2 Sperm Production and its Harvest

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Introduction

Spermatogenesis requires ~60 days in most mammals. It encompasses a series of successive mitotic divisions, two meiotic divisions and the transformation of haploid spermatids into spermatozoa. Spermatogenesis is susceptible to disruption by many physical or chemical agents, which can produce alterations in seminal quality that may be manifested either quickly or weeks thereafter. Recognition of factors that are known to alter spermatogenesis, of the time course for the first appearance of alterations in ejaculated semen, and of subsequent recovery after exposure to disruptive agents is critical for sound reproductive management. Because spermatogenesis proceeds independently of sexual activity, sexual inactivity results in the accumulation of sperm within the extragonadal ducts and subsequent losses via micturition. Consequently, large numbers of spermatozoa must be expected in ejaculates taken after lengthy sexual rest. To harvest the maximal number of spermatozoa, males must be maintained on a regular, frequent ejaculation schedule. Maintaining libido at such ejaculation frequencies can be challenging, and requires the provision of novelty. This can be accomplished for some animals (e.g. the bull) by changing teaser animals or collection locations, or by providing movement by the teaser. This chapter provides an overview of these and other important factors that contribute to the maximum reproductive potential of a given male.

Sperm Production and its Harvest

Successful reproduction requires two major contributions from the male: the production of adequate numbers of viable sperm, and the capacity to mate or to be used for semen collection, so that sperm may be used for artificial insemination. Accordingly, it is important to select and manage the male to maximize sperm production and its harvest.

Spermatogenesis

The germinal cells

The seminiferous tubules contain three major classes of germ cells: spermatogonia, spermatocytes and spermatids. The spermatogonia are the least differentiated of these cells,

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and they are distinguished from the other types by the fact that they undergo mitotic divisions that increase the number of germ cells, while also providing for stem cell renewal. By classical definition, spermatogonia with nucleoplasm of a smooth, coarse or intermediate texture are designated as type A, type B or type I (intermediate), respectively. Division of these cells yields subtypes, which are identified by subscripts denoting their order of appearance. For example, type A_1 spermatogonia would divide to produce daughter cells of type A_2. Successive divisions might proceed as follows: $A_2 \rightarrow A_3 \rightarrow I \rightarrow B_1 \rightarrow B_2$, etc. The number of divisions and, thus, the number of spermatogonial subtypes, is constant within but differs among individual species. The last in the series of spermatogonial divisions results in the production of primary (1^o) spermatocytes.

The testis contains both primary and secondary (2^o) spermatocytes, which are distinguished from the other germ cells by the fact that they undergo meiotic divisions. The primary spermatocytes are the most developmentally advanced germ cells to replicate DNA. They undergo the first reduction division to produce secondary spermatocytes, which receive duplicate copies of only one member of each pair of chromosomes. While the autosomes behave similarly, the sex chromosomes (X and Y) are particularly useful for explaining the unique manner in which chromosomes are passed to daughter cells during the meiotic divisions. Each newly formed somatic cell, or spermatogonium, receives both an X and Y chromosome; their DNA is replicated so that upon mitotic division each daughter cell receives one member of each chromosomal pair (i.e. one X plus one Y chromosome). In contrast, the replication of DNA by the primary spermatocytes is followed by the first meiotic division, through which each daughter secondary spermatocyte receives two copies of either the X or the Y chromosome, but not both. The secondary spermatocytes do not replicate DNA; their division involves separation of the identical pairs of chromosomes, resulting in haploid spermatids containing a single X or Y chromosome.

Spermatids are the most developmentally advanced of the three germinal cell types. They do not divide, but undergo transformational events that culminate with their release into the lumen of the seminiferous tubules, at which time they are considered to be spermatozoa.

Kinetics of spermatogenesis

The kinetics of spermatogenesis refers to the number and nature of the various germ cell divisions for a given species, and consists of three major components. Spermatocytogenesis encompasses the cell divisions beginning with the least differentiated spermatogonia and culminating with the production of haploid spermatids. This is followed by spermiogenesis, by which the newly formed spherical spermatids are transformed into more mature forms with a morphology closely resembling that of spermatozoa. Spermiogenesis culminates with spermiation.

Although most spermatogonial divisions yield daughter cells of a more developmentally advanced type, type A_1 spermatogonia must be replenished to prevent the supply of these cells from being exhausted. Mechanisms for accomplishing this stem cell renewal have been investigated in many species. Most proposed models are based on a combination of quantitative and morphological data. A more detailed discussion of stem cell renewal in the boar, bull, goat and ram may be found in references listed in Table 2.1. Finally, the testes contain small numbers of inactive reserve cells designated as type A_0 spermatogonia. These are uncommitted to cell division, but become mitotically active to replenish the type A_1 population when necessary (Clermont, 1962, and others).

Table 2.1. References characterizing spermatogonial stem cell renewal in the boar, bull, goat and ram.

Species	Reference
Boar	Frankenhuis *et al.*, 1980
Bull	Amann, 1962b
Bull	Hochereau-de Reviers, 1970
Bull	Berndtson and Desjardins, 1974
Goat	Bilaspuri and Guraya, 1984
Ram	Ortavant, 1958
Ram	Lok *et al.*, 1982

The cycle of the seminiferous epithelium

The division and maturation of most germ cells proceeds on a schedule that is relatively well timed in normal individuals. Thus, spermatogenesis yields a distinct number of unique combinations of cells, or cellular associations, that are observable together at any given point in time. If one could observe a given cross section of a seminiferous tubule, one would note progressive changes in the cellular associations over time. Ultimately, the cellular association that was present initially would appear once again. The series of changes beginning with the appearance of one cellular association and ending with its reappearance would constitute one cycle of the seminiferous epithelium. In most species, approximately 4.5 cycles are required for the production of sperm cells from the least developmentally advanced spermatogonia. The length of the cycle of the seminiferous epithelium and the duration of spermatogenesis (i.e. the time required to produce spermatozoa from the least developmentally advanced spermatogonia) for several relevant species are given in Table 2.2.

Although spermatogenesis is a continuous process, researchers have found it useful to divide this process into recognizable stages of the cycle of the seminiferous epithelium. The number of stages for any given species is limited only by the ability to discern distinguishing features. Although all staging systems rely on the specific cellular associations that may be observed, the two most common approaches include additional consideration of either general tubular morphology or acrosomal development. Use of the tubular morphology system includes noting whether elongated spermatids are or are not present, and whether the elongated spermatids are embedded deeply within the seminiferous epithelium or line the lumen immediately before spermiation, etc. (see Plate 1); with this system, eight stages are usually recognizable. Staging by the acrosomal system is based primarily on recognizable steps of acrosomal development during spermiogenesis (see Fig. 2.1); for most species, 12–15 stages can be identified by this approach. Publications describing criteria for staging the cycle in several economically important species are listed in Table 2.3.

All stages of the cycle of the seminiferous epithelium can be found within a single seminiferous tubule at any point in time. This feature contributes to the steady, continuous release of sperm from the testis over time. Moreover, these stages are arranged sequentially along the length of the tubule. So a segment containing stage III might be followed by segments in stage IV, V, VI, etc. This arrangement is denoted as the wave of spermatogenesis. Minor disruptions in this pattern, termed modulations, may occur (Perey *et al.*, 1961). An example of this might involve

Table 2.2. The length of the cycle of the seminiferous epithelium and the duration of spermatogenesis in selected species.

