Chapter 4
Factors Affecting Boar Reproduction, Testis Function, and Sperm Quality

Elisabeth Pinart and Marta Puigmulé

Abstract Sperm quality of boars depends on both intrinsic (genetic) factors and extrinsic (environmental/husbandry) factors. In relation to intrinsic factors, an increased reproductive efficiency of crossbred boars as compared with purebreds manifests the importance of heterosis in this context. Studies on semen traits have demonstrated that some parameters have greater heritability than others, such as semen volume. At the same time there is a poor relationship between seminal parameters and fertility that limits the sensitivity and specificity of cut-off values based on these traits to select boars. Recent studies have pointed out the importance of selecting high-fertility boars according to their testis size at pre-pubertal age. Genetic defects in testicular size and structure, such as in cases of cryptorchidism, result in partial or total arrest of spermatogenesis at post-pubertal age. In relation to extrinsic factors, the ambient temperature, photoperiod, and rhythm of semen collection are negatively correlated with the reproductive performance of boars, whereas food supplementation, social contact with other pigs and the accuracy of semen processing protocols are positively correlated with artificial insemination (AI) outcomes.Certain divergences in the effects of these factors on individuals could be mainly attributed, although not exclusively, to the nature of the breed.

4.1 Introduction

The reproductive performance of boars is usually monitored by analyzing semen quality. In assisted reproduction, threshold values for sperm concentration, sperm motility, and sperm morphology have been established in order to obtain high fertility and prolificacy rates (Bonet et al. 2000; Casas et al. 2009, 2010). Semen quality is not only a proxy measure of boar fertility, but it also has sire effects on pig production in terms or reproductive performance of progeny (Smital 2009; Huang et al. 2010).

E. Pinart (✉) · M. Puigmulé
Biotechnology of Animal and Human Reproduction (TechnoSperm),
Department of Biology, Institute of Food and Agricultural Technology,
University of Girona, Campus Montilivi, 17071 Girona, Spain
e-mail: elisabeth.pinart@udg.edu

S. Bonet et al. (eds.), *Boar Reproduction*, DOI: 10.1007/978-3-642-35049-8_4,

Monitoring and analyzing the quality of boar semen has great economic importance for pig breeders; the economic return of an artificial insemination (AI) center primarily depends on the boar's ability to produce spermatozoa (Robinson and Buhr 2005). Boars consistently producing high-quality ejaculates are crucial in AI programs due to the limited number of insemination doses that can be obtained from one ejaculate (Ciereszko et al. 2000; Estienne and Harper 2004). During the practice of AI the characteristics of collected semen are usually evaluated to ensure its quality before service (Petrunkina et al. 2007). Unfortunately, sperm production of boars can fluctuate up and down as much as 25–30 % throughout the year, forcing AI centers to keep additional boars to compensate for these shifts (Ciereszko et al. 2000; Sancho et al. 2004).

The reproductive efficiency of AI boars can be evaluated by different indicators such as semen quality (Smital 2009; Dyck et al. 2011; Yeste et al. 2010, 2011), fertility (Flowers 2002; Gadea 2005) and sexual behavior (Levis and Reciks 2005; Hemsworth and Tilbrook 2007; Frydrychová et al. 2011). A number of tests have also been developed to assess sperm functionality and in vitro fertility (Popwell and Flowers 2004; Bussalleu et al. 2005; Lovercamp et al. 2007; Foxcroft et al. 2008). Nevertheless, the limited repeatability, technical complexity, and high cost of these tests have impeded their widespread application in field conditions (Dyck et al. 2011).

Semen quality is routinely assessed by determining semen volume, sperm concentration, total number of spermatozoa per ejaculate, sperm viability, sperm motility, and sperm morphology (Pruneda et al. 2005; Casas et al. 2009, 2010). All these traits are only partial semen characteristics and it is impossible to determine semen quality on the basis of only one of them (Smital et al. 2004; Bussalleu et al. 2005). In vivo fertility of boars is currently evaluated from the farrowing rate and the litter size, the latter being expressed as the total number of piglets born or the number of piglets born alive (Gadea 2005). Sexual behavior is assessed by recording the duration of time between the boar entering the collection pen and mounting the dummy, the number of mounts before ejaculation starts, and the duration of ejaculation (Levis and Reicks 2005).

Reproductive performance of boars depends on genetic or intrinsic factors such as breed (Smital 2009; Wolf 2009), age (Huang et al. 2010) and testicular size (Clark et al. 2003; Pinart et al. 1999a), on environmental extrinsic factors like temperature (Ciereszko et al. 2000; Yeste et al. 2010) and photoperiod (Sancho et al. 2004, 2006; Yeste et al. 2010), and on husbandry extrinsic factors such as semen collection rhythm (Pruneda et al. 2005), nutrition (Yeste et al. 2010, 2011) and, social environment (Kunavongkrit et al. 2005).

4.2 Genetic or Intrinsic Factors

Little data exist about the heritability of reproductive traits. Despite the fact that heterosis (also known as hybrid vigor or outbreeding enhancement) might have an important impact on the pig industry (Smital et al. 2004), only few reports exist on the evaluation of crossbreeding effects on reproductive traits of boars and most of them are based on small datasets and/or a small number of males.

Numerous studies involving multiple genetic lines have reported large individual variations in the seminal quality of boars, whereas others have demonstrated that crossbred boars usually produce the highest seminal quality, followed by purebred terminal lines and lastly, purebred maternal lines (Sonderman and Luebbe 2008). The differences between purebreds and crossbreds also include sensitivity to seasonal infertility, ejaculate volume per age interactions, optimum collection frequency, rate of ejaculates discarded due to poor quality, age of puberty, libido, and trainability, and sperm lifespan (Sonderman and Luebbe 2008).

4.2.1 Breed

4.2.1.1 Differences Among Breeds in Reproductive Parameters

Breed Differences in Semen Quality

There are few comparative studies focusing on the seminal characteristics of boar breeds. Among these, five comprehensive trials should be mentioned since they present a good overview of the importance of the breed on seminal features (Park and Yi 2002; Smital et al. 2004; Smital 2009; Wolf 2009; Wolf and Smital 2009). Briefly, many ejaculates from boars of different purebred populations were collected and assessed over a long period of time. Data obtained from these extensive studies indicate that Large White boars appear to have the highest seminal volume, followed by other breeds in this sequential order: Yorkshire, Czech Landrace and Czech Large White, Pietrain, Czech Meat Pig, Prestice Black-Pied Hampshire, and Duroc boars. The coefficient of variation among breeds for semen volume is estimated at 30–40 % (Smital et al. 2004) (Table 4.1).

Table 4.1 Breed ranking for seminal volume

Breed	Seminal volume (ml)	References
Large white	350	Smital et al. (2004)
	270	Wolf (2009); Wolf and Smital (2009)
Yorkshire	280	Park and Yi (2002)
Czech landrace	270	Smital et al. (2004); Wolf (2009)
Czech large white	270	Smital et al. (2004); Wolf (2009)
Pietrain	270	Wolf 2009; Wolf and Smital 2009
	240	Smital et al. (2004)
Czech meat pig	250	Wolf (2009)
	215	Smital et al. (2004)
Prestice black-pied	235	Smital et al. (2004)
Hampshire	220	Smital et al. (2004)
Duroc	200	Wolf 2009; Wolf and Smital (2009)
	160	Park and Yi (2002); Smital et al. (2004)

Smital et al. (2004) reported that the Large White breed also has the highest total number of spermatozoa (120 × 10^9 spermatozoa/ejaculate), whereas in the remaining breeds the total number of spermatozoa ranges between 100 × 10^9 and 80 × 10^9 spermatozoa/ejaculate. Ranking breeds from the highest to the lowest in sperm number resulted in the following sequence: Czech Landrace, Czech Large White, Hampshire, Prestice Black-Pied, Pietrain, Czech Meat Pig, and Duroc. According to these results it was concluded that the coefficient of variation among breeds for total number of spermatozoa is of 30–40 %, the same as for semen volume. In contrast, Wolf and Smital (2009) reported that the Pietrain breed produces the highest total number of spermatozoa (118 × 10^9 spermatozoa/ejaculate) followed by Large White (101 × 10^9 spermatozoa/ejaculate) and Duroc breeds (94 × 10^9 spermatozoa/ejaculate). Such variability in records reflects a differential behavior among individuals and stresses the difficulty of validating single values for a given breed when dealing with reproductive parameters.

Differences also exist among breeds in terms of sperm concentration within the ejaculate, so that higher values have been recorded in Duroc breed and lower values in Large White breed (Wolf 2009; Wolf and Smital 2009). As for seminal volume and total number of spermatozoa per ejaculate, the coefficient of variation among breeds for sperm concentration ranges between 30 and 40 % (Smital et al. 2004) (Table 4.2).

Little data has been published about the differences among breeds in sperm membrane integrity, sperm motility, and sperm morphology. The total number of live (intact membrane) spermatozoa is about 86 × 10^9 spermatozoa/ejaculate in Large White boars, 72 × 10^9 spermatozoa/ejaculate in Czech Landrace and Prestice Black-Pied boars, 71 × 10^9 spermatozoa/ejaculate in Czech Large White boars, 68 × 10^9 spermatozoa/ejaculate in Hampshire boars, 66 × 10^9 spermatozoa/ejaculate in Czech Meat Pig, 64 × 10^9 spermatozoa/ejaculate in Pietrain boars, and 59 × 10^9 spermatozoa/ejaculate in Duroc boars (Smital et al. 2004). The coefficient of variation among breeds in sperm membrane integrity is also of 30–40 % (Smital et al. 2004). Total sperm motility (expressed as percentage of motile spermatozoa) has an average of 74–77 %, with low differences among breeds (Wolf 2009; Wolf and Smital 2009). According to this, Smital et al. (2004)

Table 4.2 Breed ranking for sperm concentration

Breed	Sperm concentration (spermatozoa/mm^3)	References
Duroc	490 × 10^3	Wolf (2009); Wolf and Smital (2009)
Czech meat pig	480 × 10^3	Wolf (2009)
Pietrain	450 × 10^3	Wolf (2009); Wolf and Smital (2009)
Czech large white	424 × 10^3	Wolf (2009)
Czech landrace	417 × 10^3	Wolf (2009)
Large white	400 × 10^3	Wolf (2009); Wolf and Smital (2009)

found a coefficient of variation for sperm motility of less than 10 % among breeds (Smital et al. 2004). On the other hand, divergences exist between studies with regard to sperm morphology. Wolf (2009) and Wolf and Smital (2009) found little differences among breeds in the percentage of aberrant spermatozoa, calculating ranges between 8.5 and 11.5 %, whereas Smital et al. (2004) found that the highest coefficient of variation among breeds for a seminal parameter was manifested in the proportion of aberrant spermatozoa, with a variation of 80 %.

In the study by Smital et al. (2004), Large White boars exceeded by far all other purebreds in all semen parameters analyzed, whereas Duroc boars had the poorest sperm output. Despite these results, most authors agree that no breed excels in all semen characteristics and that breeds differ basically in semen volume and total number of spermatozoa per ejaculate (Kennedy and Wilkins 1984; Ciereszko et al. 2000; Park and Yi 2002; Flowers 2008; Sonderman and Luebbe 2008; Smital 2009; Wolf 2009). In a more recent study, Smital (2009) also concluded that differences among breeds in semen traits correlate with sperm production, but they do not with sperm quality. Considering the total number of spermatozoa, Smital (2009) classified Pietrain, Czech Meat Pig, and Czech Large White as above-average breeds, Landrace, Hampshire, and Large White as average breeds, and Duroc as a below-average breed. In another extensive study examining the sperm production of purebred boars, Kennedy and Wilkins (1984) found that Yorkshire boars routinely produce 10–12 $\times$ 10^9 more spermatozoa than Hampshire, with Landrace and Duroc producing intermediate amounts. It is likely that these differences among studies in the sperm production of boar breeds are related to different environmental, social, and husbandry factors.

Boar breeds also differ in the refrigerability of seminal doses. Sonderman and Luebbe (2008) found that ejaculates from Hampshire boars are very sensitive to dilution and refrigeration at 15–17 °C, resulting in a significant decrease in sperm quality and fertility of refrigerated doses 24 h after semen collection. In contrast, ejaculates from both Yorkshire and Duroc are resistant to refrigeration conditions since their sperm quality and fertility are stable during the first 3 days of refrigeration, whereas Landrace boar ejaculates exhibit average refrigerability (Sonderman and Luebbe 2008). Maternal line breeds are reported to be more sensitive to storage conditions than terminal line breeds (Sonderman and Luebbe 2008).

Discrepancies exist about the freezability of semen doses from different breeds. According to Waterhouse et al. (2006), ejaculates from Duroc boars exhibit better freezability than those of Landrace boars, as confirmed by the higher percentage of spermatozoa with intact plasma membrane and intact acrosome in post-thawed samples from Duroc males. Moreover, a positive correlation exists between the proportion of viable post-thaw spermatozoa and the ratio of polyunsaturated fatty acids (PUFA), which is of 0.64 and 0.67 for Landrace and Duroc boars, respectively (Waterhouse et al. 2006). In contrast with these results, Roca et al. (2006) found that Landrace and Pietrain boars have higher post-thaw sperm motility, membrane integrity, mitochondrial membrane potential, and acrosomal integrity than Large White, Duroc, and Yorkshire boars. Nevertheless, according to this last reference the variance in post-thaw semen quality measurements is not explained

by breed due to the considerable variability among boars within the same breed. Other studies found farrowing rates 20 % greater in post-thawed spermatozoa from Large White boars as compared with Landrace boars, whereas farrowing rates of fresh semen from the same boars were higher for Landrace than for Large White males (Johnson et al. 1981, 1982).

Effect of Heterosis on Semen Quality

Heterosis is manifested in an increased reproductive efficiency of crossbred animals as a result of a faster development in comparison to purebreds, a lower age at puberty, a greater weight at a constant age, a greater testicular weight and a higher seminal quality (Smital et al. 2004; Flowers 2008; Smital 2009). Hybrid boars below 8 months of age have a stronger libido, greater semen volume, higher sperm motility, lower frequency of abnormal spermatozoa and higher pregnancy rates than purebred boars; nevertheless, these differences diminish at the age of maturity (Smital et al. 2004).

