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Role of Sertoli cell number and function on regulation of spermatogenesis☆

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Abstract

Testicular function is under the control of expression and repression of several genes and gene products, and many of these works through Sertoli cells. The capability of Sertoli cells to regulate spermatogenesis is dependent on Sertoli cell functions and Sertoli cell number. Sertoli cell number has long been thought to be stable in adults with no proliferation of Sertoli cells once adult numbers have been reached. However, adult horses do not have stable Sertoli cell numbers, and new studies indicate that adult Sertoli cells can be made to re-enter mitotic phase under certain experimental conditions. This review discusses roles of Sertoli cells in regulation of spermatogenesis and methods for estimating the number of Sertoli cells, in a testis, that overcome the problems (assumptions) associated with the indented, pear-shaped of Sertoli cell nuclei which make it difficult to estimate the volume of individual nuclei. Using several approaches to overcome the problems associated with any one method, the horse is identified as a species in which Sertoli cell number is not fixed, but it fluctuates with season. In addition to Sertoli cell numbers, the functions of Sertoli cells that are very important in signaling and controlling spermatogenesis are discussed. Recent studies have shown that "post-mitotic terminally differentiated Sertoli cells" from adult animals could, under certain conditions, re-enter the cell division cycle. Can seasonal influences be a natural set of conditions to induce the Sertoli cells of the horse testis to seasonally re-enter the cell division cycle and explain the seasonal differences in Sertoli cell number as summarized in this review? Alternatively, can seasonal differences in Sertoli cell number reflect, in the horse to a greater extent, but in adults of most species, the presence of some mitotic-capable Sertoli cells in adults? In any case, both Sertoli cell number and function are important in regulation of spermatogenesis.

Keywords: Spermatogenesis; Sertoli cell; Proliferation; Morphology; Stereology

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1. Introduction to Sertoli cell function and morphology

The Sertoli cells play a major role in regulation of spermatogenesis and altering rates of spermatozoa produced. Sertoli cell (Fig. 1) functions include providing structural support and nutrition to developing germ cells, phagocytosis of degenerating germ cells and residual bodies, release of spermatids at spermiation and production of a host of proteins that regulate and/or respond to pituitary hormone release and that influence mitotic activity of spermatogonia (Amann, 1970; Dym and Raj, 1977; Feig et al., 1980; Jutte et al., 1982, 1983; Tres et al., 1986; Buch et al., 1988; Bellve and Zheng, 1989; Johnson, 1991b; Russell and Griswold, 1993).

Testicular function in general is under the control of a host of genes and gene products which undergo both repression and expression (Maclean and Wilkinson, 2005; Tanaka and Baba, 2005; Rossi et al., 2004; Elliott, 2003; Ronfani and Bianchi, 2004; Grimes, 2004; van der Weyden et al., 2006). Many of these controlling factors exert their effects through the Sertoli cells (Varner and Johnson, 2007). Single-nucleotide polymorphisms (SNPs) have been identified in the human FSH receptor gene, which results in mutations of the FSH receptor thereby influencing Sertoli cell activity (Gromoll and Simoni, 2005). In addition, transgenic mice deficient in the estrogen receptor genes demonstrate the importance of estrogen in testicular function (Akingbemi, 2005). Furthermore, mouse studies in which the androgen receptor has been knocked-out, demonstrate that the androgen receptors of Sertoli cells are essential for normal spermatogenesis (De Gendt et al., 2004; Verhoeven, 2005). Therefore, spermatogenesis is controlled by input from a host of endocrine, paracrine, and autocrine messengers (Huleihel and Lunenfeld, 2004; Holdcraft and Braun, 2004; Abd-Elmaksoud and Sinowatz, 2005; O'Donnell et al., 2006; Perrard et al., 2007), and many of these engage the Sertoli cells.

A key feature of Sertoli cell structural support for developing germ cells is the blood testis barrier that resides in tight junctions located between adjacent Sertoli cells. This barrier segregates the spermatogonia and early preleptotene primary spermatocytes within the basal compartment. It also permits movement of preleptotene primary spermatocytes into the luminal compartment and

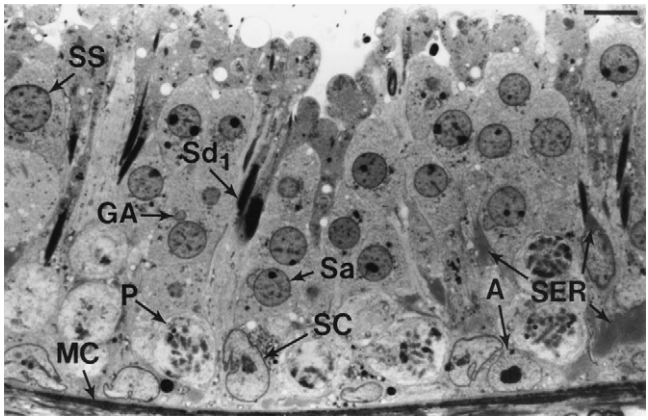


Fig. 1. Seminiferous tubule of the bull as viewed by bright field microscopy in 0.5-μm Epon sections. Myoid cells (MC) mark the outer limits of the tubule and Sertoli cells containing nuclei of varied shape (SC) provide structural support of spermatogenesis. Patches of smooth endoplasmic reticulum (SER) and the Golgi apparatus (GA; that giving rise to the acrosome of spermatids) are observable. Type A spermatogonia (A), primary spermatocyte (P), a remaining secondary spermatocyte (SS), Sa spermatids (Sa) and Sd_1 spermatids (Sd_1) characterize this Stage V seminiferous tubule. Bar length equals 12 μm. Modified from Johnson (2007).

spermatocytes and spermatids in the adluminal compartment (Setchell and Waites, 1975; Fawcett, 1975; Waites, 1977). This structural arrangement creates an immunologic barrier by isolating the more advanced germ cell types (spermatocytes and spermatids) from the immune system so that their antigens do not stimulate autoimmunity. Given that spermatocytes and spermatids first appear at puberty and not prior to the immune system distinguishing self, these cells are not recognized by the immune system as self, but rather as foreign cells. The immune system barrier continues within the epididymal ducts that transport and store spermatozoa. Given that the female has oocytes prior to the immune system recognizing itself, and females do not have cells that are counterparts to spermatids (e.g., oogonia and oocytes exist, but there are not ootids as the oocyte is fertilized to form the zygote), the female should not need nor have a counterpart to the blood–testis barrier in males.

Given that the tight junctions between Sertoli cells create the blood–testis barrier, which is the barrier between germ cells situated within the basal and adluminal compartments, molecular mechanisms must be responsible for the disassembly and assembly of the Sertoli cell membranes composing the blood–testis barrier as germ cells are transferred from the basal to the adluminal compartments of the seminiferous epithelium (Wong and Cheng, 2005; Li et al., 2006).

Sertoli cells are considered to be numerically stable in adults. In fact, if one Googled "Sertoli cell" (the somatic cell of the seminiferous epithelium; Fig. 1) in December, 2006, the first item that popped up from Wikipedia is the statement: "Once fully differentiated, the Sertoli cell is unable to proliferate." This statement was supported by studies that found that Sertoli cells failed to proliferate and enter the cell cycle even early in post-pubertal animals (Sharpe et al., 2003; Buzzard et al., 2003). However of interest is that this Wikipedia comment regarding the stable number of adult Sertoli cells, precedes statements highlighting various Sertoli cell functions which have been the main topic of the 748,000 Google citations on the topic.

Sertoli cells are columnar in shape (Fig. 1), possess long and thin mitochondria, and usually have lipofuscin and lipid droplets at the base of their cytoplasm (Johnson, 1991b; Russell, 1993). Sertoli cell nuclei exhibit a variety of shapes, but they are usually oval- or pear-shaped with significant indentations in the nuclear membranes. This indented nuclear envelope (Fig. 2), euchromatic nucleoplasm, and large distinctive nucleolus are characteristics of cells possessing

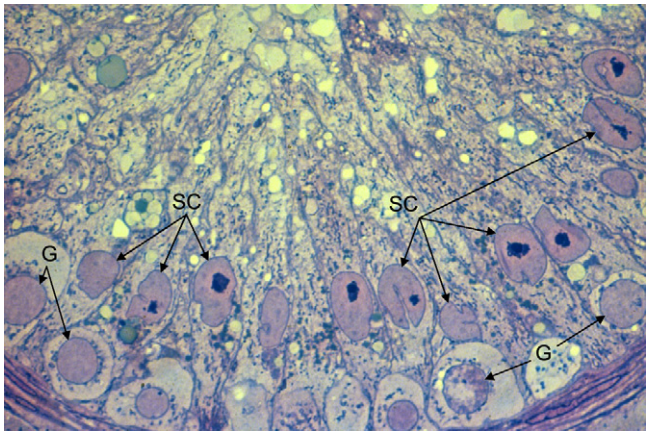


Fig. 2. Human seminiferous tubule with its Sertoli cells (SC) in an infertile man viewed by bright field microscopy in 0.5-μm Epon sections. Sertoli cell nuclei with their indented nuclear envelope, euchromatic nucleoplasm, and distinct nucleolus are located above the spermatogonia (G). Note the varied shapes of the Sertoli cell nuclei. Bar length equals 10 μm. Modified from Johnson (2007).

high metabolic rates (Johnson, 1991b). It is the non-spherical shape of Sertoli cell nuclei makes it difficult to determine their number in the testis. Methods to overcome the difficulty of estimating Sertoli cell number and the data obtained using these methods are discussed in this review along with Sertoli cell function and a little on shaping of spermatozoa. Also discussed is compelling evidence that Sertoli cells can proliferate in adults under certain conditions, and the stallion is an example of a species with natural fluctuations in Sertoli cell numbers in adults.

2. Seminiferous tubules

Sertoli cells are one of the two somatic cell types of seminiferous tubules, and together with germ cells, constitute the seminiferous epithelium (Fig. 1). The seminiferous epithelium is surrounded by one or more layers of myoid cells: these somatic cells mark the outer limits of the seminiferous tubules. The peritubular myoid cells (myofibroblasts) border the outer edge of seminiferous epithelium and touch the basal lamina of spermatogonia and Sertoli cells. Myoid cells exhibit contractions that contribute to the movement of spermatozoa and fluid through the luminal confinement of the seminiferous tubules, as well as involvement in paracrine signaling (Skinner and Fritz, 1986; Hettle et al., 1988; Maekawa et al., 1996).

A cross-section of the seminiferous epithelium (Fig. 1) shows spermatogonia located at the base of the seminiferous tubule, spermatocytes in the middle, and spermatids near the apex of the seminiferous epithelium, demonstrating the developmental progression of less mature to more mature germ cells as they move toward the lumen (Amann, 1970), where the spermatids are released, or spermiated, as spermatozoa.

In a germ cell's path to make a spermatozoon from a spermatogonium, a spermatogonium divides by mitosis in the basal compartment, of the seminiferous tubule, to produce either stem cells or committed spermatogonia that ultimately become primary spermatocytes. These cells pass through the blood–testis barrier of the Sertoli cell tight junctions as they move into the adluminal compartment. They continue their development in the immunologic-protected site of the adluminal compartment.

