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Replication Needed to Distinguish Alterations in Cell Ratios, the Frequency of Individual Stages of the
Cycle of the Seminiferous Epithelium, or the Appearance of Abnormalities in the Testes of Rodents,
Rabbits or Humans¹

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1 *Abstract*

2 The typical levels of inherent variability among untreated sexually mature rodents, rabbits or men were
3 determined for several endpoints used to characterize spermatogenesis. The latter included germ
4 cell:germ cell and germ cell:Sertoli cell ratios, the frequency of specific stages of the cycle of the
5 seminiferous epithelium, and the appearance of a variety of testicular abnormalities. Based on this
6 variability, the number of replicates needed to provide future experiments of a predictable power and
7 sensitivity was estimated. Replication requirements differed greatly as a function of species and/or
8 among specific endpoints within a single species. In addition, the replication needed to provide robust
9 experiments was substantially greater than that employed in most investigations. The application of these
10 findings during the design and interpretation of future experiments was discussed.

11 (Key words: Testis, spermatogenesis, experimental design)

12

13 *Introduction*

14 Spermatogenesis involves a lengthy, complex process that is of tremendous importance to male fertility.
15 It is also quite susceptible to disruption by a variety of agents (Amann and Berndtson, 1986; Berndtson
16 and Clegg, 1992). Accordingly, numerous studies have been undertaken to increase basic understanding
17 of this process, and there is an ongoing need to assess spermatogenic responses to known or suspected
18 environmental toxins, proposed new human or animal drugs, or other factors. A variety of endpoints has
19 been utilized for such purposes. One that has proven quite useful is the numerical ratio of various cell
20 types to each other. This endpoint has several applications. First, a doubling in the number of daughter
21 cells has been used to identify the number and timing of cell divisions when establishing models for the
22 kinetics of spermatogenesis (Berndtson and Desjardins, 1974a; Bilaspuri and Guraya, 1984; Clermont,
23 1954, 1962, 1972; Clermont and Antar, 1973; Clermont and Leblond, 1959, and others). In addition,
24 comparisons of actual vs theoretical yields of specific germ cells have permitted the efficiency of specific
25 cell divisions to be quantified (e.g., Berndtson, 1977; Berndtson and Igboeli, 1989; Castro et al., 2002;
26 Huckins, 1978). Similar comparisons among the ratio of young vs old cells of the same type at different

1 points during spermatogenesis provide insight into the magnitude of cell losses during the course of cell
2 maturation (Johnson et al., 1983). Differences between specific germ cell ratios in control vs treated
3 subjects allows quantification of treatment effects on cell losses and/or spermatogenic efficiency (Amann
4 and Berndtson, 1986; Berndtson, 1977; Berndtson and Foote, 1997; Russell et al., 1990). Finally, the
5 number of germ cells per Sertoli cell is the basis for one method for quantifying relative rates of sperm
6 production (Berndtson, 1977; Clermont and Morgentaler, 1955), and such ratios have been used to assess
7 the functional capacity or workload of individual Sertoli cells (Berndtson et al., 1987a, 1987b; Berndtson
8 and Igboeli, 1989; Berndtson and Jones, 1989; Berndtson and Thompson, 1990b; Castro et al., 2002;
9 Johnson, 1986; Jones and Berndtson, 1986; Russell and Peterson, 1984; Thompson and Berndtson, 1993).

10

11 Another useful endpoint is the frequency of a specific stage(s) of the cycle of the seminiferous epithelium.
12 Although spermatogenesis involves a continuum of events, researchers often find it useful to characterize
13 this process via the identification of stages, which constitute manmade divisions of this process. If one
14 could observe a single cross section of a seminiferous tubule over time, one would detect changes in its
15 appearance. Cell divisions and the progressive development of specific cells would produce a number of
16 distinct combinations of cells or cellular associations. These cellular associations would continue to
17 change until the seminiferous epithelium once again appeared as it did initially. The interval from the
18 appearance of one cellular association until its reappearance constitutes one cycle of the seminiferous
19 epithelium, and the different cellular associations that have been identified have been designated as stages
20 of the cycle of the seminiferous epithelium (Berndtson, 1977; Clermont, 1972).

21

22 One reason that staging is valuable is because some cells divide or are only present during specific stages.
23 If one wished, for example, to determine the number of germ cells of a particular type per seminiferous
24 tubular cross section or the ratios of one type of germ cell to another, it would be important to select
25 tubular cross sections at a stage(s) containing the cell(s) of interest. Several other applications of stage
26 frequency data are based on the expectation that, with adequate sampling, the frequency at which one

1 observed tubular cross sections at a particular stage would equal its relative duration. If the duration of
2 the cycle of the seminiferous epithelium is known, the duration of a specific stage can be determined. For
3 example, if one cycle of the seminiferous epithelium required 13.5 days and the frequency of stage I was
4 10%, one would know that stage I required 10% of the cycle, or 1.35 days. Knowledge of the duration of
5 individual stages has been useful when examining the progression of adverse treatment effects during
6 periods of exposure to antispermatogenic agents and/or to predict the time course for recovery after the
7 cessation of treatment (Foote and Berndtson, 1992). The duration of individual stages has also been
8 useful in determining time divisors that enable estimation of daily sperm production rates from data
9 generated via volume density or homogenization techniques (Amann and Almquist, 1962; Johnson et al.,
10 1980, 1981; Robb et al., 1978).

11
12 The frequency of the stages can also be a valuable endpoint in itself. Specifically, differences in the
13 frequency of individual stages between treated vs untreated subjects could provide evidence of treatment
14 effects on the timing of certain spermatogenic events. Alternatively, where extremely harsh treatments
15 result in the complete absence of germ cells of a particular type, the composition of some cellular
16 associations would be altered. This might cause some stages to appear to be of a different stage. For
17 example, both stages VIII and IX of normal male rats contain type A spermatogonia, young and old
18 primary spermatocytes and spherical spermatids (Leblond and Clermont, 1952). Stage VIII also contains
19 elongated spermatids, which distinctively line the lumen of the tubule prior to spermiation. Such
20 spermatids are absent during stage IX. In the absence of elongated spermatids, as might result from
21 exposure to a toxic agent, stage IX tubules would appear very similar to those of stage VIII. Unless these
22 stages could be distinguished by features of the remaining cells, the frequency of stages might appear
23 different because of incorrect stage identification. Consequently, the apparent frequency or duration of
24 certain stages could be altered even if the timing of spermatogenic events, per se, was unchanged. While
25 investigators need to remain cognizant of such fundamentally different mechanisms by which stage

1 frequencies might appear altered, it is clear that changes in apparent stage frequencies would, at a
2 minimum, indicate that some alteration in spermatogenesis had occurred.