For each semen trait, heterotic effects are estimated from the average value of semen characteristics of the hybrid progeny and the mid values of purebred parents (Wolf and Smital 2009). The heterotic effect of crossing on semen traits is calculated from:

$$h_{A\times B} = \text{crossing}_{A\times B} - {}^{1}/_{2}\,(\text{breed}_A + \text{breed}_B)$$

where, $\text{breed}_{A\times B}$ equal the effect for the crossbred combination A × B, and breed_A and breed_B equal the effects of breeds A and B. Heterosis can range from 0 (lack of heterotic effect) to 1 (total heterosis), the results being expressed in absolute terms or in percentage.

Although heterosis for semen traits is high, not all crossings result in increased semen quality (Smital et al. 2004; Smital 2009; Wolf and Smital 2009). The effects of heterosis on semen traits are extensively studied in two studies using large numbers of purebred and crossbred boars over a long time period. In the first study (Smital et al. 2004), a significant heterotic effect on sperm quality was found; manifestation of heterosis was less impressive in the second study (Smital 2009). The differences between both studies could probably have been related again to external ambient factors, husbandry factors, age of boars, and/or semen collection rhythm. Despite these differences, results from both studies indicate that heterosis results in increased semen volume and total number of spermatozoa in nearly all crossings, which usually ranges from 3 to 10 %. A favorable heterotic effect for sperm concentration, sperm membrane integrity, and sperm motility is only described in a few crossings. The heterotic effect on the percentage of abnormal spermatozoa in the Duroc × Large White crossing is manifested only at 5 % (Smital 2009). In the crossings of Duroc × Large White, Duroc × Pietrain, and Large White × Pietrain, the decrease in the frequency of abnormal spermatozoa ranges from 10 to 23 % as compared with their purebreds (Wolf and Smital 2009) (Table 4.3).

Table 4.3 Heterotic effect of different crossings on semen traits

Crossing	Heterotic effect		References
Duroc × Large white	Semen volume	3 %	Smital et al. (2004)
	Total number of spermatozoa/ejaculate	No heterosis	Smital (2009)
	Sperm concentration	4 %	Smital (2009)
		No heterosis	Wolf and Smital (2009)
	Sperm motility	No heterosis	Smital (2009)
Large white × Pietrain	Semen volume	4 %	Smital (2009)
		6–7 %	Wolf and Smital (2009)
	Total number of spermatozoa/ejaculate	3 %	Smital (2009)
	Sperm concentration	No heterosis	Wolf and Smital (2009)
	Sperm motility	No heterosis	Smital (2009)
Czech landrace × Duroc	Semen volume	10 %	Smital et al. (2004)
	Total number of spermatozoa/ejaculate	13 %	Smital et al. (2004)
	Sperm viability	10 %	Smital et al. (2004)
Czech meat pig × Pietrain	Semen volume	17 %	Smital et al. (2004)
		No heterosis	Smital (2009)
	Total number of spermatozoa/ejaculate	10 %	Smital et al. (2004)
	Sperm concentration	17 %	Smital (2009)
	Sperm viability	5 %	Smital et al. (2004)
	Sperm motility	No heterosis	Smital (2009)
Duroc × Hampshire	Semen volume	23 %	Smital et al. (2004)
		12 %	Smital (2009)
	Total number of spermatozoa/ejaculate	8 %	Smital et al. (2004); Smital (2009)
	Sperm viability	No heterosis	Smital et al. (2004)
	Sperm motility	2 %	Smital (2009)
Hampshire × Pietrain	Semen volume	32 %	Smital et al. (2004)
		12 %	Smital (2009)
	Total number of spermatozoa/ejaculate	18 %	Smital et al. (2004)
		6 %	Smital (2009)
	Sperm viability	10 %	Smital et al. (2004
	Sperm motility	2 %	Smital (2009)
Duroc × Pietrain	Semen volume	6–7 %	Wolf and Smital (2009)
		No heterosis	Smital (2009)
	Total number of spermatozoa/ejaculate	No heterosis	Smital et al. (2004); Smital (2009)
	Sperm concentration	No heterosis	Wolf and Smital (2009)
	Sperm viability	No heterosis	Smital et al. (2004)
	Sperm motility	No heterosis	Smital (2009)

Breed Differences in Fertility

Boars, as independent individuals, are a significant source of variation with regards to the success of both in vivo (Long et al. 1999) and in vitro (Flowers 1997) fertilization. However, owing to several technical difficulties only a few studies have attempted to investigate whether there is a genetic component for these differences. The low number of sows that can be inseminated from a single ejaculate makes it difficult to evaluate boar effects independently from those of the sow (Flowers 2008). Moreover, in commercial production, pooling ejaculates from several boars has gained widespread acceptance, especially in the USA, because it has been reported to increase the efficiency of insemination doses (Flowers 2008). Consequently, most commercial boar studs do not routinely collect data that can be used to assess individual male fertility and this practice prevents litter size and farrowing rates being used as descriptors of the fertility of individual males (Flowers 2008).

An extensive study conducted in a 200-boar stud provides some insight into the phenotypic variation of fertility among AI boars (Flowers 2002). Individuals vary in terms of the insemination dose required to reach a plateau, and the number of piglets born alive at the plateau. For some boars, there is a robust response in litter size as the sperm number in the insemination dose increases, whereas for others the change is less pronounced. Moreover, there are no clear differences among breeds in fertility patterns; and the proportion of boars that exhibit asymptotic and linear responses to increases in sperm numbers and the overall number of piglets born alive is similar among breeds (Flowers 2002, 2008). It is widely accepted that crossbred boars are more likely to produce higher litter sizes than purebred boars (Flowers 2008). On the other hand, regardless of the breed or the crossing, some boars used in AI programs are capable of producing exceptional fertility results with low numbers of spermatozoa (Flowers 2008). Therefore, development of prospective fertility tests for semen is of paramount importance for the identification of boars with extreme phenotypes.

The farrowing rate differs considerably among breeds. Sonderman and Luebbe (2008) reported that Meishan boars have the highest farrowing rates (92.2 %) followed by Duroc boars (78.1 %), whereas Yorkshire (73.8 %) and Landrace (71.8 %) boars have the lowest farrowing rates. Some studies have suggested that the differences among breeds, and even among individual boars in fertilizing ability, are related to differences in sperm head dimensions (Peña et al. 2006; Saravia et al. 2007), high-fertility boars producing spermatozoa with smaller and shorter heads than low-fertility boars (Hirai et al. 2001).

Breed Differences in Reproductive Performance

Sexual behavior in boars has not been studied to the same extent as other reproductive traits. In a trial based on the exposition of boars to gilts in estrus, the authors concluded that little differences exist within and among breeds in the

sexual behavior of males, despite crossbred boars exhibiting greater libido than their purebred counterparts (Sonderman and Luebbe 2008). Purebred Duroc boars are very docile and need a more hands-on approach in training (Sonderman and Luebbe 2008); Hampshire influence in crossbred boars makes them more aggressive, whereas boars with Duroc in their pedigrees are more reluctant to mate than crossed boars with Yorkshire (Neely and Robison 1983). These results suggest that the sexual behavior of the boar may have a heritable basis (Hemsworth and Tilbrook 2007).

Differences also exist among genetic lines in their libido and the age at which they reach puberty; maternal lines appear to be approximately 2 months delayed in contrast with terminal lines (Sonderman and Luebbe 2008). Nearly all terminal line boars reach puberty and are receptive to training to mount a dummy sow between 6 and 7 months of age, whereas the age of maternal lines is 8/9 months. However, in maternal lines a correlation exists between size and training ability; therefore, maternal boars of low size seem to lag behind in training until they attain both the minimum age and weight (Sonderman and Luebbe 2008). The trainability of boars also favors terminal lines over maternal lines. The ability to train different terminal lines is nearly the same although differences exist in how to handle them. Maternal lines need more training time, effort, and visual and competitive stimulation in order to get them properly trained; moreover, there is a greater percentage of untrainable boars within maternal lines due to lack of libido (Sonderman and Luebbe 2008).

Nevertheless, AI boars are, on rare occasions, allowed contact with females after they reach sexual maturity (Flowers 2008). Thus, the small genetic differences observed in natural mating might be magnified in AI boars whose sexual behavior is typically evaluated on the basis of their interest in a dummy sow (Flowers 2008). In swine, the receptive female assumes the dominant role in finding a sexual partner and it is reasonable to speculate that females in estrus provide more stimulation than the collection dummy (Flowers 2008). In most commercial studs, the only measure of mating behavior that is routinely monitored is the proportion of boars that could be trained for semen collection (Flowers 2008). Divergences exist among boars in their capacity to mount a dummy. While some boars quickly mount the dummy sow after entering the collection pen, others are very cautious (Levis and Reicks 2005). These divergences in mounting behavior are not dependent on the genetic line (Flowers 2008). Moreover, the phenotypic variation associated with the mounting behavior of terminal boar lines is small, so that the possibility of enhancing these traits through their identification and posterior recruitment appears to be low (Flowers 2008). Improvements in training programs seem to be the best way to enhance the sexual behavior in boars at the present time.

References about the correlation between sexual behavior traits and semen traits are scarce. In crossbred boars, the total number of spermatozoa per ejaculate does not correlate with either time for first mounting ($r = 0.03$) or number of mounts per ejaculation ($r = 0.18$), but it is positively correlated with the duration of ejaculation ($r = 0.38$) (Thiengthan 1992). Other studies have also reported a significant correlation between duration of ejaculation and semen

volume ($r = 0.58$), total number of spermatozoa ($r = 0.58$) and sperm motility ($r = 0.29$), as well as between duration of mounting time with collection frequency ($r = 0.29$), semen volume ($r = 0.29$), sperm concentration ($r = 0.30$), and sperm abnormalities ($r = 0.44$) (Kuciel et al. 1983).

Testosterone is the hormone most closely associated with male sexual behavior, so it is reasonable to expect that boars with high testosterone levels also have high libido (Flowers 2008). In the Yorkshire breed, serum testosterone levels are significantly higher than in the Duroc breed (Park and Yi 2002), whereas no differences have been found among Meishan, Fengjing, Min zhu and Duroc breeds (Borg et al. 1993).

4.2.1.2 Criteria for Boar Selection According to Reproductive Parameters

Genetic Evaluation of Boars

Selection practices for AI boars are similar throughout the global swine industry and are universally based on genetic evaluation for economically important traits. For maternal line boars selection criteria focus on the growth rate or age at a specific weight, fatness, and productivity of their daughters, whereas terminal sire boars are selected for their genetic potential to produce offspring that grow quickly and efficiently, and have commercially desirable meat qualities, with minimal emphasis placed on semen quality and fertility (Robinson and Buhr 2005; Safranski 2008; Wolf 2010).

It is appropriate to review a historical perspective of boar selection criteria. Visual selection was the practice of selecting a boar based on his appearance at a certain point in time; this method resulted in a slow rate of genetic progress for the most economically important traits (Safranski 2008). In the mid twentieth century individual boar performance for growth rate and backfat thickness was measured and used to provide objective comparisons (Safranski 2008). This approach was more effective than pure visual appraisal, but comparisons among boars were difficult unless males were in the same contemporary group; however, the development of standard guidelines for measures and selection improved this system (Miller 2000). In the 1980s, computing power led to the next step in genetic improvement by recording both phenotypic performance and pedigree information, which are used to generate genetic evaluations expressed as estimated breed values (EBV) for a number of economically important traits, expected progeny difference (EPD), and best linear unbiased predictors (BLUP) (Robinson and Buhr 2005; Safranski 2008). Using individual performance data, adjusted for environmental factors, and data from relatives of selection candidates, EBV and EPD allow objective calculation of the genetic merit of individual boars (Safranski 2008). An advantage of this procedure is the fact that it allows calculation of the genetic merit of an animal based on pedigree alone (Safranski 2008).

The use of EBVs can double the accuracy of selection for lowly heritable reproductive traits of sows, such as litter size, and it also improves the accuracy of selection for growth rate and backfat thickness (Schinckel 1991). Moreover, EBVs are combined into two different overall indices to rank individual boars and gilts on their suitability for producing fast efficient growth in their offspring (sire line index) and for producing reasonable and efficient growth along with prolificacy (dam line index) (Robinson and Buhr 2005). These indices are computed by combining EBV with economic values appropriate for the swine industry to rank the animals (Robinson and Buhr 2005). Breeding companies follow the same approach by measuring phenotypes and computing EBV, but frequently they must also compute specialized indexes that reflect the market niche in which their genetics fits (Robinson and Buhr 2005).

By utilizing objective measures and computing power, it also becomes possible to simultaneously select for multiple traits using these selection index approaches. These practices require knowledge of genetic and phenotypic correlations among traits of interest and also lead to estimate the economic value of change for each; these data are used to derive a single numeric value for selection candidates (Safranski 2008). This method is currently in use and will continue to be critical for long-term genetic improvement (Safranski 2008).

In addition to the quantitative trait selection, specific genetic markers are used in selection programs (Dekkers 2003). The marker-assisted selection (MAS) approach requires associating genetic markers with variations in phenotype (Safranski 2008). The RYR1 (PSS) gene and the RN gene are two well-known examples of genetic tests for deleterious alleles affecting the meat quality of pigs carrying one or two copies of the "bad" allele (Robinson and Buhr 2005). Nevertheless, for other genetic markers the relationship between the marker and the phenotype is not consistent in all swine populations (Safranski 2008). One of the challenges of incorporating molecular genetic markers in the selection process is deciding upon the optimal combination of selection based on MAS and conventional selection on EBV. The goal is to seek a balance between emphasizing the marker information and the EBV information (Robinson and Buhr 2005).

The next step beyond MAS is the identification of causative mutations that lead to different phenotypes. The first example in swine genetics was the identification of a mutation that changed a nucleotide in ryanodine receptor, resulting in leaky calcium channels and the condition known as Porcine Stress Syndrome (Fuji et al. 1991). The PCR tests lead to fast identification of pigs with the mutant allele and the eradication of the mutation in commercial circuits (Safranski 2008). Like MAS, a big advantage of this approach is the ability to test the animals at birth, so that it allows an assessment for traits that are only expressed late in life or that are sex-linked. Nevertheless, a major limitation of this approach is that most economically important traits are under the control of several genes (Safranski 2008). Molecular technologies are useful for traits that are measured late in life (reproduction, longevity), traits that are only expressed in one sex (litter size), or traits that are difficult to measure (disease resistance, meat quality) (Safranski 2008).