In addition to nurturing developing germ cells of different progeny, phases, or steps of development and protecting them from the immune system, Sertoli cells are involved in the release of spermatids (spermiation) into the seminiferous tubule lumen. As mature spermatids move to the lumen (Fig. 1), they are tethered to the Sertoli cells by cytoplasmic stalks connecting the cytoplasm around the middle piece of the elongated spermatids to the corresponding residual cytoplasm of these cells that is retained in the seminiferous epithelium at spermiation (Fig. 3; Johnson et al., 1978). Also seen in the lumen are the developing flagella of round spermatids with spherical nuclei (Fig. 3; also compare Fig. 11c below). The spermatids with spherical nuclei are a generation (e.g., 12.2 days in the horse; Swierstra et al., 1974) younger in developmental age than the spermatids being released. The released spermatozoa possess distinct cytoplasmic droplet at the junction of the head and middle piece. These droplets result from the excess cytoplasm that is retained by spermatozoa after the severing of the cytoplasmic stalks (Fig. 3).

3. Spermatozoa

Although epididymal spermatozoa possess cytoplasmic droplets attached to them, which migrate down the middle piece to the junction between the principal piece and middle piece of the tail, the normal, mature, ejaculated spermatozoon has no such droplet or looses it soon after ejaculation. Comparing the ultrastructure of equine spermatozoa (Fig. 4; Johnson, 1991b;

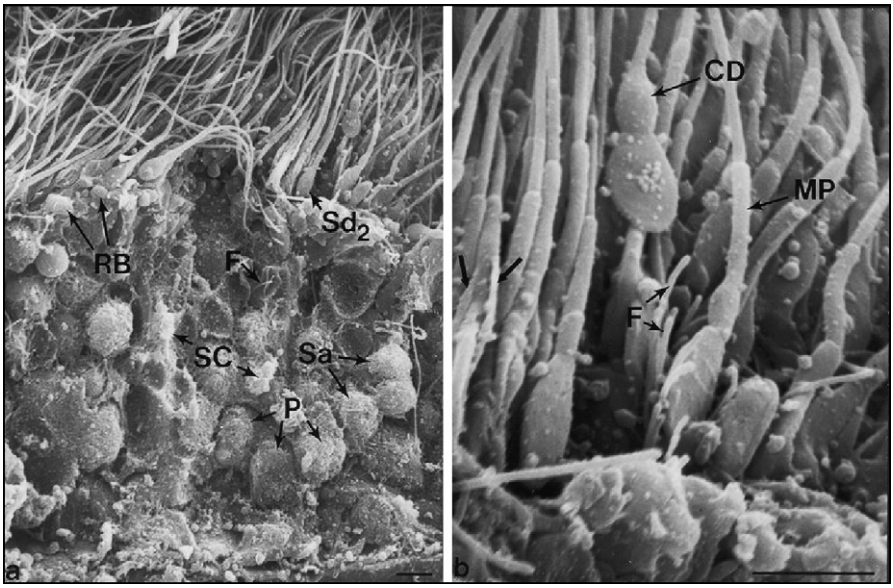


Fig. 3. Scanning electron micrographs of equine seminiferous epithelium in Stage VIII; (Swierstra et al., 1974) (a) illustrating the entire seminiferous epithelial height and (b) an enlarged luminal view of the same tubule. (a) Pachytene primary spermatocytes (P), Sa spermatids with spherical nuclei (Sa), Sd2 spermatids (Sd2) whose entire length is exposed in the lumen, Sertoli cells (SC), and residual bodies (RB) are identified by size, shape, and position within the seminiferous epithelium. (b) The middle pieces (MP) of Sd2 spermatids are enlarged with mitochondria, and they are attached to residual cytoplasm by cytoplasmic stalks (arrows). The released spermatozoon has the cytoplasmic droplet (CD). Developing flagella (F) from (a) Sa spermatids below the luminal surface project toward or (b) into the lumen. Bar lengths equal (a) 5 μm and (b) 5 μm. Modified from Johnson et al. (1978).

Johnson et al., 2001) to that first shown in drawings by Leewenhoek (who discovered spermatozoa after inventing the microscope), one can see a complex, highly differentiated genome-delivery cell. The head contains the highly packed DNA material that provides an embryo with half of its chromosomes and determines the sex of the offspring, it also has an overlaying acrosome of hydrolytic enzymes necessary to penetrate the zona pellucida of the oocyte. The tail has the typical axoneme of a flagellum (Fig. 4), with its nine outer doublets and a central pair of microtubules which are connected to the head and derived from the distal centriole. While the axoneme runs the length of the tail, the nine outer dense fibers extend varying lengths down the tail and are missing in the end piece. Helical mitochondria wrap around the dense fibers and surround the axoneme in the middle piece, but the area outside the dense fibers is covered by the flexible fibrous shaft of the principal piece. To better understand the shaping of spermatozoa by Sertoli cells, it would be useful to examine the equine spermatozoon (Fig. 4; Johnson, 1991a,b). Like the human, the head of the horse spermatozoon is tapered on two sides and is pointed at its apex. The membranes of the nucleus and the acrosome are covered by the overlying plasma membrane. Numerous mitochondria surround the dense fibers of the tail in its middle piece to the annulus. The fibrous sheath surrounds the dense fibers within the principal piece. Newly spermiated spermatozoa contain cytoplasmic droplets in the proximal position (near the head) of the middle piece. There are nine dense fibers in the middle piece, but this number is reduced as the fibers extend down the principal piece. The axoneme, however, extends down the principal piece into the end piece of the tail where the doublets become single microtubules.

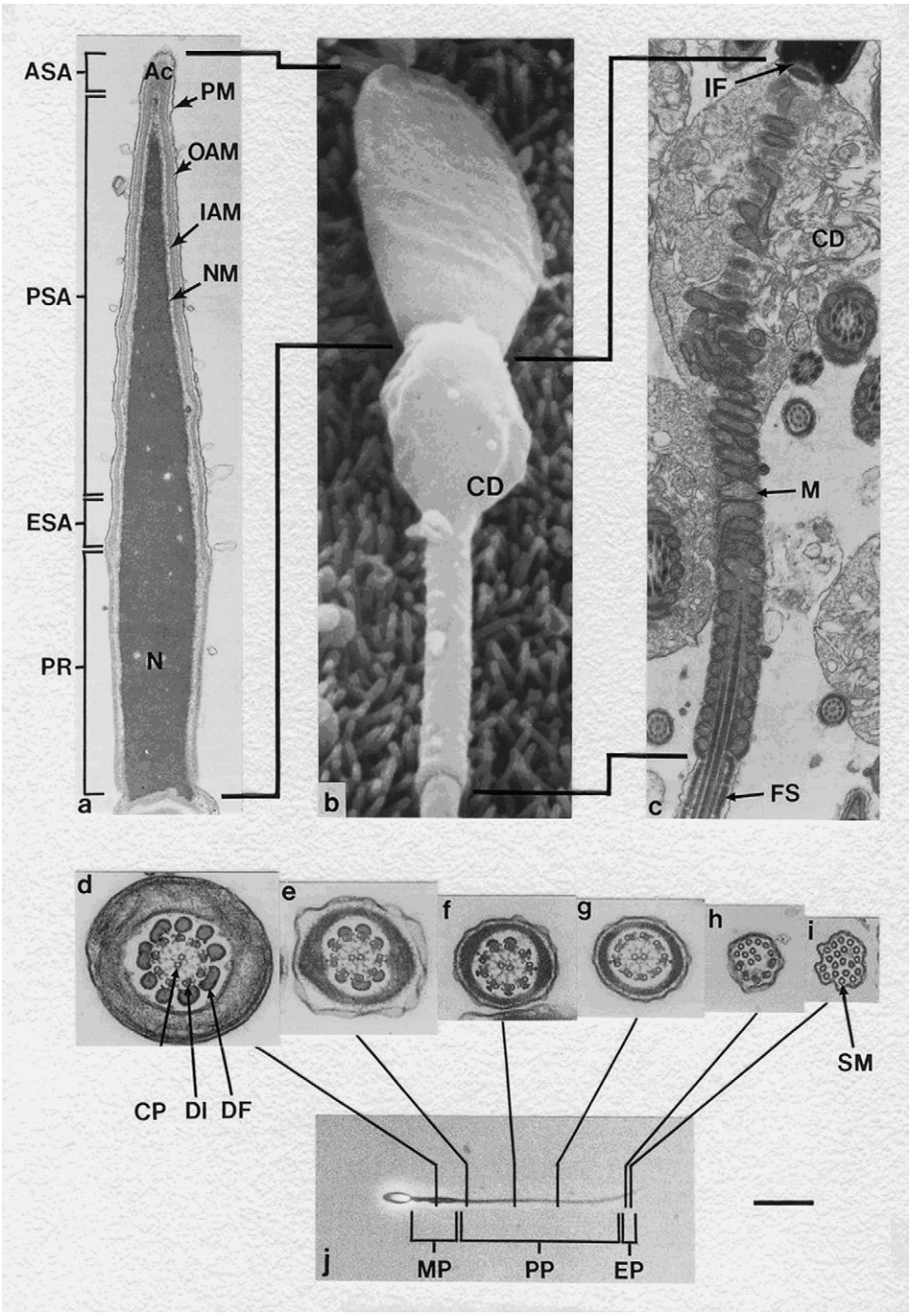


Fig. 4. Horse spermatozoa. Images and views produced by a (c–i) transmission electron; (b) scanning electron; and (j) phase contrast microscopy. Attaching lines between (a) and (b) or (b) and (c) and between (d–i) and j) correspond to regions of the spermatozoa. (a) The plasma membrane (PM), nucleus (N), overlying acrosome (A), and postacrosomal region

The cytoplasmic droplet in the proximal position of recently spermiated spermatozoa results from the remnants of the cytoplasmic stalk that attach the soon to be spermiated spermatid to its residual cytoplasm held by the Sertoli cells (Fig. 3). The residual cytoplasm which remains within the seminiferous epithelium is phagocytized by the Sertoli cells once spermiation has occurred (Figs. 3 and 4). In the segments of the seminiferous tubules at the spermatogenic stage of the cycle, when the mature spermatids (Sd2) are spermiated, developing tails of the younger generation of spermatids (Sa) with spherical nuclei are present and are already extending into the lumen (Fig. 3). In short, the spermatozoon is "an unsurpassed example of cell differentiation in the production of the self-propelled, penetrative enzyme-containing, male-genome delivery system" (Johnson, 1991b).

4. Sertoli cell number relates to magnitude of spermatogenesis

In both humans and horses, the number of Sertoli cells is related to the level of spermatogenesis as measured as daily sperm production per testis (Fig. 5; Johnson and Thompson, 1983; Johnson et al., 1984c; Johnson, 1986b). This relationship is higher for horses ($r^2 = 0.68$) than for humans ($r^2 = 0.39$). Sertoli cell number is also correlated with testicular weight ($r^2 = 0.68$) in the horse. It is not likely that Sertoli cell number in adults is related to the efficiency of spermatogenesis as has been described (Chaudhary et al., 2005), if the efficiency of spermatogenesis is daily sperm production/gram testicular parenchyma (Amann, 1970, 1986; Johnson et al., 2001). However, the total Sertoli cell number per testis is very important in determining the total daily sperm production per testis (Fig. 5; Johnson et al., 1984c; Johnson, 1991b; Johnson et al., 2001)

The relationship between Sertoli cell number per horse and sperm production rate is not only reflected in the number of mature germ cells, but the relationship also exists for A and B_1 spermatogonial populations (Fig. 5). In the stallion, spermatogonia of different types have been characterized and compared with Sertoli cell number (Fig. 5; Johnson, 1991b). The relationship between the number of spermatogonia and Sertoli cells extends to the earliest points of spermatogenesis including type A_1 (the most primitive germ cell) spermatogonium (Fig. 5; Johnson et al., 1994a). The relationship of Sertoli cell number and number of A spermatogonium is consistent with the size of one cell type influencing the population size of the other. To summarize, it implies that the relationship between Sertoli cell number and daily sperm production stems from this early relationship between the number of Sertoli cells and the number of the most primitive (A_1) spermatogonia (Fig. 5).

