.J. Schrans-Stassen, A.M.M.v. Pelt, G.A. Shuttlesworth, M.L. Meistrich, M. Okabe, and Y. Nishimune, *Regulation of the differentiation of the undifferentiated spermatogonia*, in *The Testis : From stem cell to sperm function*, E. Goldberg, Editor. 2000, Springer: New York. p. xvii, 265 p.
14. Clermont, Y. and M. Antar, *Duration of the cycle of the seminiferous epithelium and the spermatogonial renewal in the monkey Macaca arctoides.* American Journal of Anatomy, 1973. **136**(2): p. 153-165.
15. Morrison, S.J., N.M. Shah, and D.J. Anderson, *Regulatory mechanisms in stem cell biology.* Cell, 1997. **88**(3): p. 287-98.
16. Hall, P.A., *What are stem cells and how are they controlled?* J Pathol, 1989. **158**(4): p. 275-7.
17. Hall, P.A. and F.M. Watt, *Stem cells: the generation and maintenance of cellular diversity.* Development, 1989. **106**(4): p. 619-33.
18. Clermont, Y., *Two classes of spermatogonial stem cells in the monkey Cercopithecus aethiops.* American Journal of Anatomy, 1969. **126**(1): p. 57-71.
19. Kalthoff, K., *Anaylsis of biological development*. 2nd ed. 2001, Boston: McGraw-Hill.

20. Abe, K., L.S. Shen, and H. Takano, *The cycle of the seminiferous epithelium and stages in spermatogenesis in dd-mice.* Hokkaido Igaku Zasshi, 1991. **66**(3): p. 286-99.
21. Hecht, N.B., *Spermatogenesis after the millennium,* in *The testis : From stem cell to sperm function.* E. Goldberg, Editor. 2000, Springer: New York. p. xvii, 265 p.
22. Barr, A.B., D.J. Moore, and C.A. Paulsen, *Germinal cell loss during human spermatogenesis.* J Reprod Fertil, 1971. **25**(1): p. 75-80.
23. Roosen-Runge, E.C., *Germinal-cell loss in normal metazoan spermatogenesis.* J Reprod Fertil, 1973. **35**(2): p. 339-48.
24. Russell, L.D. and Y. Clermont, *Degeneration of germ cells in normal, hypophysectomized and hormone treated hypophysectomized rats.* Anat Rec, 1977. **187**(3): p. 347-66.
25. Huckins, C., *The morphology and kinetics of spermatogonial degeneration in normal adult rats: An analysis using a simplified classification of the germinal epithelium.* The Anatomical Record, 1978. **190**(4): p. 905-926.
26. Johnson, L., T.L. Blanchard, D.D. Varner, and W.L. Scrutchfield, *Factors affecting spermatogenesis in the stallion.* Theriogenology, 1997. **48**(7): p. 1199-216.
27. Zamzami, N. and G. Kroemer, *Apoptosis: Condensed matter in cell death.* Nature, 1999. **401**(6749): p. 127-128.
28. Holstein, A.F. and C. Eckmann, *Multinucleated spermatocytes and spermatids in human seminiferous tubules.* Andrologia, 1986. **18**(1): p. 5-16.
29. Miething, A., *Multinucleated spermatocytes in the aging human testis: formation, morphology, and degenerative fate.* Andrologia, 1993. **25**(6): p. 317-23.
30. Ismail, N., C. Morales, and Y. Clermont, *Role of spermatogonia in the stage-synchronization of the seminiferous epithelium in vitamin-A-deficient rats.* Am J Anat, 1990. **188**(1): p. 57-63.
31. van Pelt, A.M. and D.G. de Rooij, *Synchronization of the seminiferous epithelium after vitamin A replacement in vitamin A-deficient mice.* Biol Reprod, 1990. **43**(3): p. 363-67.
32. van Pelt, A.M. and D.G. De Rooij, *The origin of the synchronization of the seminiferous epithelium in vitamin A-deficient rats after vitamin A replacement.* Biol Reprod, 1990. **42**(4): p. 677-82.
33. Huang, H.F. and W.C. Hembree, *Spermatogenic response to vitamin A in vitamin A deficient rats.* Biol Reprod, 1979. **21**(4): p. 891-904.
34. Benson, W.R. and F.S. Clare, *Regenerative changes and spermatic granulomas in the rat testis after treatment with DL-ethionine.* Am J Pathol, 1966. **49**(5): p. 981-95.
35. Shoda, T., K. Yasuhara, M. Moriyasu, T. Takahashi, C. Uneyama, M. Hirose, and K. Mitsumori, *Testicular toxicity of nitrofurazone causing germ cell apoptosis in rats.* Arch Toxicol, 2001. **75**(5): p. 297-305.
36. Homma-Takeda, S., Y. Kugenuma, T. Iwamuro, Y. Kumagai, and N. Shimojo, *Impairment of spermatogenesis in rats by methylmercury: involvement of stage- and cell-specific germ cell apoptosis.* Toxicology, 2001. **169**(1): p. 25-35.
37. Taneli, F., S. Vatansever, C. Ulman, Ö. Yilmaz, G. Giray, A. Genç, and C. Taneli, *The effect of spermatic vessel ligation on testicular nitric oxide levels and germ cell-specific apoptosis in rat testis.* Acta Histochemica, 2005. **106**(6): p. 459-466.
38. Lue, Y., A.P. Hikim, C. Wang, M. Im, A. Leung, and R.S. Swerdloff, *Testicular heat exposure enhances the suppression of spermatogenesis by testosterone in rats: the "two-*

hit" approach to male contraceptive development. Endocrinology, 2000. **141**(4): p. 1414-24.
39. McLachlan, R.I., N.G. Wreford, S.J. Meachem, D.M. De Kretser, and D.M. Robertson, *Effects of testosterone on spermatogenic cell populations in the adult rat.* Biol Reprod, 1994. **51**(5): p. 945-55.
40. Kasinathan, S. and S.L. Basu, *Effect of steroids on the spermatogenesis in the frog Rana hexadactyla Lesson.* Indian J Exp Biol, 1975. **13**(5): p. 450-1.
41. Yazawa, T., T. Yamamoto, and S.-I. Abe, *Prolactin induces apoptosis in the penultimate spermatogonial stage of the testes in Japanese red-bellied newt (Cynops pyrrhogaster).* Endocrinology, 2000. **141**(6): p. 2027-2032.
42. Chow, S.H., W. Giglio, R. Anesetti, J.E. Ottenweller, L.M. Pogach, and H.F. Huang, *The effects of testicular denervation on spermatogenesis in the Sprague-Dawley rat.* Neuroendocrinology, 2000. **72**(1): p. 37-45.
43. Gong, Y.G., Y.R. Yang, W. Zhang, M. Gu, and C.J. Yin, *[Apoptosis in male germ cells induced by testicular denervation].* Zhonghua Nan Ke Xue, 2006. **12**(11): p. 968-73.
44. Shiraishi, K., K. Naito, and K.-I. Yoshida, *Vasectomy impairs spermatogeneis through germ cell apoptosis mediate by the p53-bax pathway in rats.* The Journal of Urology, 2001. **166**(4): p. 1565-1571.
45. Steger, K., M. Slavov, K. Failing, W. Weidner, and M. Bergmann, *Effect of vasectomy on sperm nuclear chromatin condensation in the rabbit.* J Androl, 2005. **26**(2): p. 289-295.
46. Lue, Y., A.P. Hikim, C. Wang, J.J. Bonavera, S. Baravarian, A. Leung, and R.S. Swerdloff, *Early effects of vasectomy on testicular structure and on germ cell and macrophage apoptosis in the hamster.* J Androl, 1997. **18**(2): p. 166-73.
47. Kubota, Y., S. Sasaki, H. Kubota, H. Tatsura, and K. Kohri, *[A study on the mechanism of the spermatogenic damage after vasectomy in rats].* Nippon Hinyokika Gakkai Zasshi, 2001. **92**(1): p. 13-22.
48. Inaba, Y., M. Fujisawa, H. Okada, S. Arakawa, and S. Kamidono, *The apoptotic changes of testicular germ cells in the obstructive azoospermia models of prepubertal and adult rats.* J Urol, 1998. **160**(2): p. 540-4.
49. Altay, B., M. Hekimgil, K.E. A, and N. Cikili, *Histopathological examination of both ipsilateral and contralateral testes with different obstructive models in prepubertal and adult rats.* BJU Int, 2001. **88**(1): p. 84-9.
50. Lue, Y.H., A.P. Hikim, R.S. Swerdloff, P. Im, K.S. Taing, T. Bui, A. Leung, and C. Wang, *Single exposure to heat induces stage-specific germ cell apoptosis in rats: role of intratesticular testosterone on stage specificity.* Endocrinology, 1999. **140**(4): p. 1709-17.
51. Kasinathan, S., K. Veeraraghavan, and S. Ramakrishnan, *Effect of cadmium on the spermatogenesis of Rana hexadactyla Lesson.* Acta Morphol Hung, 1987. **35**(3-4): p. 183-7.
52. Walton, A., *Spermatogenesis and nutrition.* Br J Nutr, 1949. **3**(1): p. 83-6.
53. Adams, A.E. and E.E. Rae, *An experimental study of the fat-bodies in Triturus (Diemyctylus) uiridescens.* Anat. Rec, 1929. **41**: p. 181-203.
54. Clermont, Y. and A. Rambourg, *Evolution of the endoplasmic reticulum during rat spermiogenesis.* Am J Anat, 1978. **151**(2): p. 191-211.
55. Clermont, Y., C.P. Leblond, and B. Messier, *[Duration of the cycle of the seminal epithelium of the rat.].* Arch Anat Microsc Morphol Exp, 1959. **48(Suppl)**: p. 37-55.

56. Hess, R.A., *Quantitative and qualitative characteristics of the stages and transitions in the cycle of the rat seminiferous epithelium: light microscopic observations of perfusion-fixed and plastic-embedded testes.* Biol Reprod, 1990. **43**(3): p. 525-42.
57. Heller, C.G. and Y. Clermont, *Spermatogenesis in Man: An Estimate of Its Duration.* Science, 1963. **140**(3563): p. 184-186.
58. Hess, R.A., D.J. Schaeffer, V.P. Eroschenko, and J.E. Keen, *Frequency of the stages in the cycle of the seminiferous epithelium in the rat.* Biol Reprod, 1990. **43**(3): p. 517-24.
59. Damber, J.E. and P.O. Janson, *The influence of scrotal warming on testicular blood flow and endocrine function in the rat.* Acta Physiol Scand, 1978. **104**(1): p. 61-7.
60. Rastogi, R.K., L. Iela, P.K. Saxena, and G. Chieffi, *The control of spermatogenesis in the green frog, Rana esculenta.* Journal of Experimental Zoology, 1976. **196**(2): p. 151-165.
61. Setchell, B.P., *Testicular blood supply, lymphatic drainage, and secretion of fluid*, in *The Testis*, W.R. Gomes, A.D. Johnson, and N.L. Vandemark, Editors. 1970, Academic Press: New York. p. 101-239.
62. Boron, W.F. and E.L. Boulpaep, *Medical physiology: A cellular and molecular approach* 2005, Philadelphia, PA: Elsevier/Saunders.
63. Gray, H., T.P. Pick, and R. Howden, *Gray's anatomy: The classic collector's edition*. Rev. American, from the 15th English ed. 1977, New York: Gramercy Books 1257 p.
64. Foldesy, R.G. and J.M. Bedford, *Biology of the scrotum. 1. Temperature and androgen as determinants of the sperm storage capacity of the rat cauda epididymidis.* Biol Reprod, 1982. **26**(4): p. 673-682.
65. Harrison, R.G. and J.S. Weiner, *Vascular patterns of the mammalian testis and their functional significance.* J Exp Biol, 1949. **26**(3): p. 304-16, 2 pl.
66. Dail, W.G., *Autonomic innervation of the male genitalia*, in *Nervous control of the urogenital system*, C.A. Maggi, Editor. 1993, Harwood Academic Publishers: Chur, Switzerland. p. 69-101.
67. Chiocchio, S.R., A.M. Suburo, E. Vladucic, B.C. Zhu, E. Charreau, E.E. Decima, and J.H. Tramezzani, *Differential effects of superior and inferior spermatic nerves on testosterone secretion and spermatic blood flow in cats.* Endocrinology, 1999. **140**(3): p. 1036-43.
68. Young, B., J.W. Heath, A. Stevens, J.S. Lowe, P.R. Wheater, and H.G. Burkitt, *Wheater's functional histology: A text and colour atlas*. 4th ed. 2000, Edinburgh ; New York: Churchill Livingstone. 413 p.
69. Christensen, A.K., *Leydig cells*, in *Handbook of Physiology*, D.W. Hamilton and R.O. Greep, Editors. 1975, Am. Physiological Soc.: Wash, D.C. p. 57-94.
70. Hutson, J.C., *Physiologic interactions between macrophages and Leydig cells.* Exp Biol Med (Maywood), 2006. **231**(1): p. 1-7.
71. Frungieri, M.B., R.S. Calandra, L. Lustig, V. Meineke, F.M. Kohn, H.J. Vogt, and A. Mayerhofer, *Number, distribution pattern, and identification of macrophages in the testes of infertile men.* Fertil Steril, 2002. **78**(2): p. 298-306.
72. Gnessi, L., A. Fabbri, and G. Spera, *Gonadal peptides as mediators of sevelopment and functional control of the testis: An integrated system with hormones and local environment.* Endocr Rev, 1997. **18**(4): p. 541-609.
73. Russell, L.D., *Spermiation--the sperm release process: Ultrastructural observations and unresolved problems*, in *Electron Microscopy in Biology and Medicine*, J. Van Blerkom and P.M. Motta, Editors. 1984, Plenum Press: New York. p. 46-65.