3

4 Cellular degeneration and the presence of other abnormalities represent additional endpoints of some
5 interest. Such features can be observed routinely in normal individuals (Barr et al., 1971; Berndtson and
6 Desjardins, 1974a; Clermont, 1962; Hochereau-de Reviers, 1970; Huckins, 1978; Morton et al., 1986;
7 Roosen-Runge and Leik, 1968; Russell and Clermont, 1977; Swierstra, 1966; Wing and Christensen,
8 1982). However, an increase or unusually large number of abnormalities would provide evidence of
9 spermatogenic disruption, whether naturally occurring or treatment induced. Where identification of the
10 abnormal cell type(s) is possible, one can determine which cell(s) is the target of the causative factor.

11

12 The aforementioned endpoints have many applications, and some of the information that each provides
13 can be quite unique. However, the reliability of the information resulting from their application is an
14 important consideration. Inherent among animal variability is a characteristic of all living things, and
15 such variability is also apparent among the testes of normal, sexually-mature mammals. Because of this,
16 it is important to provide adequate replication when performing experiments to assess testicular function.

17 Indeed, the likelihood that an experiment will be capable of detecting an actual treatment response or
18 difference of some magnitude is a direct function of the inherent variability in the endpoint chosen for
19 evaluation and the number of replicates used per treatment group. The inherent variability associated
20 with a number of reproductive endpoints has been characterized, and this information has been used to

21 identify the number of replicates needed to provide future experiments of predictable power and

22 sensitivity (Berndtson, 1989, 1990, 2008; Berndtson and Clegg, 1992; Berndtson et al., 1989, 1997;

23 Berndtson and Thompson, 1990a, and others). As used herein, power denotes the probability that an

24 experiment will be capable of detecting a statistically significant treatment response, if there actually is a
25 treatment effect, and sensitivity represents the minimal size of the response that would be detectable.

26 Similar information is not readily available for use in planning experiments involving cell:cell ratios, the

1 frequency of stages or the incidence of testicular abnormalities as experimental endpoints. The purpose
2 of this investigation was to characterize the inherent variability in these endpoints among rodents, rabbits
3 and humans, and to provide estimates of the number of replicates needed to provide future experiments of
4 predictable power and sensitivity when these endpoints and species are to be used.

5

6 *Methods*

7 The coefficients of variability (CV, standard deviation expressed as a percentage of the mean) associated
8 with the endpoints of interest were determined from data identified within the published literature or,
9 alternatively, from raw data sets possessed by the author. Only data for untreated, control populations
10 were used for this study. Since investigators do not normally publish CV values per se, it was usually
11 necessary to calculate these from other data. This was only possible when a publication contained the
12 mean \pm S.D., or the mean, S.E. of the mean and the number of replicates (n) in the mean. Where the latter
13 information was available, the S.D. (s) was calculated by entering the S.E. and values of n into the
14 equation: $S.E. = \sqrt{s^2/n}$ (Berndtson, 1991). CV's were identified for each endpoint for rodents, rabbits and
15 humans. To avoid the potential for introducing personal biases, CV's were determined for each study that
16 the author reviewed that contained the requisite data, and every value was reported herein. Since the
17 resulting values were based on sample populations of differing size, the typical CV associated with each
18 endpoint was determined as the weighted mean for all studies. However, since the primary objective was
19 to characterize inherent variability and replication requirements for studies with breeding-age males, data
20 for prepubertal or senescent males were excluded for calculation of the weighted mean. In such instances,
21 any exclusion(s) of specific data was cited. The number of replicates required for future studies of
22 predicted power and sensitivity was identified from tables constructed for that purpose (Berndtson, 1991).
23 To use these tables, one must provide an estimate of the anticipated CV for the experimental population to
24 be examined. The "typical" CV's determined herein as described previously were used for that purpose.
25 The investigator must also choose a power and sensitivity combination that is appropriate for the
26 experiment being planned. With this information, the required number of replicates can be read directly

1 or extrapolated from the appropriate reference table. For this study, replication requirements were
2 determined for studies that would provide a power of 80 or 90% and the sensitivity enabling detection of
3 10, 20 or 30% changes in each endpoint due to treatment with statistical significance at $P \leq 0.05$.

4

5 *Results*

6 The CV's associated with the number of spermatogonia per Sertoli cell are presented in Table 1. Several
7 types of spermatogonia have been identified in most species (e.g., hamster - Clermont, 1954; human -
8 Heller and Clermont, 1964; mouse - Oakberg, 1956; rabbit - Swierstra and Foote, 1963). By classical
9 definition, those possessing nucleoplasm having a smooth, coarse or intermediate texture have been
10 designated as Types A, B and intermediate (I), respectively. However, further morphological features and
11 kinetic data have enabled the identification of a variety of spermatogonial subtypes (e.g., Types A₁, A₂,
12 A₃, etc. in the rat). For some investigations (e.g., to establish the kinetics of spermatogenesis), it is
13 important to obtain separate counts for each subtype, whereas for others such distinctions may be
14 unnecessary. Thus, the data in Table 1 include several CV's based on the major types of spermatogonia
15 (e.g., Type A) and others based on specific subtypes or groupings of subtypes (e.g., Types A₁, A₂, etc.).
16 For one study with the mouse (Huckins and Oakberg, 1978b), all of the spermatogonia present within
17 each stage were pooled, without attempting to distinguish among those of types A, I or B, respectively.
18 Since the use of such broad groupings is uncommon, the CV's identified within that study have been
19 presented but will not be given further consideration herein.

20

21 For the rodent, the CV's for type A spermatogonia and the various type A spermatogonial subtypes
22 ranged from 7.2 to 65.4%. This is a very wide range, but as noted elsewhere for other endpoints, CV's
23 tend to be greater for those cells that are present in limited numbers, for components of the testis
24 occupying a smaller proportion of the tissue, etc. (Berndtson, 1989; 2008). Not surprisingly, the largest
25 of the foregoing reported values (65.4%) was associated with a population of undifferentiated type A
26 spermatogonia at stage III in the mouse. Undifferentiated spermatogonia are only present in very small

1 numbers in sexually-mature males. When values for undifferentiated type A spermatogonia were
2 excluded, the CV's for the remaining type A populations ranged from 8.5 to 25.6%, with a weighted
3 mean value of 18.8% (Table 8). Only a single value of 9.5% was identified for the rabbit (Table 1). This
4 value, which was based on type A spermatogonia irrespective of subtype, was within the range but lower
5 than most values reported for rodents. As the only value obtained for the rabbit, it was listed as the typical
6 CV for this endpoint within Table 8. The corresponding CV's for dark and pale type A spermatogonia in
7 one study with the human equaled 23.6 to 23.4%, respectively, for a weighted mean of 23.5% (Table 8).