Species	Cycle length (days)	Duration of spermatogenesis (days)	Reference
Boar	8.6	–	Swierstra, 1968a
Boar	–	39[a]	Amann and Schanbacher, 1983
Boar	9.0	40.6	Franca and Cardosa, 1998
Boar (wild)	9.05	41[a]	Almeida *et al.*, 2006
Bull	13.3	61	Amann and Almquist, 1962
Bull	13.5	–	Hochereau-de Reviers *et al.*, 1964
Bull	13.5	61[a]	Amann and Schanbacher, 1983
Goat	10.6	47.7	Franca *et al.*, 1999
Ram	10.6	42.3	Cardosa and Queiroz, 1988
Stallion	12.2	–	Swierstra *et al.*, 1974
Stallion	–	55[a]	Amann and Schanbacher, 1983

[a]Estimates based on an assumption that the duration of spermatogenesis requires 4.5 cycles of the seminiferous epithelium.

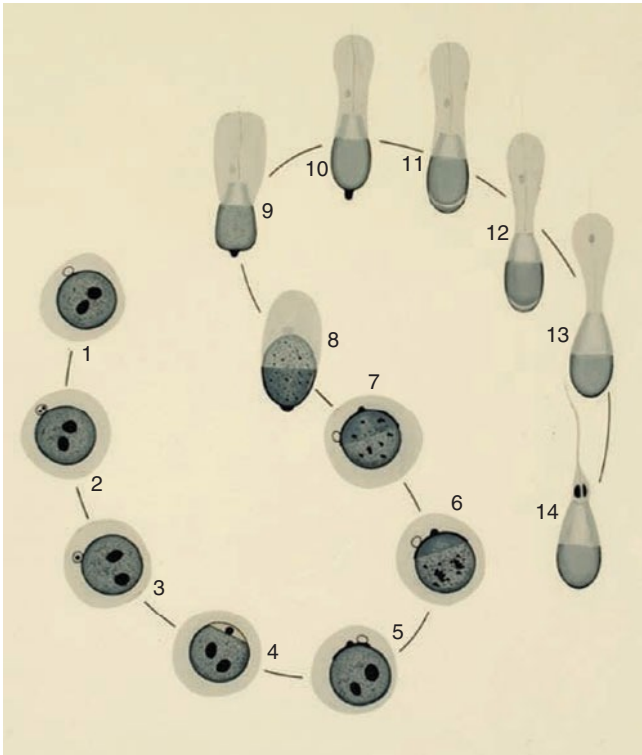

Fig. 2.1. Spermiogenesis in the bull as seen with periodic acid–Schiff (PAS) staining. Fourteen steps in the development of bovine spermatids are depicted. Changes associated primarily with acrosomal development were used by Berndtson and Desjardins (1974) to distinguish these 14 stages of the cycle of the seminiferous epithelium.

Table 2.3. Systems for classifying stages of the cycle of the seminiferous epithelium in selected species.

Species	Staging system	Reference
Boar	Tubular morphology	Swierstra, 1968a
Boar	Acrosomal	Franca *et al*., 2005
Bull	Tubular morphology	Amann, 1962b
Bull	Acrosomal	Berndtson and Desjardins, 1974
Goat	Tubular morphology	Franca *et al*., 1999
Goat	Tubular morphology	Onyango *et al*., 2000
Goat	Acrosomal	Bilaspuri and Guraya, 1984
Ram	Tubular morphology	Ortavant (1959a,b)
Ram	Acrosomal	Clermont and Lebland, 1955
Stallion	Tubular morphology	Swierstra *et al*., 1974

a spatial distribution of stages along one segment of a seminiferous tubule as follows: stage I → stage II → stage III → stage IV → stage III → stage IV → stage V → stage VI, etc. A wave of spermatogenesis is not present in man. Rather, stages are confined to small, discrete, irregular patches distributed more randomly within the seminiferous tubules (Heller and Clermont, 1964). This arrangement precludes the identification of stages within round seminiferous tubular cross sections as are applied routinely for other mammals.

By examining a large number of round seminiferous tubular cross sections in species other than man, researchers can determine the frequency of appearance for each stage of the cycle of the seminiferous epithelium. This information has several useful applications. Because the timing of spermatogenic events

is relatively constant, the frequency of a given stage will be proportional to its relative duration. For example, one cycle of the seminiferous epithelium requires 13.5 days in the bull (Table 2.2). Thus, if a particular stage appeared at a frequency of 10%, it would be apparent that the duration of that stage equalled 10% of one cycle, or 1.35 days. Similarly, estimates of the actual length of one cycle of the seminiferous epithelium or of the duration of spermatogenesis have been based on stage frequency data and the progression of radiolabelled germ cells in virtually every species for which such information has been generated.

Knowledge of the duration of individual stages is useful in predicting the time course over which a treatment that adversely impacted a particular type of cell or cell division would be expected to first become evident in an ejaculate from a treated male, or in estimating the subsequent time course for full recovery upon withdrawal of the causative agent or factor (Foote and Berndtson, 1992). Although the timing of spermatogenic events and the frequency of individual stages appear to be relatively constant in normal individuals, some variability has been reported among normal subjects. In addition, both arrested development and subsequent stage synchronization (i.e. progression of spermatogenesis with most tubular cross sections being at a single, identical stage of development at any given point in time), have been induced experimentally. These findings and their impact on the reliability of stage frequency data have been discussed elsewhere in greater detail (Berndtson, 2011).

Sperm Production Rates

Which factors have an impact on sperm production?

Sperm production is among the most important determinants of the reproductive capacity of an individual male. Indeed, although libido is also quite important with natural mating systems, the number of potential matings to a sire used for artificial insemination (AI) is generally limited only by the number of normal sperm produced per unit of time. It is, therefore, important for andrologists to recognize factors that do or do not have an impact on sperm production.

Testis size

Testis size is highly and positively correlated with sperm production in healthy postpubertal males (Table 2.4). Accordingly, scrotal circumference or width is an important

Table 2.4. Reported correlations between testis size and the daily sperm production (DSP) of sexually mature males.

Species	Correlation	Reference
Boar	0.90	Swierstra, 1968b
Boar (wild)	0.97	Almeida *et al*., 2006
Bull	0.81, 0.72, 0.64, 0.40, –0.22[a]	Hahn *et al*., 1969
Bull	0.71	Berndtson *et al*., 1987a
Bull	0.72	Berndtson *et al*., 1987b
Goat	0.94[b]	Jindal and Panda, 1980
Goat	0.50[b]	Ritar *et al*., 1992
Goat	0.98	Leal *et al*., 2004
Ram	0.97[c]	Lino, 1972
Ram	0.75–0.80[d]	Alkass *et al*., 1982
Ram	0.94	Cardosa and Queiroz, 1988
Stallion	0.77	Gebauer *et al*., 1974a
Stallion	0.89	Johnson *et al*., 1997

[a]Correlations for Holstein bulls aged 17–22, 34–42, 42–53, 56–69 and 72–150 months of age, respectively.
[b]Correlation between testicular weight and epididymal sperm number.
[c]Correlation between paired testes weight and extragonadal sperm reserves.
[d]Correlation between scrotal circumference and total sperm in the reproductive tract.

component of male breeding soundness examinations (e.g. Shipley, 1999; Eilts, 2005a,b). As discussed subsequently, testis size and sperm production increase, at least up to a point, after puberty. However, this does not diminish the importance of testis size measurements in younger males. Young bulls with relatively small testes tend to develop into adults with testes that are smaller than those of their counterparts, and vice versa (Hahn *et al.*, 1969; Coulter *et al.*, 1975). So breeders should consider testis size before enrolling young bulls in their progeny testing programmes.