(PR) are seen. The acrosome is divided into the apical segment (ASA), principal segment (PSA), and equatorial segment (ESA). The inner acrosomal membrane (IAM) is located near the nuclear membrane (NM), and the outer acrosomal membrane (OAM) is adjacent to the plasma membrane. (j) The tail is composed of the middle piece (MP), principal piece (PP), and end piece (EP). (c) The tail attaches to the nucleus at the implantation fossa (IF). The tail contains mitochondria (M) in the middle piece and the fibrous sheath (FS) in the principal piece. (d–i) The distal centriole is continuous with the outer nine doublet microtubules (Dl). The axoneme also has the characteristic central pair (CP) of microtubules. The nine dense fibers (DF) run parallel to the axoneme and extend to different lengths of the principal piece. The axonemal doublets become disorganized and ultimately separate into 20 single microtubules (SM), but still are enclosed in the plasma membrane in the end piece of the tail. The cytoplasmic droplet (CD) is located at the proximal end of the middle piece of these spermatozoa from the equine (b) efferent ducts and (c) caput epididymides. Bar length equals (a) 0.5 μm; (b) 0.85 μm; (c) 0.75 μm; (d–i) 0.24 μm; or (j) 1.28 μm. Modified from Johnson et al. (1978, 1980) and Johnson (1991b).

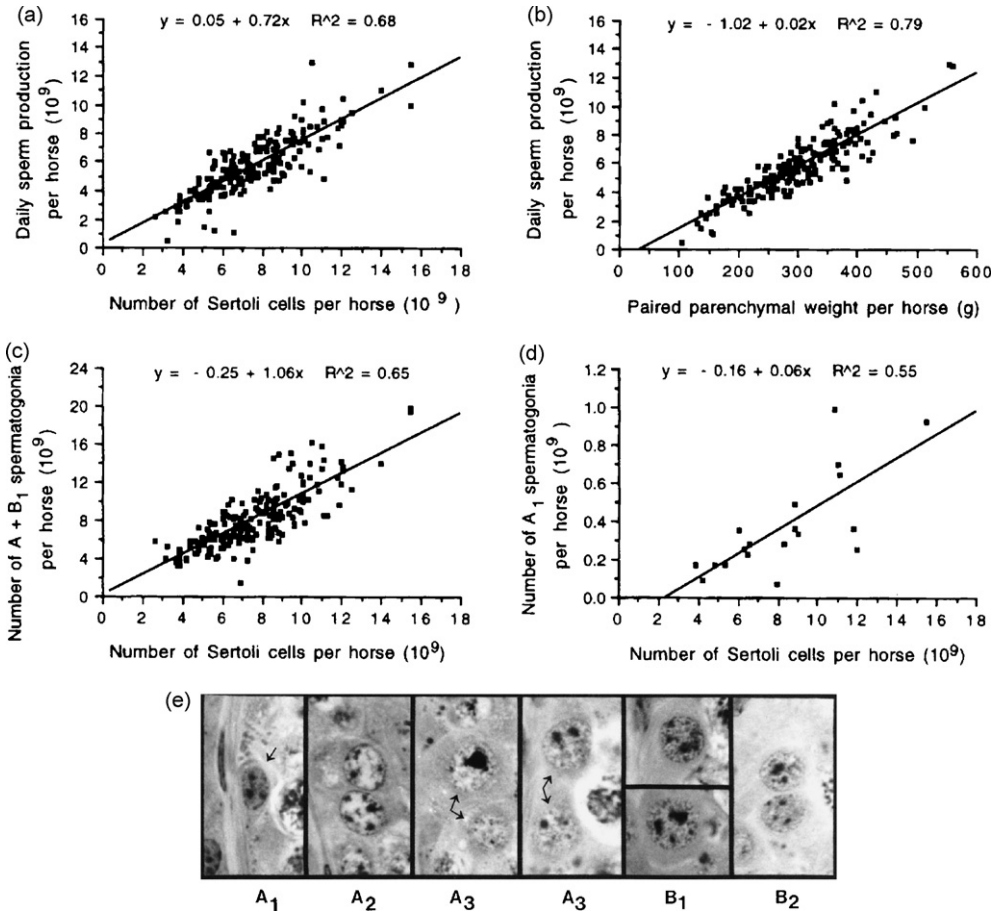


Fig. 5. Effect of Sertoli cell number and parenchymal weight (a–f) on spermatogenesis in 184 adult horses, and composite of bright-field micrographs of 5-μm methacrylate sections of spermatagonia. The (a and c) number of Sertoli cells and (b) parenchymal weight influenced (a and b) daily sperm production and (c) number of the combination of all A plus B1 spermatogonia. (d) In a subset of 19 horses representing both the breeding (June) and non-breeding (December) seasons, the effects of number of Sertoli cells on the numbers of (d) A1 spermatogonia. (e) Subtypes of spermatagonia are classified by nuclear profiles, which include the most primitive cells with a small, oval or flattened nucleus (A_1), a light center (A_2), a large single nucleolus, or two or three nucleoli (A_3), or large nucleoli plus these with fragmented nucleoli (B_1) B_1 divide into B_2. Modified from Johnson (1991b) and Johnson et al. (1994a).

5. Sertoli cells and seminiferous tubules in non-conventional views

Using Nomarski optics (that allows interior views of cells) to examine 20 μm Epon sections of equine seminiferous tubules, the shapes and sizes of nuclei and cellular structures can be focused in the optical sections (Fig. 6; Johnson et al., 1990). All eight stages of seminiferous epithelium in the horse are characterized by different types of spermatogonia, spermatocyte phases, or developmental steps of spermatids (Amann, 1970; Swierstra et al., 1974; Johnson et al., 1990). The Sertoli cell is characterized by a large nucleolus as well as linearly arranged mitochondria. Residual bodies left behind by spermiated spermatozoa can be seen at the apical end of the seminiferous

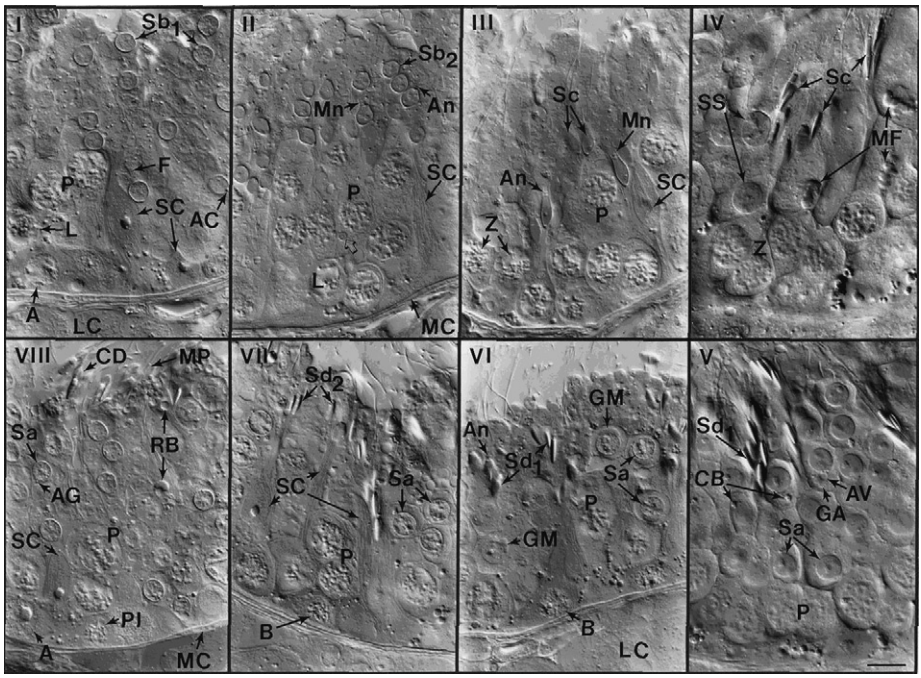


Fig. 6. Light microscopic view (Nomarski optics) of the eight stages of the horse spermatogenic cycle seen in 20-μm Epon sections. Tubules and structures identified include tubules in the eight stages of the cycle (I–VIII), A (A) and B (B) spermatogonia, preleptotene (Pl), leptotene (L), zygotene (Z), and pachytene (P) primary spermatocytes, secondary spermatocytes (SS), Sa, Sb1, Sb2, Sc, Sd1, Sd2 spermatids (Sa, Sb1, Sb2, Sc, Sd1, Sd2, respectively), Sertoli cells (SC), myoid cells (MC), Leydig cells (LC), a newly developing flagellum (F), the annulus (An), manchette (Mn), meiotic Figs. (MF), cytoplasmic droplet (CD), middle piece (MP), residual body (RB), acrosomic granule (AG), acrosomic cap (AC), group of mitochondria (GM), Golgi apparatus (GA), chromatoid body (CB), and acrosomic vesicle (AV). Optical sectioning that is possible with Nomarski optics facilitates location and observation of these structures. Bar length equals 10 μm. Modified from Johnson et al. (1990).

epithelium and down its length of Sertoli cell cytoplasm as phagocytized residual bodies move toward the base of the Sertoli cells where they disappear (Fig. 6). Also by focusing up and down (optically sectioning), one can measure the greatest diameter of a spherical germ cell nucleus (to estimate its volume by the formula for the volume of a sphere) and the greatest height and width of the Sertoli cell nuclei even though they are mostly pear-shaped.

Viewed by high voltage transmission electron microscopy, the equine Sertoli cell (Fig. 7; Johnson, 1986a) is seen to be like that first described by Enrico Sertoli in that it is a branched cell that does not transform into germ cells (Setchell, 1993). However, the “branches” were actually sheets of cytoplasm that largely surround the developing germ cells (Russell, 1993). Hence, a Sertoli cell holds germ cells in its series of pockets composed of sheets of Sertoli cell cytoplasm contained within its plasma membrane. At times, cytoplasmic bridges connecting germ cells of the same type can be seen (Fig. 7).

The closeness of Sertoli cell sheets can be seen among elongated spermatids embedded within Sertoli cells recesses formed by the plasma membrane of Sertoli cells (Fig. 7 and compare with Fig. 11 below). Even the long mitochondria can be seen extending within Sertoli cell cytoplasm among the bundles of elongated spermatids (Fig. 7).

