vasculature and lymphatic network ensures substrate supply and heat exchange, while cytokines and growth factors modulate Sertoli–germ-cell crosstalk. Peritubular myoid cells contribute contractile signaling and immune mediators, so interstitial remodeling can impair spermatogenesis even when tubules appear morphologically intact (Mayerhofer, 2013; Walenta et al., 2018).

1.1.3 Species comparisons

Comparative morphometry shows that suids generally have a higher proportion of seminiferous tubules and less interstitium than equids, reflecting higher DSP per gram in boars compared with stallions. Bulls have relatively long spermatogenic duration and are staged using 8- or 12-phase schemes, which guide biopsy interpretation and recovery expectations after heat or toxicant exposure. Carnivores such as dogs possess smaller tubule diameters and lower Vv, resulting in shorter STL and lower sperm output than large ungulates, emphasizing the need for species-specific andrology thresholds (Hafez & Hafez, 2013; Staub, 2018).

1.1.4 Developmental and seasonal changes

During puberty, testicular growth is driven by Sertoli-cell proliferation and tubular expansion; scrotal circumference in bulls correlates with semen volume and sperm number and is a practical proxy for testicular development. In seasonal breeders (small ruminants, stallions), photoperiod modulates testicular volume, tubular diameter, germ-cell layers, and circulating testosterone, leading to cyclical changes in DSP that must be considered when planning semen collection and fertility investigations. Heat stress and thermal dysregulation damage the BTB and accelerate germ-cell loss, with

effects on semen quality appearing only after the species-specific spermatogenic and epididymal transit interval (Hafez & Hafez, 2013; Aïssani et al., 2022).

1.1.5 Classical morphometric methods and DSP

Histology with Bouin's or buffered formalin fixation and PAS–hematoxylin staining enables stage identification across species. Stereology (point counting, calibrated diameter measurements) estimates Vv and STL, provided tissue shrinkage and sampling bias are controlled (Abarikwu, 2018). DSP is commonly derived from homogenization-resistant spermatid head counts (HRSH): DSP/g = HRSH/D, where D is the species-specific duration of the late spermatid stage, then scaled by parenchymal mass (Amann & Almquist, 1962; Johnson et al., 1980). Proper control of fixation, storage and counting variability is essential for reliable quantitative interpretation.

1.2 Neuroendocrine Regulation of Male Reproduction

The hypothalamic–pituitary–gonadal (HPG) axis comprises pulsatile GnRH secretion, episodic LH and FSH release, and Leydig/Sertoli-cell responses generating testosterone, estradiol and inhibin. In ruminants and other mammals, KNDy neurons in the arcuate nucleus are central to GnRH pulse generation, integrating photoperiodic signals via the suprachiasmatic nucleus and pineal melatonin, thereby linking neural timing to testicular function (Goodman et al., 2013; Moore, 2018).

1.2.1 GnRH–LH/FSH pulsatility

GnRH and LH are secreted in discrete pulses whose frequency and amplitude vary with species, age, season, and steroid feedback. In

rams, LH pulses typically occur around 0.5–1.5 pulses·h^{-1}, increasing in the breeding season; bulls show lower frequencies that increase near puberty. Stallions exhibit pulsatile testosterone dynamics entrained to LH, with higher mean levels in spring–summer. Accurate pulse analysis requires frequent sampling, validated immunoassays and strict pre-analytical control (Bielohuby et al., 2018; Nestor et al., 2023).

1.2.2 Steroid and inhibin feedback

Testosterone and estradiol reduce GnRH/LH pulse frequency and pituitary responsiveness, while inhibin B selectively suppresses FSH by antagonizing activin signaling. Experimental immunoneutralization of inhibin in bulls elevates FSH and can increase DSP, highlighting the key role of the Sertoli–inhibin axis. In stallions, seasonal changes in inhibin mirror FSH and testicular activity and decline after pharmacological HPG down-regulation (Kaneko et al., 1993; Ball et al., 2019).