74. Kerr, J.B. and D.M. de Kretser, *Proceedings: The role of the Sertoli cell in phagocytosis of the residual bodies of spermatids.* J Reprod Fertil, 1974. **36**(2): p. 439-40.
75. Brading, A. and NetLibrary Inc., *The autonomic nervous system and its effectors*. 1999, Blackwell Science: Oxford ; Malden, Mass. p. viii, 370 p.
76. Cosentino, M.J. and A.T. Cockett, *Structure and function of the epididymis.* Urol Res, 1986. **14**(5): p. 229-40.
77. Jones, R.C., *To store or mature spermatozoa? The primary role of the epididymis.* Int J Androl, 1999. **22**(2): p. 57-67.
78. Cosentino, M.J., H. Takihara, J.W. Burhop, and A.T. Cockett, *Regulation of rat caput epididymidis contractility by prostaglandins.* J Androl, 1984. **5**(3): p. 216-22.
79. Zhu, B.C., S.R. Chiocchio, A.M. Suburo, and J.H. Tramezzani, *Monoaminergic and peptidergic contributions of the superior and the inferior spermatic nerves to the innervation of the testis in the rat.* J Androl, 1995. **16**(3): p. 248-58.
80. Giuliano, F. and P. Clement, *Neuroanatomy and physiology of ejaculation.* Annu Rev Sex Res, 2005. **16**: p. 190-216.
81. Hinton, B.T., Z.J. Lan, R.J. Lye, and J.C. Labus, *Regulation of epididymal function by testicular factors: The lumicrine hypothesis*, in *The Testis : From stem cell to sperm function*, E. Goldberg, Editor. 2000, Springer: New York. p. xvii, 265 p.
82. de Kretser, D.M., *Endocrinology of male infertility.* Br Med Bull, 1979. **35**(2): p. 187-92.
83. Ryan, R.J., M.C. Charlesworth, D.J. McCormick, R.P. Milius, and H.T. Keutmann, *The glycoprotein hormones: recent studies of structure-function relationships.* FASEB J, 1988. **2**(11): p. 2661-9.
84. Schwartz, N.B. and C.E. McCormack, *Reproduction: gonadal function and its regulation.* Annu Rev Physiol, 1972. **34**: p. 425-72.
85. Amar, A.P. and M.H. Weiss, *Pituitary anatomy and physiology.* Neurosurg Clin N Am, 2003. **14**(1): p. 11-23, v.
86. Musicki, B., G.J. Pepe, and E.D. Albrecht, *Functional differentiation of placental syncytiotrophoblasts during baboon pregnancy: Developmental expression of chorionic somatomammotropin messenger ribonucleic acid and protein levels.* J Clin Endocrinol Metab, 1997. **82**(12): p. 4105-4110.
87. Combarnous, Y., *Molecular basis of the specificity of binding of glycoprotein hormones to their receptors.* Endocr Rev, 1992. **13**(4): p. 670-691.
88. Pierce, J.G. and T.F. Parsons, *Glycoprotein hormones: structure and function.* Annu Rev Biochem, 1981. **50**: p. 465-95.
89. Green, E.D. and J.U. Baenziger, *Asparagine-linked oligosaccharides on lutropin, follitropin, and thyrotropin. II. Distributions of sulfated and sialylated oligosaccharides on bovine, ovine, and human pituitary glycoprotein hormones.* J Biol Chem, 1988. **263**(1): p. 36-44.
90. Manzella, S.M., S.M. Dharmesh, M.C. Beranek, P. Swanson, and J.U. Baenziger, *Evolutionary conservation of the sulfated oligosaccharides on vertebrate glycoprotein hormones that control circulatory half-life.* J Biol Chem, 1995. **270**(37): p. 21665-71.
91. Brook, C.G.D. and N.J. Marshall, *Essential endocrinology* 4th ed. 2001, Oxford ; Malden, MA: Blackwell Science. vii, 179 p.
92. Baenziger, J.U. and E.D. Green, *Pituitary glycoprotein hormone oligosaccharides: structure, synthesis and function of the asparagine-linked oligosaccharides on lutropin, follitropin and thyrotropin.* Biochim Biophys Acta, 1988. **947**(2): p. 287-306.

93. Parsons, T.F. and J.G. Pierce, *Oligosaccharide moieties of glycoprotein hormones: bovine lutropin resists enzymatic deglycosylation because of terminal O-sulfated N-acetylhexosamines.* Proc Natl Acad Sci U S A, 1980. **77**(12): p. 7089-93.
94. Manzella, S.M., L.V. Hooper, and J.U. Baenziger, *Oligosaccharides containing beta 1,4-linked N-acetylgalactosamine, a paradigm for protein-specific glycosylation.* J Biol Chem, 1996. **271**(21): p. 12117-20.
95. Pawson, A.J. and A.S. McNeilly, *The pituitary effects of GnRH.* Anim Reprod Sci, 2005. **88**(1-2): p. 75-94.
96. Burger, L.L., D.J. Haisenleder, A.C. Dalkin, and J.C. Marshall, *Regulation of gonadotropin subunit gene transcription.* J Mol Endocrinol, 2004. **33**(3): p. 559-84.
97. Ewing, L.L., T.Y. Wing, R.C. Cochran, N. Kromann, and B.R. Zirkin, *Effect of luteinizing hormone on Leydig cell structure and testosterone secretion.* Endocrinology, 1983. **112**(5): p. 1763-9.
98. Ewing, L.L. and B. Zirkin, *Leydig cell structure and steroidogenic function.* Recent Prog Horm Res, 1983. **39**: p. 599-635.
99. Dufau, M.L., *The luteinizing hormone receptor.* Annual Review of Physiology, 1998. **60**(1): p. 461.
100. Paliogianni, F., R.L. Kincaid, and D.T. Boumpas, *Prostaglandin E2 and other cyclic AMP elevating agents inhibit interleukin 2 gene transcription by counteracting calcineurin-dependent pathways.* J. Exp. Med., 1993. **178**(5): p. 1813-1817.
101. Stocco, D.M., *The role of the StAR protein in steroidogenesis: challenges for the future.* J Endocrinol, 2000. **164**(3): p. 247-253.
102. Jo, Y., S.R. King, S.A. Khan, and D.M. Stocco, *Involvement of protein kinase C and cyclic adenosine 3',5'-monophosphate-dependent kinase in steroidogenic acute regulatory protein expression and steroid biosynthesis in Leydig cells.* Biol Reprod, 2005. **73**(2): p. 244-55.
103. Manna, P.R. and D.M. Stocco, *Regulation of the steroidogenic acute regulatory protein expression: functional and physiological consequences.* Curr Drug Targets Immune Endocr Metabol Disord, 2005. **5**(1): p. 93-108.
104. Stocco, D.M., X. Wang, Y. Jo, and P.R. Manna, *Multiple signaling pathways regulating steroidogenesis and steroidogenic acute regulatory protein expression: more complicated than we thought.* Mol Endocrinol, 2005. **19**(11): p. 2647-59.
105. Simpson, E.R., *Cholesterol side-chain cleavage, cytochrome P450, and the control of steroidogenesis.* Mol Cell Endocrinol, 1979. **13**(3): p. 213-27.
106. Klinefelter, G.R., P.F. Hall, and L.L. Ewing, *Effect of luteinizing hormone deprivation in situ on steroidogenesis of rat Leydig cells purified by a multistep procedure.* Biol Reprod, 1987. **36**(3): p. 769-83.
107. Collier, T.L., R.N. Waterhouse, and M. Kassiou, *Imaging sigma receptors: applications in drug development.* Curr Pharm Des, 2007. **13**(1): p. 51-72.
108. Monnet, F.P., *Sigma-1 receptor as regulator of neuronal intracellular Ca2+: clinical and therapeutic relevance.* Biol Cell, 2005. **97**(12): p. 873-83.
109. van Broekhoven, F. and R.J. Verkes, *Neurosteroids in depression: a review.* Psychopharmacology (Berl), 2003. **165**(2): p. 97-110.
110. Hall, P.F., *Endocrinology of the testis*, in *The Testis*, W.R. Gomes, A.D. Johnson, and N.L. Vandemark, Editors. 1970, Academic Press: New York. p. 47-92.