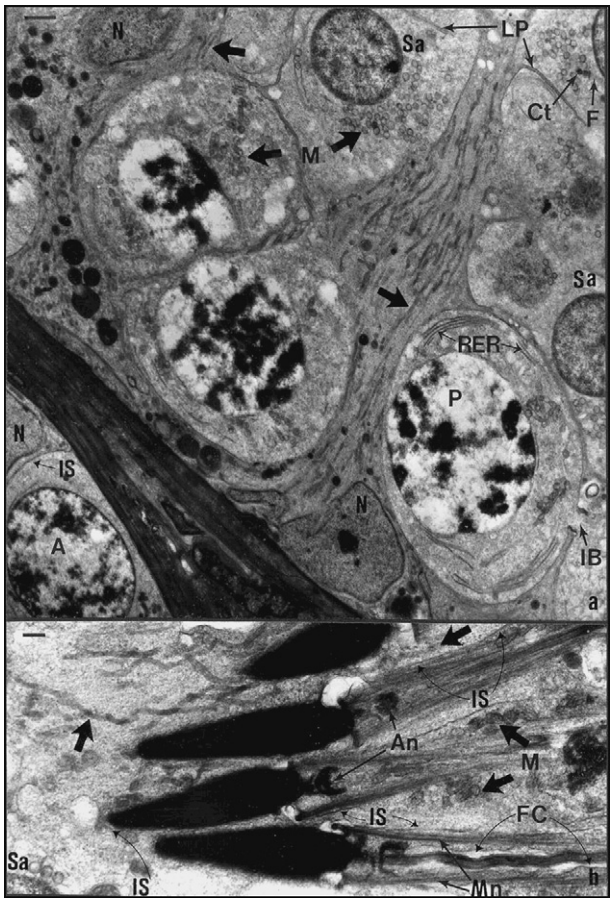


Fig. 7. High-voltage transmission electron-microscopic view of horse Sertoli and germ cells embedded in Epon 812 and sectioned at 1.0 μm. These micrographs characterize the basal (a) and apical (b) regions of Sertoli cells. (a) Laminar processes (LP) extend from the main trunk of a Sertoli cell and between germ cells. While an intercellular bridge (IB) is seen between two pachytene primary spermatocytes (PS). Long slender mitochondria (arrows) characterize Sertoli cells, and spherical mitochondria (M) and profiles of rough endoplasmic reticulum (RER) characterize germ cells. Sertoli cell nuclei (N) are indicated. An (A) spermatogonium (AS) shares the intercellular space (IS) with a Sertoli cell. All Golgi-phase Sa spermatids (Sa) developed acrosomic vesicles and the centrioles (Ct) give rise to the developing flagellum (F). The annulus (An), manchette (Mn), and mitochondria (M) characterize the Sd1 spermatids. The flagellar canal (FC) is lined by the enfolded plasma membrane and is the space between the developing tail and surrounding manchette. Bar length equals (a) 2 μm and (b) 1 μm. Modified from Johnson (1986a).

6. Spermiogenesis: shaping spermatids into spermatozoa

As spermatids develop in close contact with the Sertoli cells, their nuclei become more spear-shaped and chromatin becomes more condensed (Fig. 8; Johnson, 1991b), the tail forms from the distal centriole and mitochondria move into their position in the middle piece of the spermatozoon. The Golgi apparatus produces the acrosome whose lysosomal contents are not unlike other cellular lysosomes, but they are released by a calcium influx across the plasma membrane not unlike induced secretion (as in nerve cells). However, the acrosomal contents are released by the fusion

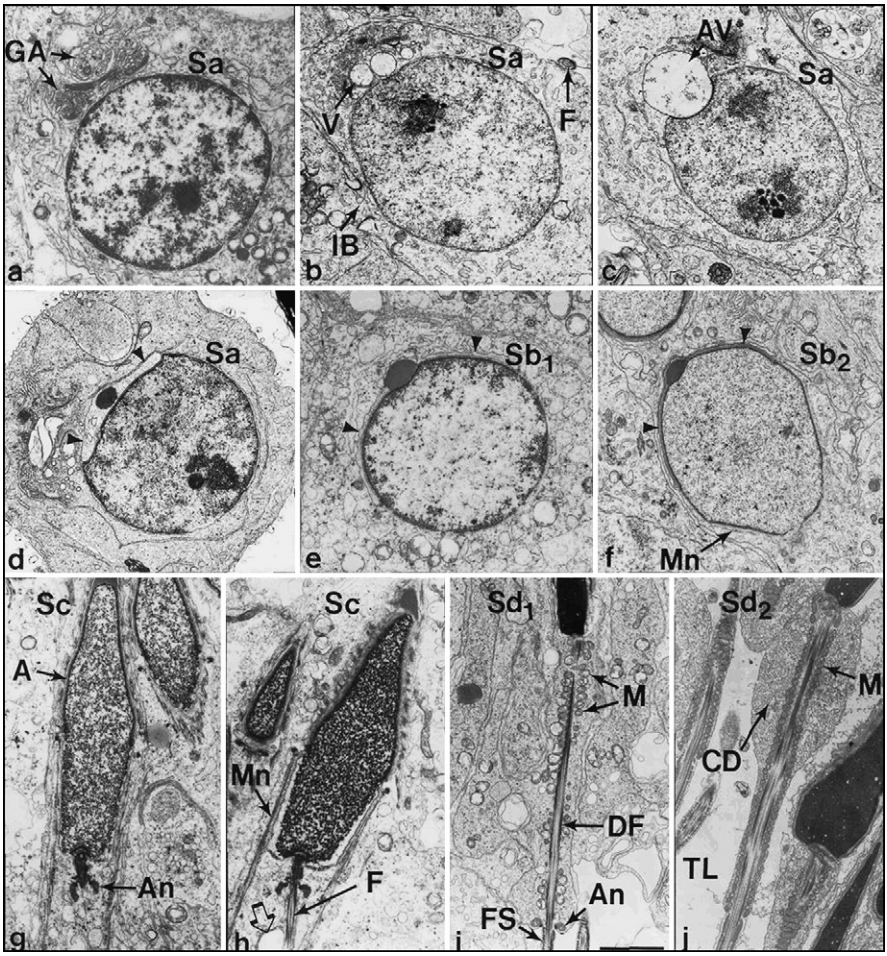


Fig. 8. Transmission electron microscopic view of development of horse spermatids during spermiogenesis. (a–d) The large Golgi apparatus (GA) in Sa spermatids (Sa) produces vesicles (V) fused to form the acrosomic vesicle (AV). (c) The nuclear envelope is indicated by the acrosomic vesicle which (d) flattens over the nucleus (arrowheads). (b) Developing tails or flagella (F) from Sa spermatids extend into the extracellular space. Intercellular bridges (IB) connect adjacent spermatids. (e) The acrosome (arrow heads) forms a head cap over the nucleus in the Sb1 spermatids (Sb1). (f) In the Sb2 spermatid (Sb2), the acrosome (arrowheads) is obvious, and the manchette (Mn) appears at the beginning of nuclear elongation. (g and h) The Sc spermatid (Sc) has a distinct manchette (Mn), an elongating and condensing nucleus, a well-defined acrosome over the anterior portion of the nucleus, and an attached flagellum with a distinct annulus (An). (i–j) The Sd1 and Sd2 spermatids (Sd1, Sd2, respectively) have further condensation of their nuclei. (b) The flagellum (F) begins in the early Sa spermatid and (g and h) appears as a growing axoneme in Sb1, Sb2 (not shown), and Sc spermatids. (i) The late Sd1 spermatid (Sd1) is characterized by the dense fibers (DF), a completed fibrous sheath (FS), and mitochondria (M) that are migrating around the flagellum. In this Sd1 spermatid, the mitochondrial migration is not complete, as small groups of mitochondria are incompletely attached around the dense fibers. Prior to mitochondrial migration in the Sd1 spermatid, the manchette is removed and the annulus (An) migrates to its permanent position at the distal end of the middle piece. (j) The late Sd2 spermatid (Sd2) is largely extended into the tubular lumen (TL) and has complete migration of mitochondria (M) around the middle piece. A cytoplasmic droplet (CD) produced by excess cytoplasm of the spermatid remains in the proximal region of the middle piece. Bar length equals 2 μm. Modified from Johnson (1991b).

of the outer acrosomal membrane and the overlaying plasma membrane (Varner and Johnson, 2007). The enzymatic contents of the acrosome, in one sense, may be considered secretions as in more conventional secretory cells (Moreno and Alvarado, 2006) with induced release (e.g., pancreatic acinar cells). Biogenesis of the acrosome in a haploid cell illustrates that the production of this complex secretory vesicle (Moreno and Alvarado, 2006) does not require the diploid chromosomal configuration for transcription (Dadoune et al., 2004). In addition to new findings and considerations of the acrosome, the mRNA of spermatozoa may play roles in fertilization and early development thereafter (Dadoune et al., 2004; Miller and Ostermeier, 2006a,b).

7. Seasonal differences in characteristics of equine Sertoli cells

Histological comparisons of seminiferous epithelium between tissues taken during the breeding season and those taken in the non-breeding season of long-day breeders (North American horse of light-weight breeds), do not yield obvious differences in cell populations between seasons (Fig. 9; Johnson et al., 1991a). However, testicular weight is significantly higher in the natural breeding season of the horse (Table 1). Using homogenates of fixed testicular equine tissue (Fig. 10; which allow enumeration of nuclei of various germ cells and Sertoli cells) from young adult horses; it was found that the number of Sertoli cells per gram parenchyma was similar between seasons as might be predicted by the similarity in the appearance of seminiferous epithelium in histologic evaluations (Table 1). However with increased testicular size, the number of Sertoli cells per testis was significantly greater in the natural breeding season (long days in the USA). This was our first indication that Sertoli cell number fluctuates with season in adult horses (Table 1; Johnson and Thompson, 1983).

To test these unique findings (which differ from the conventional thinking that Sertoli cells consist of a stable population in adults), we made additional observations using different stereological measures on the same testes (Table 1; Johnson and Thompson, 1983). Using volume density measurements of Sertoli cell nuclei, we determined that the total volume of Sertoli cell nuclei per testis was greater in the breeding season; however, the average size of individual Sertoli cell nuclei was the same. Hence, with no change in the size of individual Sertoli cell nuclei, but

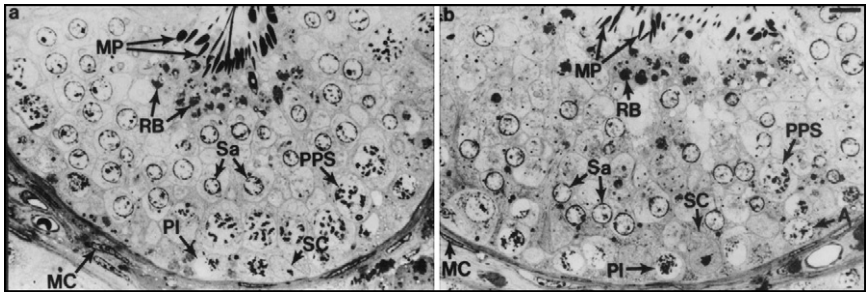


Fig. 9. Effect of season on the germ cell per Sertoli cell ratio in the Stage VIII seminiferous epithelium of the horse. Stage VIII seminiferous tubules are composed of A spermatagonia (A), preleptotene primary spermatocytes (Pl), pachytene primary spermatocytes (PPS), Sa spermatids (Sa), and the maturation-phase spermatids (MP). Sertoli cells (SC), myoid cells (MC), and residual bodies (RB) are indicated. Note the non-spherical shape of the nucleoli in Sertoli cell nuclei (SC) representing each season. Profiles of the seven Sertoli cell nuclei (small arrows) are indicated in each season. On the basis of qualitative histologic evaluation, the ratio of germ cells per Sertoli cell in the non-breeding season (a) is similar to that in the breeding season (b). For each season, there were nine or 10 profiles of different types of germ cells per Sertoli cell nuclear profile. Bar length equals 10 μm. Modified from Johnson et al. (1991a).