8
9 In the mouse, intermediate (type I) spermatogonia arise from the division of the most developmentally-
10 advanced type A spermatogonia, and they in turn divide to produce type B spermatogonia (Oakberg,
11 1956). Type I and type B spermatogonia are not present within all stages of the cycle of the seminiferous
12 epithelium. However, since each spermatogonial division has the theoretical potential to yield a doubling
13 in the number of resulting daughter cells, the numbers of type I spermatogonia should exceed the
14 corresponding numbers of type A spermatogonia present during the preceding stage(s). Similarly, the
15 numbers of type B spermatogonia should exceed the numbers of type I spermatogonia. The average
16 numbers of type A, I and B spermatogonia per Sertoli cell usually reflect these differences. Because of
17 these numerical differences, it is not surprising that the CV's for type I and type B spermatogonia per
18 Sertoli cell in the mouse were smaller than the corresponding values for type A spermatogonia, and
19 equaled 6.1 and 3.2%, respectively (Table 1). Because these were the only CV's identified for these cell
20 types, they are cited as the typical CV's within Table 8. The one CV identified for type B spermatogonia
21 per Sertoli cell in the human equaled 23.2%, which was assumed to be the typical CV for this cell type
22 (Table 8).

23
24 Spermatocytes are relatively abundant in the testes of normal, sexually-mature males. Therefore, it is not
25 surprising that the CV's associated with the spermatocyte:Sertoli cell ratio (Table 2) were generally lower
26 than the corresponding values for the less numerous type A spermatogonia (Table 1). For primary

1 spermatocytes (preleptotene, leptotene, zygotene, pachytene and/or diplotene), individual values ranged
2 from 5.4 to 16.4% for rats, 8.2 to 24.7% for hamsters, 12.7 to 14.8% for rabbits, and from 21.5 to 32.3%
3 for humans. The weighted mean values for rodents, rabbits and humans equaled 10.4, 13.5 and 28.1%,
4 respectively (Table 8). Only one value, equaling 11.5% for the rat, could be identified to serve as the
5 typical CV (Table 8) for the secondary spermatocyte:Sertoli cell ratio.

6
7 The CV's associated with the numbers of spermatids per Sertoli cell are presented in Table 3. Values for
8 round spermatids (including step 1-10 spermatids in one study) in rodents ranged from 6.8 to 17.5%. The
9 largest of these values was associated with 60-day-old rats, for which slightly greater among-animal
10 variability might be anticipated due to differential rates of post-pubertal testicular development. When
11 that value was excluded, the weighted mean based on all of the remaining studies equaled 8.7% (Table 8).
12 The corresponding CV for elongated spermatids was slightly greater, with a weighted mean of 13.8%, but
13 this value was based on a relatively small sample of animals. Only single CV's of 13.7% (regarded as
14 typical for this endpoint, Table 8) and 9.4% were identified for round and elongated spermatids per
15 Sertoli cell, respectively, in rabbits. The CV's associated with round spermatids per Sertoli cell in three
16 studies with the human ranged from 23.3 to 30.4%, with a weighted mean of 26.9% (Table 8).

17
18 Data from which the CV's associated with the ratio between the number of germ cells of one type to those
19 for another (i.e., the germ cell:germ cell ratio) could be determined were limited, but the values that were
20 identified are summarized in Table 4. For the rat, the number of round spermatids per spermatogonium in
21 stage VII (Leblond and Clermont, 1952) seminiferous tubular cross sections was associated with CV's of
22 18.3 to 22.2 among 60-, 150- and 240-day-old males, with a weighted mean value of 20.4% (Table 8).
23 For the hamster, the CV's associated with the ratios (yields) of preleptotene primary spermatocytes per
24 spermatogonium (PL:A), pachytene primary spermatocytes per preleptotene primary spermatocyte
25 (PACH:PL), and round step 7 spermatids per pachytene primary spermatocyte (Sp7:PACH) in stage VII
26 seminiferous tubular cross sections were 22.5, 4.5 and 6.2%, respectively. Although slightly different

1 germ cell populations were examined, the CV of 4.5 for the PACH:PL ratio in hamsters was relatively
2 similar to the value of 1.8% for the ratio of older (PACH + DIP) vs younger (PL + L + Z) primary
3 spermatocytes in the rat. The weighted mean value of 3.3% for these two studies would seem to represent
4 a reasonable estimate of typical variability for the ratio of young to old primary spermatocytes in
5 untreated rodents (Table 8). Similarly, the CV of 9.4% for the ratio or yield of round step 1-10
6 spermatids per older (PACH + DIP) primary spermatocyte in the rat was similar to the value of 6.2% for
7 the step 7 spermatid:pachytene primary spermatocyte ratio in the hamster. The weighted mean CV for the
8 round spermatid:primary spermatocyte ratio based on these two studies equaled 7.6% (Table 8). This
9 value was nearly identical to the one CV of 7.8% associated with the secondary spermatocyte:old primary
10 spermatocyte (PACH + DIP) observed and assumed to be typical (Table 8) for the rat. Only limited
11 comparable information could be identified for the human, for which the CV's associated with the
12 spermatid:spermatocyte ratio ranged from 18.9 to 27.8% (weighted mean = 25.0%, Table 8). CV's
13 associated with germ cell:germ cell ratios could not be identified for the rabbit.

14

15 Due to the sequential distribution of cellular associations along the length of individual seminiferous
16 tubules (Perey et al., 1961), round seminiferous tubular cross sections of most mammalian testes contain
17 only a single cellular association or stage of the cycle of the seminiferous epithelium. Although stages of
18 the cycle of the seminiferous epithelium have been identified for the human, specific cellular associations
19 (or "stages") are confined to small, discrete patches distributed along the seminiferous tubules of men
20 (Heller and Clermont, 1964). The latter pattern of distribution has precluded the traditional classification
21 of seminiferous tubular cross sections as belonging to a particular "stage". Therefore, the CV's
22 associated with the frequency of individual stages could only be determined for the rat and rabbit. Those
23 values are presented in Table 5. These data were based on staging by the acrosomal system of Leblond
24 and Clermont (1952) in the rat, which enables identification of 14 distinct stages. Staging of the rabbit
25 seminiferous epithelium was based on the tubular morphology system of Swierstra and Foote (1963),
26 which permitted identification of eight different stages. The CV's associated with a single stage of the

1 cycle in the rat (Table 5) ranged from a low of 7.0% for stage VII in the study by Van Beek and Meistrich
2 (1990) to a high of 80.2% for stage XIII in the study by Bartlett et al. (1990). The corresponding
3 weighted mean CV's associated with the frequency of stages I to XIV based on these three studies
4 equaled 18.9, 24.1, 29.7, 25.4, 17.1, 21.9, 9.0, 23.3, 32.0, 22.2, 35.2, 10.9, 43.4, and 29.0, respectively.
5 Because values differed so markedly among individual stages, both the range of 9.0 to 43.4% for
6 individual stages and a mean value of 24.4%, based on all 14 stages, are presented within the summary of
7 typical CV's given in Table 8. Similarly, the CV's associated with the frequency of individual stages in
8 the rabbit ranged from a low of 6.0% for Stage I in the study of Swierstra and Foote (1963) to a high of
9 35.8% for Stage V in the study of Amann and Lambiase (1969). When averaged over all three
10 investigations, the CV's associated with Stages I to VIII equaled 9.9, 10.2, 21.3, 17.6, 33.0, 18.2, 18.2,
11 and 23.1%, respectively (range: 9.9 - 33%, Table 8). The mean based on all eight stages equaled 18.9%
12 (Table 8).