Age

Sperm production is influenced by age. For most species, testicular size and sperm production increase to maximal levels over a period of time after puberty, and then remain at that level until ultimately declining as a result of senescence. This pattern is characteristic for the bull, as shown in Table 2.5. In contrast, sperm production appears to increase throughout life in healthy stallions (Table 2.6). Sperm production/g tissue also increases for a period of time after puberty, as illustrated by data in Table 2.6 for the stallion. Thus, young males have testes that are both smaller and less productive/g tissue than those of more sexually mature individuals.

Season

The impact of season on sperm production is species dependent. Seasonal breeding is advantageous for most wild species, in which sperm production may cease entirely during the non-breeding season. In contrast, the management of domesticated animals ensures greater consistency in the availability of feed, protection from severe weather, etc., and this, over time, has presumably diminished seasonal breeding patterns in our domesticated species. Several of our farm animals, such as the bull and boar, produce sperm at a consistent rate throughout the year. In contrast, stallions and the rams of some breeds produce sperm throughout the year, but in greater quantity during the breeding than in the non-breeding season.

Seasonal changes in sperm production are typically associated with decreases in both testicular size and the number of sperm produced per unit of testicular parenchyma. In one study, scrotal width was 11% greater (101 versus 91 mm) at the onset of the breeding season (27 April–26 May) than for the same stallions during the non-breeding season 180 days later (Squires *et al.*, 1981). Based on the imperfect assumption that testes are precise spheres, a 10% difference in testicular diameter would be associated with a corresponding difference of approximately 37% in testicular volume. Testicular tissue must be removed to quantify sperm production by direct methods. This precludes the quantification of sperm production within the same individuals at each season. None the less, sperm production per volume of testicular parenchyma is clearly lower in stallions during the non-breeding season. For example, in one study, daily sperm production (DSP)/g of tissue for stallions ≥4 years old averaged 14.8 versus 18.8 million during non-breeding versus breeding seasons, respectively (Johnson and Thompson, 1983). Thus, although stallions produce sperm throughout the year, sperm production occurs at a markedly reduced rate during the non-breeding season. Hence, season must be considered when estimating the breeding capacity of seasonally breeding males from scrotal measurements.

Table 2.5. Changes in reproductive characteristics with age in the bull. Adapted from Hahn *et al.*, 1969.

	Age (months)				
Variable	17–22	34–42	42–53	56–59	72–150
Scrotal circumference (cm)	35.1	40.0	40.3	42.0	42.6
DSO[a]/male (billions)	4.1	5.9	5.4	6.1	4.0
DSO/g (millions)	8.7	8.4	7.6	7.5	4.9

[a]Daily sperm output.

Table 2.6. Changes in reproductive characteristics with age in the stallion. Adapted from Johnson and Neaves, 1981.

	Age (years)		
Variable	2–3	4–5	13–20
Testicular weight (g)	117	161	213
DSP[a]/testis (billions)	1.27	2.67	3.18
DSP/g (millions)	11.4	18.8	17.0

[a]Daily sperm production.

Environmental factors

Normal spermatogenesis requires a testicular temperature slightly below normal core body temperature. It is for that reason that the testes of all mammals other than the elephant and whale reside within a superficial scrotum. Heat can be dissipated from the scrotal surface, which can be increased or decreased as necessary via the contraction or relaxation of the external cremaster muscles of the spermatic cord and/or the tunica dartos muscle at the base of the scrotum. These muscles also serve to position the testes closer to or further from the rest of the body. In addition, the spermatic cord contains an extensive vasculature of closely intertwined arterial and venous blood vessels known as the pampiniform plexus, which provides a countercurrent exchange mechanism by which warm arterial blood is cooled before reaching the testis by the cooler, returning venous blood, and vice versa.

The importance of testicular thermoregulation is evident in cases of cryptorchidism, in which one or both testes fail to descend into the scrotum. Whereas cryptorchid testes continue to produce near-normal levels of androgens, they do not produce sperm. Although the scrotal testis of a unilaterally cryptorchid individual will continue to produce sperm, cryptorchidism is a heritable condition, and such individuals should not be used for breeding. The disparity in testicular size within one unilaterally cryptorchid stallion is depicted in Plate 2.

Thermoregulatory mechanisms may be incapable of maintaining an appropriate testicular temperature when ambient temperatures are excessive, or during periods of illness accompanied by fever. Shade, fans or other methods for preventing overheating can be very helpful during such times. Beyond that, it is important to recognize the potential for transient depressions in seminal quality or fertility as a result of elevated temperature. As with any insult to the testis, the severity of an effect will reflect both its magnitude and duration. Moreover, the interval required for any resulting negative impacts to be manifested via depressions in the fertility of mated females or the quality of ejaculated semen, and the time required subsequently for full recovery can vary.

A simple assembly line concept is useful for illustrating the reasons for this variability in seminal quality or fertility as a result of elevated temperature. Imagine an assembly line for producing wristwatches consisting of ten stations at which each watch remains for 1 h as different components are added. Now, imagine that a new employee assigned to station number three began to insert components in a manner that would cause the hands of the watch to move counterclockwise rather than clockwise. At the time of the first error, stations 4–10 would contain partially but correctly assembled watches. These would continue to move through the assembly process. Some 7–8 h would elapse before the first defective watch had advanced through the remaining stations and undergone a final quality control inspection. Had an error occurred instead at station 10, defective watches would have been detected soon thereafter, but in this example the problem was not immediately apparent. Indeed, depending on the point in the assembly process at which a problem arose, detection via evaluation of the completed product could take anywhere from 1 to 10 h in this hypothetical example.

By analogy, spermatogenesis requires approximately 60 days in most mammals. Several more days are required for epididymal transit before sperm are available for ejaculation (Table 2.7). Therefore, a problem(s) with spermatogenesis might be reflected in an ejaculate almost immediately, or might require more than 60 days, depending on the particular types of germ cell or spermatogenic events affected. Recovery times are subject to similar variability. Referring once

Table 2.7. Total epididymal transit time.

Species	Total (days)	Reference
Boar	10.2	Swierstra, 1971
Boar	7.94	Franca and Cardosa, 1998
Bull	5.6–8.3	Weisgold and Almquist, 1979
Goat	Unknown	–
Ram	12	Lino, 1972
Stallion	4.9	Gebauer *et al*., 1974b

again to the wristwatch illustration, at the moment that defective watches were detected, stations 3–10 would already contain watches with components inserted incorrectly. An additional 7–8 h would be required after correction of the problem before properly assembled watches would once again begin coming off the assembly line. Had the problem occurred at station ten, correction would have produced a more immediate remedy.