The EBVs for growth and carcass traits are generally high; however, the litter size has a low heritability (about 10 %) and is not measured on the boar himself (Robinson and Buhr 2005). The EBV of the litter size of a boar has a low accuracy compared with growth traits as it is necessary to accumulate data on the litter size produced by a number of his daughters (Robinson and Buhr 2005). Selection is therefore more effective on growth and fatness traits than for litter size (Robinson and Buhr 2005).

Boar selection has become increasingly important in parallel with AI (Robinson and Buhr 2005; Safranski 2008; Dyck et al. 2011). Nevertheless, and as stated above, this selection is based on genetic evaluation of economically important traits and minimal emphasis is placed on semen quality and fertility (Robinson and Buhr 2005; Safranski 2008; Wolf 2010); this practice results in the culling of valuable selected boars because of poor semen quality (Robinson and Buhr 2005). This fact highlights the importance of including reproductive traits in boar selection, even though the incorporation of additional traits into a selection index will reduce the establishment of other traits (Safranski 2008). There are few reports on genetic correlations among male reproductive traits and growth or carcass traits, which show a low correlation with growth and fatness or even a negative correlation with muscle depth (Oh et al. 2006). This is clearly an area where more data are needed to allow effective inclusion in selection decisions (Safranski 2008).

Heritability of Semen Traits

As for heterosis, values of heritability of any specific trait range from 0 (lack of heritability) to 1 (total heritability) and results can be expressed either in absolute terms or in percentage. Little data exist about the heritability of reproductive traits, despite preliminary results suggesting that selection based upon EBVs is possible (Safranski 2008; Wolf 2010). Semen volume and sperm concentration (Robinson and Buhr 2005), together with the proportion of morphologically normal spermatozoa (Roca et al. 2006), are heritable traits, but estimates of heritabilities and repeatabilities for these parameters vary widely. Large variations in semen traits are probably related to the number of ejaculates and/or boars used; recent data suggest that large numbers of ejaculates (>26,000) and boars (>600) have to be analyzed in order to obtain robust results about the heritabilities of semen traits (Wolf and Smital 2009; Wolf 2010). Moreover, the heritability of sperm quality parameters is difficult to evaluate since they are affected by several extrinsic factors (Robinson and Buhr 2005; Wolf 2009, 2010).

Heritabilities for semen traits are reported to be similar among breeds. Comparative studies show that semen volume has the greatest heritability, followed by sperm concentration and total number of spermatozoa. Moreover, some studies state that the heritability estimates for total sperm cells tend to increase according to boar age (Huisman et al. 2002; Oh et al. 2006). The heritability of sperm motility, sperm viability and sperm morphology is reported to be low (Wolf

Table 4.4 Heritabilities of semen traits in different breeds

Semen trait	Breed	Heritability	References
Semen volume	Pietrain	0.28	Wolf and Smital (2009)
	Large white	0.28	Wolf and Smital (2009)
	Duroc	0.28	Wolf and Smital (2009)
	Czech landrace	0.25–0.24	Wolf 2009, (2010)
	Czech large white	0.20	Wolf (2010)
		0.14	Wolf (2009)
Sperm concentration	Pietrain	0.20	Wolf and Smital (2009)
	Large white	0.20	Wolf and Smital (2009)
	Duroc	0.20	Wolf and Smital (2009)
	Czech landrace	0.20–018	Wolf (2009), (2010)
	Czech large white	0.18	Wolf (2010)
		0.13	Wolf (2009)
Total number of spermatozoa/ejaculate	Pietrain	0.18–0.17	Wolf and Smital (2009)
	Large white	0.18–0.17	Wolf and Smital (2009)
	Duroc	0.18–0.17	Wolf and Smital (2009)
	Czech landrace	0.10	Wolf (2010)
	Czech large white	0.10	Wolf (2010)
Sperm motility	Czech landrace	0.16	Wolf (2009)
		0.10	Wolf (2010)
	Czech large white	0.10	Wolf (2010)
		0.06	Wolf (2009)
	Pietrain	0.05	Wolf and Smital (2009)
	Large white	0.05	Wolf and Smital (2009)
	Duroc	0.05	Wolf and Smital (2009)
Frequency of aberrant spermatozoa	Pietrain	0.16	Wolf and Smital (2009)
	Large white	0.16	Wolf and Smital (2009)
	Duroc	0.16	Wolf and Smital (2009)
	Czech landrace	0.12	Wolf (2009)
		0.10	Wolf (2010)
	Czech large white	0.10	Wolf (2010)
		0.04	Wolf (2009)

2009, 2010). This low heritability may explain the treatment of these traits as variable parameters in several studies (Table 4.4).

In boars, a negative genetic correlation exists between semen volume and sperm concentration of about −0.68 and −0.69 (Smital et al. 2005; Wolf 2009); in cattle (Basso et al. 2005) and sheep (David et al. 2006) a negative relationship but of lower magnitude has also been reported. Both sperm motility and proportion of aberrant spermatozoa are considered genetically independent from both semen volume and sperm concentration (Smital et al. 2005; Wolf 2009). In contrast, a high negative correlation exists between sperm motility and frequency of aberrant spermatozoa (Wolf 2009); this fact agrees with previous studies that noticed significant differences in sperm movement in relation to head shape (Thurston et al. 2001), and negative correlations between head width and flagellum length (Kondracki et al. 2005). Sperm length is positively correlated with sperm velocity

(Gomedio and Roldan 1991), and negatively correlated with total number of spermatozoa per ejaculate (Wysokinska et al. 2009).

Taken together, the results obtained by different researchers show that heritability values for semen traits, especially those for semen volume, sperm concentration and sperm morphology, allow their effective selection using genetic evaluations. Nevertheless, the negative genetic correlation between semen volume and sperm concentration is unfavorable for selection for total number of sperm (Smital et al. 2005; Oh et al. 2006; Wolf and Smital 2009; Wolf 2009, 2010). On the other hand, the negative correlation between sperm motility and the percentage of aberrant spermatozoa is favorable for selection to improve male reproduction (Smital et al. 2005; Wolf and Smital 2009; Wolf 2009, 2010).

Despite the fact that several investigations have been focused on detecting reproductive boars producing high quality ejaculates (Lovercamp et al. 2007), others studies have demonstrated the low predictive value of seminal parameters in terms of fertility outcomes (farrowing rate and litter size) (Gadea et al. 2004; Popwell and Flowers 2004; Gadea 2005; Sancho et al. 2006). In commercial AI, threshold values for seminal parameters have been established for boar selection in order to ensure high fertility rates (Briz et al. 1996; Bonet et al. 2000). However, the poor relationship between seminal parameters and fertility limits the sensitivity and specificity of such a cut-off (Gadea 2005).

Negative correlations have been reported between semen volume and number of piglets born (Gadea et al. 2004; Smital et al. 2005), and between total number of spermatozoa and litter size (Gadea et al. 2004; Smital et al. 2005; Wolf 2010). Correlations for the litter size are near zero in relation with sperm concentration (Gadea et al. 2004; Wolf 2010), and either positive (from 0.12 to 0.35) (Gadea et al. 2004; Smital et al. 2005), near zero or negative (Wolf 2010) with sperm motility. In contrast, sperm motility has a low positive but significant correlation with the farrowing rate ($r = 0.05$) (Gadea et al. 2004). Contradictory results regarding the relation between sperm motility and litter size may be caused by differences in the experimental procedures to measure sperm motility. Sperm morphology has usually been related to fertility; in standard semen analysis this parameter provides information on the status of spermatogenesis and it can facilitate the selection of boars for AI programs (Gadea 2005). An inverse relationship exists between the percentage of sperm abnormalities and fertility; Xu et al. (1998) reported that sperm morphology explains 59 % of variation in litter size in a commercial setting, whereas Hirai et al. (2001) established a significant relationship between sperm head dimensions and fertility. Lack of correlation between sperm viability and fertility is supported by several studies (Gadea et al. 2004; Roca et al. 2006; Yeste et al. 2010). Sperm viability is currently measured by assessing the integrity of the sperm plasma membrane, which is not related to fertility (Gadea et al. 2004). Intactness of the sperm plasma membrane is a prerequisite for correct sperm metabolism and function (Harrison 1997); therefore, functional assays on sperm plasma membrane have to be performed in order to correlate sperm viability with fertility.

In conclusion, sperm traits are poor predictors of male fertility outcome in AI (Gadea 2005); they are good indicators only in cases of altered testicular and/or

epididymal function, which is manifested in low quality ejaculates and poor fertility rates (Xu et al. 1998; Rodríguez-Martínez 2003; Gadea 2005; Pruneda et al. 2005). In contrast, this is not an accurate way to distinguish samples with excellent fertility from those with medium fertility (Gadea 2005). This could be related to the highly limited variation in these parameters in mature fertile boars (Xu et al. 1998) or to the fact that these tests do not properly evaluate the functionality of spermatozoa (Bussalleu et al. 2005). According to Flowers and Turner (1997) the common estimates of semen quality are good qualitative, but poor quantitative, indicators. To solve this problem, new procedures have been developed which include multiple functional tests (Bussalleu et al. 2005) or in vitro fertilization tests (Selles et al. 2003), but they are expensive and time-consuming, and cannot be applied under commercial field conditions.

Selection of Artificial Insemination (AI) Boars for Testis Size

According to Flowers (2008) the best way to improve semen quality of AI boars is to select them according to testis size. In boars the existence of a gene or group of genes on the X chromosome that affect testicular size has been confirmed (Rohrer et al. 2000; Ford et al. 2001).

Testis size correlates not only with daily sperm production but also with testosterone levels (Ford et al. 2001; Hemsworth and Tilbrook 2007; Flowers 2008). Therefore, boars with a large testicular size show high sperm concentration and testosterone levels, as well as high libido (Flowers 2008) and large epididymis (Walker et al. 2004). Greater epididymal weight may result in a larger capacity for sperm storage, which can improve overall fertility (Walker et al. 2004). A threshold concentration of testosterone is required to elicit sexual behavior; above this threshold, testosterone concentration is ineffectual at increasing copulatory behavior (Hemsworth and Tilbrook 2007). In addition to maintaining sexual behavior, androgens stimulate the latter stages of spermatogenesis and prolong the lifespan of epididymal spermatozoa (Hemsworth and Tilbrook 2007).

Testis size also affects the age of the onset of puberty; therefore, young boars with large testicular size begin puberty at an early age and also reach sexual maturity 2–3 months earlier, according to the breed; besides, these boars exhibit a higher daily sperm production (Johnson et al. 1994; Harder et al. 1995; Rathje et al. 1995; Huang and Johnson 1996), superior mating efficiency (Schinckel et al. 1983), and a longer reproductive longevity (Flowers 2008). Moreover, a significant positive response occurs in ovulation rate from selection for testis weight not only in swine (Schinckel et al. 1983, 1984), but also in sheep (Hanrahan and Quirke 1977) and mice (Islam et al. 1976). In swine, the genetic correlation between testis size and ovulation rate varies from 0.10 (Johnson et al. 1994) to 0.65 (Schinckel et al. 1983), whereas the genetic correlations between testis size and both female traits (Schinckel et al. 1983) and age of gilt puberty (Johnson et al. 1994) are negligible. Nevertheless, these findings suggest that it might be

possible to base genetic selection for increased fertility in the female on the reproductive characteristics of the male (Johnson et al. 1994).

Recent reports also highlight the relevance of male selection for increased testosterone levels as it results in increased estrogen levels of daughters (Walker et al. 2004). The heritability of testosterone production is reported to be similar to other male reproductive traits (Lubritz et al. 1991). However, at the present time, there are technical limitations associated with collecting and using testosterone concentrations in selection programs (Flowers 2008). As boars selected for increased testis size also have elevated testosterone concentrations, selection for testis size might be the best way to enhance sexual behavior in boars (Flowers 2008). Consequently, there is an economic value for AI studs in producing boars with larger testes (Ford and Wise 2011).

Weight of testes at a constant age may be a useful indicator trait to select for increasing reproductive efficiency of boars (Harder et al. 1995; Rathje et al. 1995). Determination of testicular diameter from three to 5 months of age would provide a method to rank boars of a specific genetic line (Ford and Wise 2011). Little data exist about the heritability of testis size, nor other characteristics related with boar reproductive capacity; estimated heritability ranges from 0.6 to 0.3 (Schinckel et al. 1983; Young et al. 1986; Johnson et al. 1994). Flowers (2008) stated that the heritability of testis size could be similar to other corporal traits. Estimates of genetic correlations between testis size and body weight range between 0.4 and 0.5, between testis size and backfat thickness are of 0.25, and between testicular size and age of puberty of 0.16 (Johnson et al. 1994). Daily sperm production of adult boars is highly correlated with testicular weight (Ford et al. 2001); correlations between 0.50 and 0.65 (Schinckel et al. 1983; Young et al. 1986) and even 0.90 (Rathje et al. 1995) have been reported between testis size and daily sperm production.

Boars selected for large testis size produce 6×10^9 more spermatozoa per ejaculate than control boars (Huang and Jonhson 1996); this represents almost a 10 % increase in sperm production. Daily sperm production shifts more rapidly and reaches its plateau at younger ages in the lines selected for testis size as compared with controls (Rathje et al. 1995; Ford and Wise 2011). Replacement rates in terminal sire lines are high, so that swine production companies can take advantage of superior genetics. Consequently, boars that can produce large quantities of semen at young ages and maintain them over their productive life are of premium value (Flowers 2008). Selection for testis size appears to be a valid approach for enhancing spermatogenesis and can be applied to any type of genetics (Flowers 2008; Ford and Wise 2011).

Very little is known about the impact of the boar on the farrowing rate (Robinson and Buhr 2005). In bulls, increasing pregnancy rates after AI were reported positive for a fertility-associated antigen (FAA) in semen samples, a 31 kDa protein present on the sperm surface (Sprott et al. 2000). In contrast, recent data have demonstrated that the identification of FAA-positive and FAA-negative status is not a successful procedure to select AI bulls (Dalton et al. 2012). In mice the activin receptor gene II and estrogen receptor beta (ESR) have been directly related to fertility. Knock-out males lacking the activin receptor gene

are fertile, although they reach puberty later and with smaller testes than healthy males; in contrast, knock-out females are completely infertile (Matzuk and Lamb 2002). Male mice lacking ESR are phenotypically normal and completely fertile, although their prostates do enlarge in later life, whereas females lacking this receptor are subfertile (Krege et al. 1998). These results indicate that an apparently normal fertile male can transmit a genetic cause of subfertility to his female offspring (Robinson and Buhr 2005). All these studies provide great evidence of male genetic influence on pregnancy rate, which warrants further investigations on pigs.