13
14 Table 6 contains the CV's identified for the numbers of degenerating germ cells per 100 Sertoli cells (or
15 Sertoli nucleoli) in the rat, and for rates of cellular attrition during the post-prophase of meiosis in the
16 human. For the rat, the CV's differed by several orders of magnitude due to the specific type of germ cell
17 examined and/or among individual studies. Six of ten CV's exceeded 100%, with one high value of
18 316%. Potential explanations for these large and sometimes inconsistent values are discussed
19 subsequently. However, such inconsistencies precluded the identification of one single level of
20 variability that one might consider as typical or reliable for estimating the power and sensitivity of future
21 investigations. The range of CV's identified herein (Table 6) will be considered for the latter purpose,
22 and is presented among the typical values summarized in Table 8. The CV's associated with cellular
23 attrition during the progression of primary spermatocytes to round spermatids ranged from 37.6 to 51.7%
24 in the human, for which the weighted mean CV was 44.4% (Table 8).

25

1 The CV's associated with the incidence of spermatid giant cells, hypospermatogenesis, spermatogonial
2 swelling and cytoplasmic vacuoles in rabbits of different ages are depicted in Table 7. For males greater
3 than 52 weeks of age, the CV's associated with those variables and reported as typical CV's (Table 8)
4 were 112.0, 173.2, 28.7 and 37.1%, respectively.

5
6 The typical CV's identified herein have been summarized in Table 8. Those values, in turn, were used to
7 estimate the approximate number of replicates needed to provide future experiments of 80 or 90% power
8 for detecting statistically significant treatment responses of 10, 20 or 30% at $P \leq 0.05$. Those replication
9 requirements are summarized in Tables 9-11.

10

11 *Discussion*

12 Despite extensive literature on spermatogenesis, the characterization of the inherent variability
13 associated with some endpoints proved quite challenging. In many instances, means were reported,
14 but the additional data needed to calculate CV's was unavailable. Identifying the variability
15 associated with the ratio of one cell type to another proved especially difficult. In most studies,
16 researchers first determined the group mean for the number of cells of each type per unit of tissue.
17 Those means, rather than the values for each male, were used to calculate the ratios between one cell
18 type and another. In such instances, it was not possible to characterize the variability among the
19 individual males in the sample population. Therefore, to expand the present database, the author
20 found it necessary to calculate and include several CV's from sets of raw data in his possession.
21 Where this was done, the reference for the original studies from which these data were taken has been
22 cited.

23

24 Different approaches have been used to quantify daily sperm production or to determine the number of
25 cells of a specific type per seminiferous tubular cross section, per gram of tissue, or per testis (Berndtson,
26 1977). Of these, only two have been used extensively to determine cell:cell ratios. One is the classical

1 approach of Clermont and Morgentaler (1955) by which one performs direct counts of the number of
2 germ cells and Sertoli cells per round seminiferous tubular cross section at a given stage of the cycle of
3 the seminiferous epithelium. The second is the volume density approach, in which one employs a system
4 of random “hits” of a pointer to determine the percentage of the testis occupied by specific nuclei. The
5 total volume of those nuclei is calculated, and the total number of cells of that type is estimated by
6 dividing the total volume of those nuclei by the volume of a single nucleus (Berndtson, 1977). A third,
7 and more specialized approach, has been to examine the number of germ cells embedded in the apex of a
8 single Sertoli cell via reconstruction of serial sections examined by electron microscopy (Johnson, 1986;
9 Russell and Peterson, 1984). The fundamental basis for these approaches and a critical examination of
10 the technical assumptions required with each have been described elsewhere (Berndtson, submitted
11 manuscript). The inherent variability and replication requirements associated with many endpoints
12 derived with these methods have also been reported (Berndtson, 1989, 1990, 2008; Berndtson and Clegg,
13 1992; Berndtson et al., 1989, 1997; Berndtson and Thompson, 1990a, and others). Despite their relative
14 merits and shortcomings, each would appear to provide data that are both accurate and reliable when
15 applied to assess spermatogenesis in normal males. Accordingly, the CV's associated with cell ratios
16 have been pooled across assessment methods for presentation and discussion herein.

17

18 The CV's associated with numbers of germ cells per Sertoli cell included a very wide range of values
19 (Tables 1-3). Although the cause(s) may not be understood in their entirety, some of this variability
20 does have a probable or obvious explanation. First, the among-animal variability in the ratio of type A
21 spermatogonia (Table 1), preleptotene and pachytene primary spermatocytes (Table 2) and round
22 spermatids (Table 3) per Sertoli cell was greater for 60-day-old rats than among counterparts aged
23 150 or 240 days. Potential differences in the rate of testicular maturation could account for the
24 greater variability among younger rats during the immediate post-pubertal period. Similarly, the
25 challenge in obtaining testicular tissues from humans has undoubtedly necessitated the use of subjects
26 of different ages, unknown environmental backgrounds and much less genetic homogeneity in

1 comparison to that for the more highly-inbred laboratory species. These factors probably contribute
2 to the greater variation among men. Differences in the size of the reported CV's (Tables 1-3) are also
3 apparent among the specific populations of cells being evaluated within the same study. In the
4 authors experience (Berndtson, In Prep), CV's tend to be larger for those cells that are less prevalent
5 and for specific tissue components that occupy only a small portion of the testis, and such trends are
6 generally apparent among the typical CV's identified herein. The reason for this relationship can be
7 readily appreciated by simple illustration, as follows. Imagine that the mean percentage of the
8 testicular parenchyma occupied by seminiferous tubules and interstitial tissue for a group of males
9 equaled 90 and 10%, respectively. Since these two components must collectively constitute 100% of
10 the testicular parenchyma, any deviation from this mean would cause a proportionately larger change
11 for the smaller component. For example, a male in which these two components occupied 81 and
12 19% of the testis, respectively, would deviate from the hypothetical mean by possessing 10% less
13 seminiferous tubular tissue and 90% more interstitial tissue. Accordingly, a much larger CV should
14 be expected for the smaller of these two components.