111. Cohen, P.E., K. Nishimura, L. Zhu, and J.W. Pollard, *Macrophages: important accessory cells for reproductive function.* J Leukoc Biol, 1999. **66**(5): p. 765-772.
112. Bischoff, P.L., V. Holl, D. Coelho, P. Dufour, D. Weltin, and B. Luu, *Apoptosis at the interface of immunosuppressive and anticancer activities: the examples of two classes of chemical inducers, oxysterols and alkylating agents.* Curr Med Chem, 2000. **7**(7): p. 693-713.
113. Yokoyama, S., F. Nozawa, N. Mugita, and M. Ogawa, *Suppression of rat liver tumorigenesis by 25-hydroxycholesterol and all-trans retinoic acid: differentiation therapy for hepatocellular carcinoma.* Int J Oncol, 1999. **15**(3): p. 565-9.
114. Chen, J.J., Y. Lukyanenko, and J.C. Hutson, *25-hydroxycholesterol is produced by testicular macrophages during the early postnatal period and influences differentiation of Leydig cells in vitro.* Biol Reprod, 2002. **66**(5): p. 1336-41.
115. Lukyanenko, Y.O., A.M. Carpenter, D.E. Brigham, D.M. Stocco, and J.C. Hutson, *Regulation of Leydig cells through a steroidogenic acute regulatory protein-independent pathway by a lipophilic factor from macrophages.* J Endocrinol, 1998. **158**(2): p. 267-75.
116. Heinlein, C.A. and C. Chang, *The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions.* Mol Endocrinol, 2002. **16**(10): p. 2181-2187.
117. Olefsky, J.M., *Nuclear Receptor Minireview Series.* J. Biol. Chem., 2001. **276**(40): p. 36863-36864.
118. Evans, R.M., *The steroid and thyroid hormone receptor superfamily.* Science, 1988. **240**(4854): p. 889-95.
119. Parker, M.G., *Structure and function of nuclear hormone receptors.* Semin Cancer Biol, 1990. **1**(1): p. 81-7.
120. Heemers, H.V. and D.J. Tindall, *Androgen receptor (AR) coregulators: A diversity of functions converging on and regulating the AR transcriptional complex.* Endocr Rev, 2007. **28**(7): p. 778-808.
121. Dieudonne, M.N., R. Pecquery, A. Boumediene, M.C. Leneveu, and Y. Giudicelli, *Androgen receptors in human preadipocytes and adipocytes: regional specificities and regulation by sex steroids.* Am J Physiol Cell Physiol, 1998. **274**(6): p. C1645-1652.
122. Xu, X., G. De Pergola, and P. Bjorntorp, *The effects of androgens on the regulation of lipolysis in adipose precursor cells.* Endocrinology, 1990. **126**(2): p. 1229-1234.
123. Tsai, M.Y., S.D. Yeh, R.S. Wang, S. Yeh, C. Zhang, H.Y. Lin, C.R. Tzeng, and C. Chang, *Differential effects of spermatogenesis and fertility in mice lacking androgen receptor in individual testis cells.* Proc Natl Acad Sci U S A, 2006. **103**(50): p. 18975-80.
124. Huang, H.F., M.T. Li, R. Anesetti, W. Giglio, J.E. Ottenweller, and L.M. Pogach, *Effects of spinal cord injury on spermatogenesis and the expression of messenger ribonucleic acid for Sertoli cell proteins in rat Sertoli cell-enriched testes.* Biol Reprod, 1999. **60**(3): p. 635-41.
125. Komori, S., H. Kasumi, K. Sakata, and K. Koyama, *The role of androgens in spermatogenesis.* Soc Reprod Fertil Suppl, 2007. **63**: p. 25-30.
126. Zhang, C., S. Yeh, Y.T. Chen, C.C. Wu, K.H. Chuang, H.Y. Lin, R.S. Wang, Y.J. Chang, C. Mendis-Handagama, L. Hu, H. Lardy, and C. Chang, *Oligozoospermia with normal fertility in male mice lacking the androgen receptor in testis peritubular myoid cells.* Proc Natl Acad Sci U S A, 2006. **103**(47): p. 17718-23.

127. Simpson, E.R., C. Clyne, G. Rubin, W.C. Boon, K. Robertson, K. Britt, C. Speed, and M. Jones, *Aromatase: A brief overview.* Annual Review of Physiology, 2002. **64**(1): p. 93-127.
128. Carreau, S. and J. Levallet, *Testicular estrogens and male reproduction.* News Physiol Sci, 2000. **15**(4): p. 195-198.
129. Lambard, S., D. Silandre, C. Delalande, I. Denis-Galeraud, S. Bourguiba, and S. Carreau, *Aromatase in testis: expression and role in male reproduction.* J Steroid Biochem Mol Biol, 2005. **95**(1-5): p. 63-9.
130. Carreau, S., D. Silandre, S. Bourguiba, K. Hamden, L. Said, S. Lambard, I. Galeraud-Denis, and C. Delalande, *Estrogens and male reproduction: a new concept.* Braz J Med Biol Res, 2007. **40**(6): p. 761-8.
131. Oliveira, C.A., R. Nie, K. Carnes, L.R. Franca, G.S. Prins, P.T. Saunders, and R.A. Hess, *The antiestrogen ICI 182,780 decreases the expression of estrogen receptor-alpha but has no effect on estrogen receptor-beta and androgen receptor in rat efferent ductules.* Reprod Biol Endocrinol, 2003. **1**: p. 75.
132. Hess, R.A., *Estrogen in the adult male reproductive tract: a review.* Reprod Biol Endocrinol, 2003. **1**: p. 52.
133. Huddleston, G.G., R.P. Michael, D. Zumpe, and A.N. Clancy, *Estradiol in the male rat amygdala facilitates mounting but not ejaculation.* Physiol Behav, 2003. **79**(2): p. 239-46.
134. Huddleston, G.G., J.C. Paisley, and A.N. Clancy, *Effects of estrogen in the male rat medial amygdala: infusion of an aromatase inhibitor lowers mating and bovine serum albumin-conjugated estradiol implants do not promote mating.* Neuroendocrinology, 2006. **83**(2): p. 106-16.
135. Huddleston, G.G., J.C. Paisley, S. Graham, M.S. Grober, and A.N. Clancy, *Implants of estradiol conjugated to bovine serum albumin in the male rat medial preoptic area promote copulatory behavior.* Neuroendocrinology, 2007. **86**(4): p. 249-59.
136. Huddleston, G.G., C.K. Song, J.C. Paisley, T.J. Bartness, and A.N. Clancy, *Gonadal steroid receptors colocalize with central nervous system neurons projecting to the rat prostate gland.* Am J Physiol Regul Integr Comp Physiol, 2007. **292**(6): p. R2196-205.
137. Robaire, B., L.L. Ewing, D.C. Irby, and C. Desjardins, *Interactions of testosterone and estradiol-17 beta on the reproductive tract of the male rat.* Biol Reprod, 1979. **21**(2): p. 455-63.
138. Ewing, L.L., R.A. Gorski, R.J. Sbordone, J.V. Tyler, C. Desjardins, and B. Robaire, *Testosterone-estradiol filled polydimethylsiloxane subdermal implants: effect on fertility and masculine sexual and aggressive behavior of male rats.* Biol Reprod, 1979. **21**(4): p. 765-72.
139. Eddy, E.M., T.F. Washburn, D.O. Bunch, E.H. Goulding, B.C. Gladen, D.B. Lubahn, and K.S. Korach, *Targeted disruption of the estrogen receptor gene in male mice causes alteration of spermatogenesis and infertility.* Endocrinology, 1996. **137**(11): p. 4796-805.
140. Gronning, L.M., J.E. Wang, A.H. Ree, T.B. Haugen, K. Tasken, and K.A. Tasken, *Regulation of tissue inhibitor of metalloproteinases-1 in rat Sertoli cells: induction by germ cell residual bodies, interleukin-1alpha, and second messengers.* Biol Reprod, 2000. **62**(4): p. 1040-6.

141. Gomez, D.E., D.F. Alonso, H. Yoshiji, and U.P. Thorgeirsson, *Tissue inhibitors of metalloproteinases: structure, regulation and biological functions.* Eur J Cell Biol, 1997. **74**(2): p. 111-22.
142. Hales, D.B., *Cytokines and testicular function*, in *Cytokines in human reproduction*, J.A. Hill, Editor. 2000, Wiley: New York. p. xii, 341 p.
143. Huleihel, M. and E. Lunenfeld, *Involvement of intratesticular IL-1 system in the regulation of Sertoli cell functions.* Mol Cell Endocrinol, 2002. **187**(1-2): p. 125-32.
144. Huleihel, M. and E. Lunenfeld, *Regulation of spermatogenesis by paracrine/autocrine testicular factors.* Asian J Androl, 2004. **6**(3): p. 259-68.
145. Petersen, C., C. Boitani, B. Froysa, and O. Soder, *Interleukin-1 is a potent growth factor for immature rat sertoli cells.* Mol Cell Endocrinol, 2002. **186**(1): p. 37-47.
146. Svechnikov, K., C. Petersen, T. Sultana, A. Wahlgren, C. Zetterstrom, E. Colon, C. Bornestaf, and O. Soder, *The paracrine role played by interleukin-1 alpha in the testis.* Curr Drug Targets Immune Endocr Metabol Disord, 2004. **4**(1): p. 67-74.
147. McCorry, L.K., *Physiology of the autonomic nervous system.* Am J Pharm Educ, 2007. **71**(4): p. 78.
148. Hamill, R.W., *Peripheral Autonomic Nervous System*, in *Primer on the autonomic nervous system*, D. Robertson, P.A. Low, and R.J. Polinsky, Editors. 1996, Academic Press: San Diego. p. xv, 343 p., [1] leaf of plates.
149. Nadelhaft, I., W.C. de Groat, and C. Morgan, *The distribution and morphology of parasympathetic preganglionic neurons in the cat sacral spinal cord as revealed by horseradish peroxidase applied to the sacral ventral roots.* J Comp Neurol, 1986. **249**(1): p. 48-56.
150. Strosberg, A.D., *Structure, function, and regulation of adrenergic receptors.* Protein Sci, 1993. **2**(8): p. 1198-209.
151. Bylund, D.B., *Subtypes of alpha 1- and alpha 2-adrenergic receptors.* FASEB J, 1992. **6**(3): p. 832-9.
152. McGraw, D.W. and S.B. Liggett, *Molecular mechanisms of beta2-adrenergic receptor function and regulation.* Proc Am Thorac Soc, 2005. **2**(4): p. 292-6; discussion 311-2.
153. Skeberdis, V.A., *Structure and function of beta3-adrenergic receptors.* Medicina (Kaunas), 2004. **40**(5): p. 407-13.
154. Trendelenburg, U., *The interaction of transport mechanisms and intracellular enzymes in metabolizing systems.* J Neural Transm Suppl, 1990. **32**: p. 3-18.
155. Haddad, P.M. and S.M. Dursun, *Neurological complications of psychiatric drugs: clinical features and management.* Hum Psychopharmacol, 2008. **23 Suppl 1**: p. 15-26.
156. Cinti, S., *The adipose organ.* Prostaglandins Leukot Essent Fatty Acids, 2005. **73**(1): p. 9-15.
157. Large, V., O. Peroni, D. Letexier, H. Ray, and M. Beylot, *Metabolism of lipids in human white adipocyte.* Diabetes Metab, 2004. **30**(4): p. 294-309.
158. Gerendai, I., *Supraspinal connections of the reproductive organs: structural and functional aspects.* Acta Physiol Hung, 2004. **91**(1): p. 1-21.
159. Stewart, J.D., *Sexual function*, in *Primer on the autonomic nervous system*, D. Robertson, P.A. Low, and R.J. Polinsky, Editors. 1996, Academic Press: San Diego. p. xv, 343 p., [1] leaf of plates.