In contrast, evidence exists in pigs for genetic impact on litter size (Robinson and Buhr 2005). Certainly, sows can be selected for improved litter size (Bolet et al. 2001), but males also contribute (Robinson and Buhr 2005). Reciprocal translocation occurs when different chromosomes exchange pieces, and subsequent segregation during meiosis produces gametes that can be balanced or unbalanced with respect to the chromosomes carrying the translocations; the unbalanced gametes carrying a chromatid that is either too long or too short (Robinson and Buhr 2005). If such a spermatozoon penetrates an egg, the unbalanced spermatid cannot pair properly with the female partner chromatid, resulting in early embryonic death and therefore smaller litter size (Robinson and Buhr 2005). A number of such translocations has been identified in boars; in Finnish York boars these translocations are reported to produce a reduction of two pigs per litter as compared with the breed average (Makinen et al. 1999). Furthermore, although the offspring that receives the unbalanced chromosome dies, half of the living offspring carries the balanced translocation; thus, a spontaneous translocation can be perpetuated in subsequent generations (Robinson and Buhr 2005). AI stations have been strongly recommended to perform cytogenetic assays to detect and exclude boars with genetic abnormalities (Makinen et al. 1999; Robinson and Buhr 2005).

Recent studies also highlight the importance of boar selection for semen freezability (Safranski 2008; Casas et al. 2010). Although it has been demonstrated that genetics is responsible for freezability in boars (Thurston et al. 2002a, b; Roca et al. 2006) no data exist about the heritability of this semen trait. Whereas ejaculates collected from the same boar tend to maintain freezability (Roca et al. 2006), heritability could explain the large differences among breeds and among boars in this parameter (Waterhouse et al. 2006; Safranski et al. 2011). In the Pietrain breed, 75–79 % of freezability features of ejaculates can be predicted by using different linear combinations of sperm quality parameters in the first steps of the cryopreservation process (17º and 5º C) (Casas et al. 2009). This range of percentages emphasizes the weight of individual boar genetics on sperm freezability (Casas et al. 2009).

Today, boar selection criteria for sperm freezability are only based on pre-freeze and post-thaw sperm quality (Casas et al. 2009, 2010). Nevertheless, it seems unlikely that conventional measures of sperm quality result in accurate prediction of freezability, and therefore the identification of genetic markers for freezability might be the most efficient approach (Safranski 2008). In this sense, Thurston et al. (2002a) have identified 16 candidate gene markers presumably linked to genes associated with sperm freezability. This again suggests the potential of genetic improvement in sperm chilling resistance (Safranski 2008).

In conclusion, identifying and enhancing the genetics of sperm production and quality will benefit the reproductive performance of AI boars (Safranski 2008).

4.2.2 Cryptorchidism and Testicular Activity

4.2.2.1 Testicular Activity

Testicular activity is frequently assessed by measuring the daily sperm production per gram of testis or the total number of spermatozoa per ejaculate, both parameters being correlated with the age of boars (Kondracki et al. 2005; Huang et al. 2010), testicular weight (Ford et al. 2001; Lunstra et al. 2003; Almeida et al. 2006; Ford and Wise 2011) and number of Sertoli cells (Lunstra et al. 2003; Caires et al. 2008; Ford and Wise 2009). The ratio spermatid-to-Sertoli cell is a measure of the Sertoli cell efficiency based on the assumption that relative changes in daily sperm production would alter the number of spermatids supported by a stable Sertoli cell population (Okwun et al. 1996). Despite its accuracy such a ratio is not routinely used due to technical limitations in field conditions, so available data are scarce. Another ratio, the germ cell-to-Sertoli cell ratio, is reported to be significantly higher in the Meishan breed than in Whitecross and West African breeds (Okwn et al. 1996); these results indicate that Meishan boars are more efficient in supporting spermatogenesis than Whitecross and West African boars, due both to a lower degeneration index of germ cells and to the accommodation of more germ cells by an equal population size of Sertoli cells (Okwun et al. 1996).

Despite significant differences in total number of spermatozoa per ejaculate and testosterone levels, daily sperm production per gram of testis in adult boars is fairly constant within a breed and also among breeds, being approximately 21.7×10^6 in West African (Okwun et al. 1996), 23.6×10^6 in Yorkshire (Swierstra 1970), 24.1×10^6 in Lacombe (Swierstra 1970), 24.5×10^6 in Meishan (Okwun et al. 1996), 24.8×10^6 in Whitecross (Okwun et al. 1996), 25.9×10^6 in Landrace (Egbunike et al. 1975), and 27.3×10^6 in Piau breeds (França 1992). This indicates that although spermatogenesis requires testosterone, there may be a threshold effect whereby further increases do not result in higher sperm production once a certain level of testosterone is achieved (Walker et al. 2004). This could explain the low correlation ($r = 0.12$) between sperm production and testosterone levels in the ejaculate of boars (Peter et al. 1980). As the duration of spermatogenesis (Almeida et al. 2006) and the rate of germ cell apoptosis during both spermatogonia and spermatocyte stages (De Rooij and Rusell 2000) do not differ substantially among breeds and genetic lines, variations among boars in daily sperm production are related with testicular size, as well as the number of A spermatogonia and Sertoli cells per testis (Orth et al. 1988; Huang and Jonhson 1996; Okwun et al. 1996; Lunstra et al. 2003).

The number of A spermatogonia per testis is highly positively correlated with the number of Sertoli cells per testis ($r = 0.95$) and with the parenchymal mass

($r = 0.88$), and it also accounts for 77 % of variability in daily sperm production among breeds (Okwun et al. 1996). The number of A spermatogonia which initiate the spermatogenic cycle depend on: (1) the number of stem cells per testis, (2) the process of stem cell renewal, (3) the number of cell divisions from stem cells to primary spermatocytes, and (4) the degeneration index of spermatogonial subtypes (Okwun et al. 1996). Given that (2), (3) and (4) are similar among breeds, differences in daily sperm production are partly related with the number of A spermatogonia (Okwun et al. 1996).

It has been suggested that Sertoli cells establish a ceiling in the rate of sperm production (Johnson 1986; Berndtson et al. 1987). Sertoli cells can place this limit in spermatogenic potential by controlling testis size (Kluin et al. 1984), modulating type A spermatogonial populations (Hochereau-de Reviers 1981; Johnson 1986), and/or limiting the number of germ cells supported (Almeida et al. 2006). In boars the number of Sertoli cells correlates with parenchymal mass ($r = 0.87$), number of A spermatogonia ($r = 0.95$) and daily sperm production ($r = 0.87$) (Okwun et al. 1996). Therefore, the number of Sertoli cells is responsible for 76 % of variability in daily sperm production among breeds. Breed differences in the total number of Sertoli cells and A spermatogonia per testis are manifested in divergences in sperm production (Okwun et al. 1996; Almeida et al. 2006).

Although the testicular activity of boars is measured after the onset of puberty, the daily sperm production is dependent on changes occurring during the prepubertal period (Caires et al. 2008).

In boars a significant increase in both testis weight and Sertoli cell number occurs during the first 3–4 weeks of neonatal life (McCoard et al. 2003; Caires et al. 2008; Huang et al. 2011). Throughout this period, Sertoli cells maintain an immature appearance and secrete high levels of anti-Mullerian hormone (AMH) (Rey et al. 2003; Caires et al. 2008); the germ cell population consists of mitotically quiescent prespermatogonia, located centrally in the seminiferous tubules, among immature Sertoli cells (Caires et al. 2008). Prespermatogonia start to migrate towards the basal lamina after 14 days of age and begin to express progesterone receptors (Kohler et al. 2007; Caires et al. 2008); the expression of progesterone receptors is a key process for further maturation of prespermatogonia into spermatogonia and later mitosis (Kohler et al. 2007). In 60-day-old boars, the seminiferous tubules are constituted by immature Sertoli cells and prespermatogonia (Rey et al. 2003); 85 % of prespermatogonia that have reached the basal lamina express progesterone receptors, whereas only 18 % of preespermatogonia remaining in the center of the seminiferous epithelium express this receptor (Kohler et al. 2007). The signals inducing the expression of progesterone receptors in the prespermatogonia in the way to or upon reaching the basal lamina remain obscure, but it might result from a paracrine stimulation of Leydig cells mediated by estrogens (Kohler et al. 2007). In sexually mature boars expression of progesterone receptors is maintained in 78 % of A and B spermatogonia, regardless of age and stage of spermatogenesis; this expression disappears when spermatogonia enter in the developmental stage of primary spermatocytes (Kohler et al. 2007). Therefore, progesterone is not just an intermediary in the synthesis of testicular androgens

and estrogens by Leydig cells, but it also acts as a paracrine factor involved in the control of spermatocytogenesis (Kohler et al. 2007).

It is well known that Sertoli cell numbers established before puberty determine adult testis size, spermatogenic activity, and lifetime in boar fertility (Caires et al. 2008). In the porcine testis, maximum Sertoli cell proliferation occurs during the first 2 weeks of life under follicular stimulating hormone (FSH) stimulus, resulting in a four-fold increase in Sertoli cell numbers during this period (McCoard et al. 2001, 2003); nevertheless, porcine Sertoli cells still express markers of proliferating cells around 4 months of age (Klobucar et al. 2003). In vivo studies have suggested the potential to manipulate the establishment of Sertoli cell populations in rodents (Orth et al. 1988), bulls (Madjdic et al. 1998) and boars (Lunstra et al. 2003). In order to obtain a notable impact on boar lifetime fertility, manipulations must be performed during the first 2 weeks of neonatal life (Caires et al. 2008).

In response to testosterone, Sertoli cells cease proliferation and initiate maturation (Buzzard et al. 2003). The maturation of Sertoli cells starts at about 60 and 90 days of age, depending on the breed (Ford and Wise 2009; Huang et al. 2011), and is characterized by the arrest of proliferation (Ahmed et al. 2009), the progressive decrease in AMH expression (Rey et al. 2003; Ford and Wise 2009), the increase in the diameter of the seminiferous tubules (Rey et al. 2003; França et al. 2005; Ford and Wise 2009), the formation of the blood-testis barrier (França et al. 2005; Ford and Wise 2009), the secretion of tubular fluids (Ford and Wise 2009, 2011), and the onset of spermatogenesis in some seminiferous areas (Kohler et al. 2007; Ford and Wise 2009). Increased androgen secretion by early pubertal testes marks the onset of sexual maturity (Ford and Wise 2009). The development of Leydig cells in boar testes follows a different pattern than that of Sertoli cells, and it can be divided into three stages (Huang et al. 2011): the first stage occurs before the first 35 days of gestation; the second stage begins 3 weeks before parturition and continues for approximately 3 weeks postnatally; and the third stage starts at approximately 90 days of age throughout puberty. Intratesticular androgens act as negative regulators of AMH secretion (Rey et al. 2003). In humans, the lack of androgen receptors on Sertoli cells during neonatal development (Chemes et al. 2008) offers a means whereby Sertoli cells avoid the negative influence of androgen on AMH expression (Ford and Wise 2009). Then, at the onset of puberty, acquisition of androgen receptors within Sertoli cells provides a link for suppressing AMH production (Ford and Wise 2009). In neonatal boars, published reports disagree regarding the presence of androgen receptors in Sertoli cells (Ramesh et al. 2007; Caires et al. 2008), leaving unresolved the role of androgens in the regulation of AMH production during early postnatal development.

Differences exist among breeds in the rate of decrease of AMH expression in Sertoli cells; in this sense, Ford and Wise (2009) found that AMH expression increases from seven to 28 days of age in Meishan and crossbred boars; after 28 days AMH declines in both genetic lines but at a more rapid rate in Meishan than in crossbred boars (Ford and Wise 2009). In Meishan boars, AMH expression is nearly absent at 70 days of age, whereas in crossbreds it is absent at 112 days of age (Ford and Wise 2009). These findings correlate with an earlier onset of pubertal

development in Meishan boars as compared with crossbred boars (Kanematsu et al. 2006). Longer expression of AMH in crossbred boars reflects a less mature status of their Sertoli cells, and a prolonged potential to proliferate (Ford and Wise 2009).

In boars, Sertoli cell maturation begins next to the mediastinum and progresses outwardly towards the interior of the testis (Ford and Wise 2009, 2011). This outwardly progressing pattern supports the hypothesis of a paracrine regulation of Sertoli cell maturation within a given tubule (Ford and Wise 2009). Adjacent sections of the same seminiferous tubules have a defined sequence of change, with AMH production decreasing before much expansion of seminiferous tubules, followed by a change in gene expression in Sertoli cells before the formation of a distinct lumen and the onset of spermatogenesis (Ford and Wise 2009).

A progressive transformation of spermatogonia into spermatocytes begins at approximately 100–115 days of age, which is followed by the transformation of spermatocytes into immature spermatids and the differentiation of immature spermatids into mature spermatids with advancing age (Kohler et al. 2007). A low concentration of spermatozoa can be found in the epididymal cauda at 125 days of age (Andersson et al. 1998a, b). The germ cell differentiation is followed by an extremely rapid increase in the number of germ cells together with the organization of the seminiferous epithelium, which acquires a sexually mature appearance at approximately 180 days of age (Andersson et al. 1998a, b). In domestic boars, puberty starts at 150 days of age with the completion of the first spermatogenic wave (Caires et al. 2008; Ford and Wise 2009), and it involves rapid expansion of seminiferous tubules that manifests itself in a marked increase in testicular size (França et al. 2005). Thereafter, the quantity and quality of spermatozoa improve over time (Malgrem et al. 1996) and reach their maximum at approximately 24 months of age (Kennedy and Wilkins 1984).

Testicular weight increases significantly from 3 to 10 months of age, the monthly progression being of 25 g (3 months), 100 g (4 months), 205 g (5 months), 300 g (6 months), 350 g (7 months) and 375 g (10 months) (Ford and Wise 2011). The percentage of testicular parenchyma occupied by seminiferous tubules increases at 3 months (38 %) and reaches its maximum by 5 months of age (68 %); at 10 months of age it decreases slightly to 65 % (Ford and Wise 2011). The mean diameter of seminiferous tubules is also greater at 7 months than at 4 or 5 months of age (Ford and Wise 2011). A high coefficient of correlation exists between testis weight and volume, although it decreases progressively with age, from 0.95 at 4 months to 0.78 at 7 months of age (Ford and Wise 2011). The correlation coefficients of the mean diameter of seminiferous tubules with testicular diameter and weight decrease with advancing age and become non-significant at 6 months for testicular diameter, and at 7 months for testicular weight; the values of both coefficients were 0.71 and 0.63 at 4 months, 0.75 and 0.66 at 5 months, 0.15 and 0.35 at 6 months, and 0.14 and 0.10 at 7 months (Ford and Wise 2011). The coefficient of variation of the mean seminiferous tubule diameter decreases from 21.6 % at 3 months and 20.6 % at 4 months to 11.2 % at 5 months, 10.9 % at 6 months and 6.6 % at 7 months of age, thus indicating less variation in testicular diameter as the boar progresses throughout puberty (Ford and Wise 2011).