Once again by analogy, the time required for semen quality to recover after a transient disruption of spermatogenesis could be brief or might require more than 2 months. For AI, it is customary to evaluate every ejaculate for the number and percentage of motile sperm and, occasionally, for spermatozoal morphology. This provides an opportunity for detecting some forms of damage and thereby some safeguard against the use of semen of low quality. Similar evaluations are not performed routinely for males used for natural mating. Although breeding soundness evaluations would certainly be indicated upon detection of herd fertility problems, full recovery during the interval between the unsuccessful matings and the detection of low fertility could render such evaluations inconclusive.

Although severe nutritional deficiencies or imbalances can be disruptive, the nutritional requirements for normal spermatogenesis in sexually mature males do not appear to differ from those for maintaining good general health and body condition (Foote, 1978). The author is unaware of any dietary supplements that are effective in increasing sperm production or seminal quality of normal, healthy males. It should be noted that there has been some recent interest in assessing whether dietary supplementation might prove effective for altering the composition of sperm membranes, thus rendering these cells more resistant to the stresses of cooling or freezing (e.g. Brinsko *et al.*, 2005; Harris *et al.*, 2005). Research in this area is likely to continue.

Exogenous agents

Spermatogenesis is readily disrupted by a variety of exogenous agents. While an extensive description of known anti-spermatogenic agents is beyond the scope of this undertaking, several groups of compounds are of current relevance to the animal industries. As cited previously, one of the main roles of the male is either to mate with receptive females or to ejaculate semen that can be harvested for use via AI. This requires that the male have adequate libido. Because libido is under endocrine control, exogenous hormones have been evaluated as potential treatments for enhancing libido or for treating impotence or other breeding issues (Berndtson *et al.*, 1979; McDonnell, 1999). Such applications are likely to be of greater interest for companion animals of intrinsically high value than for most food-producing species. None the less, from the author's perspective, any use of exogenous hormones should be preceded by a thorough evaluation of the physical and endocrine status of the male. If an endocrine disorder is confirmed, an informed decision would be required to determine whether endocrine therapy might be helpful and advisable.

The administration of testosterone to normal, healthy individuals is not indicated. Exogenous testosterone does not appear to be effective for enhancing the libido of normal males, and higher dosages are clearly detrimental to sperm production (Berndtson *et al.*, 1974, 1979). For example, Berndtson *et al.* (1979) administered 0, 50 or 200 μg of testosterone propionate/kg body weight to normal sexually mature stallions every other day for 88 days. Treatment did not influence the time to erection, interval from first mount to ejaculation or the number of mounts required per ejaculation. However, the highest dosage reduced sperm production/g testis and per stallion by 41 and 57%, respectively, while also reducing sperm motility by ~10 percentage points. In contrast to these findings,

McDonnell *et al.* (2003) suggested that injections of testosterone every other day could be used to boost the sexual arousal of stallions without affecting spermatogenesis. The 80 mg dosage used, which reportedly was without effect on sperm production (McDonnell, 1999), was similar to that eliciting pronounced decreases in sperm production and seminal quality in the aforementioned investigation with normal stallions (Berndtson *et al.*, 1979). The reason for the discrepancy between these two studies is unclear. However, the potential for exogenous androgens to depress sperm production is apparent (Berndtson *et al.*, 1974, 1979), and this should be recognized whenever the administration of exogenous androgens is under consideration.

Anabolic steroids

Anabolic steroids are synthetic hormones with growth-promoting properties. Because they enhance muscle development and physical performance, they have been used to provide a competitive advantage for animals shown at halter or used as athletes. Although such uses are prohibited, anabolic steroid use has probably continued at some level, as it has with professional human athletes. Most, if not all, of the anabolic steroids possess androgenic properties. For the male reproductive system, anabolic steroids produce responses closely mimicking those with exogenous androgens. In one study, in which stallions were injected with Equipoise (the anabolic steroid boldenone undecylenate) at a dosage of 4.4 mg/kg body weight once every 3 weeks over a 15 week period, testis size, sperm production/g testis, sperm production per testis and sperm motility were decreased to approximately 55, 65, 28 and 62% of control values, respectively (Squires *et al.*, 1982). The use of anabolic steroids to provide a competitive advantage should be prohibited for both ethical and physiological justifications.

Exercise

Although the benefits of exercise to male fertility are often cited in the popular literature, results from well-designed scientific studies appear limited. Inconsistencies among the reported benefits may, at least in part, reflect shortcomings in experimental design, such as treatment periods that were shorter than the duration of spermatogenesis and/or the absence of reliable pretreatment baseline data. Other potential contributors include differences in the intensity of exercise, provisions for prior conditioning or the use of field data devoid of suitable controls.

However, Snyder and Ralston (1955) conducted an excellent study to assess seminal characteristics and non-return rates for non-exercised versus exercised dairy bulls. Non-exercised bulls were confined to box stalls. The remaining bulls were exercised on a mechanical walker at a speed of 1.24 miles/h on 6 days/week. Exercise was limited to 15 min/day for the first 35 days, but was increased to 23 min for the next 14 days and to 30 min/day for the remainder of the 6 month study period. Exercise was without effect on seminal characteristics or fertility. The total number of sperm per ejaculate averaged 8.53 versus 7.68 billion in non-exercised versus exercised bulls, respectively. The corresponding values for initial sperm motility, abnormal sperm and 60–90 day non-return rates equalled, 73.2 versus 71.5%, 15.0 versus 13.4% and 65.0 versus 63.8%, respectively. This was a large study involving 32 bulls (16/exercise group), a total of 33,292 inseminations and a treatment period exceeding the duration of spermatogenesis.

One of the first studies to assess the effects of exercise on the stallion was conducted by Dinger and Noiles (1986). This two-phase study involved eight 2-year-old stallions. During phase 1, four stallions were assigned either to confinement in box stalls or to a 6x day/week regimen of either 18 min (12 trotting and 6 walking) or 24 min (16 trotting and 8 walking) of forced exercise. After 16 weeks, treatment assignments were reversed for an additional 16 week period. Semen was collected once every 14 days, at which time libido was scored on a scale of 0–4 (with 4 being highest). Libido declined during the exercise periods. For stallions placed initially in the exercised group, libido scores averaged 2.88, 2.06 and 2.81 before treatment and at the end of the 16 week exercised and non-exercised

periods, respectively. For stallions placed first in the non-exercised group, libido scores during the pretreatment, non-exercised and exercised periods averaged 3.00, 3.5 and 2.00, respectively. Treatment was without effect on daily sperm output or seminal quality (Dinger *et al.*, 1986).

Based on the foregoing studies, moderate exercise does not appear to have a substantive impact on spermatogenesis or on the seminal quality of bulls (Snyder and Ralston, 1955) or stallions (Dinger and Noiles, 1986; Dinger *et al.*, 1986). However, whereas intense exercise would be unexpected for most farm animals, its effects are of interest relative to stallions used simultaneously for breeding and athletic competition.