160. Maruch, S.M., H.J. Alves, and C.R. Machado, *Sympathetic innervation of the reproductive organs of the male opossum, Didelphis albiventris (Lund, 1841).* Acta Anat (Basel), 1989. **134**(3): p. 257-62.
161. Saleh, A.M., M.A. Alameldin, M.E. Abdelmoniem, E.M. Hassouna, and K.H. Wrobel, *Immunohistochemical investigations of the autonomous nerve distribution in the testis of the camel (Camelus dromedarius).* Ann Anat, 2002. **184**(3): p. 209-20.
162. Tainio, H., *Peptidergic innervation of the human testis and epididymis.* Acta Histochem, 1994. **96**(4): p. 415-20.
163. Rauchenwald, M., W.D. Steers, and C. Desjardins, *Efferent innervation of the rat testis.* Biol Reprod, 1995. **52**(5): p. 1136-43.
164. Hill, R.T. and P.T. Toivola, *Effect of sacral cordotomy on the reproductive organs of the male guinea pig.* Biol Reprod, 1978. **19**(1): p. 64-8.
165. Lakomy, M., J. Kaleczyc, and M. Majewski, *Noradrenergic and peptidergic innervation of the testis and epididymis in the male pig.* Folia Histochem Cytobiol, 1997. **35**(1): p. 19-27.
166. Wrobel, K.H. and A. Gurtler, *The nerve distribution in the testis of the cat.* Ann Anat, 2001. **183**(4): p. 297-308.
167. Lamano-Carvalho, T.L., W.G. Kempinas, and A.L. Favaretto, *Morphometric evaluation of the rat testis, epididymis and vas deferens following chemical sympathectomy with guanethidine.* Ann Anat, 1993. **175**(5): p. 453-7.
168. Suburo, A.M., S.R. Chiocchio, M.V. Canto Soler, A. Nieponice, and J.H. Tramezzani, *Peptidergic innervation of blood vessels and interstitial cells in the testis of the cat.* J Androl, 2002. **23**(1): p. 121-34.
169. McKenna, K.E. and I. Nadelhaft, *The organization of the pudendal nerve in the male and female rat.* J Comp Neurol, 1986. **248**(4): p. 532-49.
170. Dail, W.G., *The pelvic plexus: Innervation of pelvic and extrapelvic visceral tissues.* Microscopy Research and Technique, 1996. **35**(2): p. 95-106.
171. Hodson, N., *The nerves of the testis, epididymis and scrotum*, in *The Testis*, W.R. Gomes, A.D. Johnson, and N.L. Vandemark, Editors. 1970, Academic Press: New York. p. 47-99.
172. Standring, S., *Gray's anatomy: The anatomical basis of clinical practice*. 2004, Oxford, UK: Churchill Livingstone.
173. Kuo, D.C., T. Hisamitsu, and W.C.D. Groat, *A sympathetic projection from sacral paravertebral ganglia to the pelvic nerve and to postganglionic nerves on the surface of the urinary bladder and large intestine of the cat.* The Journal of Comparative Neurology, 1984. **226**(1): p. 76-86.
174. Standring, S., *Gray's anatomy: The anatomical basis of clinical practice*. Rev. American, from the 15th English ed. 2004, Oxford, UK: Churchill Livingstone. 1257 p.
175. Clement, P. and F. Giuliano, *Neuroanatomy and physiology of ejaculation.* Annual Review of Sex Research, 2005. **16**: p. 190-216.
176. Bell, C. and J.R. McLean, *The autonomic innervation of the rat testicular capsule.* J Reprod Fertil, 1973. **32**(2): p. 253-8.
177. Wrobel, K.H. and E. Schenk, *Immunohistochemical investigations of the autonomous innervation of the cervine testis.* Ann Anat, 2003. **185**(6): p. 493-506.
178. Wrobel, K.H. and M.N. Moustafa, *On the innervation of the donkey testis.* Ann Anat, 2000. **182**(1): p. 13-22.

179. Wrobel, K.H. and B. Brandl, *The autonomous innervation of the porcine testis in the period from birth to adulthood.* Ann Anat, 1998. **180**(2): p. 145-56.
180. Hodson, N., *The nerves of the testis, epididymis and scrotum* in *The Testis*, W.R. Gomes, A.D. Johnson, and N.L. Vandemark, Editors. 1970, Academic Press: New York. p. 47-92.
181. Linsenmeyer, T.A., *Male infertility following spinal cord injury.* J Am Paraplegia Soc, 1991. **14**(3): p. 116-21.
182. Linsenmeyer, T.A., *Sexual function and infertility following spinal cord injury.* Phys Med Rehabil Clin N Am, 2000. **11**(1): p. 141-56, ix.
183. Linsenmeyer, T.A. and I. Perkash, *Infertility in men with spinal cord injury.* Arch Phys Med Rehabil, 1991. **72**(10): p. 747-54.
184. Hirsch, I.H., B. Huang, M.B. Chancellor, D.A. Rivas, S.K. Salzman, L.K. Jost, and D.P. Evenson, *Spermatogenesis in early and chronic phases of experimental spinal cord injury in the rodent model.* J Androl, 1999. **20**(1): p. 63-71.
185. Huang, H.F., M.T. Li, W. Giglio, R. Anesetti, J.E. Ottenweller, and L.M. Pogach, *The detrimental effects of spinal cord injury on spermatogenesis in the rat is partially reversed by testosterone, but enhanced by follicle-stimulating hormone.* Endocrinology, 1999. **140**(3): p. 1349-55.
186. Huang, H.F., M.T. Li, S. Wang, B. Barton, R. Anesetti, and J.A. Jetko, *Effects of exogenous testosterone on testicular function during the chronic phase of spinal cord injury: dose effects on spermatogenesis and Sertoli cell and sperm function.* J Spinal Cord Med, 2004. **27**(1): p. 55-62.
187. Huang, H.F., M.T. Li, S. Wang, L.M. Pogach, and J.E. Ottenweller, *Alteration of cyclic adenosine 3',5'-monophosphate signaling in rat testicular cells after spinal cord injury.* J Spinal Cord Med, 2003. **26**(1): p. 69-78.
188. Huang, H.F., T.A. Linsenmeyer, R. Anesetti, W. Giglio, J.E. Ottenweller, and L. Pogach, *Suppression and recovery of spermatogenesis following spinal cord injury in the rat.* J Androl, 1998. **19**(1): p. 72-80.
189. Huang, H.F., T.A. Linsenmeyer, M.T. Li, W. Giglio, R. Anesetti, J. von Hagen, J.E. Ottenweller, C. Serenas, and L. Pogach, *Acute effects of spinal cord injury on the pituitary-testicular hormone axis and Sertoli cell functions: a time course study.* J Androl, 1995. **16**(2): p. 148-57.
190. Huang, H.F., S. Wang, C.A. Molina, and J.E. Ottenweller, *Preservation of spermatogenesis in spinal cord injured rats with exogenous testosterone. Relationship with serum testosterone levels and cellular localization of cAMP responsive element modulator.* J Androl, 2004. **25**(1): p. 95-103.
191. Kempinas, W.D., J.D. Suarez, N.L. Roberts, L.F. Strader, J. Ferrell, J.M. Goldman, M.G. Narotsky, S.D. Perreault, D.P. Evenson, D.D. Ricker, and G.R. Klinefelter, *Fertility of rat epididymal sperm after chemically and surgically induced sympathectomy.* Biol Reprod, 1998. **59**(4): p. 897-904.
192. Linsenmeyer, T.A., Q. Chang, J. Ottenweller, R. Anesetti, L. Pogach, and H.H. Huang, *Testicular blood flow following spinal cord injury in the Sprague Dawley rat.* J Spinal Cord Med, 1996. **19**(3): p. 183-5.
193. Linsenmeyer, T.A., L.M. Pogach, J.E. Ottenweller, and H.F. Huang, *Spermatogenesis and the pituitary-testicular hormone axis in rats during the acute phase of spinal cord injury.* J Urol, 1994. **152**(4): p. 1302-7.

194. Wang, S., G. Wang, B.E. Barton, T.F. Murphy, and H.F. Huang, *Impaired sperm function after spinal cord injury in the rat is associated with altered cyclic adenosine monophosphate signaling.* J Androl, 2005. **26**(5): p. 592-600.
195. Campos, M.B., M.L. Vitale, M.N. Ritta, S.R. Chiocchio, and R.S. Calandra, *Catecholamine distribution in adult rat testis.* Andrologia, 1990. **22**(3): p. 247-50.
196. Campos, M.B., M.L. Vitale, R.S. Calandra, and S.R. Chiocchio, *Serotonergic innervation of the rat testis.* J Reprod Fertil, 1990. **88**(2): p. 475-9.
197. Gong, Y., Y. Yang, H. Li, Y. Lu, Q. Wei, and D. Liu, *[Effect of removal of superior spermatic nerve on spermatogenesis].* Sichuan Da Xue Xue Bao Yi Xue Ban, 2003. **34**(3): p. 462-4.
198. Gong, Y.G., Y.R. Yang, W. Zhagn, M. Gu, and C.J. Yin, *[Expression of Bax and Bcl-2 gene in rat testis tissues induced by testicular denervation].* Zhonghua Nan Ke Xue, 2006. **12**(12): p. 1063-5.
199. Lamano Carvalho, T.L., N.P. Hodson, M.A. Blank, P.F. Watson, P.K. Mulderry, A.E. Bishop, J. Gu, S.R. Bloom, and J.M. Polak, *Occurrence, distribution and origin of peptide-containing nerves of guinea-pig and rat male genitalia and the effects of denervation on sperm characteristics.* J Anat, 1986. **149**: p. 121-41.
200. Sato, K., K. Kihara, M. Ando, T. Sato, and H. Oshima, *Seminal emission by electrical stimulation of the spermatic nerve and epididymis.* Int J Androl, 1991. **14**(6): p. 461-7.
201. Kihara, K. and W.C.D. Groat, *Sympathetic efferent pathways projecting bilaterally to the vas deferens in the rat.* The Anatomical Record, 1997. **248**(2): p. 291-299.
202. Kihara, K., K. Sato, M. Ando, T. Morita, and H. Oshima, *Control of bilateral seminal emissions from ejaculatory ducts by a lumbar splanchnic nerve.* Am J Physiol, 1993. **265**(4 Pt 2): p. R743-8.
203. Kihara, K., K. Sato, M. Ando, T. Sato, and H. Oshima, *Ability of each lumbar splanchnic nerve and disability of thoracic ones to generate seminal emission in the dog.* J Urol, 1992. **147**(1): p. 260-3.
204. Kihara, K., K. Sato, M. Ando, T. Sato, and H. Oshima, *A mechanism of retrograde ejaculation after bilateral hypogastric nerve transections in the dog.* J Urol, 1992. **148**(4): p. 1307-9.
205. Cosentino, M.J., S.R. Schoen, and A.T. Cockett, *Effect of sympathetic denervation of rat internal genitalia on daily sperm output.* Urology, 1984. **24**(6): p. 587-90.
206. Kihara, K., K. Sato, and H. Oshima, *Sympathetic efferent pathways projecting to the vas deferens.* Microsc Res Tech, 1998. **42**(6): p. 398-408.
207. Sato, K. and K. Kihara, *Spinal cord segments controlling the canine vas deferens and differentiation of the primate sympathetic pathways to the vas deferens.* Microscopy Research and Technique, 1998. **42**(6): p. 390-397.
208. Michel, M.C., *a1-Adrenoceptors and ejaculatory function.* British Journal of Pharmacology, 2007. **152**: p. 289-290.
209. Yonese, J., K. Kihara, K. Sato, H. Fukuda, S. Kamata, and H. Oshima, *Sympathetic efferent pathways projecting to the prostate in the dog.* The Prostate, 2000. **44**(3): p. 225-232.
210. Kepper, M.E. and J.R. Keast, *Location, immunohistochemical features, and spinal connections of autonomic neurons innervating the rat seminal vesicles.* Biol Reprod, 1997. **57**(5): p. 1164-74.