Similarly, the coefficient of variation for testicular diameter decreases from 16.1 % at 4 months to 12.6 % at 5 months, 8.1 % at 6 months, and 5.6 % at 7 months of age, thus reflecting the significant correlation between seminiferous tubule diameter and testicular diameter in pubertal boars (Ford and Wise 2011).

The stage of pubertal development is determined by the mean diameter of seminiferous tubules (Ford and Wise 2011). Due to the high correlation between mean diameter of seminiferous tubules and testicular weight and diameter (Ford and Wise 2011), it is possible to obtain an accurate estimation of testicular development of pubertal males by evaluating the testis size (Clark et al. 2003; Rawlings et al. 2008). Ford and Wise (2011) proposed determining the testis size as an estimator of tubular diameter following a two-phase protocol: (1) ranking boars by testicular size at 4 months of age, the period of greatest variation in diameter of seminiferous tubules, and (2) ranking boars by testicular size at 8 months of age. At both evaluations, testicular traits would be adjusted for age and weight of the boar within a defined genetic line.

The progressive maturation of the seminiferous epithelium during the pubertal period leads to the onset of spermatogenesis between around 5 and 8 months of age, with a gradual increase in daily sperm production and also in semen volume until boars reach 18 months of age (Kondracki et al. 2005). In a tropical environment, the progressive maturation of testicular function in Duroc boars occurs from nine to 33 months (Suriyasomboon et al. 2005). In general terms, at 18 months of age, the total number of spermatozoa per ejaculate ranges between 20 and 80 $\times$ 10^9 and the seminal volume between 200 and 400 ml depending on the breed (Park and Yi 2002); this level of production is maintained until a gradual decline starts at the age of 60 months (Park and Yi 2002; Wolf and Smital 2009; Huang et al. 2010). Testosterone levels also increase during puberty, reflecting the high activity of steroid synthesis by Leydig cells (Andersson et al. 1998b) and remain fairly constant at >2 mg/ml during adult life (Tan and Raeside 1980). There are significant breed differences in sperm production and heavy breeds such as Yorkshire and Large White generally tend to produce greater semen volume and total number of spermatozoa over a period, although it is not clear how weight of boars at maturity affects sperm concentration (Park and Yi 2002). The maximum quality of boar semen may last for a certain period but the duration has not yet been established (Huang et al. 2010). In temperate environments the highest total number of spermatozoa per ejaculate has been set at the 32nd month of age (Swierstra 1973), whereas the maximum semen volume, sperm concentration and number of seminal doses is obtained in 24–28-month-old boars (Kennedy and Wilkins 1984). Nonetheless, beyond a certain optimal age semen quality inevitably declines at an unknown rate to a level that is no longer acceptable for service. Little data exist about the longevity of a boar's reproductive performance in terms of acceptable semen quality for AI. According to Huang et al. (2010) it ranges between 60 and 70 months of age.

The decrease in semen quality with boar age is due to a reduction in the number and the impairment of Leydig cell function, and to the narrowing and sclerosing of seminiferous tubules. All these alterations result in decreased spermatogenic activity and increased degeneration of germ cells (Johnson 1986), as well as in increased frequency of spermatozoa with altered chromatin condensation, gene

mutations and aneuploidies (Wyrobek et al. 2006). In Duroc boars, semen volume declines at a slower rate than sperm parameters (Huang et al. 2010), thus indicating that the spermatogenic function is more vulnerable to aging effects than accessory glands and chiefly seminal vesicles, since they produce the bulk of semen volume (Badia et al. 2006). A different declining rate of testes and accessory gland function as a result of aging has also been reported in other mammals (Huang et al. 2010).

4.2.2.2 Types of Cryptorchidism: Unilateral or Bilateral and Abdominal or Inguinal

Cryptorchidism is the most frequent male sexual disorder in mammals, arising from a failure in the descent of one testis towards the scrotum (unilateral cryptorchidism) or of both testes (bilateral cryptorchidism). The alteration of the testicular descent can be either total, the ectopic testes remaining in the abdominal cavity (abdominal cryptorchidism), or partial, with the ectopic testes lodged at different levels of the inguinal canal (inguinal cryptorchidism) (Mieusset et al. 1995, 1997; Pinart et al. 2000). The etiology of cryptorchidism is not clear; it can develop as a result of genetic defects, disrupted endocrine regulation, or anatomical lesions (Demircan et al. 2006; Matuszczak et al. 2011).

Several authors have reported anomalies in the seminiferous tubules, lamina propria and interstitial tissue of ectopic testes that become apparent at pre-puberty (Paninagua et al. 1990; Regadera et al. 1991; Lee 1993; Antich et al. 1995; Sasagawa and Yanagimachi 1997; Matuszczak et al. 2011). In humans, the purpose of early treatment of cryptorchidism is to recover testicular activity but it does not always restore complete function, leading to decreased semen quality at adulthood (Mieusset et al. 1995; Rozanski and Bloom 1995; Foresta et al. 1996; Lee and Coughlin 2001; Kvist et al. 2006; Hutson et al. 2010; Thorup et al. 2010; Marchetti et al. 2012). It has been established that up to 30 and 54 % of patients treated during childhood of unilateral (Thorup et al. 2010) and bilateral (Lee and Coughlin 2001) cryptorchidism, respectively, are subfertile in their adulthood. Subfertile patients with a history of pre-pubertal cryptorchidism usually show hyperthermia on the cryptorchid side, so in these patients treatment of cryptorchidism does not result in normalization of testicular temperature (Mieusset et al. 1995; Hutson et al. 2010). In other cases, treatment does not warrant the maintenance of the testes in a scrotal position; these patients exhibit a retractile testis (Mieusset et al. 1995, 1997) or an ascended testis (Gracia et al. 1997) on the cryptorchid side. A significant relationship exists between spontaneous ascent and high localization of the ectopic testis before treatment (Mieusset et al. 1995, 1997). Therefore, subfertile cryptorchid men treated at pre-puberty show similar anatomopathological alterations to those of untreated cryptorchid men (Gracia et al. 1997).

Cryptorchid testes also show an increased risk of cancer at adulthood; in humans, early successful correction of cryptorchidism does not appear to reduce the incidence of malignant transformation of the testes (Mieusset et al. 1995, 1997; Hutson et al. 2010; Ma et al. 2011). The majority of testicular tumors, usually originated by malignant transformation of germ cells (Ma et al. 2011), occur during adolescence and after puberty (James et al. 2009; Hutson et al. 2010).

Malignant degree risk is proportional to the higher position of the testis; in unilateral cryptorchidism, the scrotal testis also shows increased probabilities of developing cancer (Ma et al. 2011).

Great controversy exists about the severity of testicular lesions in cryptorchid testes, not only in humans but also in other mammals (Pinart et al. 1999a). Similar abnormalities have been reported in the scrotal testes of unilateral cryptorchid males by some authors (Mieusset et al. 1995), but not by others (Nistal et al. 1990). Divergences are due to the different conditions in which the study of cryptorchidism is performed (Pinart et al. 1999a). This pathology has been studied in humans (Mieusset et al. 1995, 1997; Demircan et al. 2006; Matuszczak et al. 2011) and in rodents under experimentally-induced conditions (Antich et al. 1995; Sasagawa and Yanaguimachi 1997). Spontaneous cryptorchidism brings about a hormonal imbalance that is not found in the same way or in the same intensity in experimental cryptorchidism; this makes both types of cryptorchidism not comparable (Heyns and Hutson 1995; McMahon et al. 1995; Pinart et al. 1998).

Most studies focused on the alterations induced by spontaneous cryptorchidism in mammals do not discriminate between inguinal or abdominal, and unilateral or bilateral, although the effects are different (Paniagua et al. 1990; Lee 1993; Mieusset et al. 1995; Pinart et al. 1998, 2000, 2001d). Additionally, in unilateral cryptorchidism, significant differences have been described between right- and left-sided cryptorchidism (Mieusset et al. 1995). Moreover, the effects of this pathology in post-pubertal males are different depending on age; thus, differences exist between young adults and elderly males in the alterations of the ectopic testes (Lee 1993). Likewise, data in humans are not comparable between men with cryptorchidism and men with a history of pre-pubertal cryptorchidism (Lee 1993). Besides, in men with a history of cryptorchidism the abnormalities correlate to the age of the orchidopexy (Lee 1993; Mieusset et al. 1995).

These discrepancies lead us to focus on the study of cryptorchidism using the boar as an animal model (Pinart et al. 1998). The main advantages of using this species are: (1) spontaneous cryptorchidism in boars has an incidence of 6–10 % (McMahon et al. 1995), so there is availability of individuals; (2) cryptorchid conditions both in boars and humans involve similar hormonal disorders and morpho-functional abnormalities of the ectopic testes (Heyns and Hutson 1995; McMahon et al. 1995); (3) parallelism in the time of testicular descent between humans and boars, as testicular descent influences semen quality (Heyns and Hutson 1995; McMahon et al. 1995); (4) the structural analogy between human and boar spermatozoa (Guraya 1987); and (5) the correspondence in the mechanisms of testicular thermoregulation between humans and boars (Dadoune and Demoulin 1993).

An extensive study was carried out using three groups of post-pubertal boars of the same age; one group was composed of three boars with spontaneous unilateral abdominal cryptorchidism on the right side, another group was formed by three boars with spontaneous bilateral abdominal cryptorchidism, and the third group was formed by healthy boars. For each male group we performed an analysis of the alterations in the testicular structure (Pinart et al. 1999a, b, 2001d), ultrastructure (Pinart et al. 2000, 2001a), lectin histochemistry (Pinart et al. 2001b, c,

2002), and of the alterations in semen quality (Bonet et al. 1998; Pinart et al. 1998, 1999c). It resulted in a thorough description of the effects on spermatogenesis and steroidogenesis of unilateral abdominal cryptorchidism on the right side and bilateral abdominal cryptorchidism at post-pubertal age.

4.2.2.3 Structural and Ultrastructural Alterations of Cryptorchid Testes

Macroscopic Characteristics

Despite the fact that few differences exist in size and weight between the right and the left testis in post-pubertal healthy boars, in unilateral boar cryptorchidism on the right side, the left (scrotal) testis is two-fold more voluminous and 3.35 times heavier than the right (abdominal) testis (Pinart et al. 1999a). Moreover, it is 1.29 times longer, 1.47 times wider, and 1.24 times heavier than the left testis of healthy boars; the right testis of unilateral cryptorchid boars is smaller (×0.73 in length and ×0.68 in width) and lighter (×0.38 in weight) than the right testis of healthy boars (Pinart et al. 1999a). In bilateral abdominal cryptorchid boars the left testis is slightly more voluminous (×1.70 in length and ×1.12 in width) and heavier (×1.13 in weight) than the right (abdominal) testis; moreover, the right testis has a degenerative appearance. Left and right testes of bilateral abdominal cryptorchid boars are smaller and lighter than the left (×0.29 in length, ×0.52 in width, and ×0.29 in weight) and right (×0.38 in length, ×0.52 in width, and ×0.26 in weight) testes of healthy boars. Differences also exist between unilateral and bilateral cryptorchidism in testicular appearance (Pinart et al. 1999a). The left testis of the bilateral abdominal cryptorchid boars is significantly smaller (×0.49 in length and ×0.40 in width) and lighter (×0.23 in weight) than the left (scrotal) testis of unilateral cryptorchid boars. The right testis of bilateral cryptorchid boars is also smaller (×0.52 in length and 0.77 in width) and lighter (×0.68 in weight) than the right (abdominal) testis of unilateral cryptorchid boars. These findings are congruent with those found in dogs, in which paired testicular weight is also significantly lower in bilateral than in unilateral cryptorchidism (Pathirana et al. 2011).

Therefore, at post-pubertal age unilateral and bilateral cryptorchidism induces hypotrophia of the abdominal testes, whereas unilateral cryptorchidism results in hypertrophia of the scrotal testes of boars (Pinart et al. 1999a, 2001d). Abnormalities in the volume and weight have also been reported in the abdominal testes of unilateral and bilateral cryptorchid men (Paniagua et al. 1990; Regadera et al. 1991) and in the testes of men with a history of unilateral and bilateral cryptorchidism (Mieusset et al. 1995). Supporting the above-mentioned findings some authors reported that alterations are more severe in the bilateral disease (Mieusset et al. 1995), whereas others do not find differences between unilateral and bilateral cryptorchidism in testicular appearance (Paniagua et al. 1990). Both reduced size of abdominal testes and increased size of scrotal testes are early phenomena that develop during the pre-pubertal period and become progressively more severe with age (Antich et al. 1995; Mieusset et al. 1995; Matuszczak et al. 2011). In the

abdominal testes of unilateral and bilateral cryptorchid boars the low testicular weight results from the defective development of the seminiferous tubules, whereas in the scrotal testis of unilateral cryptorchid boars the increased weight correlates with a compensatory hypertrophy (Pinart et al. 1999a). This latter malformation has not been described in the scrotal testis of unilateral cryptorchid men. Some authors have reported a significant decrease in the volume of the left testis in the case of unilateral cryptorchidism on the right side, whereas unilateral cryptorchidism on the left side is not believed to alter the volume of the right testis (Mieusset et al. 1995).

Testicular Structure and Histochemistry

Left (scrotal) and right (abdominal) testes of unilateral cryptorchid boars are surrounded by the tunica albuginea, which extends from the large fibrous septa to the testicular parenchyma in the right testis. The seminiferous tubules occupy 48 % of the testicular parenchyma in the left testis and the 44 % in the right testis, the amount of interstitial tissue being, respectively, of 52 and 56 % (Pinart et al. 1999a). These differences between both testes in the proportion of seminiferous tubules and interstitial tissue are not significant; nevertheless, in comparison to healthy boars, the left and right testes show a significant reduction in the amount of seminiferous tubules in unilateral cryptorchid boars of 10 and 20 %, respectively (Pinart et al. 1999a).