Janett *et al.* (2006) assessed the impact of repeated strenuous exercise on the seminal characteristics of 11 stallions 7–19 year old. They collected one ejaculate/week during each of four consecutive 4 week pretreatment (Period 1), exercise (Period 2) and post-exercise (Periods 3 and 4) periods. Exercise consisted of two sessions/week for a total of eight sessions. For the first two sessions, stallions walked on a treadmill for 5 min at 1.5 m/s at 0% inclination, followed by two intervals of trotting at 3.5 m/s at inclinations of 3 and 6%, respectively. For the subsequent sessions, stallions trotted for 3 min at 3.5 m/s and walked for 1 min at 1.5 m/s as the inclination was increased sequentially from 0 to 9%. At the 9% inclination, trotting speed was increased to 4 and 4.5 m/s. This regimen resulted in increases in average heart rate values ranging from 70.4 to 97.4 beats per minute (bpm) before exercise to between 166.8 and 194.0 bpm immediately thereafter. Strenuous exercise had a negative effect on semen quality; the incidence of acrosome defects and nuclear vacuoles began to rise 3 weeks after the beginning of the exercise period, while the percentage of viable frozen–thawed sperm decreased from 53.8% during the pretreatment period to 49.2% during the exercise period. The number of sperm per ejaculate averaged 7.0, 6.8, 6.1 and 6.6 billion during Periods 1–4, respectively. The decline in sperm number per ejaculate during the 4 week period subsequent to the discontinuation of exercise (i.e. during Period 3) is consistent with a negative effect of strenuous exercise on spermatogenesis, which requires approximately 2 months for completion.

Conflicting reports on the effects of intensive training can be found (e.g. Lange *et al.*, 1997; Davies Morel and Gunarson, 2000). For example, Davies Morel and Gunarson (2000) reported average fertility rates of 74.1, 63.7 and 66.9% for Icelandic stallions receiving intensive, moderate or no training, respectively. However, these findings were based on survey data obtained from a variety of breeding associations and individual stallion owners, rather than from a well-controlled experiment. Intense exercise training has also been associated with negative reproductive consequences in humans (e.g. Arce and De Souza, 1993). Accordingly, intense, stressful exercise is probably detrimental to male fertility. As a practical matter, it is possible that moderate physical conditioning might have a beneficial impact on levels of mating activity for males in a natural mating system, although it does not appear to have a beneficial effect on seminal quality per se.

Frequency of ejaculation

Frequency of ejaculation appears to be without effect on quantitative rates of sperm production. For example, Carson and Amann (1972) conducted a well-replicated study in which daily sperm production of ~8-month-old rabbits was assessed after 40 days of sexual rest or ejaculation either daily or twice daily on an every other day basis. Selected data from that study are presented in Table 2.8. Treatment was without effect on testicular weight or DSP/male or DSP/g of testicular tissue.

Amann (1962a) also studied the effect of frequency of ejaculation on spermatogenesis in dairy bulls. For this study, nine young Holstein bulls were assigned to treatments consisting of either sexual rest or ejaculation 2× or 8×/wk for 20 wk. Five older bulls were either sexually rested or ejaculated 6–8×/week. The results of this study are summarized in Table 2.9.

Frequency of ejaculation was without effect on testicular weight, the percentage of the testicular parenchyma occupied by

seminiferous tubules, or sperm production as judged from the number of germ cells/stage I seminiferous tubular cross section. Although the frequency of ejaculation does not appear to influence sperm production rates, it does exert a profound effect on the number of sperm per ejaculate, as described later.

How to measure daily sperm production?

A number of approaches may be used to measure sperm production. Choosing from among these requires consideration of several factors, including whether the testes can be removed or whether sperm production is to be estimated in a prospective breeding animal. In the former case, one should also consider whether one needs to know how many sperm are being produced per unit of time, or whether it is sufficient to simply assess relative changes in sperm production due to an experimental treatment. In addition to this, every method for quantifying sperm production requires one or more technical assumptions. Readers with an interest in quantifying sperm production should familiarize themselves with these and other considerations of importance during the selection of an evaluation method. Such considerations have been presented elsewhere in greater detail (Amann, 1981; Berndtson, 2011). Several useful and popular methods for quantifying sperm production are described briefly below.

Table 2.8. Daily sperm production of sexually rested (SR) versus frequently ejaculated rabbits. Adapted from Carson and Amann, 1972.

	Frequency of ejaculation[a]		
Characteristic	1×/24 h	2×/48 h	SR
Paired testes weight (g)	6.29	6.58	6.40
DSP[b]/male (10^6)	225	247	247
DSP/g testis	37.4	38.9	39.0

[a]Rabbits were sexually rested for 30 days, followed by 40 days of once daily ejaculation, twice every other day ejaculation or sexual rest.
[b]Daily sperm production.

Enumeration of homogenization-resistant spermatids

During spermiogenesis, the chromatin of elongating spermatids condenses and becomes resistant to homogenization (Amann and Almquist, 1961a). These nuclei remain intact during homogenization, while other components of the tissue are destroyed. Thus, after homogenization of a given quantity of tissue in a known volume of fluid, haemocytometry can be used to determine the number of resistant

Table 2.9. Testicular characteristics and sperm production of sexually rested (SR) or ejaculated bulls. Adapted from Amann, 1962a.

Treatment	No. of bulls	Testes weight (g)	Seminiferous tubules (% testis)	Germ cells/cross section			
				A[a]	Young (1°)[b]	Old (1°)[b]	Spermatids
			Young bulls[c]				
SR[d]	3	301.5	76.5	2.75	55.5	54.5	214.0
2×/wk[d]	3	286.5	77.5	3.30	54.5	52.5	186.0
8×/wk[d]	3	290.5	77.0	2.85	52.5	49.5	188.5
			Mature bulls				
SR[e]	2	411.0	73.8	2.90	55.0	52.0	189.0
6–7×/wk[f]	3	396.3	71.7	3.70	64.0	62.0	226.0

[a]Type A spermatogonia (smooth).
[b]Primary spermatocytes.
[c]Animals 36 months old when sacrificed.
[d]Treatment duration 20 weeks.
[e]5 or 20 weeks sexual rest.
[f]Individual bulls ejaculated 6×/wk for 26 wk, ≥6×/wk for 40 wk, followed by 7×/wk for 7 wk and 8×/wk for 17 wk, respectively.

spermatids/g tissue, or per testis or male. The numbers of such spermatids can be compared directly to provide a relative comparison of sperm production rates in control versus treated subjects (Berndtson, 1977). Alternatively, if an estimate of actual daily sperm production is needed, this can be determined by dividing the spermatid number by a time divisor, equivalent to the number of days of sperm production represented by these cells. For example, if spermatids became resistant to homogenization 6.5 days before spermiation, the total number of homogenization-resistant spermatids would be divided by a time divisor of 6.5 days to obtain an estimate of daily sperm production. Table 2.10 reports time divisors used with the homogenization method for several farm species.