Table 1
First horse Sertoli cell data

Effect of season on the number of Sertoli cells in 4–5 year old horses

Season	*n*	Number	
		10^6 per g	10^9 per testis
Non-breeding	10	25.5 ± 3.3	2.74 ± 0.29
Breeding	10	22.8 ± 2.2	4.06 ± 0.54
		NS	$P < 0.05$

Effect of season on the total nuclear volume of Sertoli cells per testis and average height and width of Sertoli cell nuclei in 4–5 year old male horses

Season	*n*	Total nuclear volume (ml)	Average size (μm)
Non-breeding	10	1.80 ± 0.09	11.35 ± 0.12
Breeding	12	3.20 ± 0.52	11.65 ± 0.19
		$P < 0.05$	NS

Effect on season on the number of Sertoli cell nuclei containing nucleoli per seminiferous tubule cross-section and length of tubules

Season	*n*	Number/cross-section	Tubular length (km)
Non-breeding	10	4.1 ± 0.2	1.9 ± 0.1
Breeding	10	4.0 ± 0.1	2.9 ± 0.3
		NS	$P < 0.01$

Modified from Johnson and Thompson (1983).

with a significant change in the total volume of Sertoli cell nuclei per testis a larger number of Sertoli cells/testis must occur in the breeding season of adult horses. In further support, the number of Sertoli cells containing nucleoli per tubular cross-section was similar between seasons; however, the total length of seminiferous tubules are significantly longer during the breeding season compared to the non-breeding season. If there is no difference in the number of Sertoli cells per cross-section and no difference in size of individual Sertoli cell nuclei, but the total length of the seminiferous tubules is greater in the breeding season, then the number of Sertoli cells must be significantly greater in the breeding season (Table 1; Johnson and Thompson, 1983). Although the data obtained using these two methods of are consistent with a larger number of Sertoli cells in the testis during the natural breeding season, they do not themselves estimate the actual number of Sertoli cells in the testis or pair of testes.

8. Direct counts to calculate Sertoli cell numbers

In subsequent studies, the number of Sertoli cell nuclei was determined for a large number of horses at different ages during the breeding season and the non-breeding seasons (Johnson and Thompson, 1983; Johnson, 1986a; Johnson and Nguyen, 1986; Johnson and Tatum, 1989; Johnson et al., 1991a). Because of the large number of horses that needed to be assessed, the enumeration of Sertoli cell nuclei in homogenates of fixed testicular tissue (Fig. 10) was used as this provided a rapid evaluation. It was found that the Sertoli cell number per horse increased with age until years 4–5 and then remained relatively stable to 20 years of age (Fig. 10; Johnson and Thompson, 1983). Seasonal differences in Sertoli cell number were noted for horses

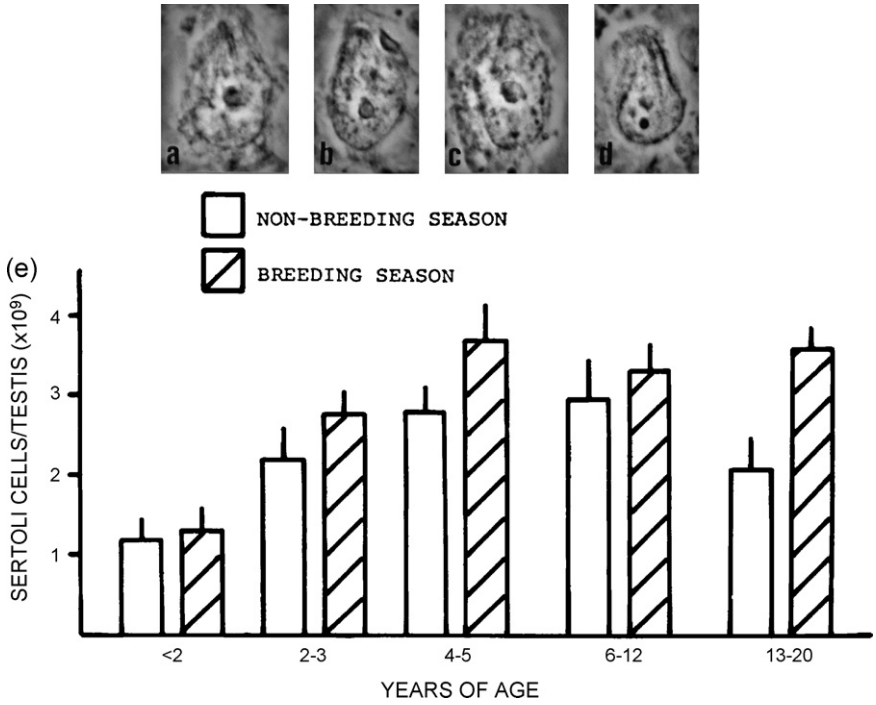


Fig. 10. The effect of age and season on the Sertoli cell number as determined by enumerating Sertoli cell nuclei in homogenates of fixed equine testicular tissue. (a) The number of Sertoli cells per testis increased with age to 4–5 years, but leveled off at age 20. However, there was a significantly larger number of Sertoli cells in the breeding season. (b) Phase-contrast microscopic view of typical nuclear morphology of Sertoli cells in homogenates of glutaraldehyde-fixed horse testes. (a–d) Sertoli cells often retained some granular cytoplasm. Nuclei were identified by their large size, overall pear shape, and irregular, indented nuclear envelopes. Modified from Johnson and Thompson (1983).

aged 2–20 years with a significantly higher Sertoli cell numbers occurring during the breeding season.

9. Development of new methods to determine Sertoli cell number and function

Given the uniqueness of enumerating Sertoli cell nuclei in fixed testicular homogenates (Fig. 10) and that the finding that seasonal differences in their numbers, in adult horses, was inconsistent with the common dogma that the Sertoli cell population remains unchanged in adults, further methods were developed. These were developed to count the number of Sertoli cells in the testis, to confirm or refute the notion that Sertoli cell numbers are not stable in adult horses, as they appear to fluctuate with season.

In 1984, Dr. Lonnie D. Russell estimated the functional capacity of Sertoli cells in a host species by counting the number of elongated spermatids embedded in the apex of individual Sertoli cells (Russell and Peterson, 1984). Hence, he calculated the ratio of elongated spermatids per Sertoli cells as a measure of the function of Sertoli cells. We employed this functional measure, germ cell: Sertoli cell ratio, to estimate the number of Sertoli cells/testis (Johnson, 1986a). The number of Sertoli cells was estimated as the number of elongated spermatids (easily counted in testicular

Table 2
Effect of season on number of spermatids per Sertoli cell apex, number of Sertoli cells per gram of parenchyma, and number of Sertoli cells per testis

Item	Season		Significance
	Non-breeding	Breeding	
Parenchymal volume (ml)	103.2 ± 7.6[a]	144.1 ± 8.4	$P < 0.01$
Number of spermatids per cross-sectional apex of the single Sertoli cells[b]	7.54 ± 0.19	9.36 ± 0.41	$P < 0.01$
Number of Sertoli cells/gram (10^6) based on			
Spermatids with round nuclei and spermatids per Sertoli cell apex	23.8 ± 1.7	26.0 ± 1.1	NS
Spermatids with elongated nuclei and spermatids per Sertoli cell apex	25.4 ± 1.2	24.7 ± 0.8	NS
Number of Sertoli cells/testis 10^9) based on			
Spermatids with round nuclei and spermatids per Sertoli cell apex	2.77 ± 0.30	3.96 ± 0.29	$P < 0.01$
Spermatids with elongated nuclei and spermatids per Sertoli cell apex	2.92 ± 0.29	3.77 ± 0.28	$P < 0.05$

Number of spermatids embedded in the apex of individual Sertoli cells and number of Sertoli cells per testis were greater in the breeding season. Modified from Johnson (1986a).

[a] Mean ± S.E.M.

[b] Based on three stallions per season.

homogenates or by stereology) divided by the ratio of elongated spermatids embedded in the apex of Sertoli cells as determined histologically (Figs. 11 and 12; Tables 2 and 3).

Dr. Russell established his germ cell: Sertoli cell ratio at the electron microscopic level. He used electron microscopy because it allowed the observation of individual membranes of elongated spermatids embedded in the recesses of Sertoli cells as well a verification that some cross-sections of developing tails of both round and elongated spermatids are in the lumen and are not surrounded by Sertoli cells as seen in the profile of these seasonal breeder (Fig. 11: Johnson et al., 2001). However, we counted the elongated spermatids embedded in the apex of individual Sertoli cells

Table 3
Effect of season on number of Sertoli cells and on the number of germ cells accommodated by individual Sertoli cells

Item	Season		Significance
	Non-breeding	Breeding	
Number of Sertoli cells per testis[b] (10^9)	2.6 ± 0.2[a]	3.6 ± 0.2	$P < 0.01$
Germ cell type			
Type A spermatogonia	1.1 ± 0.1	1.5 ± 0.1	$P < 0.01$
Preleptotene and leptotene plus zygotene primary spermatocytes	2.9 ± 0.4	2.9 ± 0.2	NS
Pachytene plus diplotene primary spermatocytes	2.6 ± 0.2	3.0 ± 0.2	NS
Spermatids with round nuclei	8.1 ± 0.8	10.9 ± 0.8	$P < 0.05$
Spermatids with elongated nuclei	8.0 ± 0.8	10.2 ± 0.7	$P < 0.05$
All germ cell types combined	22.8 ± 2.1	28.5 ± 1.7	$P < 0.05$

Modified from Johnson (1986a).

[a] Means + S.E.M.

[b] Based on homogenates of fixed testes (Johnson and Thompson, 1983).

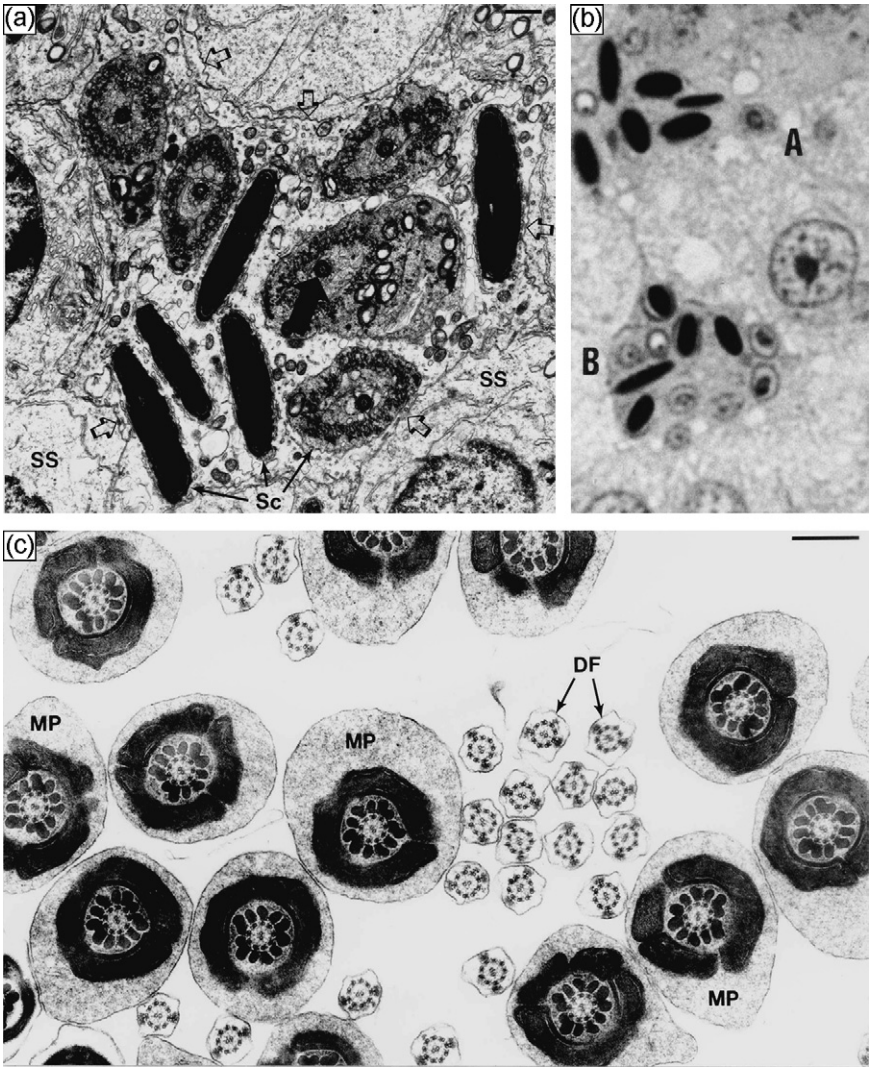


Fig. 11. Cross-sections of the middle apical region of a Sertoli cell and embedded Sc spermatids (Sc) in the horse viewed by (a) transmission electron microscopy or (b) bright field light microscopy, and (c) cross-sections of developing tails of early (spherical) spermatids and cross-sections of developed tails in late (elongated) spermatids in the hamster viewed by electron microscopy. (a) The plasma membrane of the Sertoli cell (large open arrows), surrounded by 8–10 secondary spermatocytes (SS), can be traced around the entire cluster of Sc spermatids (Sc). The axoneme with dense fibers (large arrow) is within a cross-section of the developing tail. (b) Clusters of spermatids with elongated nuclei separated by cytoplasm of germ cells with round nuclei, Sa spermatids (Sa) in this case, were located within 50 serial sections. Only clusters containing at least two spermatids cross-sectioned through their tails were enumerated to ensure the Sertoli cells were sectioned near the lumen. Spermatids within clusters not meeting these criteria were not enumerated (right side). This is the final size at which prints were evaluated. Clusters A and B contain 10 and 12 spermatids. (c) Middle pieces (MP) seen here in cross-sections of Sd_2 spermatids run parallel to the developing flagellum (DF) of younger Sa spermatids portraying the plasma membrane and axoneme. Bar lengths equal (a) 1 μm; (b) 0.3 μm; and (c) 0.4 μm. From Johnson (1986a, 1991b, 2007) and Johnson et al. (2001). Modified from Johnson et al. (1999a,b, 2001).