In the left (scrotal) testis of unilateral cryptorchid boars the tubular diameter (averaging 515 μm) and the height of the seminiferous epithelium are similar to that of healthy boars, whereas the tubular lumen is greater (Pinart et al. 1999a); the lamina propria shows the same structural (Pinart et al. 1999a) and ultrastructural (Pinart et al. 2001c) pattern as in healthy boars. The seminiferous epithelium, despite showing a normal structural appearance (Pinart et al. 1999a), is characterized by a low density of germ cells (Pinart et al. 1999b) and altered sugar composition in the apical compartment of the seminiferous epithelium (Pinart et al. 2001b). As compared with healthy boars, the apical cytoplasm of Sertoli cells displays a decreased content of fucosyl and galactosyl residues; spermatogonia have similar sugar residues as in healthy boars, but spermatocytes exhibit higher amounts of α-D-glucose. Spermatids present an altered nature of both glucosyl and galactosyl residues (Pinart et al. 2001b). On the other hand, although all four stages of the seminiferous epithelium cycle are found in the scrotal testis of unilateral cryptorchid boars, stages I and II occur most frequently (Pinart et al. 1999a). Increased frequency of stages I and II results in impaired maturation of spermatids (Pinart et al. 1999a) and corresponds to the incidence of spermatozoa with primary abnormalities in the ejaculate (Pinart et al. 1998). The analysis of the meiosis indicates that in the scrotal testis of unilateral cryptorchid boars transformation from primary to secondary spermatocytes (meiosis I) is low, whereas transformation from secondary spermatocytes to round spermatids (meiosis II) shows similar values to healthy boars (Fig. 4.1a–h) (Pinart et al. 1999b). Moreover, pathological germ cells under such conditions correspond to giant-cell forms of primary

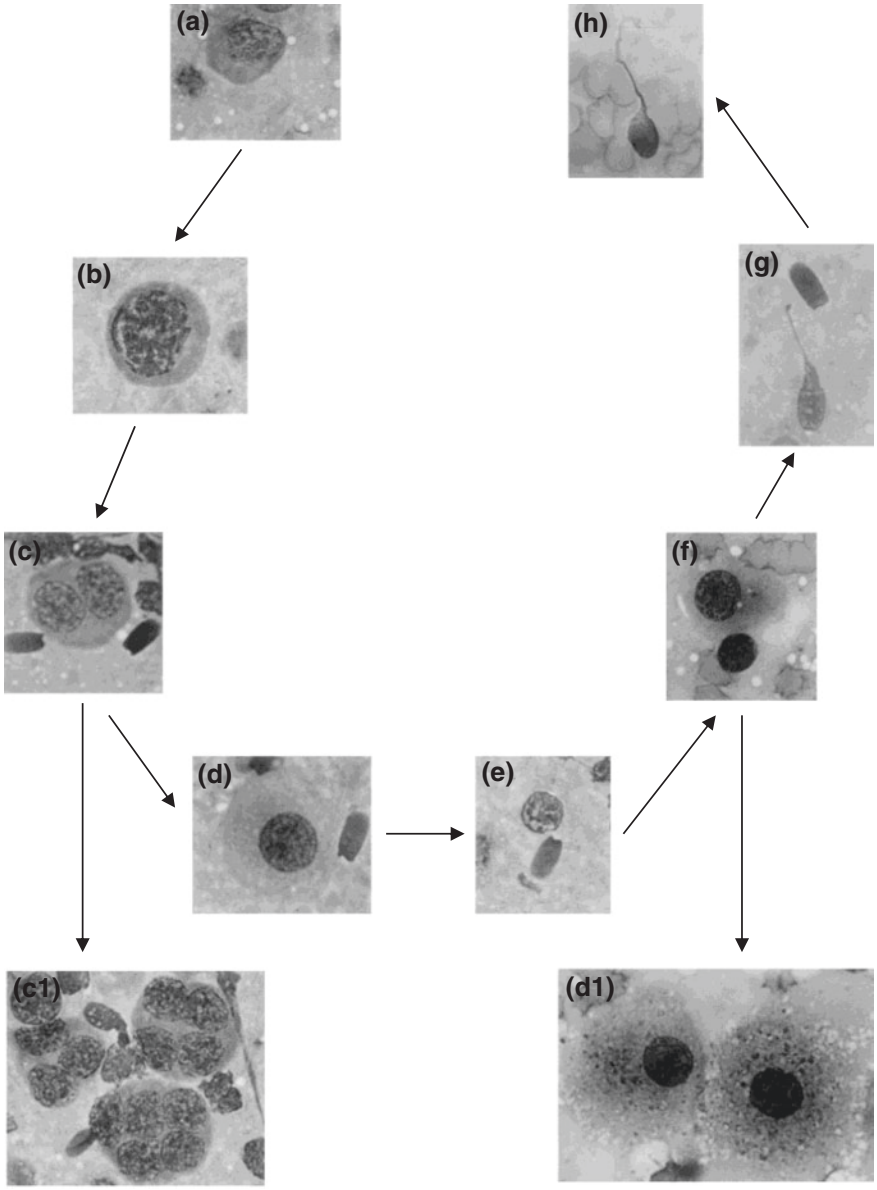


Fig. 4.1 Appearance of germ cells in the scrotal testis of post-pubertal boars with spontaneous unilateral abdominal cryptorchidism on the *right side* by light microscopy Quick Panoptic Method (*QCA*). **a** Ap type spermatogonium. **b** Pachytene prophase stage of a primary spermatocyte. **c** Advanced telophase of a primary spermatocytes. $\mathbf{c_1}$ Pathological giant-cell forms of primary spermatocytes showing a tetranucleated cytoplasm. **d** Secondary spermatocyte. **e** Early spermatid. **f** Intermediate spermatid (Golgi phase). $\mathbf{f_1}$ Pathological giant-cell forms of intermediate spermatids, showing a granular cytoplasm. **g** Elongating spermatid. **h** Elongated spermatid. ×500. Reproduced from Pinart et al. (1999b) with permission

spermatocytes with a tetranuclear cytoplasm (Fig. 4.1c$_1$), and to aberrant intermediate spermatids with a granular cytoplasm (Fig. 4.1d$_1$). Impaired meiosis I leads to a low production of round spermatids in the scrotal testis of unilateral cryptorchid boars, which is 50 % lower than in the left testis and 30 % lower than in the right testis of healthy boars (Pinart et al. 1999b).

Taken together, boar unilateral abdominal cryptorchidism on the right side affects sperm production of the scrotal testis by inducing a partial arrest of spermatogenesis at both the primary spermatocyte stage (Pinart et al. 1999b) and the spermatid stage (Pinart et al. 1999a). Mieusset et al. (1995) state that unilateral abdominal cryptorchidism affects the spermatogenic potential of the scrotal testis more severely in the case of right-sided than of left-sided cryptorchidism.

Partial arrest of spermatogenesis at the primary spermatocyte stage has been related to several testicular disorders associated with oligospermia and azoospermia (Gargiulo et al. 1991; Foresta et al. 1996; Jannes et al. 1998). Defective transformation of primary spermatocytes could appear as a result of disturbed mitosis in spermatogonia, which leads to the formation of spermatocytes with an abnormal DNA content (Setchell et al. 1998), or to anomalies in the metabolic activity and in the organization of the cytoskeleton of primary spermatocytes (Kojima 1991; Jannes et al. 1998). Moreover, defective meiosis can lead to the formation of aberrant spermatids that are not capable of achieving the process of spermiogenesis, resulting in arrest at the spermatid stage (Pinart et al. 1998, 1999a), as well as in the presence of degenerative spermatids in the scrotal testis of unilateral cryptorchid boars (Pinart et al. 1999b). The degeneration of intermediate spermatids correlates with an abnormal activity of both endoplasmic reticulum and Golgi complex that is manifested in an increased granule amount in the cytoplasm (Kojima 1991). It has been suggested that these granules have a defective content of glycoproteins implicated in the formation of the acrosome vesicle (Kojima 1991). Multinucleated spermatocytes have also been detected in men with severe oligospermia (Hofmann et al. 1992; Miething 1995). These cells are not capable of further development, so it has been suggested that they are forms of spermatogenic arrest at the spermatocyte and spermatid levels (Miething 1995). According to Miething (1995), multinuclearity comes from the confluence of cell membranes of neighboring spermatocytes, whereas Hofmann et al. (1992) suggest that it appears from disturbed mitosis.

In adult males, FSH and testosterone are required for the maintenance of quantitatively and qualitatively normal spermatogenesis (Chemes et al. 2008; Pathirana et al. 2011). Receptors for these hormones are present in Sertoli cells but not in germ cells (Kohler et al. 2007; Caires et al. 2008); therefore, the effects of both hormones are indirect and mediated by Sertoli cells, which secrete factors that regulate germ-cell differentiation in a paracrine manner (Ford and Wise 2009). In the scrotal testis of unilateral cryptorchid boars defective spermatogenesis correlates with abnormalities in the activity of Sertoli cells (Kojima 1991; Miething 1995; Pinart et al. 1999a); altered Sertoli cells produce abnormal paracrine signals that damage germ-cell differentiation, leading to spermatogenic arrest at different stages (Antich et al. 1995; Jannes et al. 1998). Anomalies in Sertoli-cell function

could be related to a genetic origin of unilateral cryptorchidism (Lee 1993; Foresta et al. 1996) or an underlying endocrinopathy generated by the cryptorchid testis (Antich et al. 1995; Rozanski and Bloom 1995).

Altered lectin affinity of the seminiferous epithelium is also an indicator of both impaired Sertoli cell function and spermatogenic arrest in scrotal testes of unilateral cryptorchid boars (Pinart et al. 2001b). Therefore, the increased content of glucosyl conjugates in spermatocytes and spermatids suggests that an abnormal ion transport could be implicated in the arrest of spermatogenesis at spermatocyte and spermatid steps; according to Santi et al. (1998), adequate ion transport is crucial for normal spermatogenesis. The decrease in galactose residues in spermatids and Sertoli cells is indicative of anomalies in cell-to-cell adhesion; defective attachment of germ cells to Sertoli cells has also been reported in subfertile rats (Courtens and Ploen 1999). The low galactosyl content of spermatids in case of partial arrest agrees with the notion that these sugars are important for spermiogenesis (Pinart et al. 2001b). In healthy boars, the residues of α-fucose at the apical cytoplasm of Sertoli cells are markers of lysosomal enzymes implicated in the degradation of residual bodies formed during spermiation (Ueno et al. 1991; Pinart et al. 2001b); in the scrotal testis of unilateral cryptorchid boars, the low amount of α-fucose residues in these cells correlates with the partial spermatogenic arrest, which results in low numbers of mature spermatids and residual bodies, and therefore in low content of lysosomes (Pinart et al. 2001b).

As in healthy boars, the tail region of testicular spermatozoa in the left (scrotal) testis of unilateral cryptorchid boars shows a poor lectin affinity. However, differences exist in the affinity of the acrosome, with a decreased content of galactosyl residues and increased content of glucosyl residues (Pinart et al. 2001b). This abnormal content of sugar residues may interfere with sperm maturation during epididymal transit and also with sperm fertilizing ability (Töpfer-Petersen 1999). Both abnormal testicular differentiation and defective epididymal maturation of the acrosome correlate with the increased frequency of spermatozoa with acrosomal anomalies in the ejaculate of post-pubertal boars affected by unilateral abdominal cryptorchidism (Pinart et al. 1998, 1999a).

The interstitial tissue of the left (scrotal) testis of unilateral cryptorchid boars has a normal appearance, with small blood and lymph vessels (Pinart et al. 1999a) and a similar Leydig cell, fibroblast and mast cell density as in healthy boars (Pinart et al. 2001d). Structure and ultrastructure of both Leydig cells and fibroblasts are also similar to scrotal testis of healthy boars (Pinart et al. 1999a, 2001d). Controversies exist about the effects of unilateral cryptorchidism on the Leydig cell population of the scrotal testis. Some authors describe anomalies either in Leydig cell number or in steroid production (Ezeasor 1985; Sirvent et al. 1989), whereas others link an increase in both Leydig cell number and steroidogenic activity to a compensatory mechanism for the low testosterone production of the abdominal testis (Jansz and Pomerantz 1986). Besides, some authors describe similar, but less severe anomalies than in the ectopic testis, in the scrotal testis of unilateral cryptorchid adults (Zakaria et al. 1998). These divergences are probably correlated with age; according to Kawakami et al. (1993) the testosterone

production of the scrotal testis of unilateral cryptorchid males peaks in the postpubertal period, and then decreases progressively in adulthood.

Although structural (Pinart et al. 1999a) and ultrastructural (Pinart et al. 2001d) evaluations do not show abnormalities in Leydig cells of scrotal testes of unilateral cryptorchid boars, the histochemical approach reveals anomalies in the sugar nature of O-linked glycans (Pinart et al. 2002). Therefore, as compared with healthy boars, Leydig cells of scrotal testes of unilateral cryptorchid boars have an altered content of glucosyl and galactosyl residues, which are indicative of disturbances in the transport of fluid and ions and in membrane permeability, and also of defective steroidogenesis (Arenas et al. 1998; Pinart et al. 2002). Alterations in substrate transport and in membrane permeability of Leydig cells are described in ectopic (either abdominal or inguinal) testes but not in the scrotal testes of unilateral cryptorchid males (Tanigawa et al. 1990). On the other hand, divergent views exist about the effects of unilateral cryptorchidism on the steroidogenic activity of the scrotal testis; thus, whereas some authors describe unaffected androgen synthesis (Ezeasor 1985; Sirvent et al. 1989; Toppari et al. 2006), others report increased (Jansz and Pomerantz 1986) or even decreased (Pinart et al. 2001d, 2002) testosterone production. This variability in testosterone production probably reflects the different aetiology of cryptorchidism (Kvist et al. 2006). In normal and pathological conditions, Leydig cell function depends on the presence of specific cell populations in the seminiferous tubules (Wu and Murono 1996). Pachytene spermatocytes and spermatids modulate the secretion of specific paracrine factors by Sertoli cells, which are involved in the regulation of Leydig cell activities (Jegou and Sharpe 1993; Wu and Murono 1996). In several disturbances, including varicocele, X-irradiation, vitamin A deficiency, efferent duct ligation or heat stress, damage of spermatogenesis results in impaired testosterone production (Jegou and Sharpe 1993). In the scrotal testes of unilateral abdominal cryptorchid boars, alterations in Leydig cells correlate with partial spermatogenic arrest at the spermatocyte and spermatid stages (Pinart et al. 1999a, b, 2000).