Germ cells per Sertoli cell or per round seminiferous tubular cross section

In addition to germinal cells, seminiferous tubules contain somatic cells known as Sertoli cells. These perform functions that include the formation of a blood–testis barrier, secretion of androgen-binding protein and others (Russell and Griswold, 1993). Sertoli cells form at an early age, and were once believed to remain as a numerically stable population thereafter (Gondos and Berndtson, 1993). It is now apparent that the number of Sertoli cells can change in response to age and/or season in some species (Johnson and Thompson, 1983; Johnson and Nguyen, 1986; Johnson *et al.*, 1991). None the less, these cells appear to be quite resistant to harsh treatments, as evident from their persistence after exposure to some treatments that cause almost complete obliteration of the germ cells (Oakberg, 1959). Because of this resistance, the germ cell:Sertoli cell ratio can be used to assess relative changes in sperm production. For example, if a treatment reduced sperm production by 50%, this would be expected to cause a corresponding 50% reduction in the spermatid:Sertoli cell ratio. With this technique, direct counts of the number of germ cells and Sertoli cells are made in a predetermined number of round seminiferous tubular cross sections. For most studies, one stage, usually containing spherical spermatids, is chosen as representing spermatogenesis as a whole.

Table 2.10. Reported time divisors used for estimating daily sperm production from the number of homogenization-resistant spermatids.

Species	Time divisor (days)	Reference
Boar	5.86	Okwun *et al.*, 1996
Bull	3.27	Amann and Almquist, 1962
Goat	3.56	Ritar *et al.*, 1992
Ram	5.0	Cardosa and Queiroz, 1988
Stallion	6.0	Johnson and Neaves, 1981

The histological specimens that are examined contain some nuclei residing entirely within the section, and also fragments produced by sectioning. The proportion of fragments will increase as the section thickness is decreased, and it will be greater for nuclei of larger versus smaller diameter. Therefore, the resulting crude counts, which include both whole and partial nuclei, must be converted to true counts or whole-cell equivalents. Several equations have been developed for this purpose. That used by the author is Abercrombie's equation (Abercrombie, 1946), which is as follows:

True count = Crude count × (section thickness/section thickness + nuclear diameter)

Abercrombie's equation and other similar equations are only applicable for spherical nuclei. Thus, it is customary to select tubules at a stage containing spherical spermatids for counting; irregularly shaped elongated spermatids are not counted. The shape of the Sertoli nuclei is also irregular, so it is also customary to count only those Sertoli nuclei containing a nucleolus. Abercrombie's formula can be applied to the spherical nucleoli to obtain a true count for the Sertoli nuclei. True counts are then used to determine germ cell:Sertoli cell ratios. Alternatively, some investigators calculate the average number of Sertoli cells per tubular cross section in the control subjects or for all animals in

the experiment. The number of germ cells in this average is then expressed as the number of germ cells per tubular cross section. This technique is useful for assessing relative changes in sperm production due to treatment, but it does not provide an estimate of the actual number of sperm being produced/day per unit of tissue or per male.

Volume density approaches for estimating sperm production

For the volume density approach, the volume of the testicular tissue is first recorded. This is usually determined by measuring its fluid displacement or, alternatively, is simply assumed to equal testis weight minus the weight of the testicular capsule, because the specific gravity of the testis in mammals is very close to 1.0 (Swierstra, 1966; Gebauer *et al.*, 1974a; de Jong and Sharpe, 1977; Johnson and Neaves, 1981; Johnson *et al.*, 1981; Mori *et al.*, 1982). The tissue is then processed for histological evaluation. Tissue shrinkage should be recorded, to permit adjustment as necessary (Berndtson, 2011). Next, the eyepiece of a microscope is fitted with a fixed pointer or pointers. The slide of testicular tissue is moved at random, after which the identity of the structure at the tip of the pointer is recorded. This process is repeated many times. With sufficient sampling, the frequency with which a particular structure is 'hit' will be proportional to its volume density. For example, if the nuclei of the spherical spermatids occupied 10% of the tissue, one would expect these nuclei to be 'hit' 10% of the time.

With these data, the total volume of the nuclei of each type of germ cell can be determined as the product of its volume density and the total testicular parenchymal volume. By dividing the total volume of these nuclei by the volume of a single nucleus, an estimate is obtained of the total number of cells of each type. The volume of spherical nuclei is usually determined by entering nuclear diameter into the equation for calculating the volume of a sphere. Reconstructon of serial sections and other more sophisticated procedures have been used to estimate the volume of nuclei with an irregular outline (e.g. elongated spermatids and Sertoli cells; Johnson *et al.*, 1984; Sinha Hikim *et al.*, 1988). Once the total number of cells of a given type has been determined, the data can be used to calculate germ cell:germ cell or germ cell:Sertoli cell ratios. Alternatively, an estimate can be obtained of daily sperm production by dividing the total number of spermatids by a time divisor (the number of days of sperm production represented by these cells). DSP may also be estimated from the numbers of younger germ cells, but this requires correction for subsequent cell divisions. Such estimates are subject to greater potential errors due to normal or treatment-induced cellular attrition.

Estimating daily sperm production (DSP) from daily sperm output (DSO)

Sexual rest results in sperm losses in the urine (Holtz and Foote, 1972), but such losses are minimal for males maintained on a regular, frequent schedule of ejaculations (Amann, 1981). Thus, sperm output that is frequently collected from a male provides an excellent method for estimating DSP. It offers the advantage of not requiring the removal of testicular tissue. In addition, by collecting semen continuously over time, there is the opportunity to assess temporal changes in sperm output and seminal quality that might follow acute, experimental exposure of males to an agent or experimental treatment of interest (e.g. assessment of seminal quality after a single dosage with an anthelmintic).

Typical sperm output of economically important species

Sperm production varies greatly among individuals both within and among species. Several factors contributing to such variability within species have already been described, including inherent differences in testis size and the influences of variables such as age, season (in some species), environmental factors, drugs, etc. The typical sperm production of breeding-age males of several species is summarized in Table 2.11.

Most male mammals of reproductive age other than humans produce and ejaculate

Table 2.11. Daily sperm production (DSP) of sexually mature males (range in parentheses).

Species	DSP/g (million)	DSP/male (billion)	Reference
Boar	–	31.3	Kennelly and Foot, 1964
Boar	24.7	16.5	Swierstra, 1968b
Bull	17.7	11.5	Amann and Almquist, 1962
Bull	16.9	5.3	Swierstra, 1966
Bull	11.2 (7.25–16.67)	–	Berndtson *et al.*, 1987a
Bull	9.31	4.30	Berndtson and Igboeli, 1988
Bull	–	3.79 (1.99–7.86)	Berndtson and Igboeli, 1989
Goat	23.8	4.0–6.4	Ritar *et al.*, 1992
Goat	30.3	5.54	Leal *et al.*, 2004
Ram	~27[a]	12.9	Schanbacher and Ford, 1979
Ram		8.06[b]	Dacheux *et al.*, 1981
Ram	22.8	4.4	Cardosa and Queiroz, 1988
Stallion	21.2	8.0	Gebauer *et al.*, 1974a
Stallion	11.9[c]	3.6[c]	Berndtson *et al.*, 1979
Stallion	17.0–18.8	5.3–6.4	Johnson and Neaves, 1981

[a]Estimate calculated by the author by dividing DSP by testicular weight, without correction for the weight of the tunica albuginea.
[b]Ile-de-France rams.
[c]Estimates derived by dividing the number of homogenization-resistant spermatids by a time divisor of 6.0 days.

sperm in great excess of the numbers required for normal fertility. This does not diminish the importance of considering sperm production during an examination of breeding soundness or during the selection of males for breeding. As discussed later, sperm number declines in successive ejaculates taken on a single day (Amann, 1981). Thus, the potential for sperm numbers per mating to decrease to below an optimal level is always possible for males required to breed large numbers of females within a short period of time via natural mating. However, the latter possibility should be less for males producing large numbers of sperm than for those for which sperm production is more limited. The potential for sperm number to become limited may also differ among species. Foote (1978) reported that rams tend to ejaculate a relatively smaller number of sperm per semen sample than bulls or boars, and that they are capable of ejaculating repeatedly (11×/day in the study of Salamon, 1962). In contrast, boars ejaculate a large number of sperm in each ejaculate, and can deplete their epididymal sperm reserves quite rapidly (Foote, 1978).