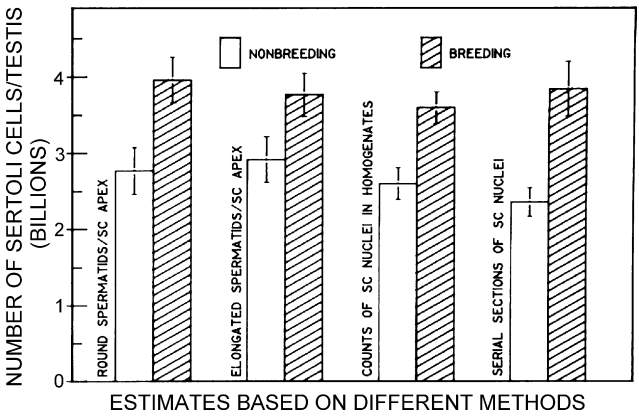


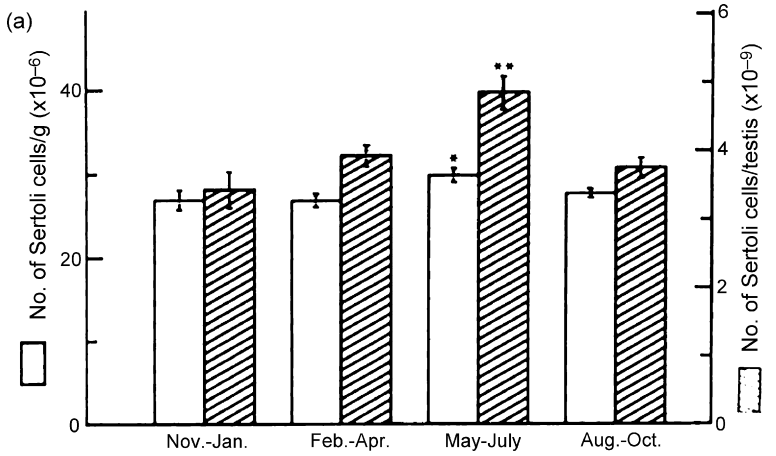
Fig. 12. Number of Sertoli cells per testis in 28 adult stallions in each season as determined by different methods. These methods include: enumerating spermatids with round nuclei or with elongated nuclei and spermatid–Sertoli cell apex ratio, direct counts made from testicular homogenates (Johnson and Thompson, 1983), and nuclear volume density and volume of the individual Sertoli cell nucleus determined by reconstruction of serial sections (Johnson and Nguyen, 1986). No difference among methods was detected; however, each method detected a seasonal difference. Modified from Johnson (1986a).

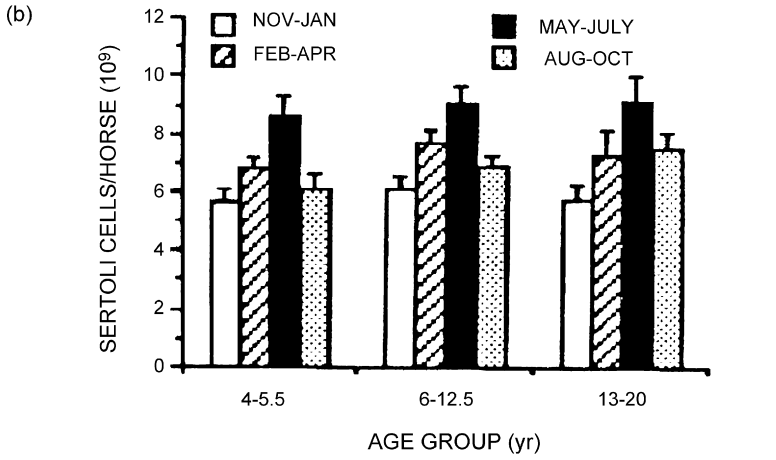
at the light microscope level (Fig. 11) to permit counting more Sertoli cells from each horse (Johnson, 1986a). This method, using serial sections, facilitated the observation of the apical end of individual Sertoli cells containing a group of embedded elongated spermatids separated from other Sertoli cells by a continuous group of surrounding germ cells with spherical nuclei (Fig. 11). It was found that Stage IV of the cycle, which has secondary spermatocytes or new spermatids, allowed separation of individual Sertoli cell profiles by surrounding germ cells with spherical nuclei (Fig. 11).

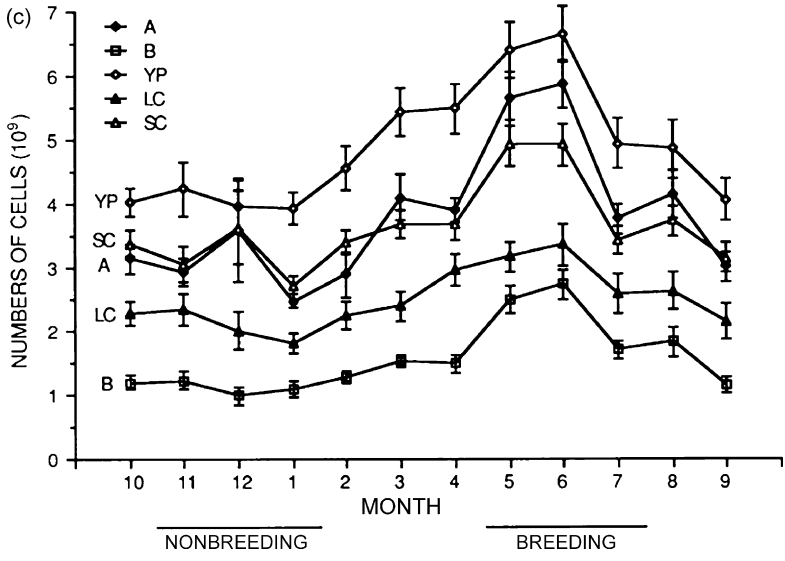
Using the ratio of the number of elongated spermatids embedded in a single Sertoli cell apex as the germ cell: Sertoli cell ratio, for 28 adult horses (4–20 years of age); it was determined that both testicular parenchymal weight and the number of Sertoli cells per testis were significantly greater during the breeding season (Table 2; Johnson, 1986a). As expected and consistent with Sertoli cell numbers dogma, there was no difference in number of Sertoli cells per gram of testicular parenchyma between seasons. However, there was a significant seasonal difference in the number of spermatids accommodated by individual Sertoli cells with an average of two more germ cells being accommodated in the breeding season (Fig. 11; Table 2).

Sertoli cell numbers, estimated using the number of germ cells in the apex of individual Sertoli cells (Fig. 11; Table 2), were compared to Sertoli cell numbers based on enumeration of Sertoli cell nuclei in testicular homogenates of fixed tissue (Fig. 10) or determined by volume density and reconstruction of individual Sertoli cell nuclei (Fig. 12; Johnson, 1986a). Not only did all methods discern seasonal differences in the Sertoli cell number of adult horses, they also yielded a remarkable similarity in absolute Sertoli cell number per horse, within each season (Fig. 12).

Given that the numbers of various types of germ cells were determined by stereological analysis, the number of germ cells of various types in Stage VIII per Sertoli cell (Table 3) could be calculated using the number of elongated spermatids embedded in Sertoli cell apex (germ cell: Sertoli cell ratio; Johnson, 1986a). It was found that the number of type A spermatogonia, the number of round spermatids or elongated spermatids per Sertoli cell, and the number of all germ cell per


(a)
No. of Sertoli cells/g (x10⁻⁶)
No. of Sertoli cells/testis (x10⁻⁹)
0
20
40
0
2
4
6
*
**
Nov.-Jan.
Feb.-Apr.
May-July
Aug.-Oct.


(b)
NOV-JAN
FEB-APR
MAY-JULY
AUG-OCT
SERTOLI CELLS/HORSE (10⁹)
0
2
4
6
8
10
12
4-5.5
6-12.5
13-20
AGE GROUP (yr)


(c)
A
B
YP
LC
SC
NUMBERS OF CELLS (10⁹)
0
1
2
3
4
5
6
7
10
11
12
1
2
3
4
5
6
7
8
9
MONTH
NONBREEDING
BREEDING

Sertoli cell were greater in the breeding season (Table 3). Sertoli cells in the breeding season accommodated six more germ cells than did Sertoli cells in the non-breeding season. Hence, both the function of individual Sertoli cells (Table 3; measured by ratio of germ cells accommodated) and number of Sertoli cells per horse were higher in the breeding season (Fig. 12).

10. Sertoli cell numbers fluctuate with season in a "dose-dependent" effect

If the number of Sertoli cells is different during the breeding and non-breeding seasons, one would anticipate intermediate values during the transitional periods from one season to the other or a "dose-dependent" effect of season on Sertoli cell number. Using large numbers of adult horses (Fig. 13), it was found that the number of Sertoli cells per horse was greater in the breeding season than the non-breeding season and that transitional periods between seasons yielded intermediate values (e.g., a "dose-dependent" effect of season). This "dose-dependent" effect was found to be true for all horses 4–20 years of age and for horses in different age groups (Fig. 13; Johnson and Nguyen, 1986). The fact that different age groups showed the same seasonal effect is consistent with season fluctuation in Sertoli cell number as a yearly event for stallions from 4 to 20 years of age and possibly throughout adult life (Fig. 13; Johnson et al., 1991a).

Fig. 13 also depicts changes in total number of germ cells and Sertoli cells over a 12-month period (Johnson and Tatum, 1989). Increases in the number of type A spermatogonia appear to precede the corresponding increases in Sertoli cell number. It is as if there is a need for more nurse cells (e.g., Sertoli cells) as spermatogonial numbers increase early in the breeding season. In addition to Sertoli cells and spermatogonia, numbers of spermatocytes and Leydig cells vary with the month of the year.