211. Kaiho, Y., H. Nakagawa, A. Ito, M. Satoh, Y. Ikeda, S. Saito, and Y. Arai, *Ipsilateral seminal emission generated by electrostimulation of the lumbar sympathetic nerve during nerve sparing laparoscopic retroperitoneal lymph node dissection for testicular cancer.* The Journal of Urology, 2004. **172**(3): p. 928-931.
212. Campos, M.B., S.R. Chiocchio, R.S. Calandra, and M.N. Ritta, *Effect of bilateral denervation of the immature rat testis on testicular gonadotropin receptors and in vitro androgen production.* Neuroendocrinology, 1993. **57**(2): p. 189-94.
213. Frungieri, M.B., H.F. Urbanski, B. Hohne-Zell, and A. Mayerhofer, *Neuronal elements in the testis of the rhesus monkey: ontogeny, characterization and relationship to testicular cells.* Neuroendocrinology, 2000. **71**(1): p. 43-50.
214. Mayerhofer, A., M. Danilchik, K.Y. Pau, H.E. Lara, L.D. Russell, and S.R. Ojeda, *Testis of prepubertal rhesus monkeys receives a dual catecholaminergic input provided by the extrinsic innervation and an intragonadal source of catecholamines.* Biol Reprod, 1996. **55**(3): p. 509-18.
215. Mayerhofer, A., M.B. Frungieri, A. Bulling, and S. Fritz, *Sources and function of neuronal signalling molecules in the gonads.* Medicina (B Aires), 1999. **59**(5 Pt 2): p. 542-5.
216. Mayerhofer, A., M.B. Frungieri, S. Fritz, A. Bulling, B. Jessberger, and H.J. Vogt, *Evidence for catecholaminergic, neuronlike cells in the adult human testis: changes associated with testicular pathologies.* J Androl, 1999. **20**(3): p. 341-7.
217. Kuntz, A. and R.E. Morris, *Components and distribution of the spermatic nerves and the nerves of the vas deferens.* J Comp Neurol, 1946. **85**: p. 33-44.
218. Santamaria, L., A. Reoyo, J. Regadera, and R. Paniagua, *Histochemistry and ultrastructure of nerve fibres and contractile cells in the tunica albuginea of the rat testis.* Acta Anat (Basel), 1990. **139**(2): p. 126-33.
219. Hodson, N., *Sympathetic nerves and reproductive organs in the male rabbit.* J Reprod Fertil, 1965. **10**(2): p. 209-20.
220. Zhu, B.C., S.R. Chiocchio, J.H. Han, and J.C. Cavicchia, *Neural control of the compensatory increase of testosterone in hemicastrated adult male rats.* Sheng Li Xue Bao, 2000. **52**(1): p. 10-6.
221. Nagai, K., S. Murano, Y. Minokoshi, H. Okuda, and M. Kinutani, *Effects of denervation and local 6-hydroxydopamine injection on testicular growth in rats.* Experientia, 1982. **38**(5): p. 592-4.
222. Zhu, B., J.C. Cavicchia, and S.R. Chiocchio, *Testicular denervation-induced nuclear changes in Leydig cell of hemicastrated adult rats.* Tissue Cell, 1998. **30**(5): p. 485-91.
223. Mayerhofer, A., A.G. Amador, R.W. Steger, and A. Bartke, *Testicular function after local injection of 6-hydroxydopamine or norepinephrine in the golden hamster (Mesocricetus auratus).* J Androl, 1990. **11**(3): p. 301-11.
224. Trasler, J.M., L. Hermo, and B. Robaire, *Morphological changes in the testis and epididymis of rats treated with cyclophosphamide: a quantitative approach.* Biol Reprod, 1988. **38**(2): p. 463-479.
225. Boekelheide, K. and H.A. Schoenfeld, *Spermatogenesis by Sisyphus: proliferating stem germ cells fail to repopulate the testis after 'irreversible' injury.* Adv Exp Med Biol, 2001. **500**: p. 421-8.
226. Kuntz, A., *Experimental degeneration in the testis of the dog.* Anat Rec, 1919. **17**: p. 221-234.

227. Coujard, R., *[Effects on the testis and epididymis of the phenolization of nerve fibers.].* C R Seances Soc Biol Fil, 1952. **146**(9-10): p. 704-6.
228. Frankel, A.I. and E.L. Ryan, *Testicular innervation is necessary for the response of plasma testosterone levels to acute stress.* Biol Reprod, 1981. **24**(3): p. 491-5.
229. Allen, L.F., R.J. Lefkowitz, M.G. Caron, and S. Cotecchia, *G-protein-coupled receptor genes as protooncogenes: constitutively activating mutation of the alpha 1B-adrenergic receptor enhances mitogenesis and tumorigenicity.* Proc Natl Acad Sci U S A, 1991. **88**(24): p. 11354-8.
230. Mhaouty-Kodja, S., A. Lozach, R. Habert, M. Tanneux, C. Guigon, S. Brailly-Tabard, J.P. Maltier, and C. Legrand-Maltier, *Fertility and spermatogenesis are altered in {alpha}1b-adrenergic receptor knockout male mice.* J Endocrinol, 2007. **195**(2): p. 281-92.
231. Michell, R.H., L.A. Conroy, P.J. French, C.M. Bunce, J.M. Lord, G. Brown, M.A. Baxter, F. McConnell, and J.A. Creba, *Inositol lipids and phosphates in the differentiation of blood cells.* Adv Second Messenger Phosphoprotein Res, 1990. **24**: p. 147-51.
232. Bergh, A., H. Blom, J.E. Damber, and R. Henriksson, *The effect of long-term variation in sympathetic activity on testicular morphology in immature rats.* Andrologia, 1987. **19**(4): p. 448-51.
233. Lamano Carvalho, T.L., M.A. Guimaraes, W.G. Kempinas, S.O. Petenusci, and A.A. Rosa e Silva, *Effects of guanethidine-induced sympathectomy on the spermatogenic and steroidogenic testicular functions of prepubertal to mature rats.* Andrologia, 1996. **28**(2): p. 117-22.
234. Guimaraes, M.A., L.O. Lunardi, C.H. Pellizzon, W.G. Kempinas, and A.A. Rosa e Silva, *Male gonadal denervation by guanethidine at pre-puberty: different doses, different results. The intervention of the pineal deafferentation.* Cell Mol Biol (Noisy-le-grand), 1997. **43**(3): p. 383-91.
235. Kempinas, W.D., J.D. Suarez, N.L. Roberts, L. Strader, J. Ferrell, J.M. Goldman, and G.R. Klinefelter, *Rat epididymal sperm quantity, quality, and transit time after guanethidine-induced sympathectomy.* Biol Reprod, 1998. **59**(4): p. 890-6.
236. Schneider, J.E., *Energy balance and reproduction.* Physiol Behav, 2004. **81**(2): p. 289-317.
237. Wade, G.N., J.E. Schneider, and H.Y. Li, *Control of fertility by metabolic cues.* Am J Physiol, 1996. **270**(1 Pt 1): p. E1-19.
238. Schneider, J.E., C.A. Buckley, R.M. Blum, D. Zhou, L. Szymanski, D.E. Day, and T.J. Bartness, *Metabolic signals, hormones and neuropeptides involved in control of energy balance and reproductive success in hamsters.* Eur J Neurosci, 2002. **16**(3): p. 377-9.
239. Schneider, J.E., M.D. Goldman, N.A. Leo, and M.E. Rosen, *Central vs. peripheral metabolic control of estrous cycles in Syrian hamsters. II. Glucoprivation.* Am J Physiol, 1997. **272**(1 Pt 2): p. R406-12.
240. Schneider, J.E. and G.N. Wade, *Availability of metabolic fuels controls estrous cyclicity of Syrian hamsters.* Science, 1989. **244**(4910): p. 1326-8.
241. Wade, G.N. and J.E. Jones, *Neuroendocrinology of nutritional infertility.* Am J Physiol Regul Integr Comp Physiol, 2004. **287**(6): p. R1277-96.
242. Cinti, S., *Anatomy of the adipose organ.* Eat Weight Disord, 2000. **5**(3): p. 132-42.

243. Berthoud, H.-R., E.A. Fox, and W.L. Neuhuber, *Vagaries of adipose tissue innervation.* Am J Physiol Regul Integr Comp Physiol, 2006. **291**(5): p. R1240-1242.
244. Bronson, F.H. and J.M. Manning, *The energetic regulation of ovulation: a realistic role for body fat.* Biol Reprod, 1991. **44**(6): p. 945-50.
245. Patton, J., D.A. Kenny, J.F. Mee, F.P. O'Mara, D.C. Wathes, M. Cook, and J.J. Murphy, *Effect of milking frequency and diet on milk production, energy balance, and reproduction in dairy cows.* J Dairy Sci, 2006. **89**(5): p. 1478-87.
246. Thong, F.S., C. McLean, and T.E. Graham, *Plasma leptin in female athletes: relationship with body fat, reproductive, nutritional, and endocrine factors.* J Appl Physiol, 2000. **88**(6): p. 2037-44.
247. van Knegsel, A.T., H. van den Brand, J. Dijkstra, and B. Kemp, *Effects of dietary energy source on energy balance, metabolites and reproduction variables in dairy cows in early lactation.* Theriogenology, 2007. **68 Suppl 1**: p. S274-80.
248. van Knegsel, A.T., H. van den Brand, J. Dijkstra, S. Tamminga, and B. Kemp, *Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle.* Reprod Nutr Dev, 2005. **45**(6): p. 665-88.
249. Frisch, R.E., *Body fat, menarche, fitness and fertility.* Hum Reprod, 1987. **2**(6): p. 521-33.
250. Bronson, F.H., *Food-restricted, prepubertal, female rats: rapid recovery of luteinizing hormone pulsing with excess food, and full recovery of pubertal development with gonadotropin-releasing hormone.* Endocrinology, 1986. **118**(6): p. 2483-2487.
251. Wade, G.N. and J.E. Schneider, *Metabolic fuels and reproduction in female mammals.* Neuroscience & Biobehavioral Reviews, 1992. **16**(2): p. 235-272.
252. McInroy, J.K., D.G. Brousmiche, and K.E. Wynne-Edwards, *Fathers, fat, and maternal energetics in a biparental hamster: paternal presence determines the outcome of a current reproductive effort and adipose tissue limits subsequent reproductive effort.* Horm Behav, 2000. **37**(4): p. 399-409.
253. Bartness, T.J., *Food hoarding is increased by pregnancy, lactation, and food deprivation in Siberian hamsters.* Am J Physiol, 1997. **272**(1 Pt 2): p. R118-25.
254. Goumenou, A.G., I.M. Matalliotakis, G.E. Koumantakis, and D.K. Panidis, *The role of leptin in fertility.* Eur J Obstet Gynecol Reprod Biol, 2003. **106**(2): p. 118-24.
255. De Souza, M.J. and N.I. Williams, *Physiological aspects and clinical sequelae of energy deficiency and hypoestrogenism in exercising women.* Hum Reprod Update, 2004. **10**(5): p. 433-448.
256. Bronson, F.H., P.D. Heideman, and M.C. Kerbeshian, *Lability of fat stores in peripubertal wild house mice.* J Comp Physiol [B], 1991. **161**(1): p. 15-8.
257. Lujan, M.E., A.A. Krzemien, R.L. Reid, and D.A. Van Vugt, *Developing a model of nutritional amenorrhea in rhesus monkeys.* Endocrinology, 2006. **147**(1): p. 483-92.
258. Srinivasan, V., D.P. Thombre, S. Lakshmanan, and A.S. Chakrabarty, *Effect of removal of epididymal fat on spermatogenesis in albino rats.* Indian J Exp Biol, 1986. **24**(8): p. 487-8.
259. Hull, E., R.L. Meisel, and B.D. Sachs, *Male sexual behavior*, in *Hormons, brain and behavior*, D.W. Pfaff, et al., Editors. 2002, Academic Press; Elsevier Science: New York. p. 3-137.
260. Cohen, H., O. Zinder, and B. Shapiro, *Effect of excision of one epididymal fat pad on its contralateral.* Life Sci, 1977. **20**(12): p. 1979-81.