Sperm Maturation and Transport through the Excurrent Ducts

Sperm transit time

Upon release from the testis, sperm undergo transit through the efferent ducts and the epididymis. The time required for epididymal transit was summarized in Table 2.7 for several of the economically important species. Whereas the transit time through the caput epididymis and corpus epididymis is relatively constant, transit through the cauda epididymis is more rapid in males ejaculating at a higher frequency (e.g. daily; Amann, 1981).

Maturational changes and the acquisition of fertility

Spermatozoa are not fertile upon release from the testis, but undergo maturation within the epididymis. Epididymal secretions appear to play a critical role in the maturation process, which includes the acquisition of motility, changes in sperm membranes that permit binding to the zona pellucida of the oocyte,

nuclear decondensation and other changes (Cooper, 1995). The sperm of most species do not attain fertility until reaching the corpus epididymis.

Extragonadal sperm reserves (EGR)

The epididymides and vas deferentia typically contain the equivalent of several days of sperm production, which collectively constitute the EGR. It is from these reserves, and especially from the tail of the epididymis, that sperm are emitted during ejaculation. During periods of sexual rest, sperm accumulate and reach maximal levels. Degeneration and resorption of aged sperm appears to be minimal in normal males (Amann, 1981). Rather, as additional sperm leave the testis and enter the EGR, others enter the urethra and are flushed from the body via the urine (Holtz and Foote, 1972). The size of the EGR in sexually rested males of several economically important species is summarized in Table 2.12.

Only a portion of the EGR is available for ejaculation. Some sperm reside within the head and initial segment of the body of the epididymis, from which they are unavailable for ejaculation, while the lining of the epididymal duct can also preclude complete removal. Based on studies with rats, bulls and stallions, Amann (1981) estimated that about 55–65% of the sperm within the cauda epididymis and vas deferens of sexually rested males can be removed by harvesting 5–20 ejaculates in succession on a single day.

Table 2.12. Extragonadal sperm reserves (EGR) of sexually rested males.

Species	EGR (billion)	Reference
Boar	166[a]	Swierstra, 1971
Bull	69	Almquist and Amann, 1961
Goat	17.5	Jindal and Panda, 1980
Goat	47.8	Ritar *et al.*, 1992
Ram	93.5	Lino, 1972
Stallion	89	Amann *et al.*, 1979

[a]Values 72 h post depletion of EGR via the collection of one ejaculate in the morning, a second in the afternoon and a third on the following morning.

Several important management concepts are easy to understand by drawing an analogy between the EGR and a reservoir from which water might be obtained. As sperm are released from the testis and enter the extragonadal ducts at a constant rate, one could imagine that water is also entering the reservoir at a constant rate. During times when water is not being used, its level will continue to rise until, upon reaching maximal capacity, it simply overflows the dam. This would be comparable to the filling of the EGR during periods of sexual rest, which would continue until sperm overflowed into the urinary tract. At other times, water might be drawn from a reservoir at a rate exceeding that at which it was being replenished. This might continue for a while, but would be accompanied by a progressive decrease in the level of water behind the dam. This would be analogous to the collection of several successive ejaculates from a male after sexual rest. Each ejaculate would reduce the number of residual sperm within the EGR. Ultimately, if water continued to be drawn from a reservoir at a rate exceeding its rate of replenishment, the reservoir would be depleted. At that point, the rate at which additional water could be drawn would be limited by and equal to its rate of replenishment. Similarly, if a male continues to ejaculate at a high frequency, the EGR will ultimately be depleted. At that point, DSO will be limited by and equal to DSP. These concepts have important implications relative to conventional breeding soundness examinations, and for the management of males to maximize the sperm harvest, which are discussed below.

Sperm Harvest – Maximising DSO and Seminal Quality

Seminal quality and sperm number in ejaculates taken after sexual rest

The first ejaculate(s) taken after sexual rest usually contains very large numbers of sperm. The data of Squires *et al.* (1979) illustrate the decline in sperm output in successive ejaculates taken after sexual rest. In their

study, the sperm output of 2–3 year old stallions averaged 4.5, 2.4, 0.9, 0.6 and 0.5 billion for the first to fifth ejaculates, respectively. The corresponding sperm output for 4–6 year old stallions averaged 9.5, 3.5, 2.6, 1.3 and 1.1 billion, while that for 9–16 year old stallions averaged 11.4, 5.5, 2.4, 1.8 and 1.2 billion, respectively. A decline in sperm output in successive ejaculates is to be expected, based on the concept of the EGR described above. For this reason, the number of sperm within the first few ejaculates taken after sexual rest does not provide a reliable estimate of the average DSO to be expected once a male is used on a regular frequent basis. This is an important point relative to routine breeding soundness examinations, for which only one or two ejaculates are often collected after a lengthy period of sexual inactivity. Such collections are useful, because they demonstrate a willingness or ability of the male to ejaculate, while confirming recent spermatogenic activity and providing an opportunity to assess seminal quality. However, they are inadequate for characterizing actual levels of sperm production. Unless there are the time and resources with which to maintain the male on a regular, frequent seminal collection schedule, at which DSO would approach DSP, measurements should be made of scrotal width or circumference, as these allow an estimate of testis size, which is highly correlated with DSP (Table 2.4).

While initial ejaculates taken after sexual rest usually contain large numbers of sperm, the quality of these first ejaculates may be reduced, although this appears species dependent. For example, in one study (Pickett and Voss, 1973), initial motility averaged 56% for ejaculates from stallions collected either once or six times/wk. In contrast, Almquist (1973) collected two ejaculates from bulls on 1 day/wk. Initial motility averaged 48 versus 60% for first and second ejaculates of beef bulls collected without sexual preparation. The corresponding motility for dairy bulls collected after one false mount averaged 63 versus 68%, respectively. Much greater decreases in semen quality are possible during extended periods of inactivity. With respect to a breeding soundness examination, retesting at a later date and/or the collection of additional ejaculates on the same date would be advisable if the quality of an initial ejaculate(s) was questionable. It is equally important to consider that spermatogenesis requires approximately 2 months in most mammals, and that additional time is required for sperm transport through the epididymis (Table 2.7). Thus, the semen that is harvested on any given day represents the culmination of a process that for most species extended over at least the previous 60–70 days. Low semen quality could reflect problems with spermatogenesis or epididymal function arising at any time during that period, from which recovery could require a similar length of time.