15
16 In most studies, germ cells were quantified at a specific stage of the cycle. However, whereas some
17 investigators simply counted all of the spermatogonia that were present (e.g., per seminiferous tubular
18 cross section), others performed separate counts for each of the spermatogonial types or subtypes.
19 Because some cells are present only in very low numbers, one would expect those cell types to be
20 accompanied by larger CV's. Such a relationship between the numerical size of a population and its
21 relative CV is apparent by comparing data in Tables 1 – 3. Although the number of cells of each type
22 is not stated, each successive mitotic or meiotic division has the potential to produce a theoretical
23 doubling in the number of daughter cells. Thus, spermatogonia should be less numerous than primary
24 or secondary spermatocytes, and spermatids should be the most numerous cells within the
25 seminiferous epithelium. The CV's for many of the spermatogonial populations were often in the
26 20's, while those for the spermatocytes and spermatids were more frequently in the teens or single

1 digits. Also undifferentiated spermatogonia are typically the least numerous spermatogonia in the
2 testes of sexually mature males, and the CV's associated with those cells tended to be very large
3 (Table 1).

4
5 Another factor that may have contributed to the variation among CV's in different studies is the
6 sampling intensity employed. For example, due to rigorous technical requirements, the direct
7 enumeration of germ cells embedded in the Sertoli cell apex has often been limited to a small number
8 of Sertoli cells per male. Unless the number of germ cells per Sertoli cell is highly consistent within a
9 single male, the examination of only a few Sertoli cells could not be expected to yield an accurate
10 characterization of each male (Berndtson et al., 1989; Berndtson and Thompson, 1990a). Similarly,
11 some studies involved a relatively small number of experimental subjects. Thus, some of the
12 apparent variability among males observed in Tables 1-3 might be attributable to limited replication
13 and/or sampling intensity.

14
15 The variability associated with the ratio of old to young primary spermatocytes in the rodent was
16 quite small (CV=3.3%, Table 8). Accordingly, it is apparent that rates of attrition during the
17 progressive development of the primary spermatocytes are quite similar among normal, sexually-
18 mature rodents. Although the CV's were still relatively small, slightly greater among-male variability
19 was recorded (Table 8) for the progression of events by which primary spermatocytes produce
20 secondary spermatocytes (CV=7.8%) or round spermatids (CV=7.6%). A larger CV of 20.4% was
21 noted for the ratio of round spermatids to type A spermatogonia (Table 8). This might be anticipated,
22 since the progression from spermatogonia to round spermatids would encompass a number of mitotic
23 and meiotic divisions extending over multiple cycles of the seminiferous epithelium. Since
24 comparable data for other species was either limited or unavailable, the potential for or magnitude of
25 species differences remain a matter of conjecture. However, the one CV for the human of 25.0% for
26 the ratio of round spermatids per primary spermatocyte was approximately three times greater than

1 the corresponding CV in the rodent. The greater variability associated with the human was consistent
2 with that noted for most other endpoints.

3
4 Data presented for the rat in Table 5 were based on the acrosomal system for identifying stages
5 developed by Leblond and Clermont (1952), which enables identification of 14 distinct stages,
6 whereas the 8-stage tubular morphology system of classification of Swierstra and Foote (1963) was
7 employed for the rabbit. Because increases in the number of identifiable stages will, in general,
8 decrease the frequency of most individual stages, one would expect to encounter larger CV's with the
9 14- vs 8-stage systems. In general, the CV's for most stages could be considered to be moderate to
10 large, and it is clear that the values for specific stages sometimes differed substantially among the
11 different studies.

12
13 It is interesting to note that the study by Hess et al. (1990) with rats involved a much greater number
14 of rats and observations per rat than were used in the other investigations with this species. This
15 greater replication and sampling intensity (i.e., number of observations per male) may have
16 contributed to the absence of CV's for individual stages of the great magnitude noted in each of the
17 other studies (e.g., 80.2%, 63.6%, etc.). Nonetheless, the CV's associated with most stages, even in
18 the study of Hess et al. (1990), were in the teens to 30 percentile range. Corresponding values appear
19 to be slightly lower for the rabbit, as would be expected at least in part because only eight different
20 stages were distinguished.

21
22 At one time, it was thought that the length of one cycle of the seminiferous epithelium, the duration of
23 spermatogenesis (time required to produce a sperm cell from the least differentiated spermatogonium)
24 and the timing of various spermatogenic events was constant and rigidly controlled (Clermont, 1972).
25 If so, the frequency of stages should also be identical and constant among all males. However,
26 moderate to large differences in the frequency of individual stages was apparent even among normal,

1 untreated males (Table 5). When using this endpoint, the importance of taking a sufficient number of
2 observations per male and of including an adequate number of males per treatment group should be
3 apparent. This issue also has important implications for the validity of time divisors used to estimate
4 daily sperm production. Time divisors are intended to represent the life span of specific testicular
5 germ cells (i.e., the length of time during which specific cells are present within the testis). For
6 example, one might determine the total number of round spermatids per testis or per gram of
7 testicular parenchyma. An estimate of DSP could then be derived by dividing the total number of
8 such cells by the appropriate time divisor. Since the frequency of a given stage is directly
9 proportional to its duration, most time divisors are determined from stage frequency data and
10 knowledge of the duration of one cycle of the seminiferous epithelium. Due to inherent variability in
11 stage frequencies (Table 5), a time divisor derived by examination of a relatively large number of
12 males would be expected to yield a reliable estimate of the mean for a given population, from which
13 the actual time divisor for some individual males might deviate. In addition to the inherent variability
14 noted herein, recent evidence has documented the potential for arrested development and stage
15 synchronization (i.e., most tubular cross sections containing the same stage) in response to specific
16 experimental treatments (Bartlett et al., 1990; Morales and Griswold, 1987; VanBeek and Meistrich,
17 1990). The implications of these findings on the validity of time divisors have been described
18 elsewhere at length (Berndtson, submitted manuscript).

19
20 Many of the CV's associated with rates of germ cell degeneration were huge, with a value for Step 7
21 spermatids in one study with rats determined to equal 316%. Even the smallest recorded CV of
22 21.8% was of moderate size. Collectively, these data (Table 6) reinforce how a characteristic that is
23 normally observed only at a low frequency will typically be characterized by a large CV. This
24 relationship was further evident from the CV's for a number of different abnormalities observed in
25 one study with the rabbit (Table 7).

26

1 The importance of considering inherent variability while designing and interpreting results of a given
2 experiment should be apparent from the data in Tables 9-11. Whereas the use of only 4 rats per
3 treatment group would be expected to provide an experiment with a 90% probability (i.e., power) for
4 detecting an actual 10% change in the number of type B spermatogonia per Sertoli cell, 89 rats would
5 be required to provide equivalent power for detecting an alteration of similar magnitude in the ratio of
6 round spermatids per type A spermatogonium. Indeed, the number of replicates needed to provide
7 experiments of equivalent power and sensitivity differed by several orders of magnitude among the
8 various endpoints one might wish to utilize, and replication requirements also differed for the same
9 endpoint among species.