11. Initiation of spermatogenesis in colts

When 1–5 year-old horses (Fig. 14) were evaluated for seasonal differences in Sertoli cell number and for the timing of the initiation of spermatogenesis, it was found that seasonal differences in Sertoli cell number existed and the relationship between Sertoli cell number and level of spermatogenesis was established at an early age of pubertal horses (Fig. 14; Johnson et al., 1991b). As the horse reaches puberty, its small testis with dark shaded parenchyma locally develops a light shade as the seminiferous tubules expand and create a lumen and the number of Sertoli cells/gram parenchyma is reduced as germ cells increase in number (Fig. 15; Clemmons et al., 1995). With maturation and aging, the interstitium between seminiferous tubules becomes packed with Leydig cells (Johnson and Neaves, 1981), which use their abundance of cytoplasm (largely composed of smooth endoplasmic reticulum (Fig. 16)) to support the Sertoli cell and spermatogenesis.

Fig. 13. The number of Sertoli cells in the horse testis at different times of the year, effect of season on the number of Sertoli cells in different age groups of adult horses, and effect of season on the numbers of A spermatogonia (A). Number of Sertoli cells found in 43–48 adult horses during each 3-month period throughout 1 complete year illustrates more ($P < 0.05$) Sertoli cells per gram parenchyma during May to July (the natural breeding season of the horse) than in other periods. The number of Sertoli cells per testis is greater during May to July compared with the value during August to October or February to April ($P < 0.05$) or compared with the value for November to January ($P < 0.01$). B spermatogonia (B), preleptotene and leptotene plus zygotene primary spermatocytes (YP). Leydig cells (LC), and Sertoli cells (SC) per testis in 13–17 adult (4–20 years old) stallions each month. Modified from Johnson and Nguyen (1986), Johnson and Tatum (1989) and Johnson et al. (1991a).

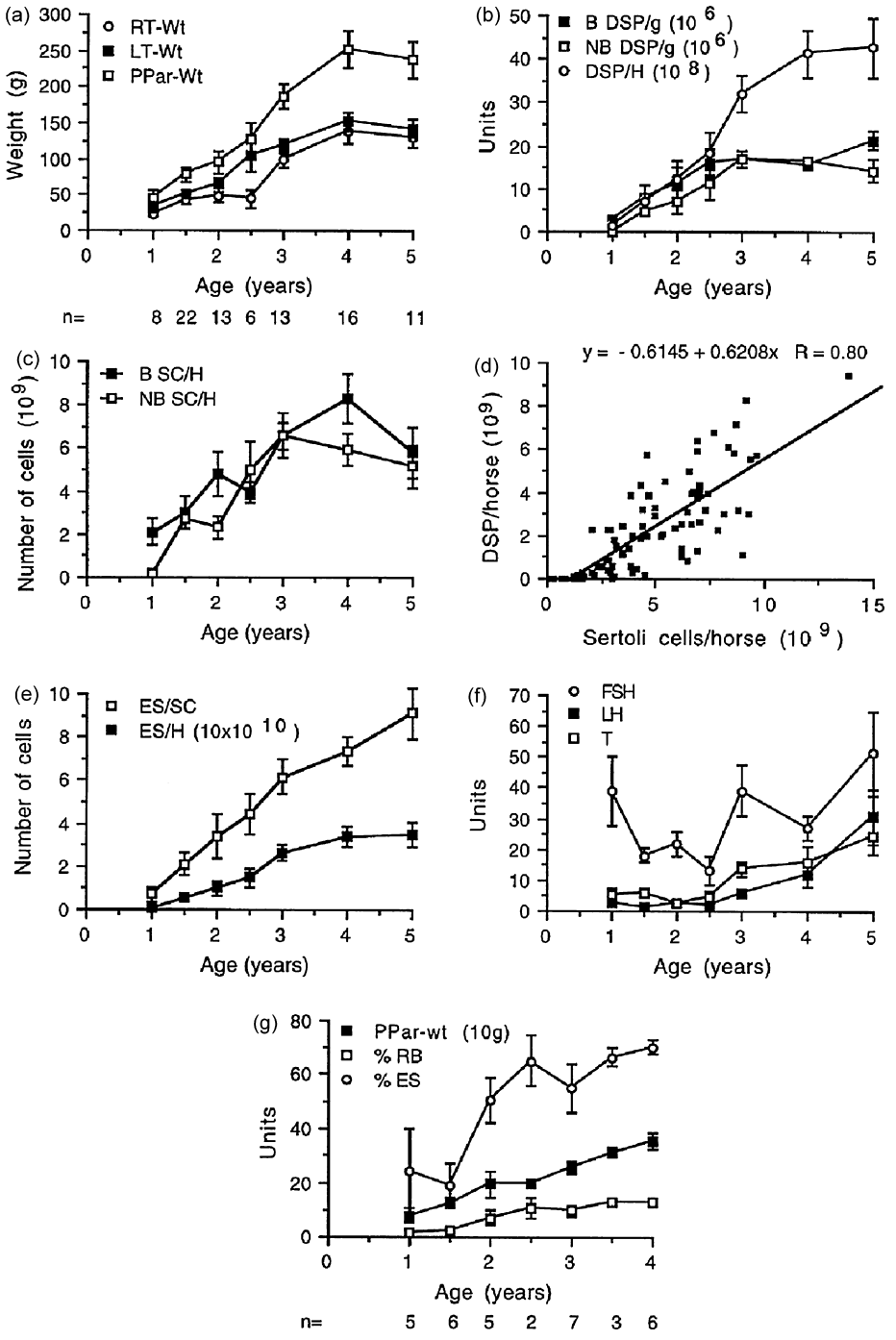


Fig. 14. Pubertal maturation of the horse testis as measured by testicular weight, daily sperm production (DSP), number of Sertoli cells, elongated spermatids per Sertoli cell and serum hormonal concentrations in a group of young and adult

12. Species comparisons in Sertoli cell numbers and function

Compared to other species, the horse has intermediate numbers of Sertoli cells per gram testicular parenchyma and numbers per testis (Fig. 17; Okwun et al., 1996; Johnson et al., 2001). However, the function of individual Sertoli cells, as measured by the ratio of germ cells (spermatocytes and especially round spermatids) per Sertoli cell, was greater in the horse than other species (Fig. 17) even though the horse exhibits considerable germ cell degeneration during spermatogenesis (Fig. 18).

13. Evidence of Sertoli cell proliferation in adults

Returning to the Wikipedia description of Sertoli cells, it states that the Sertoli cell number could be augmented in adults outside the body by experimentation. Hence, adult Sertoli cell numbers are generally stable, but they can be augmented in adults under certain conditions. This statement is supported by several lines of research indicating that the Sertoli cell population may not be numerically stable. Proliferation of Sertoli cells is influenced by FSH (Orth et al., 1988), thyroid hormone and hypothyroidism (Cooke and Meisami, 1991; Van Haaster et al., 1992; Van Haaster et al., 1993; Cooke et al., 1994), testicular transplantation and the number of testes transplanted (Johnson et al., 1996a,b), hypophysectomy (Johnson et al., 1996b), and by removal of DBKO or p27KO in knock-out mice, but it did not alter the age of proliferation (Holsberger et al., 2005). Using delayed growth in testicular transplants (Johnson et al., 1996a) or temporary removal of hormonal stimulation of transplanted testes by hypophysectomy of the host (Johnson et al., 1996b), Sertoli cell numbers increased in rats beyond age 15–20 days of age when rapid Sertoli cell proliferation typically stops (Johnson et al., 1996a).

Transformed SV 40 T antigen alters adult post-mitotic Sertoli cell proliferation (Roberts et al., 1995); however, adult Sertoli cells (in typical research species) appear to be unable to proliferate without cellular transformation (Chaudhary et al., 2005). Along these lines, over-expression of ID1 and ID2 caused post-mitotic, terminally differentiated Sertoli cells (e.g., from 60-day old rats) to re-enter the cell division cycle and increase their numbers (Chaudhary et al., 2005). These authors suggest that a possible mechanism, though currently unknown, would likely include alteration of cell cycle control genes (e.g., p27, p21, c-myc, p16, and Rd are possible candidates; Chaudhary et al., 2005). In non-Sertoli cells systems, ID proteins are considered to be negative regulators of cellular differentiation (Barone et al., 1994; Hara et al., 1994; Moldes et al., 1997); but they are considered to be positive regulators of cellular proliferation (Chaudhary et al., 2005). Is there a natural mechanism to alter cell cycle genes in the breeding horse to account for seasonal differences

horses whose (a) numbers in each age group are indicated. (a) Left (LT-Wt) and right (RT-Wt) testicular weights and paired parenchymal weight (PPara-Wt) increased ($P < 0.01$) with age and sexual development. (b) DSP/gram parenchyma in the breeding season (B DSP/gram) and non-breeding season (NB DSP/gram) as well as DSP per horse (DSP/H) increased ($P < 0.01$) with age. (c) The number of Sertoli cells per horse in the breeding (B SC/H) or non-breeding (NB SC/H) season increased with age. Over all age groups, B SC/H was greater ($P < 0.01$) than NB SC/H. (d) In spite of the lack of complete spermatogenesis in some of the youngest stallions, DSP/horse was directly related ($P < 0.01$) with the number of Sertoli cells per horse (SC/H). (e) Numbers of elongated spermatids per horse (ES/H) and elongated spermatids accommodated by a single Sertoli cell (ES/SC) increased with age. (f) Serum concentrations of FSH, LH and testosterone (T) increased with age. FSH and LH levels are ng/ml of serum, T in 10 pg/ml serum and ITT in 10 μg/g testicular parenchyma. (g) Adult values for the elongated spermatids (%ES) and residual bodies (%RB) are established by 2–5 years of age, as noted by a second group of horses whose numbers for each age group. Paired parenchymal weight (PPar-wt) is reached by 4 years of age. Modified from Johnson et al. (1991b).