The relationship between frequency of ejaculation and DSO

As cited previously, sperm production is not influenced by frequency of ejaculation. During sexual rest, sperm may be lost in the urine (Holtz and Foote, 1972). Higher frequencies of ejaculation can eliminate such losses, while at any frequency above that level, increasing frequencies of ejaculation will reduce the number of sperm per ejaculate. The results of several representative studies conducted to examine the influence of frequency of ejaculation on the DSO of dairy bulls and stallions are given in Tables 2.13 and 2.14, respectively. For most

Table 2.13. Frequency of ejaculation and daily sperm output (DSO) of Holstein bulls.

Frequency of ejaculation	Sperm/ ejaculate (billion)	DSO (billion)	Reference
1×/wk	10.9[a]	1.56[a]	Almquist, 1982
6×/wk	5.1[a]	4.41[a]	
1×/wk	17.8	2.5	Hafs *et al.*, 1959
7×/wk	4.8	4.8	
2×/day on M, W, F	5.3	4.6	Amann and Almquist, 1961b
2×/day on Th, F	7.6	4.3	Seidel and Foote, 1969

[a]Means for 5–9-year old bulls.

species, daily or every other day ejaculation will enable essentially all of a male's sperm production to be harvested.

Sexual preparation and sperm output

To collect semen with an artificial vagina, one need only provide conditions that will arouse the male and permit him to mount. However, the number of sperm per ejaculate from rams (Knight, 1974), bulls (Hale and Almquist, 1960) and boars (Hemsworth, 1979) can be increased by appropriate sexual preparation. Sexual preparation entails administering procedures such as teasing, false mounting or active restraint before ejaculation. Such procedures cause a release of oxytocin and possibly other hormones, which, in turn, enhance sperm transport or emission from the extragonadal ducts (Sharma and Hays, 1973, Berndtson and Igboeli, 1988).

False mounting consists of allowing the male to mount a teaser animal or dummy while the penis is deflected to prevent penetration of the reproductive tract or artificial vagina. Without tactile stimulation, the male will dismount without ejaculating. This procedure is usually repeated 2–3 times. Active restraint involves providing conditions that encourage the male to mount, but restraining him when he attempts to do so. The latter is called teasing when applied to the stallion, for which it is customary to provide a physical barrier between the mare and stallion to prevent injury. The impact of sexual preparation on the sperm output of dairy bulls is illustrated by the data in Table 2.15. Sexual arousal does not increase sperm output from stallions, although teasing increases gel and total seminal volume (Pickett and Voss, 1973).

While sexual preparation is effective in increasing the number of sperm per ejaculate in most species, it is important to recall that ejaculation does not affect sperm production. Rather, by increasing the efficiency of ejaculation, sexual preparation simply allows maximal DSO to be achieved via ejaculation at a lower frequency than would be required in its absence. This is beneficial for the AI industry, because it reduces the total time required to collect and process the maximal quantity of semen for a given male.

Table 2.14. Frequency of ejaculation and daily sperm output (DSO) of stallions.

Frequency of ejaculation	Sperm/ejaculate (billion)	DSO (billion)	Reference
Daily	4.5	4.5	Gebauer *et al.*, 1974a
Every other day	7.0	3.5	Swierstra *et al.*, 1975
1×/wk	13.5	1.93	Pickett and Voss, 1973
3×/wk	12.7	5.43	
6×/wk	7.2	6.16	
1×/wk	11.4	1.63	Pickett *et al.*, 1975
3×/wk	11.7	5.03	
6×/wk	5.9	5.04	

Table 2.15. Increase in sperm output attributable to sexual preparation of bulls.

Ejaculations/wk	No. of false mounts	Active restraint (min)	Sperm/first ejaculation (% increase)	Reference
1	1	2–3	36	Collins *et al.*, 1951
2	1	0	50	Branton *et al.*, 1952
2	2	0	67	
4	0	1	129	Crombach, 1958
4	10	0	147	
4	5	1	251	
6	3	–	~30	Almquist *et al.*, 1958

Stimulus pressure to maintain libido

Although the frequencies of ejaculation required to maximize sperm harvest are not excessive, males maintained on such schedules can experience reduced libido. This is often manifested via increases in reaction time – the time between presentation to a teaser animal and mounting or ejaculation.

This issue was examined extensively for dairy bulls in the classical studies of Hale and Almquist (1960), who developed strategies for maintaining libido that remain the standard of the bovine AI industry. Their research showed that the key to maintaining good libido is to provide novelty, or what they defined as stimulus pressure. In one of their studies, bulls were maintained on a 1×/wk collection schedule. Identical procedures were followed for each collection, and reaction times were recorded. This process was repeated each week until the reaction time for a bull exceeded 10 min. On the subsequent week, the bull was presented to the same teaser animal, which had simply been moved a distance of 3 ft from the location used previously. Reaction time decreased to an average of ~2 min. A similar response was obtained without moving the location of the teaser animal, but by simply rocking the teaser back and forth in place. In another experiment, these authors (Hale and Almquist, 1960) allowed bulls to mount and ejaculate at will for 1 h, during which time individual bulls ejaculated an average of 10.6 times. The interval between successive ejaculates increased during this time, and most bulls were satiated by the end of the first hour. The authors then introduced a new teaser animal, again allowing the bulls to mount at will. During the second hour, bulls ejaculated an additional 8.4 times. These data demonstrated that satiation was associated with a specific teaser animal or set of conditions, rather than with ejaculation per se. The novelty created by a change of teasers, movement and/or a change of locations is exploited routinely by the AI industry.

Almquist and Hale (1956) demonstrated further that the frequency with which teasers, locations, etc. needs to be changed to maintain good libido is related to the frequency of ejaculation. This is evident from the data in Table 2.16. In this study, new teasers were introduced as necessary to maintain minimal reaction times, which averaged ~6 min. The frequency at which new teaser animals needed to be introduced was three times greater for bulls maintained on a 6× versus a 2×/wk collection schedule. Novelty appears to have a similar beneficial impact on libido in many other species (Price, 1985).

Conclusions

Spermatogenesis is a truly remarkable process, resulting in levels of sperm production in most species that enable successful mating with large numbers of females or the potential for insemination of an even greater number of females via AI. Because sperm production is not influenced by frequency of ejaculation, higher frequencies of ejaculation are accompanied by corresponding decreases in the number of sperm per ejaculate. The implications of this relationship for the management of males used for natural mating or for AI have been described. In addition to this, libido must be maintained if producers are to make maximal utilization of valuable sires. Novelty appears to be a key factor affecting libido in bulls, and presumably also in many other species. Several approaches for providing novelty have been described. Judicious selection and management are critical for allowing each male to realize its maximum reproductive potential.

Table 2.16. Stimulus pressure to maintain sexual activity of bulls ejaculated 2× or 6×/wk. From Almquist and Hale, 1956.

Reaction time/Stimulus pressure	Frequency of ejaculation	
	2×/wk	6×/wk
Reaction time (min)	6.4	6.2
Stimulus pressure[a]		
Per 24 wk period	4.5	12.5
Per 48 ejaculates	4.5	4.2

[a]No. stimulus animals or combinations of stimulus animals presented.

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