1.2.3 Seasonal breeders and melatonin

Photoperiod is encoded as melatonin duration and transmitted to KNDy–GnRH networks. Short-day breeders (rams, bucks) increase GnRH/LH pulsatility when nights lengthen, whereas long-day breeders (stallions) peak in spring–summer. Exogenous melatonin or controlled photoperiod can advance reproductive activity and improve semen parameters in rams, providing management tools for out-of-season breeding and emphasizing the need to interpret endocrine tests in a seasonal context (Malpaux et al., 2001; Abecia et al., 2018; Pool et al., 2020).

1.2.4 Pharmacologic modulation and diagnostic tests

GnRH and hCG stimulation tests probe pituitary and Leydig function respectively and are widely used in clinical andrology, provided pre-analytical conditions and species-specific protocols are respected (Bielohuby et al., 2018). GnRH tests assess LH/FSH reserve and downstream testosterone responses; hCG acts directly on LH receptors to evaluate Leydig capacity, especially in stallions and valuable studs (Bollwein et al., 2008; Esteller-Vico et al., 2021). Melatonin implants or photoperiodic manipulation in rams require sufficient time to reach new steady states before endocrine sampling.

1.3 Spermatogenesis and Spermiogenesis

Spermatogenesis is the ordered production of haploid gametes from spermatogonia through meiosis and spermiogenesis within the seminiferous epithelium. It includes a mitotic phase (spermatogonial proliferation), a meiotic phase (chromosome synapsis, recombination and reductional divisions) and a post-meiotic remodeling phase (spermiogenesis). Total duration is ≈40 days in boars, ≈57–61 days in bulls and stallions, and shorter in rodents, determining the delay between testicular insults and changes in semen phenotype (França & Russell, 1998; Swierstra, 1974; Staub, 2018).

1.3.1 Spermatogonial stem-cell dynamics

Spermatogonial stem cells (SSCs) maintain spermatogenesis by balancing self-renewal with differentiation. Their fate is governed by Sertoli-cell paracrine cues (e.g. GDNF, FGF2) and basal-lamina niche geometry (de Rooij, 2001; Oatley & Brinster, 2008). Rodents use the As–Apr–Aal classification, whereas domestic species are described as A1–A4 → Intermediate → B spermatogonia, reflecting species

differences in pre-meiotic amplification. Sertoli-cell efficiency—round spermatids per Sertoli cell—varies among species and provides a quantitative indicator of Sertoli function and maximal DSP (França & Russell, 1998; Griswold, 2018). Classical stereology on PAS-hematoxylin sections is used to estimate Sertoli-cell number and efficiency in teaching and diagnostic laboratories.

1.3.2 Meiotic checkpoints

Meiosis is monitored by checkpoints that survey homologous synapsis and DNA double-strand break repair. Defects in synaptonemal complex formation or recombination activate apoptosis, reducing downstream spermatids and DSP (Handel & Schimenti, 2010; Baudat et al., 2013). Heat and toxicants frequently induce pachytene arrest and subsequent loss of germ-cell cohorts. Histologically, meiotic arrest appears as an accumulation of primary spermatocytes with loss of later stages; interpretation must consider species-specific stage frequencies and cycle lengths (França & Russell, 1998; Staub, 2018).

1.3.3 Spermiogenesis

Spermiogenesis transforms round spermatids into polarized spermatozoa through Golgi, cap, acrosome and maturation phases, coordinated by the acrosome–acroplaxome–manchette complexes (Kierszenbaum & Tres, 2004). The manchette orchestrates head shaping and tail implantation; its disruption leads to severe teratozoospermia. Nuclear condensation involves sequential replacement of histones by transition proteins and protamines; incomplete protamination compromises chromatin integrity and correlates with reduced motility and fertilizing ability (Lehti & Sironen, 2016; O'Donnell & O'Bryan, 2014). Acrosome development

and residual cytoplasm removal can be followed using PAS–hematoxylin and routine semen smears, linking testicular pathology to ejaculate morphology (Hafez & Hafez, 2013; Griswold, 2018).