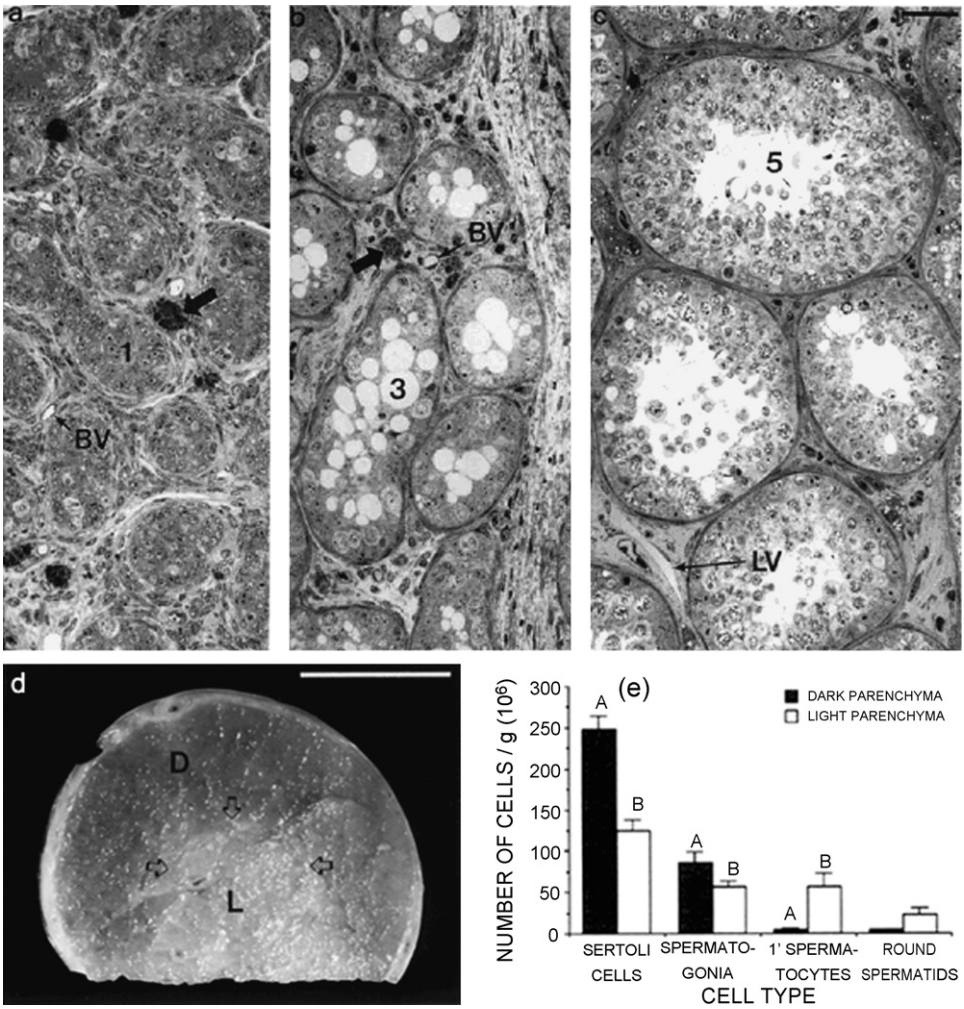


Fig. 15. Histologic view of seminiferous tubules in both (a) dark and (b and c) light parenchyma, gross view of an equine testis revealing both dark and light, differential shading characteristics, and effect of shade of parenchyma on number of Sertoli cells, spermatogonia (gonocytes), primary spermatocytes, and spermatids with spherical nuclei (round). Luminal scores for tubular development are (a) 1 (no lumen); (b) 3 (vacuoles between Sertoli cells); and (c) 5 (complete lumen). Macrophages (arrow), lymphatic vessels (LV), and blood vessels (BV) are indicated. Regions of dark parenchyma (dark) in periphery and light parenchyma (light) in center as well as tunica albuginea (TA) are indicated. This testis was from a 2-year-old horse and measured 2.6 cm in diameter. Light parenchyma had greater number/gram parenchyma of primary spermatocytes. Dark parenchyma had larger number/gram of Sertoli cells and of spermatogonia (gonocytes). Paired means with different superscripts (A and B) are different ($P < 0.05$). Bar lengths equal (a) 50 μm and (b) 5 mm. Modified from Clemmons et al. (1995).

in the Sertoli cell population size of adult horses? Alternatively, are there undifferentiated (mitotic-capable) Sertoli cells in adults (perhaps to a greater degree in the horse than other species)?

In seasonal breeding hamsters, it was shown by co-localization labeling (for proliferation and for Sertoli cell nuclei independently on the same Sertoli cell) that FSH treatment would induce Sertoli cell proliferation in adult hamsters with seasonally regressed testes (Tarulli et al., 2006).

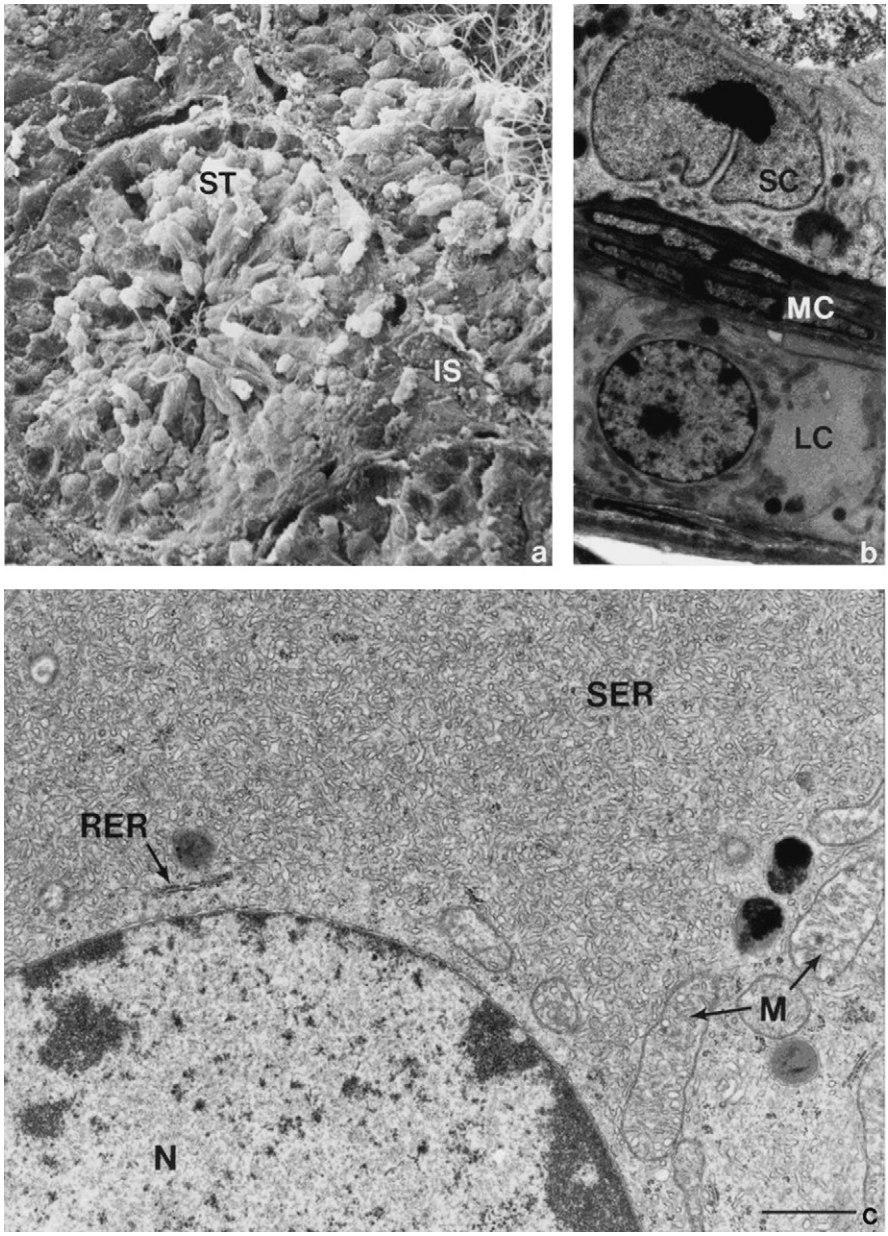


Fig. 16. The adult equine testis 1 μm Epon section viewed by (a) scanning electron microscopy; (b) high-voltage transmission electron microscopy; and (c) conventional transmission electron microscopy. (a) Adult equine testes have larger clusters of Leydig cells in the interstitium (IS) surrounding the seminiferous tubules (ST). (b) Sertoli cells (SC) portray their indented nuclear envelope, euchromatic nucleoplasm with a distinct nucleolus, and proximity to myoid cells (MC) creating the boundary of the seminiferous tubule. Adjacent Leydig cells (LC) display numerous mitochondria, spherical nucleus, and large regions of clear cytoplasm. (c) The clear Leydig cell cytoplasm seen under (b) high-voltage microscopy is identified as an abundance of smooth endoplasmic reticulum (SER). While mitochondria (M) with tubular cristae are relatively numerous, the rough endoplasmic reticulum (RER) and ribosomes in general are sparsely scattered within the cytoplasm. The nucleoplasm of the Leydig cell nucleus (N) is largely euchromatic. Bar length equals (a) 46 μm; (b) 6 μm; and (c) 1 μm. Modified from Johnson and Thompson (1987) and Johnson et al. (2001).

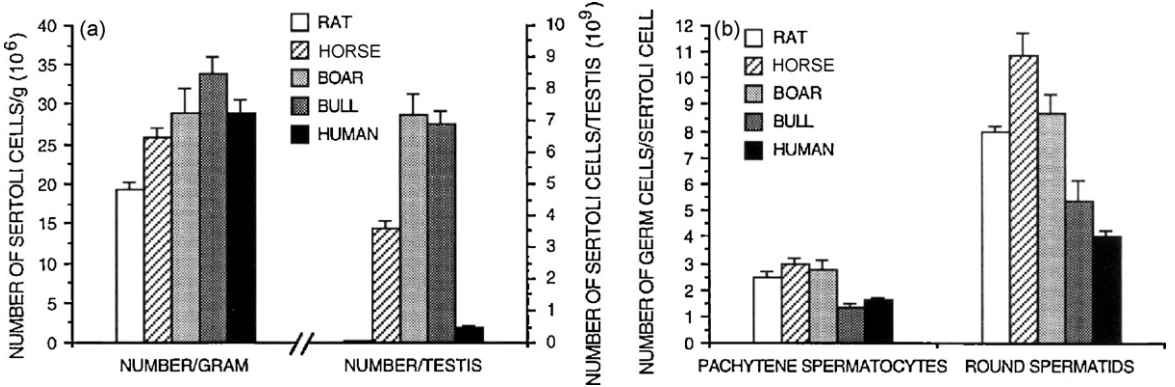


Fig. 17. Species comparison in the number of Sertoli cells and in the germ cell:Sertoli cell ratio. (a) The number of Sertoli cells per gram parenchyma or per testis for the rat, horse, boar, bull, and human; and (b) the number of germ cells per Sertoli cell. The bull and human have fewer germ cells supported by each Sertoli cell than does the rat, horse, or boar (from Johnson (1986b), Johnson et al. (1984c, 1994b, 1999a, 1999b, 2001), Okwun et al. (1996)). Modified from Johnson et al. (2000).

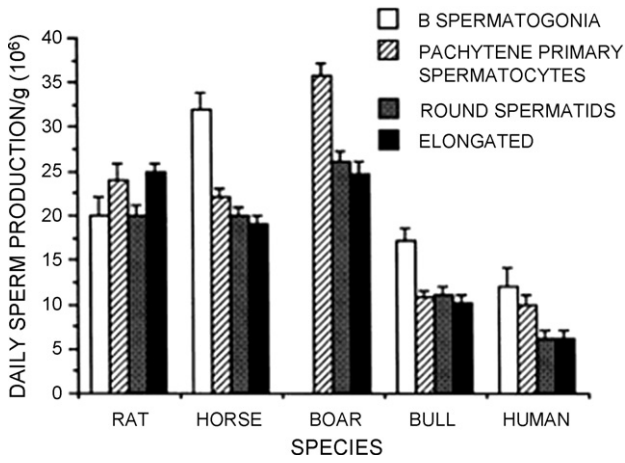


Fig. 18. Efficiency of spermatogenesis in various species based on potential daily sperm production per gram parenchyma at different developmental steps in spermatogenesis of the rat, bull, horse, boar, and human (Johnson et al., 1981, 1984a,b, 1994b; Johnson, 1986b; Okwun et al., 1996). Modified from Johnson et al. (2000).

These data are consistent with the data we produced, but never published. With two of three different quantitative approaches, the number of Sertoli cells was greater in the recrudesced adult hamster testes than dark-regressed adult testes. These data were not published because the findings of all three methods did not agree, as they did in the horse.

14. Implications for other species

Proliferation of Sertoli cells is greatly reduced in the adult compared to the early proliferation rates prior to puberty (Johnson et al., 1996a). The data described herein constitutes compelling evidence that the Sertoli cell population is augmented seasonally in the adult stallion and that Sertoli cell proliferation can be restarted by various treatments in rodents. Hence, proliferation of Sertoli cell number in adults may be a general phenomenon of mammals (albeit a greater extent in the horse). Regardless of the actual mechanism by which Sertoli cells in adults support spermatogenesis year after year, the end product of the Sertoli cell support is a remarkable cell, the spermatozoon.

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