10

11 Although a relatively large number of replicates are needed to yield powerful and sensitive
12 experiments for assessing treatment effects via most of the endpoints examined herein, many of the
13 studies reviewed for this investigation involved a relatively small number of replicates (see Tables 1-
14 4 and 6). Such investigations are clearly of very limited power and sensitivity. This conclusion is not
15 intended to imply that certain studies were poorly designed or inadequately replicated. Indeed,
16 decisions about the number of replicates to use in a given study will continue to require consideration
17 of cost and many other factors (Berndtson, 1991, 2008). However, it is important to remain cognizant
18 of the relationship between levels of replication and the power and sensitivity of each experiment. In
19 that regard, it is especially important to realize that most statistical analyses focus only on type I error
20 (i.e., the probability of error when declaring the existence of a treatment effect). In the absence of
21 statistical significance, typically at $P \leq 0.05$, many investigators conclude inappropriately that a
22 treatment(s) was without effect. The failure to detect a treatment effect at $P \leq 0.05$ simply indicates
23 that one cannot say with 95% certainty that the treatment has had an effect. It does not assure the
24 absence of a treatment effect, especially if the capacity of the experiment to detect a treatment effect
25 was very limited. Indeed, if one did not detect a treatment response in an experiment possessing 95%
26 power for detecting a 5% change, one could be relatively confident that the treatment was either

1 without effect or that any treatment effect was likely to be quite small. In contrast, if the experiment
2 provided a power of only 80% for detecting a 50% change, the need for a more cautious interpretation
3 would be evident. For this reason, the author strongly recommends that investigators adopt the
4 practice of considering and presenting the power and sensitivity of their experiments when
5 interpreting and publishing their results.

6
7 As discussed elsewhere in detail (Berndtson, 2008), readers should not attempt to judge the relative
8 ability of specific endpoints to detect treatment effects based on replication requirements alone. First,
9 the responses to a given treatment usually differ among various endpoints, and such differences may
10 be difficult to predict in advance. For example, a treatment that depressed sperm production would
11 be likely to reduce the number of spermatids per Sertoli cell, but might be without effect on the
12 frequency of specific stages of the cycle of the seminiferous epithelium. Accordingly, one should
13 select from among those endpoints that are most meaningful and appropriate for the intended
14 objectives of a given experiment. In addition, the endpoints characterized by larger CV's are
15 sometimes associated with treatment responses of substantially greater relative magnitude. One
16 hypothetical example presented previously illustrated how a 10% change in the volume density of the
17 seminiferous tubules would produce a 90% change in the volume density of the interstitial tissue. A
18 simple comparison of the number of replicates needed to detect changes of equal magnitude with
19 these two endpoints would be clearly misleading. For such reasons, the identification of the
20 endpoint(s) by which one would be most likely to detect a response, if there were an actual treatment
21 effect, is a somewhat complex and challenging exercise. In another investigation (Berndtson, 2008),
22 the author ranked several methods for quantifying sperm production rates based on their expected
23 relative ability to detect actual treatment responses. The approach used in that investigation may serve
24 as a model for others wishing to develop similar rankings for other endpoints.

25

1 Because the CV's identified within the literature (Tables 1-8) often differed for the same endpoint
2 among individual studies, the replication requirements cited in Tables 9-11 should be regarded as
3 approximate values intended to serve as a general guide. Investigators possessing data sets of their
4 own or with access to other published studies may readily characterize the replication requirements
5 that might be most appropriate for future investigations with similar or different populations of
6 experimental subjects or endpoints via the general approach utilized herein. This same approach may
7 also be used to confirm the actual power and sensitivity of completed studies, which can be
8 invaluable during the interpretation of the results.

9

10 *Conclusions*

11 The reliability of one's results and conclusions is of great importance for most experiments. This
12 investigation has identified and characterized large differences in the inherent variability associated
13 with several endpoints that may be used for assessing testicular function in rodents, rabbits and men,
14 and the impact of this variability on the number of replicates needed to provide future experiments of
15 a given power and sensitivity. Hopefully, this information will contribute to greater awareness and
16 appreciation of the value of power and sensitivity considerations, while serving as a valuable resource
17 for other investigators during the design and interpretation of future studies with these endpoints and
18 species.

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26

1 Table 1. CV's associated with the number of spermatogonia per Sertoli cell.
2

Subject	n	Spermatogonia	CV (%)	Ref. ¹
Rat				1
60-day-old	25	Type A	25.6	
150-day-old	23	Type A	23.4	
240-day-old	24	Type A	18.0	
Mouse ²	10			2
Stage I		Type A ₁ + A	13.2	
Stage II		Type A ₂ + A	18.2	
Stage III		Type A ₃ + A	9.8	
Stage III		Undiff. A	65.4	
Stage IV		Type A ₄	8.5	
Stage IV		Undiff. A	35.6	
Stage V		Type I	6.1	
Stage V		Undiff. A	35.4	
Stage VI		Type B	3.2	
Stage VI	Undiff. A	16.0		
Mouse ³	3	Stage I	7.2	3
		Stage III	35.8	
		Stage IV	19.5	
		Stage V	19.4	
		Stage VI	8.8	
Rabbit ⁴	7	Type A	9.5	4
Human ⁵	21	Type A – dark	23.6	5
		Type A – pale	23.4	
		Type B	23.2	
		All types	21.6	

3
4 ¹Reference: 1 – Berndtson and Thompson (1990a), 2 – Huckins and Oakberg (1978a), 3 - Huckins and
5 Oakberg (1978b), 4 – Thompson and Berndtson (1993), 5 – Shakkebaek and Heller (1973).
6

7 ² 12 week old mice.

8 ³ Adult mice.

9 ⁴ 8-mo-old New Zealand rabbits.

10 ⁵ 19- to 39-yr-old men.
11
12
13
14
15

1 Table 2. CV's associated with the number of spermatocytes per Sertoli cell.
2

Subject	n	Spermatocyte	CV (%)	Ref. ¹
Rat	13-15	L	11.1	1
Rat				2
60-day-old	25	PL	15.5	
150-day-old	23	PL	6.7	
240-day-old	24	PL	8.3	
60-day-old	25	PACH	16.4	
150-day-old	23	PACH	7.7	
240-day-old	24	PACH	7.8	
Rat ²	4	PL + L + Z	7.0	3
		PACH + DIPL	5.4	
		Secondary	11.5	
Hamster ³	5	PL	11.8	4
		PACH	8.2	
Hamster ⁴	7	Primary	24.7	5
Rabbit ⁵	5	L	14.8	6
Rabbit ⁶	7	Young primary	12.7	7
		Old primary	13.3	
Human ⁷	21	PL	30.5	8
		L	32.3	
		Z + PACH	21.5	

3
4 ¹References: 1 – Berndtson, Neefus, Foote and Amann (1989), 2 – Berndtson and Thompson (1990a), 3 –
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6 (1971), 6 - Berndtson, Neefus, Foote and Amann (1989), 7 – Thompson and Berndtson (1993), 8 –
7 Shakkebaek and Heller (1973).
8

9 ² Sprague Dawley rats aged 54, 64, 77 and 77 days.