In the right (abdominal) testis of unilateral cryptorchid boars a significant reduction of the diameter of seminiferous tubules has been found (average 129 μm) (Pinart et al. 1999a). Moreover, spermatogenesis is totally inhibited in all seminiferous tubules, as evidenced by the structural appearance of the seminiferous epithelium (Pinart et al. 1999a); therefore, in most tubules, the seminiferous epithelium is pseudostratified and constituted by Sertoli cells and few spermatogonia (Fig. 4.2a), whereas in others a complete absence of germ cells is shown (Fig. 4.2b). The nucleus of Sertoli cells is located in the basal cytoplasm and the apical cytoplasm fills the lumen of seminiferous tubules (Pinart et al. 2000). The lamina propria displays a variable degree of thickening (from 4.5 to 10 μm) and collagenization; this thickness usually being greater in the seminiferous tubules exhibiting only Sertoli cells (Pinart et al. 1999a, 2001c). Peritubular cells of inner and outer layers of the lamina propria appear as fibroblasts of immature appearance, whereas myoid cells are not observed (Pinart et al. 2001c). The interstitial tissue shows a heterogeneous pattern, with three different areas irregularly distributed: areas with abundant Leydig cells, areas crossed by large fibrous septa, and areas with abundant blood vessels and erythrocytes (Pinart et al. 1999a, 2001d).

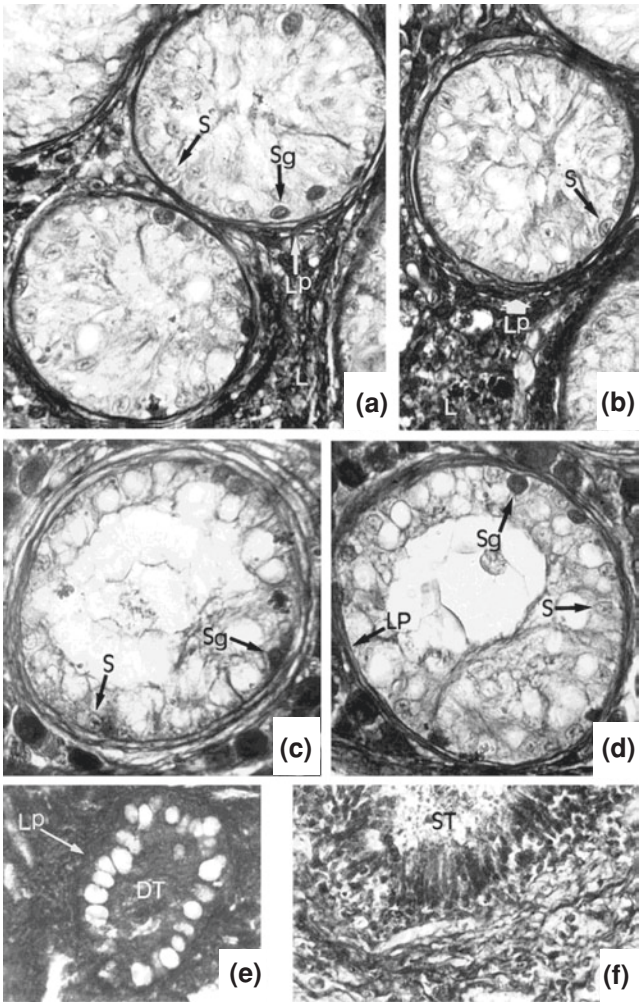


Fig. 4.2 Structure of the seminiferous tubules in abdominal testes of post-pubertal boars with spontaneous unilateral and bilateral cryptorchidism. Mallory's trichrome stain. **a** Seminiferous tubule with Sertoli cells (*S*) and spermatogonia (*Sg*) of the *right* (abdominal) testis of unilateral cryptorchid boars. **b** Seminiferous tubule with only Sertoli cells (*S*) of the *right* (abdominal) testis of unilateral cryptorchid boars. **c, d** Seminiferous tubule with Sertoli cells (*S*) and spermatogonia (*Sg*) of the *left* (abdominal) testis of bilateral cryptorchid boars; note the height of Sertoli cells and the considerable thickening of the lamina propria (*LP*). **e** Degenerative seminiferous tubule (*DT*) of the *right* (abdominal) testis of bilateral cryptorchid boars. **f** Sclerotic seminiferous tubule (*ST*) of the *right* (abdominal) testis of bilateral cryptorchid boars. ×170. L, Leydig cells. Reproduced from Pinart et al. (1999a) with permission

Therefore, unilateral abdominal cryptorchidism provokes an important regression of the Leydig cell population, which appears in an unpatterned distribution, and a great vascularization of the abdominal testis. Under these circumstances, Leydig cells can have either a mature but degenerative appearance or an immature

appearance (Pinart et al. 2001d). The fibroblast population, constituted by immature cells, displays a higher density than in healthy boars.

The left and right testes of bilateral abdominal cryptorchid boars are covered by a thickened tunica albuginia; the testes show marked abnormalities in the structural organization of the seminiferous tubules, lamina propria and interstitial tissue. In the left testis, seminiferous tubules and interstitial tissue occupy 38 and 62 % of the testicular parenchyma, respectively. In the right testis, scattered seminiferous tubules occupy about 6 % of the testicular parenchyma; thus, the amount of interstitial tissue is of 94 % (Pinart et al. 1999a). Compared to healthy boars, both testes of bilateral cryptorchid boars show a significant reduction in the amount of seminiferous tubules that is of 20 % in the left testis and practically complete in the right one. In both abdominal testes of bilateral cryptorchid boars, spermatogenesis is inhibited. In the left testis, the diameter of seminiferous tubules is 181 μm, significantly greater than in the abdominal testis of unilateral cryptorchid boars (Pinart et al. 1999a). The seminiferous epithelium, about 50 μm high, is simple cuboidal and contains Sertoli cells and few spermatogonia (Fig. 4.2c–d) or only Sertoli cells; in this last case the tubules usually display a degenerative pattern. The lamina propria shows a great thickening, which can double or even triple the thickness of the lamina propria of scrotal testes from either healthy or unilateral cryptorchid boars. Inner and outer peritubular cell layers of the lamina propria are composed of fibroblasts of immature appearance; myoid cells are lacking (Pinart et al. 2001c). The right testis contains few seminiferous tubules without germ cells, which can exhibit either a degenerative (Fig. 4.2e) or sclerotic (Fig. 4.2f) appearance; the diameter of these tubules is 182 μm. The lamina propria is composed of a thickened basal lamina (2–3 μm thick) with a high density of collagen fibers and low glycoconjugate content; peritubular cells are lacking (Pinart et al. 2001c).

Regadera et al. (1991) have also reported anomalies in the seminiferous tubules of ectopic testes from unilaterally cryptorchid men. However, their results are quite divergent:

(a) 10 % of cryptorchid testes contain seminiferous tubules with germ cells, mainly spermatogonia, but also some spermatocytes and occasionally spermatids,
(b) 57 % of cryptorchid testes show seminiferous tubules with only Sertoli cells, and
(c) 33 % of cryptorchid testes have sclerotic tubules (Regadera et al. 1991).

These divergent results may be attributed to the different positions (abdominal or inguinal) in which the ectopic testes can be found and also to the age of the patients at the time of the experiment, which ranged from 16 to 63 years. Probably the most severe abnormalities are related to a higher position of ectopic testes and/or advanced age (Pinart et al. 1999a). The structural alterations of the Sertoli cells in cryptorchid testes can be due to either a primary defect or acquired damage provoked by high temperatures (Paniagua et al. 1990; Lee 1993; Antich et al. 1995). In any case, abnormalities in Sertoli cells produce the absence of spermatogenesis in abdominal testes (Nistal et al. 1990; Paniagua et al. 1990; Pinart et al. 1999a).

Lectin histochemistry indicates that few differences exist in sugar content among non-degenerating, degenerating and degenerated immature Sertoli cells, as well as between the basal and apical cytoplasm of immature Sertoli cells, and between immature Sertoli cells and spermatogonia. Compared to healthy boars, the seminiferous epithelium of abdominal testes displays a decreased content of fucosyl residues and an increased content of glucosyl and galactosyl residues. A similar sugar composition has been found in the degenerating testes of rodents (Vanha-Perttula and Arya 1985). These results indicate that degeneration of the seminiferous epithelium may induce increased ion transport and high membrane permeability (Pinart et al. 2001b). Some studies report that in abdominal testes the degeneration of spermatogonia occurs by apoptosis whereas the degeneration of Sertoli cells is necrotic (De Rooij and Russell 2000); contrarily, more recent data suggest that abdominal cryptorchidism, both unilateral and bilateral, does not result in an increased apoptotic degeneration of spermatogonia as compared with the scrotal testes of healthy boars (Bernal-Mañas et al. 2005).

Increased thickness of the lamina propria has also been described in men with unilateral and bilateral cryptorchidism (Paniagua et al. 1990; Santamaria et al. 1990), in men with a history of cryptorchidism (Gotoh et al. 1987), in bulls with testicular hypoplasia (Veeramachaneni et al. 1987), and in bulls with azoospermia (Gargiulo et al. 1991). The thickening of the lamina propria is due to abnormalities in the cooperation between damaged Sertoli cells and peritubular cells that lead to both increased synthesis and secretion of extracellular components by peritubular cells and decreased turnover of glycoconjugates (Gargiulo et al. 1991; Richardson et al. 1995). Besides, the thick lamina propria impairs interactions between tubular and interstitial compartments, further interfering with testicular functions (Gargiulo et al. 1991; Pinart et al. 2001c).

The interstitial tissue of the right (abdominal) testis of unilateral cryptorchid boars is composed of mature but degenerative Leydig cells, immature Leydig cells and fibroblasts, and few mast cells. In the left testis of bilateral abdominal cryptorchid boars the interstitial cell population is composed of mature and immature Leydig cells, immature fibroblasts and mast cells, but also of lymphocytes and erythrocytes and few plasma cells (Pinart et al. 2001d). The numerical density of Leydig cells does not differ from the right (abdominal) testis of unilateral cryptorchid boars, whereas fibroblast and mast cell density is higher than in unilateral cryptorchid boars. The interstitial tissue of the right testis of bilateral abdominal cryptorchid boars exhibits large fibrous connective tissue areas with immature fibroblasts and mast cells, and also adipose areas. The Leydig cell population shows notable regression with scarce immature cells in advanced pignosis. Both fibroblasts and mast cell density are higher than in healthy boars (Pinart et al. 2001d).

Regression of the Leydig cell population and steroid production is also observed in ectopic testes of unilateral and bilateral cryptorchid men (Regadera et al. 1991; Sheth et al. 1996) and dogs (Kawakami et al. 1993), in unilateral abdominal cryptorchid boars and stallions (Raeside et al. 1988), and in men with a history of pre-pubertal cryptorchidism (Mieusset et al. 1995, 1997). Instead,

divergences exist regarding the degree of alteration in Leydig cell numbers; in cryptorchid testes of post-pubertal men some authors describe Leydig cell hyperplasia (Sirvent et al. 1989; Regadera et al. 1991), whereas others report a reduction in the number of Leydig cells that begins in the first months of life and becomes progressively more severe with age (Sasagawa et al. 1994). These divergences reflect different etiologies of cryptorchidism (Pinart et al. 1999a, 2000). Leydig cells from adult cryptorchid testes have impaired testosterone production (Regadera et al. 1991). The anomalies in Leydig cells are due to defective stimulation during the pre-pubertal period (Rozanski and Bloom 1995) as a result of abnormal paracrine influence from altered Sertoli cells (Antich et al. 1995). The abnormal differentiation of Leydig cells may be the cause of the marked fibrosis in the abdominal testes of unilateral and bilateral cryptorchid boars (Pinart et al.1999a).

The presence of adipocytes in the interstitial tissue has also been observed by Ezeasor (1985) in abdominal testes of adult goats. Testicular adipocytes develop from an abnormal differentiation of mesenquimatic precursors of Leydig cells during the pre-pubertal period (Huhtaniemi and Pelliniemi 1992) and/or from immature fibroblasts (Hill et al. 1999). Both the anomalies in Sertoli cell population (Antich et al. 1995) and impaired blood irrigation (Setchell et al. 1995) result in a defective differentiation of interstitial cells.

Therefore, in post-pubertal boars the abnormalities in the seminiferous tubules and the interstitial tissue of the abdominal testes are more severe in bilateral cryptorchidism than in unilateral cryptorchidism, and in the case of bilateral cryptorchidism, alterations are more marked in the right than in the left testis (Pinart et al. 1999a). In young adult men, Paniagua et al. (1990) do not find differences in testicular alterations between the unilateral and the bilateral disease.