261. Mauer, M.M. and T.J. Bartness, *Fat pad-specific compensatory mass increases after varying degrees of lipectomy in Siberian hamsters.* Am J Physiol, 1997. **273**(6 Pt 2): p. R2117-23.
262. Mauer, M.M., R.B. Harris, and T.J. Bartness, *The regulation of total body fat: lessons learned from lipectomy studies.* Neurosci Biobehav Rev, 2001. **25**(1): p. 15-28.
263. Zancanaro, C., F. Merigo, M. Digito, and G. Pelosi, *Fat body of the frog Rana esculenta: an ultrastructural study.* J Morphol, 1996. **227**(3): p. 321-34.
264. Kasinathan, S., A. Balasubramanian, S. Ramakrishnan, and S.L. Basu, *The role of fat body in testicular spermatogenesis and steroidogenesis in Rana hexadactyla Lesson.* J. Biosci., 1979. **1**(2): p. 207-213.
265. Chieffi, G., R.K. Rastogi, L. Iela, and M. Milone, *The function of fat bodies in relation to the hypothalamo-hypophyseal-gonadal axis in the frog, Rana esculenta.* Cell and Tissue Research, 1975. **161**(2): p. 157-165.
266. Das, I., *Reproductive strategy and lipid mobilization in Rana hexadactyla at a seasonal locality.* Ethology Ecology & Evolution, 1996. **8**: p. 125-133.
267. Dubois, A.M., *[The relationship between the testicular function and the fat bodies among ambibians]* Rev. Suisse Zool., 1927. **34**: p. 499-581.
268. Chieffi, G., C. Lupo di Prisco, and G. Delrio, *[Identification of sex hormones in the fat bodies of the male Triturus cristatus carnifex].* Boil. Zool., 1967. **34**: p. 104-105.
269. Pierantoni, R., B. Varriale, C. Simeoli, L.D. Matteo, M. Milone, R.K. Rastogi, and G. Chieffi, *Fat body and autumn recrudescence of the ovary in Rana esculenta.* Comp. Biochem. Physiol., 1983. **76A**: p. 31-25.
270. Chieffi, G. and R.K. Rastogi, *Interrelationships between hypothalamus, par distalis, testis and fat body of Rana esculenta.* Gen Comp Endocr, 1974. **22**: p. 356.
271. Kasinathan, S., A.G. Singh, and S.L. Basu, *The gonadal fat body--a major reproductive strategy in Rana hexadactyla.* Comp Physiol Ecol, 1979. **4**: p. 201-206.
272. Best, C.H. and J. Campbell, *Anterior pituitary extracts and liver fat.* J Physiol, 1936. **86**(2): p. 190-203.
273. Best, C.H. and J. Campbell, *The effect of anterior pituitary extracts on the liver fat of various animals.* J Physiol, 1938. **92**(1): p. 91-110.
274. Seifter, J. and D.H. Baeder, *Lipid mobilization by a crystalline peptide isolated from plasma of horses administered cortisone.* Proc Soc Exp Biol Med, 1956. **95**(3): p. 469-71.
275. Baeder, D.H., G.M. Miller, J. Seifter, W.A. Steiger, and C.J.D. Zarafonetis, *Preliminary report on the effects of a plasma lipid mobilizing factor in man.* Am J Med Sci, 1956. **232**(6): p. 605-12.
276. Rondinone, C.M., *Adipocyte-derived hormones, cytokines, and mediators.* Endocrine, 2006. **29**(1): p. 81-90.
277. Trayhurn, P. and J.H. Beattie, *Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ.* Proc Nutr Soc, 2001. **60**(3): p. 329-39.
278. Seifter, J. and D.H. Baeder, *Lipid mobilizer (LM) from posterior pituitary of hogs.* Proc Soc Exp Biol Med, 1957. **95**(2): p. 318-20.
279. Kastin, A.J., T.W. Redding, R. Hall, G.M. Besser, and A.V. Schally, *Lipid mobilizing hormones of the hypothalamus and pituitary.* Pharmacol Biochem Behav, 1975. **3**(1 Suppl): p. 121-6.
280. Schwandt, P., *Special lipid mobilizing hormones from the pituitary gland (lipotropins).* Acta Endocrinol Suppl (Copenh), 1973. **173**: p. 177-8.

281. Schwandt, P., P. Weisweiler, and R. Eicher, *[Comparative studies on a lipotropin from human hypophyses]*. Klin Wochenschr, 1969. **47**(21): p. 1174-5.
282. Knudtzon, J., A. Winsnes, S. Oyasaeter, I. Foss, and O. Trygstad, *Acute in vivo stimulatory effects of human pituitary lipid-mobilizing factor on plasma levels of glucagon and insulin in rabbits.* Horm Metab Res, 1984. **16 Suppl 1**: p. 1-6.
283. Pawan, G.L., *Extraction and assay of the fat-mobilizing substance from urine of normal adult human subjects in various physiological states.* Biochem J, 1968. **110**(4): p. 52P-53P.
284. Beaton, J.R., A.J. Szlavko, B.M. Box, and J.A. Stevenson, *Biological effects of anorexigenic and fat-mobilizing substance from rat urine.* Can J Physiol Pharmacol, 1964. **42**: p. 657-64.
285. Redding, T.W. and A.V. Schally, *Lipid mobilizing factor from the hypothalamus.* Metabolism, 1970. **19**(9): p. 641-52.
286. Shah, P.P., A.R. Tee, P.D. English, B.T. Redman, and J. Bunyan, *The identification of a lipid-mobilizing factor from sheep midbrain.* Biochem J, 1972. **130**(2): p. 467-73.
287. Larsen, L.O., *Fat bodies in Bufo bufo with auto transplanted pars distalis.* Gen Comp Endocr, 1963. **3**: p. 713-714.
288. Trygstad, O., *The lipid-mobilizing effect of some pituitary gland preparations. 3. A purified human pituitary lipid-mobilizing factor (LMF) with hyperglycaemic activity.* Acta Endocrinol (Copenh), 1968. **58**(2): p. 277-94.
289. Trygstad, O. and I. Foss, *Inhibition by human serum of the adipokinetic effect of a human pituitary lipid-mobilizing factor (LMF) in rabbits.* Acta Endocrinol (Copenh), 1967. **56**(4): p. 649-60.
290. Trygstad, O. and I. Foss, *Inhibition by human serum of the adipokinetic effect of a human pituitary lipid-mobilizing factor. II. Characterization of an inhibitory protein.* Acta Endocrinol (Copenh), 1972. **71**(3): p. 443-53.
291. Trygstad, O. and I. Foss, *Inhibition by human serum of the adipokinetic effect of a human pituitary lipid-mobilizing factor. 3. Influence of different serum albumins on in vitro lipolysis.* Acta Endocrinol (Copenh), 1973. **72**(2): p. 226-34.
292. Li, C.H., *Lipotropin, a new active peptide from pituitary glands.* Nature, 1964. **201**(4922): p. 924-924.
293. Seidah, N.G., C. Gianoulakis, P. Crine, M. Lis, S. Benjannet, R. Routhier, and M. Chretien, *In vitro biosynthesis and chemical characterization of beta-lipotropin, gamma-lipotropin, and beta-endorphin in rat pars intermedia.* Proc Natl Acad Sci U S A, 1978. **75**(7): p. 3153-7.
294. Spiess, J., C.D. Mount, W.E. Nicholson, and D.N. Orth, *NH2-terminal amino acid sequence and peptide mapping of purified human beta-lipotropin: comparison with previously proposed sequences.* Proc Natl Acad Sci U S A, 1982. **79**(16): p. 5071-5.
295. Liotta, A.S., T. Suda, and D.T. Krieger, *β-Lipotropin is the major opioid-like peptide of human pituitary and rat pars distalis: Lack of significant β-endorphin.* Proceedings of the National Academy of Sciences, 1978. **75**(6): p. 2950-2954.
296. Kawauchi, H. and S.A. Sower, *The dawn and evolution of hormones in the adenohypophysis.* General and Comparative Endocrinology, 2006. **148**(1): p. 3-14.
297. Tyrell, J.B., J.W. Findling, and D.C. Aron, *Hypothalamus and pituitary*, in *Basic & clinical endocrinology*, F.S. Greenspan and J.D. Baxter, Editors. 1994, Appleton & Lange: Norwalk, Conn. p. 95-156.

298. Norman, N. and R. Turter, *Radioimmunoassay studies with human growth hormone and a pituitary lipid mobilizing factor.* Acta Endocrinol (Copenh), 1968. **58**(2): p. 318-28.
299. Trygstad, O., *The lipid-mobilizing effect of some pituitary gland perparations. II. Preparation of a human pituitary lipid-mobilizing factor (LMF) with hypocalcaemic and hyperglycaemic effects in rabbits.* Acta Endocrinol (Copenh), 1968. **57**(1): p. 81-108.
300. Hadley, M.E. and C. Haskell-Luevano, *The proopiomelanocortin system.* Annals of the New York Academy of Sciences, 1999. **885**(1): p. 1-21.
301. O'Connell, Y., T.J. McKenna, and S.K. Cunningham, *beta-Lipotropin-stimulated adrenal steroid production.* Steroids, 1996. **61**(5): p. 332-6.
302. O'Connell, Y., T.J. McKenna, and S.K. Cunningham, *Effects of pro-opiomelanocortin-derived peptides on adrenal steroidogenesis in guinea-pig adrenal cells in vitro.* J Steroid Biochem Mol Biol, 1993. **44**(1): p. 77-83.
303. Solomon, S., *POMC-derived peptides and their biological action.* Ann N Y Acad Sci, 1999. **885**: p. 22-40.
304. Valenca, M.M. and A. Negro-Vilar, *Proopiomelanocortin-derived peptides in testicular interstitial fluid: characterization and changes in secretion after human chorionic gonadotropin or luteinizing hormone-releasing hormone analog treatment.* Endocrinology, 1986. **118**(1): p. 32-7.
305. Bardin, C.W., C. Shaha, J. Mather, Y. Salomon, A.N. Margioris, A.S. Liotta, I. Gerendai, C.L. Chen, and D.T. Krieger, *Identification and possible function of pro-opiomelanocortin-derived peptides in the testis.* Ann N Y Acad Sci, 1984. **438**: p. 346-64.
306. Chen, C.L. and M.B. Madigan, *Regulation of testicular proopiomelanocortin gene expression.* Endocrinology, 1987. **121**(2): p. 590-6.
307. Forman, L.J. and S. Estilow, *Administration of gonadal steroids to neonatal rats affects beta-endorphin levels in the adult.* Life Sci, 1987. **40**(7): p. 687-95.
308. Boston, B.A., *The role of melanocortins in adipocyte function.* Annals of the New York Academy of Sciences, 1999. **885**(1): p. 75-84.
309. Burgi, W. and K. Schmid, *Preparation and properties of Zn-alpha2-glycoprotein of normal human plasma.* J. Biol. Chem., 1961. **236**(4): p. 1066-1074.
310. Bao, Y., C. Bing, L. Hunter, J.R. Jenkins, M. Wabitsch, and P. Trayhurn, *Zinc-alpha2-glycoprotein, a lipid mobilizing factor, is expressed and secreted by human (SGBS) adipocytes.* FEBS Lett, 2005. **579**(1): p. 41-7.
311. Bing, C., Y. Bao, J. Jenkins, P. Sanders, M. Manieri, S. Cinti, M.J. Tisdale, and P. Trayhurn, *Zinc-{alpha}2-glycoprotein, a lipid mobilizing factor, is expressed in adipocytes and is up-regulated in mice with cancer cachexia.* Proceedings of the National Academy of Sciences, 2004. **101**(8): p. 2500-2505.
312. Russell, S.T., T.P. Zimmerman, B.A. Domin, and M.J. Tisdale, *Induction of lipolysis in vitro and loss of body fat in vivo by zinc-alpha2-glycoprotein.* Biochim Biophys Acta, 2004. **1636**(1): p. 59-68.
313. Sanders, P.M. and M.J. Tisdale, *Effect of zinc-alpha2-glycoprotein (ZAG) on expression of uncoupling proteins in skeletal muscle and adipose tissue.* Cancer Lett, 2004. **212**(1): p. 71-81.
314. Coimbra, C.C. and R.H. Migliorini, *Evidence for a longitudinal pathway in rat hypothalamus that controls FFA mobilization.* Am J Physiol, 1983. **245**(4): p. E332-7.