10 ³ Golden (Syrian) hamsters.

11 ⁴ Characterized as “normal” hamsters.

12 ⁵ ~2kg Dutch Belted rabbits.

13 ⁶ 8-mo-old New Zealand rabbits.

14 ⁷ 19- to 39-yr-old men.
15
16
17

1 Table 3. CV's associated with the number of spermatids per Sertoli cell.

Subject	n	Spermatids	CV (%)	Ref. ¹
Rat				
60-day-old	25	Round	17.5	1
150-day-old	23	Round	7.0	
240-day-old	24	Round	7.7	
Rat	3	Elongated	15.7	2
Rat ²	4	Step 1-10	6.8	3
Rat	15 ³	Round	11.9	4
	15 ⁴	Round	8.1	
Hamster	5	Elongated	12.7	2
Hamster ⁵	5	Round	15.3	5
Rabbit	4	Elongated	9.4	2
Rabbit ⁶	7	Round	13.7	6
Human ⁷	15	Spermatids ⁸	23.3	7
Human				8
20 – 48 yrs	37	Round	30.4	
50 - 85 yrs	34	Round	27.1	
Human ⁹	21	Early	23.6	9
		Late	26.4	

2 ¹Reference: 1 – Berndtson and Thompson (1990a), 2 – Russell and Peterson (1984), 3 – Wing and
3 Christensen (1982), 4 – Berndtson et al., 1989, 5 – Sinha Hikim, Bartke and Russell (1988), 6 –
4 Thompson and ‘Berndtson (1993), 7 – Barr, Moore and Paulsen (1971), 8 – Johnson, Zane, Petty
5 and Neaves (1984), 9 – Shakkebaek and Heller (1973).

6

7 ² Sprague Dawley rats aged 54, 64, 77 and 77 days.

8 ^{3,4} Values for ~350g Sprague Dawley rats assigned to groups of 15 non-gavaged
9 and gavaged control animals, respectively.

10 ⁵ Golden (Syrian) hamsters.

11 ⁶ 8-mo-old New Zealand rabbits.

12 ^{7,8} Values for round and elongating spermatids in “normal” males.

13 ⁹ 19- to 39-yr-old men.

1 Table 4. CV's associated with germ cell:germ cell ratios.

2

Subject	n	Cell ratio	CV (%)	Ref ¹
Rat				1
60-day-old	25	R. sp.: Sp-gonium	20.7	
150-day-old	23	R. sp.: Sp-gonium	22.2	
240-day-old	24	R. sp.: Sp-gonium	18.3	
Rat ²	4	PACH + DIP: PL + L + Z	1.8	2
		2 ⁰ : PACH + DIP	7.8	
		Step 1-10: 2 ⁰	16.0	
		Step 1-10: PACH + DIP	9.4	
Hamster	5	PL:A	22.5	3
		PACH:PL	4.5	
		Sp 7:PACH	6.2	
Human ³	7	Sp-tids:Sp-cyte	18.9	4
	15	Sp-tids:Sp-cyte	27.8	

3

4 ¹Reference: 1 – Berndtson and Thompson (1990a), 2 – Wing and Christensen (1982), 3-
5 Berndtson and Desjardins, 1974b; 4– Barr et al. (1971).

6

7 ² Sprague Dawley rats aged 54, 65, 77 and 77 days.

8

³ Males described as reproductively “normal”.

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Table 5. Coefficients of variability associated with the frequency of stages of the seminiferous epithelium.

Stage of the cycle	Reference ¹					
	Rat			Rabbit		
	1	2	3	4	5	6
I	22.7	17.0	18.5	8.7	6.0	10.6
II	14.8	29.2	23.7	18.4	11.9	7.3
III	35.6	33.7	12.0	18.4	8.4	23.5
IV	25.0	23.7	30.4	11.7	12.9	20.0
V	17.8	17.1	16.3	27.6	22.6	35.8
VI	26.8	20.7	18.2	18.4	11.5	18.7
VII	13.5	7.4	7.0	21.6	7.3	18.1
VIII	26.6	25.5	13.5	16.8	19.3	25.6
IX	36.9	25.8	40.9			
X	32.9	12.1	33.3			
XI	31.7	25.8	63.6			
XII	12.4	8.9	14.1			
XIII	80.2	31.2	25.0			
XIV	47.6	17.1	34.0			

¹ Reference identities and population characteristics are as follows:

1 = Bartlett et al. (1990); n = 8 Sprague-Dawley rats (2 rats each at ages of 174, 195, 216 and 251 days); 200 tubules were examined per male.

2 = Hess et al. (1990a); n = 15 Sprague-Dawley rats aged 90 to 110 days; an average of 645 tubules (range:513 to 735) was examined per rat.

3 = Van Beek & Meistrich (1990); n = 6 Sprague-Dawley rats (2 rats each at ages of 75, 110 and 203 days); a total of 2081 tubules were examined in the study.

4 = Amann (1970); n = 22 testes from eleven 54-wk-old New Zealand rabbits (coefficients are the values among 22 testes from 11 rabbits); 8.00 tubules were examined per testis.

5 = Swierstra & Foote (1963); n = 6 Dutch Belted rabbits aged 32 to 40 weeks; 240 tubules were examined per rabbit.

1 6 = Amann & Lambiase (1969); n = 66 testes from 33 New Zealand rabbits (22 rabbits were
2 about 10 months old, while 11 rabbits are those cited under reference number 4); values are for
3 the variability among 66 testes.
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1 Table 6. Coefficients of variability (CV) associated with numbers of degenerating
 2 germ cells.

3	4	5	6	7	8
Species	Description	n	CV (%)	Reference ¹	
6	Rat	Sprague-Dawley (225-300g)	10		1
7		Pachytene sp-cytes (Stage VII) ²		158.1	
8		Dividing sp-cytes (Stage I) ²		112.9	
9		Step 7 sp-tids (Stage VII) ²		316.2	
10		Step 19 sp-tids (Stages VIII-IX) ²		126.5	
11		Unidentified cells (Stage VII) ²		158.1	
12					
13	Rat	250-300g	8		2
14		Sp-cytes (Stage XIV)		127.3 (132.0) ³	
15		Sp-cytes (Stage VII)		57.5 (59.7) ³	
16		Step 7 sp-tids		21.8 (27.9) ³	
17		Step 19 sp-tids		38.3 (45.7) ³	
18		Unidentified cells (Stage VII)		23.0 (25.4) ³	
19					
20	Human	26- to 53-yr-old			3
21		Histometric	10	47.2 ⁴	
22		Homogenization	10	51.7 ^{4,5}	
23		Homogenization	15	37.6 ^{4,5}	
24					

25 ¹ Reference: 1 - Russell and Clermont (1977), 2 – Russell et al. (1981), 3 – Johnson et al. (1983).