1.3.4 Cycle time, DSP, and Sertoli ratios

The cycle of the seminiferous epithelium multiplied by the number of cycles required to reach spermiation (≈4–4.5) gives the total duration of spermatogenesis. Representative values are ≈13.5 days per cycle and ≈61 days total in bulls, ≈9 days and 40 days in boars, ≈12 days and 57–60 days in stallions, with shorter cycles in rodents (Oakberg, 1956; Zeng et al., 2006; Staub, 2018). Classical HRSH-based methods and histological staging provide complementary estimates of DSP and help distinguish Sertoli-cell dysfunction (low efficiency) from post-testicular losses when DSP is reduced despite normal endocrine profiles (França & Russell, 1998; Johnson et al., 1980).

1.4 Epididymal Maturation and Sperm Reservoirs

Epididymal maturation comprises post-testicular modifications that confer progressive motility and fertilizing competence as sperm transit the caput–corpus–cauda segments. This process depends on a precisely regulated luminal milieu and epithelial secretions that remodel sperm membranes and signaling pathways in the absence of new transcription (Dacheux & Dacheux, 2014; Hinton & Robaire, 2015). Proton secretion and bicarbonate handling maintain an acidic, low-bicarbonate environment that preserves quiescence in the cauda reservoir; activation occurs after ejaculation when alkaline, bicarbonate-rich accessory-gland fluids trigger cAMP signaling and motility.

1.4.1 Regional specialization

The caput initiates surface remodeling and volume regulation, the corpus consolidates motility potential, and the cauda provides long-term storage under conditions preventing premature activation (Hinton & Robaire, 2015). Luminal pH is mildly acidic and osmolality exceeds plasma, reflecting specialized solute transport. Species-specific epididymal transit times (on the order of 1–2 weeks in large domestic mammals) determine when testicular insults are first expressed in ejaculates and guide re-examination schedules (Zeng et al., 2006; Hafez & Hafez, 2013). The cauda reservoir buffers short-term fluctuations in testicular output and explains why semen quality may remain normal for several days after transient testicular injury (Cooper, 2011).

1.4.2 Luminal milieu and extracellular vesicles

Epididymal epithelial cells release epididymosomes—small extracellular vesicles carrying proteins and microRNAs—that fuse with sperm and drive acquisition of motility, zona-binding capacity and membrane stability. Their cargo includes CRISP proteins, tetraspanins, lipid-handling proteins, ion transporters and regulatory RNAs, with region-specific patterns along the duct (Gatti et al., 2004; Sullivan, 2016). Proper handling of epididymal sperm for diagnostic purposes (warm, isotonic conditions, minimal cold shock) is essential to preserve these modifications and avoid artifactual loss of loosely bound proteins (Dacheux & Dacheux, 2014).

1.4.3 Decapacitation factors

Decapacitation factors—mainly glycoconjugates and sialylated glycans—stabilize sperm membranes and mask receptors, preventing premature capacitation. They accumulate during epididymal transit and, in some species, are reinforced by accessory-gland proteins at ejaculation (Cohen et al., 2001; Manjunath & Thérien, 2002). In bovines, binder-of-sperm proteins coat ejaculated sperm and both stabilize membranes and facilitate cholesterol efflux during capacitation. Glycan remodeling is reversed in the female tract, where enzymatic activity and albumin-mediated lipid extraction unmask fertilization ligands. Over-vigorous semen handling can strip these factors and overestimate “capacitated-like” sperm (Hafez & Hafez, 2013; Cooper, 2011).

1.4.4 Storage and release

The cauda epididymidis stores mature quiescent sperm at high concentration and low metabolism. During ejaculation, adrenergic stimulation of the cauda and ductus deferens mobilizes this reserve, while alkaline seminal plasma raises intracellular pH and activates soluble adenylyl cyclase–PKA signaling to initiate progressive motility (Acott & Carr, 1984; Hinton & Robaire, 2015). Retrieval protocols for epididymal sperm (e.g. in obstructive azoospermia or post-mortem) must respect temperature, osmolality and time limits to avoid iatrogenic damage (Hafez & Hafez, 2013).

1.5 Accessory Sex Glands and Seminal Plasma

Accessory sex glands (vesicular glands, prostate, bulbourethral glands, ampullae) provide fluids that dilute epididymal sperm and supply buffers, electrolytes, energy substrates and regulatory proteins