315. Hirai, K., H.J. Hussey, M.D. Barber, S.A. Price, and M.J. Tisdale, *Biological evaluation of a lipid-mobilizing factor isolated from the urine of cancer patients.* Cancer Res, 1998. **58**(11): p. 2359-65.
316. Todorov, P.T., T.M. McDevitt, D.J. Meyer, H. Ueyama, I. Ohkubo, and M.J. Tisdale, *Purification and characterization of a tumor lipid-mobilizing factor.* Cancer Res, 1998. **58**(11): p. 2353-8.
317. Tada, T., I. Ohkubo, M. Niwa, M. Sasaki, H. Tateyama, and T. Eimoto, *Immunohistochemical localization of Zn-alpha 2-glycoprotein in normal human tissues.* J Histochem Cytochem, 1991. **39**(9): p. 1221-6.
318. Russell, S.T., K. Hirai, and M.J. Tisdale, *Role of beta3-adrenergic receptors in the action of a tumour lipid mobilizing factor.* Br J Cancer, 2002. **86**(3): p. 424-8.
319. Russell, S.T. and M.J. Tisdale, *Effect of a tumour-derived lipid-mobilising factor on glucose and lipid metabolism in vivo.* Br J Cancer, 2002. **87**(5): p. 580-4.
320. Kitada, S., E.F. Hays, and J.F. Mead, *A lipid mobilizing factor in serum of tumor-bearing mice.* Lipids, 1980. **15**(3): p. 168-74.
321. Kitada, S., E.F. Hays, and J.F. Mead, *Characterization of a lipid mobilizing factor from tumors.* Prog Lipid Res, 1981. **20**: p. 823-6.
322. Kasinathan, S., A.G. Singh, and S.L. Basu, *Fat bodies and spermatogenesis in South Indian green frog Rana hexadactyla Lesson.* Bull. Zool., 1978. **45**: p. 15-22.
323. Kasinathan, S., A.G. Singh, and S.L. Basu, *Adenohypophysial histophysiology of gonadectomized, fatectomized and steroid treated male frogs, Rana hexadactyla (Lesson).* Acta Morphol Acad Sci Hung, 1978. **26**(3-4): p. 187-96.
324. Biswas, N.M., S. Sanyal, and P.B. Patra, *Antispermatogenic effect of aspirin and its prevention by prostaglandin E2.* Andrologia, 1978. **10**(2): p. 137-41.
325. Charbonnel, B., *[Prostaglandins and reproduction].* Sem Hop, 1975. **-51**(46): p. 2793-2804.
326. Abbatiello, E.R., M. Kaminsky, and S. Weisbroth, *The effect of prostaglandins F1 alpha and F2 alpha on spermatogenesis.* Int J Fertil, 1976(2): p. 82-8.
327. Didolkar, A.K. and D. Roychowdhury, *Effect of prostaglandins A-1, E-2 and F-2 alpha on spermatogenesis in rats.* J Reprod Fertil, 1980. **58**(1): p. 275-8.
328. Conte, D., M. Nordio, F. Romanelli, F. Manganelli, P. Giovenco, F. Dondero, and A. Isidori, *Role of seminal prostaglandins in male fertility. II. Effects of prostaglandin synthesis inhibition on spermatogenesis in man.* J Endocrinol Invest, 1985. **8**(4): p. 289-91.
329. Frungieri, M.B., S.I. Gonzalez-Calvar, M.E. Matzkin, A. Mayerhofer, and R.S. Calandra, *Sources and functions of prostaglandins in the testis: evidence for their relevance in male (in)fertility.* Anim. Reprod., 2007. **4**(3/4): p. 63-69.
330. Marsh, J.M., *The role of cyclic AMP in gonadal function.* Adv Cyclic Nucleotide Res, 1975. **6**: p. 137-99.
331. Marsh, J.M., *The role of cyclic AMP in gonadal steroidogenesis.* Biol Reprod, 1976. **14**(1): p. 30-53.
332. Didolkar, A.K., A. Gurjar, U.M. Joshi, A.R. Sheth, and D. Roychowdhury, *Effect of prostaglandins A_1, E_2 and F_2-alpha on blood plasma levels of testosterone, LH and FSH in male rats.* Andrologia, 1981. **13**(1): p. 50-5.
333. Singh, S.K. and C.J. Dominic, *Prostaglandin F2 alpha-induced changes in the sex organs of the male laboratory mouse.* Exp Clin Endocrinol, 1986. **88**(3): p. 309-15.

334. Veeraragavan, K., S. Kasinathan, and S. Ramakrishnan, *Biosynthesis of cholesterol in fat body of the male frog Rana hexadactyla.* Indian Journal of Experimental Biology, 1985. **23**(7): p. 410-411.
335. Kadas, L. and D. Nagy, *[Investigations on direct acting lipid mobilizers of the organism. (I). Investigations on the lipid mobilizing factor present in the human posterior pituitary lobe and in the hypothalamus].* Endokrinologie, 1965. **48**(1): p. 1-8.
336. Anselmino, K.J. and F. Hoffmann, *[On the lipotropic hormone of the anterior pituitary gland (lipotropin) and its significance in fat metabolism under physiologic and pathologic conditions].* Dtsch Med Wochenschr, 1965. **90**(39): p. 1697-701.
337. Kasinathan, S. and S.L. Basu, *Effect of pituitary extracts on the spermatogenesis of the green frog Rana hexadactyla Lesson.* Endocrinol Jpn, 1972. **19**(1): p. 59-67.
338. Kasinathan, S., A. Balasubramanian, S. Ramakrishnan, and S.L. Basu, *3'5' cyclic AMP phosphodiesterase activity in the testes & fat body & the effect of prostaglandins & cAMP in the green frog Rana hexadactyla Lesson.* Indian J Exp Biol, 1980. **18**(1): p. 62-4.
339. Huleihel, M., E. Lunenfeld, A. Blindman, I. Prinsloo, and G. Potashnik, *Over expression of interleukin-1alpha, interleukin-1beta and interleukin-1 receptor antagonist in testicular tissues from sexually immature mice as compared to adult mice.* Eur Cytokine Netw, 2003. **14**(1): p. 27-33.
340. Gustafsson, K., T. Sultana, C.K. Zetterstrom, B.P. Setchell, A. Siddiqui, G. Weber, and O. Soder, *Production and secretion of interleukin-1alpha proteins by rat testis.* Biochem Biophys Res Commun, 2002. **297**(3): p. 492-7.
341. Gerendai, I., P. Banczerowski, V. Csernus, and B. Halasz, *Innervation and serotoninergic receptors of the testis interact with local action of interleukin-1beta on steroidogenesis.* Auton Neurosci, 2007. **131**(1-2): p. 21-7.
342. Potashnik, H., M.A. Elhija, E. Lunenfeld, G. Potashnik, S. Schlatt, E. Nieschlag, and M. Huleihel, *Interleukin-6 expression during normal maturation of the mouse testis.* Eur Cytokine Netw, 2005. **16**(2): p. 161-5.
343. Brech, W.J. and E.S. Gordon, *[The physiological role of adipose tissue].* Klin Wochenschr, 1967. **45**(18): p. 905-17.
344. MacDougald, O.A. and C.F. Burant, *The rapidly expanding family of adipokines.* Cell Metab, 2007. **6**(3): p. 159-61.
345. Guerre-Millo, M., *Adipose tissue and adipokines: for better or worse.* Diabetes Metab, 2004. **30**(1): p. 13-9.
346. Flickinger, C.J., M.L. Baran, S.S. Howards, and J.C. Herr, *Epididymal obstruction during development results in antisperm autoantibodies at puberty in rats.* J Androl, 1998. **19**(2): p. 136-44.
347. Witkin, S.S., G. Zelikovsky, A.M. Bongiovanni, N. Geller, R.A. Good, and N.K. Day, *Sperm-related antigens, antibodies, and circulating immune complexes in sera of recently vasectomized men.* J Clin Invest, 1982. **70**(1): p. 33-40.
348. Shahani, S., *The effects of vasectomy on health.* Journal of Biosciences, 1985. **7**(2): p. 197-201.
349. Shahani, S.K. and N.S. Hattikudur, *Immunological consequences of vasectomy.* Arch Androl, 1981. **7**(2): p. 193-9.
350. Azim, A.A., S. Fayad, A.A. Fattah, and M. Habeib, *Immunologic studies of male infertility.* Fertil Steril, 1978. **30**(4): p. 426-9.

351. Ford, W.C., K.M. Williams, E.A. McLaughlin, S. Harrison, B. Ray, and M.G. Hull, *The indirect immunobead test for seminal antisperm antibodies and fertilization rates at in-vitro fertilization.* Hum Reprod, 1996. **11**(7): p. 1418-22.
352. Haas, G.G., Jr., R. Weiss-Wik, and D.P. Wolf, *Identification of antisperm antibodies on sperm of infertile men.* Fertil Steril, 1982. **38**(1): p. 54-61.
353. Flickinger, C.J., J.C. Herr, S.S. Howards, J.R. Sisak, J.M. Gleavy, T.J. Fusia, L.D. Vailes, and H.H. Handley, *Early testicular changes after vasectomy and vasovasostomy in Lewis rats.* Anat Rec, 1990. **227**(1): p. 37-46.
354. Herr, J.C., C.J. Flickinger, S.S. Howards, S. Yarbro, D.R. Spell, D. Caloras, and T.N. Gallien, *The relation between antisperm antibodies and testicular alterations after vasectomy and vasovasostomy in Lewis rats.* Biol Reprod, 1987. **37**(5): p. 1297-305.
355. Alexander, N.J. and D.J. Anderson, *Vasectomy: consequences of autoimmunity to sperm antigens.* Fertil Steril, 1979. **32**(3): p. 253-60.
356. Herr, J.C., C.J. Flickinger, S.S. Howards, E.S. Yarbro, D.R. Spell, D. Caloras, and T.N. Gallien, *Temporal appearance of antisperm autoantibodies in Lewis rats following vasectomy.* J Androl, 1987. **8**(4): p. 253-8.
357. Flickinger, C.J., S.S. Howards, P.O. Carey, D.R. Spell, S.J. Kendrick, D. Caloras, T.N. Gallien, and J.C. Herr, *Testicular alterations are linked to the presence of elevated antisperm antibodies in Sprague-Dawley rats after vasectomy and vasovasostomy.* J Urol, 1988. **140**(3): p. 627-31.
358. Flickinger, C.J., S.S. Howards, and J.C. Herr, *Effects of vasectomy on the epididymis.* Microsc Res Tech, 1995. **30**(1): p. 82-100.
359. Neaves, W.B., *The effect of vasectomy on the testes of inbred Lewis rats.* J Reprod Fertil, 1978. **54**(2): p. 405-11.
360. Flickinger, C.J., E.S. Yarbro, S.S. Howards, J.C. Herr, D. Caloras, T.N. Gallien, and D.R. Spell, *The incidence of spermatic granulomas and their relation to testis weight after vasectomy and vasovasostomy in Lewis rats.* J Androl, 1986. **7**(5): p. 285-291.
361. Flickinger, C.J., J.C. Herr, S.S. Howards, D. Caloras, E.S. Yarbro, D.R. Spell, and T.N. Gallien, *The influence of vasovasostomy on testicular alterations after vasectomy in Lewis rats.* Anat Rec, 1987. **217**(2): p. 137-45.
362. Flickinger, C.J., J.C. Herr, D. Caloras, J.R. Sisak, and S.S. Howards, *Inflammatory changes in the epididymis after vasectomy in the Lewis rat.* Biol Reprod, 1990. **43**(1): p. 34-45.
363. Sarrat R, W.J., Torres A, LostalÃ© F, DÃ-az MP., *Experimental vasectomy and testicular structure. [Histol Histopathol. 1996] - PubMed Result.* Histol Histopathol, 1996. **11**(1): p. 1-6.
364. Kong, L.S., A.P. Huang, X.Z. Deng, and Z.W. Yang, *Quantitative (stereological) study of the effects of vasectomy on spermatogenesis in rabbits.* J Anat, 2004. **205**(2): p. 147-56.
365. Hattikudur, N.S., S.R. Shanta, S.K. Shahani, P.R. Shastri, P.V. Thakker, and A.D. Bordekar, *Immunological and clinical consequences of vasectomy.* Andrologia, 1982. **14**(1): p. 15-22.
366. Hellema, H.W., T. Samuel, and P. Rumke, *Sperm autoantibodies as a consequence of vasectomy. II. Long-term follow-up studies.* Clin Exp Immunol, 1979. **38**(1): p. 31-36.