26 ² Degenerating cells/100 Sertoli cells.

27 ³ Values in parentheses are for degenerating cells per 100 Sertoli nucleoli, while
 28 others are per 100 Sertoli nuclei.

29 ⁴ Degeneration expressed as potential losses in sperm production during
 30 the post-prophase of meiosis, calculated as the ratio of daily sperm production
 31 estimates based on primary spermatocytes vs round spermatids.

32 ⁵ Cells were enumerated in fixed, homogenized tissue samples.

1 Table 7. Coefficients of variability (CV) associated with the incidence of testicular
 2 abnormalities¹ in New Zealand rabbits of different ages²:

-----Abnormality-----				
Age (wk)	Spermatid giant cells	Hypospermatogenesis	Spermatogonial swelling	Cytoplasmic vacuoles
8	193.9	_____	44.2	123.3
15	100.9	110.4	31.1	35.3
18	58.3	261.5	42.1	37.1
26	82.9	213.0	37.1	55.2
>52	112.0	173.2	28.7	37.1

14 ¹Abnormalities were expressed as the number of altered seminiferous tubules per cm² of cross-
 15 sectional area.

16 ²Calculated from data of Morton et al. (1986).

1 Table 8. Typical CV's associated with selected endpoints.

Variable	Rodent	Rabbit	Human
Type A/Sertoli	18.8	9.5	23.5
Type I/Sertoli	6.1	----	----
Type B/Sertoli	3.2	----	23.2
1 ⁰ sp-cytes/Sertoli	10.4	13.5	28.1
2 ⁰ sp-cytes/Sertoli	11.5	----	----
R. sp-tids/Sertoli	8.7	13.7	26.9
R. sp-tids/type A	20.4	----	----
Old 1 ⁰ /Young 1 ⁰ sp-cyte	3.3	----	----
R. sp-tids/1 ⁰ sp-cyte	7.6	----	25.0
2 ⁰ sp-cytes/old 1 ⁰ sp-cyte	7.8	----	----
Freq. of stages:			
Mean for all stages	24.4	18.9	----
Range for indiv. stages	9.0 – 43.4	9.9 – 33.0	----
Degen. germ cells	21.8 – 316.2	----	44.4
Sp-tid giant cells	----	112.0	----
Hypospermatogenesis	----	173.2	----
Spermatogonial swelling	----	28.7	----
Cytoplasmic vacuoles	----	37.1	----

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- 1 Table 9. Approximate number of rats needed per treatment group to provide experiments of a given
 2 level of power and sensitivity¹.

Endpoint	80 % Power			90% Power		
	Sensitivity			Sensitivity		
	10	20	30	10	20	30
Type A / Sertoli	57	15	8	76	21	10
Type I / Sertoli	7	3	3	9	4	3
Type B / Sertoli	3	2	2	4	2	2
1 ⁰ sp-cytes / Sertoli	19	7	4	25	8	5
2 ⁰ sp-cytes / Sertoli	23	7	4	30	9	5
R. sp-tids / Sertoli	14	5	3	18	6	4
R. sp-tids / type A	66	18	10	89	24	12
Old/young 1 ⁰ sp-cyte	3	2	2	4	2	2
R. sp-tids / 1 ⁰ sp-cyte	11	4	3	14	5	3
2 ⁰ / 1 ⁰ sp-cyte	12	4	3	15	5	3
Freq. of indiv. Stages ²	95	25	12	127	33	16
Degen. germ cells	$\geq 76^3$	$\geq 21^3$	$\geq 10^3$	$\geq 102^3$	$\geq 27^3$	$\geq 13^3$

1 ¹For two-tailed tests with two-treatment experiments. For experiments with a one-tailed test, the
2 replication shown for experiments of 80 and 90% power would provide the power of 90 and 95%,
3 respectively, at $P \leq 0.025$.

4 ² Values based on the mean CV of 24.4% based on all stages. Replication requirements will vary as a
5 function of the specific stage chosen for evaluation (Table 8).

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7 ³ Values based on the lowest observed CV of 21.8%. The mean CV's for degenerating germ cells in
8 several studies ranged from 21.8 to 316.2% (Table 8).

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1 Table 10. Approximate number of rabbits needed per treatment group to provide experiments of a
 2 given level of power and sensitivity¹.

Endpoint	80% Power			90% Power		
	Sensitivity			Sensitivity		
	10	20	30	10	20	30
Type A/Sertoli	16	6	4	21	7	4
1 ⁰ sp-cytes/Sertoli	30	9	5	41	12	6
Round sp-tids/Sertoli	31	9	5	42	12	6
Freq. of stages ²	57	16	8	77	21	10
Sp-tid giant cells ³	>1,571	>393	>17 5	>2,10 3	>52 6	>23 4
Hyospermatogenesis ³	>1,571	>393	>17 5	>2,10 3	>52 6	>23 4
Spermatogonial swelling	131	35	16	175	46	22
Cytoplasmic vacuoles	218	56	26	292	74	34

3 ¹For two-tailed tests with two-treatment experiments. For experiments with a one-tailed test, the
 4 replication shown for experiments of 80 and 90% power would provide the power of 90 and 95%,
 5 respectively, at $P \leq 0.025$.

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7 ² Values based on the mean CV of 18.9% based on all stages. Replication requirements will vary as a
 8 function of the specific stage chosen for evaluation (Table 8).

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10 ³For spermatid giant cells and hyospermatogenesis, the values shown are for CV's of 100% (i.e., the
 11 maximum CV provided in the published replication requirement tables).

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1 Table 11. Approximate number of men needed per treatment group to provide experiments of a given
 2 level of power and sensitivity¹.

Endpoint	80% Power			90% Power		
	Sensitivity			Sensitivity		
	10	20	30	10	20	30
Type A/Sertoli	89	24	11	118	31	15
Type B/Sertoli	86	23	11	115	30	14
1 ⁰ sp-cytes/Sertoli	126	33	15	168	44	21
R. sp-tids/Sertoli	116	31	14	154	40	19
R. sp-tids/1 ⁰ sp-cyte	99	26	12	132	34	16
Degen. germ cells	305	78	36	416	105	48

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4 ¹For two-tailed tests with two-treatment experiments. For experiments with a one-tailed test, the
 5 replication shown for experiments of 80 and 90% power would provide the power of 90 and 95%,
 6 respectively, at $P \leq 0.025$.

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