367. Fisch, H., E. Laor, N. BarChama, S.S. Witkin, B.M. Tolia, and R.E. Reid, *Detection of testicular endocrine abnormalities and their correlation with serum antisperm antibodies in men following vasectomy.* J Urol, 1989. **141**(5): p. 1129-32.
368. Sotolongo, J.R., Jr., *Immunological effects of vasectomy.* J Urol, 1982. **127**(6): p. 1063-6.
369. McVicar, C.M., D.A. O'Neill, N. McClure, B. Clements, S. McCullough, and S.E. Lewis, *Effects of vasectomy on spermatogenesis and fertility outcome after testicular sperm extraction combined with ICSI.* Hum Reprod, 2005. **20**(10): p. 2795-800.
370. McDonald, S.W., *Cellular responses to vasectomy.* Int Rev Cytol, 2000. **199**: p. 295-339.
371. O'Neill, D.A., C.M. McVicar, N. McClure, P. Maxwell, I. Cooke, K.M. Pogue, and S.E.M. Lewis, *Reduced sperm yield from testicular biopsies of vasectomized men is due to increased apoptosis.* Fertility and Sterility, 2007. **87**(4): p. 834-841.
372. Raleigh, D., L. O'Donnell, G.J. Southwick, D.M. de Kretser, and R.I. McLachlan, *Stereological analysis of the human testis after vasectomy indicates impairment of spermatogenic efficiency with increasing obstructive interval.* Fertility and Sterility, 2004. **81**(6): p. 1595-1603.
373. Peng, B., Y.P. Wang, Y. Shang, Y. Guo, and Z.W. Yang, *Effect of vasectomy via inguinal canal on spermatogenesis in rabbits.* Asian J Androl, 2008. **10**(3): p. 486-93.
374. Whyte, J., R. Sarrat, A.I. Cisneros, A. Whyte, R. Mazo, A. Torres, and J. Lazaro, *The vasectomized testis.* Int Surg, 2000. **85**(2): p. 167-74.
375. Larsen, A., O. Stamnes, and M. Majak, *[Testicular trauma].* Tidsskr Nor Laegeforen, 1993. **113**(15): p. 1853-5.
376. Champy, C., J. Chevreau, and R. Coujard, *[Mechanism of testicular atrophy in fistulas and transections of the vas deferens.].* C R Seances Soc Biol Fil, 1952. **146**(23-24): p. 1886-90.
377. Silber, S.J., *Microsurgical TESE and the distribution of spermatogenesis in non-obstructive azoospermia.* Hum Reprod, 2000. **15**(11): p. 2278-84.
378. Itoh, N., K. Akagashi, Y. Kumamoto, T. Suzuki, and Y. Ohta, *Influence of hypertensive vascular changes in intratesticular arteries on spermatogenesis in SHRSP* Clinical and Experimental Pharmacology and Physiology, 1995. **22**(s1): p. S134-S135.
379. Kotani, M., K. Seiki, and M. Hattori, *Retardation of spermatogenesis and testosterone secretion after ligature of lymphatics draining the testes of rabbits.* Endocrinol Jpn, 1974. **21**(1): p. 1-8.
380. Mansfeld, G. and F. Muller, *[The influence of the nervous system in the mobilization of fat].* Arch. Physiol., 1913. **152**: p. 61-27.
381. Bartness, T.J. and M. Bamshad, *Innervation of mammalian white adipose tissue: implications for the regulation of total body fat.* Am J Physiol Regul Integr Comp Physiol, 1998. **275**(5): p. R1399-1411.
382. Bartness, T.J., C. Kay Song, H. Shi, R.R. Bowers, and M.T. Foster, *Brain-adipose tissue cross talk.* Proc Nutr Soc, 2005. **64**(1): p. 53-64.
383. Bartness, T.J. and C.K. Song, *Brain-adipose tissue neural crosstalk.* Physiol Behav, 2007. **91**(4): p. 343-51.
384. Bartness, T.J. and C.K. Song, *Thematic review series: adipocyte biology. Sympathetic and sensory innervation of white adipose tissue.* J Lipid Res, 2007. **48**(8): p. 1655-72.
385. Weiss, B. and R.P. Maickel, *Sympathetic nervous control of adipose tissue lipolysis.* International Journal of Neuropharmacology, 1968. **7**(4): p. 395-403.

386. Bamshad, M., V.T. Aoki, M.G. Adkison, W.S. Warren, and T.J. Bartness, *Central nervous system origins of the sympathetic nervous system outflow to white adipose tissue.* Am J Physiol, 1998. **275**(1 Pt 2): p. R291-9.
387. Bowers, R.R., W.T. Festuccia, C.K. Song, H. Shi, R.H. Migliorini, and T.J. Bartness, *Sympathetic innervation of white adipose tissue and its regulation of fat cell number.* Am J Physiol Regul Integr Comp Physiol, 2004. **286**(6): p. R1167-75.
388. Shi, H. and T.J. Bartness, *Neurochemical phenotype of sympathetic nervous system outflow from brain to white fat.* Brain Res Bull, 2001. **54**(4): p. 375-85.
389. Youngstrom, T.G. and T.J. Bartness, *Catecholaminergic innervation of white adipose tissue in Siberian hamsters.* Am J Physiol, 1995. **268**(3 Pt 2): p. R744-51.
390. Slavin, B.G. and K.W. Ballard, *Morphological studies on the adrenergic innervation of white adipose tissue.* Anat Rec, 1978. **191**(3): p. 377-89.
391. Fredholm, B.B., *Nervous control of circulation and metabolism in white adipose tissue*, in *New perspectives in adipose tissue : structure, function, and development*, A. Cryer and R.L.R. Van, Editors. 1985, Butterworths: London; Boston. p. 45-64.
392. Laszlo, J., W.R. Harlan, R.F. Klein, N. Kirshner, E.H. Estes, Jr., and M.D. Bogdonoff, *The effect of 2-deoxy-D-glucose infusions on lipid and carbohydrate metabolism in man.* J Clin Invest, 1961. **40**: p. 171-6.
393. Hokfelt, B. and S. Bydgeman, *Increased adrenaline production following administration of 2-deoxy-D-glucose in the rat.* Proc Soc Exp Biol Med, 1961. **106**: p. 537-9.
394. Brown, J., *Effects of 2-deoxyglucose on carbohydrate metablism: review of the literature and studies in the rat.* Metabolism, 1962. **11**: p. 1098-112.
395. Teixeira, V.L., J. Antunes-Rodrigues, and R.H. Migliorini, *Evidence for centers in the central nervous system that selectively regulate fat mobilization in the rat.* J Lipid Res, 1973. **14**(6): p. 672-7.
396. Giordano, A., Song, C. Kay, Bowers, Robert R., Ehlen, Christopher J., Frontini, Andrea, Cinti, Saverio, and Bartness, Timothy J., *Letter to the editor: Reply to Kreier and Buijs: no sympathy for the claim of parasympathetic innervation of white adipose tissue* Am J Physiol Regulatory Integrative Comp Physiol, 2007. **293**: p. R550–R552.
397. Kreier, F. and R.M. Buijs, *Evidence for parasympathetic innervation of white adipose tissue, clearing up some vagaries.* Am J Physiol Regul Integr Comp Physiol, 2007. **293**(1): p. R548-9; author reply R550-2, discussion R553-4.
398. Kreier, F., E. Fliers, P.J. Voshol, C.G. Van Eden, L.M. Havekes, A. Kalsbeek, C.L. Van Heijningen, A.A. Sluiter, T.C. Mettenleiter, J.A. Romijn, H.P. Sauerwein, and R.M. Buijs, *Selective parasympathetic innervation of subcutaneous and intra-abdominal fat--functional implications.* J Clin Invest, 2002. **110**(9): p. 1243-50.
399. Giordano, A., C.K. Song, R.R. Bowers, J.C. Ehlen, A. Frontini, S. Cinti, and T.J. Bartness, *White adipose tissue lacks significant vagal innervation and immunohistochemical evidence of parasympathetic innervation.* Am J Physiol Regul Integr Comp Physiol, 2006. **291**(5): p. R1243-55.
400. Ballantyne, B., *Histochemical and biochemical aspects of cholinesterase activity of adipose tissue.* Arch Int Pharmacodyn Ther, 1968. **173**(2): p. 343-50.
401. Rosell, S. and E. Belfrage, *Blood circulation in adipose tissue.* Physiol Rev, 1979. **59**(4): p. 1078-1104.

402. Comu, S., W. Weng, S. Olinsky, P. Ishwad, Z. Mi, J. Hempel, S. Watkins, C.F. Lagenaur, and V. Narayanan, *The murine P84 neural adhesion molecule is SHPS-1, a member of the phosphatase-binding protein family*. J Neurosci, 1997. **17**(22): p. 8702-10.
403. Jiang, P., C.F. Lagenaur, and V. Narayanan, *Integrin-associated protein is a ligand for the P84 neural adhesion molecule.* J Biol Chem, 1999. **274**(2): p. 559-62.
404. Schuppe, H.C. and A. Meinhardt, *Immune privilege and inflammation of the testis.* Chem Immunol Allergy, 2005. **88**: p. 1-14.
405. Shi, H. and T.J. Bartness, *White adipose tissue sensory nerve denervation mimics lipectomy-induced compensatory increases in adiposity.* Am J Physiol Regul Integr Comp Physiol, 2005. **289**(2): p. R514-R520.
406. Kullendorff, C.M., H. Ivarsson, M. Ekelund, and T. Holmin, *A method for testicular denervation in rats.* J Microsurg, 1982. **3**(4): p. 228-30.
407. Dailey, M.E. and T.J. Bartness, *Fat pad-specific effects of lipectomy on foraging, food hoarding, and food intake.* Am J Physiol Regul Integr Comp Physiol, 2008. **294**(2): p. R321-8.
408. Hausman, D.B., J. Lu, D.H. Ryan, W.P. Flatt, and R.B. Harris, *Compensatory growth of adipose tissue after partial lipectomy: involvement of serum factors.* Exp Biol Med (Maywood), 2004. **229**(6): p. 512-20.
409. Mefford, I.N., M.M. Ward, L. Miles, B. Taylor, M.A. Chesney, D.L. Keegan, and J.D. Barchas, *Determination of plasma catecholamines and free 3,4-dihydroxyphenylacetic acid in continuously collected human plasma by high performance liquid chromatography with electrochemical detection.* Life Sci, 1981. **28**(5): p. 477-83.
410. Brito, M.N., N.A. Brito, D.J. Baro, C.K. Song, and T.J. Bartness, *Differential activation of the sympathetic innervation of adipose tissues by melanocortin receptor stimulation.* Endocrinology, 2007. **148**(11): p. 5339-47.
411. Siegel, S. and N.J. Castellan, *Nonparametric statistics for the behavioral sciences*. 2nd ed. 1988, New York: McGraw-Hill. xxiii, 399 p.
412. Kirk, R.E., *Experimental design : Procedures for the behavioral sciences*. 3rd ed. ed. 1995, Pacific Grove, CA: Brooks/Cole.
413. Flickinger, C.J., M.L. Baran, S.S. Howards, and J.C. Herr, *Degeneration of the seminiferous epithelium following epididymal obstruction in prepubertal rats.* Anat Rec, 1999. **254**(1): p. 76-86.