Ultrastructure of the Seminiferous Epithelium

Sertoli cells of the right (abdominal) testis of unilateral cryptorchid boars show an oval or roundish nucleus, smaller than in healthy boars (averaging 10×8 μm), placed in the basal or medio-apical cytoplasm and forming few small infoldings on the nuclear envelope; the long axis lies parallel to the basal lamina (Pinart et al. 2000). Sertoli cell nuclei contain homogeneously distributed euchromatin with small heterochromatic areas and one or two prominent centrally placed nucleoli (Fig. 4.3a). Sertoli cells adhere to the basal lamina by numerous but poorly developed hemidesmosomes. Neighboring Sertoli cells form abundant small desmosomes and a few gap and occludens junctions located at different levels of the seminiferous epithelium. The defective establishment of junctional complexes leads to an incomplete development of the blood-testis barrier (Pinart et al. 2000). Few junctions also exist between Sertoli cells and germ cells, and they are usually small desmosomes. The cytoplasm has low organelle content, with perinuclear mitochondria, flat cisternae of rough endoplasmic reticulum, and polysomes. Regularly outlined nuclei and apical cytoplasm filling the tubular lumen are considered markers of Sertoli cell immaturity

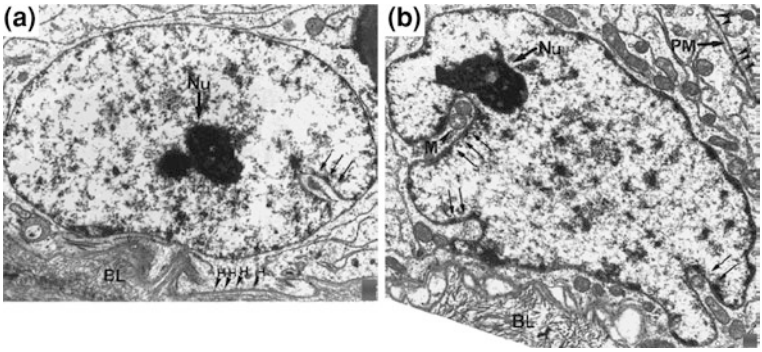


Fig. 4.3 Ultrastructural appearance of Sertoli cells in abdominal testes of post-pubertal boars with spontaneous unilateral and bilateral cryptorchidism. **a** Ultrastructure of Sertoli cells in the *right* (abdominal) testis of unilateral cryptorchid boars. The oval nucleus contains one prominent and centrally located nucleolus (*Nu*) and one small infolding on the nuclear surface (*arrows*). **b** Ultrastructure of Sertoli cells in abdominal testes of bilateral cryptorchid boars. The nuclear envelope forms deep infoldings (*arrows*) giving rise to nuclear crypts; neighboring Sertoli cells attach by poorly developed desmosomes (*arrowheads*). ×10,080. *BL* basal lamina, *ER* endoplasmic reticulum, *M* mitochondria, *PM* plasma membrane. Reproduced from Pinart et al. (2000) with permission

(Paniagua et al. 1990; Bruning et al. 1993). These ultrastructural features have also been observed in young men with unilateral and bilateral cryptorchidism (Paniagua et al. 1990) and in other testicular diseases such as hypogonadotropic hypogonadism (Nistal et al. 1990), tubular hypoplasia (Bruning et al. 1993), Klinefelter's syndrome (Regadera et al. 1991), and ovotestis (Bruning et al. 1993). Thus, different etiological factors may induce the persistence of immature Sertoli cells in adulthood.

In bilateral cryptorchid boars, Sertoli cells are cuboidal and have an elongated basal nucleus, smaller than in healthy boars (averaging 12 × 6 μm); it encloses granular euchromatin and one large nucleolus (Fig. 4.3b). The nuclear envelope forms abundant deep infoldings that produce nuclear crypts (Pinart et al. 2000). The basal cell membrane adheres to the basal lamina by abundant but poorly developed hemidesmosomes. Neighboring Sertoli cells form abundant small desmosomes, and gap and occludens junctions are scarce; as in unilateral cryptorchid boars the development of the blood-testis barrier is defective (Pinart et al. 2000). The apical cytoplasm forms lateral processes that are superimposed and attached by small desmosomes with lateral processes of adjacent Sertoli cells. The cytoplasm contains flat cisternae of endoplasmic reticulum, scattered vesicles and polysomes, and abundant mitochondria usually forming perinuclear aggregates lying inside the nuclear crypts. Elongated nuclei with deep membrane indentations and lateral cytoplasmic processes have been identified in the degenerative Sertoli cells of pre-pubertal cryptorchid testes of goats (Singh and Ezeasor 1989) and humans (Rune et al. 1992), and of post-pubertal cryptorchid testes of humans (Nistal et al. 1990). In cryptorchid testes, degeneration of immature Sertoli cells involves, in initial stages, the formation of deep infoldings of the nuclear

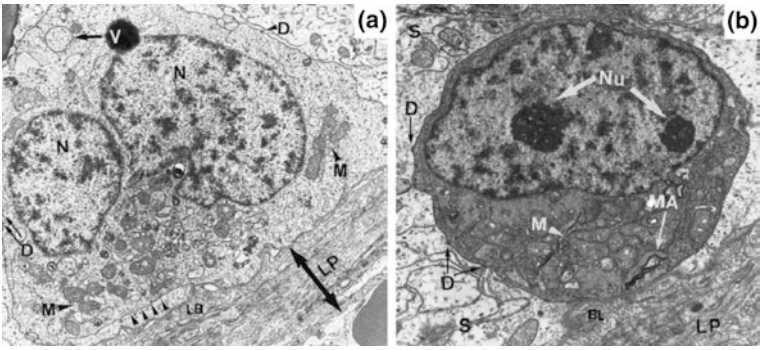


Fig. 4.4 Ultrastructural appearance of spermatogonia in abdominal testes of post-pubertal boars with spontaneous unilateral cryptorchidism. **a** Pyramidal spermatogonium; the cell is partially attached (*arrowheads*) to the basal laminal (BL). ×8,000. Ultrastructure of Sertoli cells in the *right* (abdominal) testis of unilateral cryptorchid boars. **b** Rounded spermatogonium. ×12,800. *D* desmosomes, *LP* lamina propria, *M* mitochondria, *MA* membrane aggregates, *N* nucleus, *Nu* nucleolus, *S* Sertoli cell, *V* vesicles. Reproduced from Pinart et al. (2000) with permission

membrane and retraction and convolution of the apical cytoplasm, thus forming lateral processes. More advanced degeneration includes dilation of endoplasmic reticulum cisternae, increased lipid content, and atrophy of Golgi complex and mitochondria (Singh and Ezeasor 1989; Rune et al. 1992).

Spermatocytes and spermatids are not observed in the abdominal testes of unilateral and bilateral cryptorchid boars (Pinart et al. 1999a, 2000). Seminiferous tubules contain only scattered spermatogonia with an abnormal appearance. Reduction of germ cell number is more severe in bilateral cryptorchidism than in unilateral cryptorchidism. In unilateral cryptorchid boars, two different types of spermatogonia have been distinguished (Pinart et al. 2000). Some appear as large cells (averaging 16 × 15 μm) with pyramidal profiles and partially attached to the basal lamina (Fig. 4.4a). The large nuclei (averaging 12 × 8 μm) usually show irregular profiles, with one or two deep infoldings dividing them into separate lobes. Nuclei are euchromatic and the cytoplasm contains abundant mitochondria aggregated basally, poorly developed endoplasmic reticulum, electrolucent vesicles and polysomes. Other spermatogonia appear as highly electrodense cells, with roundish profiles (averaging 10 × 8 μm) that have practically lost their contact with the basal lamina (Fig. 4.4b). The apical oval nucleus (averaging 7 × 5 μm) is strongly electrodense and contains granular euchromatin with scattered heterochromatic areas and one or two prominent nucleoli. The cytoplasm has abundant mitochondria, scattered cisternae of endoplasmic reticulum and membrane aggregates. In some cases, these spermatogonia have completely lost their attachment to the basal lamina and can be found at different levels of the seminiferous epithelium; some detached cells show degenerative patterns.

Spermatogonia with round profiles are also observed in bilateral cryptorchid boars; however, the cells are slightly larger (averaging 11 × 9.5 μm) than in

unilateral cryptorchid boars. The nucleus contains granular euchromatin with abundant heterochromatic areas and one large peripherically placed nucleolus, and the cytoplasm encloses few cellular organelles and displays a degenerative appearance (Pinart et al. 2000).

Therefore, abdominal testes of both unilateral and bilateral cryptorchid boars at post-pubertal age are likewise constituted by immature Sertoli cells, showing degenerative signs in bilateral cryptorchid boars (Pinart et al. 2000). The alterations of Sertoli cells of post-pubertal abdominal testes are attributed to an abnormal differentiation during the pre-pubertal period (Antich et al. 1995). In normal testes, proliferation and differentiation of Sertoli cells occur post-natally under FSH and testosterone stimulus, respectively (McCoard et al. 2001, 2003; Ford and Wise 2009). Differentiated Sertoli cells produce inhibin, which is involved in feedback control of FSH levels; as FSH levels decrease, Sertoli cell replication declines (McCoard et al. 2001, 2003). Testosterone produced by perinatal Leydig cells under LH stimulus also prevents enhancement of Sertoli cell proliferation and promotes their differentiation (Buzzard et al. 2003). In abdominal testes the hormonal cascade that stimulates Sertoli cell differentiation is blunted (Antich et al. 1995; Rozanski and Bloom 1995), leading to the persistence of immature Sertoli cells. Divergences between unilateral and bilateral cryptorchidism could be due to different alterations in endocrine and paracrine factors implicated in the regulation of these cells (Mieusset et al. 1995; Pinart et al. 1999a; Pinart et al. 2000). It has been reported that endocrine and paracrine disfunctions are more severe in the bilateral disease (Mieusset et al. 1995; Foresta et al. 1996; Matuszczak et al. 2011).

The degeneration of the seminiferous epithelium in abdominal testes has been extensively described in humans, boars, and rams (Rune et al. 1992; Courtens and Ploen 1999; Pinart et al. 1999a, 2000). At an initial degeneration stage, the tubules exhibit immature Sertoli cells with regularly outlined nuclei and apical cytoplasm filling the tubular lumen, and few degenerating spermatogonia placed at different levels of the seminiferous epithelium (Pinart et al. 1999a, 2000). More advanced degeneration involves nuclear elongation, formation of deep infoldings in the nuclear envelope, and retraction and convolution of the apical cytoplasm in immature Sertoli cells, as well as the disappearance of degenerated spermatogonia, the tubules being composed of a cuboidal epithelium (Rune et al. 1992; Courtens and Ploen 1999; Pinart et al. 1999a, 2000). Total degeneration includes disappearance of the nucleus, atrophy of organelles, and increased lipid content in Sertoli cells forming a disorganized nodule (Rune et al. 1992; Pinart et al. 1999a). Therefore, the right (abdominal) testis of unilateral cryptorchid boars, and the left and right testes of bilateral abdominal cryptorchid boars are representative of these three different stages of seminiferous epithelium degeneration (Pinart et al. 2001b).

In cryptorchidism, abnormal development of the blood-testis barrier has also been described by several authors (Cinti et al. 1993; Maekawa et al. 1995; Rozanski and Bloom 1995). Paniagua et al. (1990) and Nistal et al. (1990) reported that the lack of Sertoli cell maturation in abdominal testes of post-pubertal men does not hinder the development of junctional specializations, which

contradicts with explanations above. Some studies suggest that the establishment of the blood-testis barrier requires the complete maturation of Sertoli cells, the onset of spermatogenesis, and the development of the tubular lumen (França et al. 2005; Ford and Wise 2009, 2011). In unilateral and bilateral cryptorchid boars, the basal membrane of immature Sertoli cells forms small hemidesmosomes. Anomalies in hemidesmosomes have been correlated with the increased thickness of the basal lamina in cases of cryptorchidism (Paniagua et al. 1990; Maekawa et al. 1995; Rozanski and Bloom 1995). Such alterations in basal and lateral junctions of Sertoli cells are attributed to an abnormal development of actin and intermediate filaments (Maekawa et al. 1995).

The seminiferous epithelium of abdominal testes from unilateral and bilateral cryptorchid boars contains few spermatogonia; the decrease in germ cell number is greater in the case of bilateral cryptorchidism (Pinart et al. 2000). Differences also exist between the unilateral and bilateral disease in the ultrastructural appearance of spermatogonia. In abdominal testes, abnormally differentiated Sertoli cells may produce abnormal paracrine signals that damage germ cell differentiation (Antich et al. 1995). Alterations in germ cell number are attributed to delayed and defective maturation of prespermatogonia into spermatogonia, decreased proliferation of spermatogonia, and complete failure of the transformation of spermatogonia into primary spermatocytes during the perinatal period (Kohler et al. 2007; Caires et al. 2008). Untransformed gonocytes and abnormal spermatogonia degenerate progressively throughout the pre-pubertal period (Codesal et al. 1993; Rozanski and Bloom 1995), resulting in decreased numbers of spermatogonia in the abdominal testes of post-pubertal males with unilateral and bilateral cryptorchidism.

The presence of roundish spermatogonia with electrodense nuclei and large nucleoli has also been observed in cryptorchid testes of children and men (Codesal et al. 1993). It has been suggested that these cells have abnormal DNA content as a result of disturbances in DNA replication at above-scrotal temperatures of 35 °C (Codesal et al. 1993; Jannes et al. 1998). Giant pyramidal spermatogonia have been described in cryptorchid and retractile testes of children (Cinti et al. 1993; Codesal et al. 1993), and in testes of sub-fertile men (Hofmann et al. 1992). Some studies state that these cells are multinucleate and develop as a result of complete DNA replication but abnormal cell division (Hofmann et al. 1992; Cinti et al. 1993; Codesal et al. 1993).

In conclusion, the seminiferous epithelium of abdominal testes from unilateral and bilateral cryptorchid boars is constituted by immature Sertoli cells and few spermatogonia. Differences in the ultrastructural appearance of immature Sertoli cells indicate that testicular degeneration is initiated earlier in bilateral cryptorchidism than in unilateral cryptorchidism. Sertoli cell degeneration may be the cause of the degenerative appearance of germ cells and also of their lower number in the abdominal testes of bilateral cryptorchid boars (Pinart et al. 2000). These results differ from those reported by Singh and Ezeasor (1989) and Rune et al. (1992), who found Sertoli cell degeneration of cryptorchid testes at pre-pubertal age. These divergences reflect different etiologies of cryptorchidism. Tubular degeneration at pre-pubertal age could be due to an intrinsic defect of Sertoli cells,

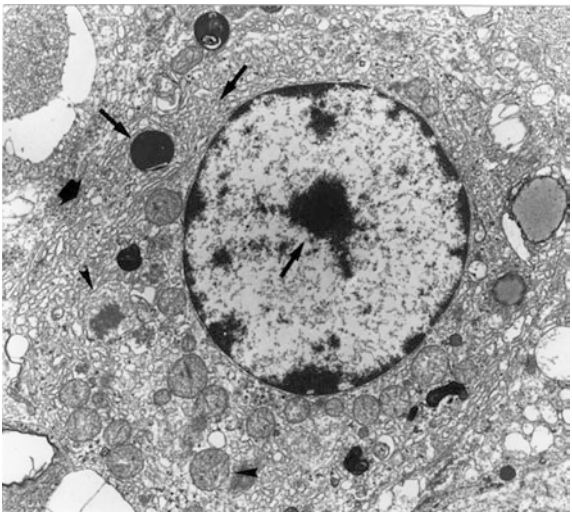


Fig. 4.5 Ultrastructure of mature Leydig cells in initial degeneration stage in abdominal testes of post-pubertal boars with spontaneous unilateral and bilateral abdominal cryptorchidism. ×10,080. *ER* endoplasmic reticulum, *Ly* lysosomes, *M* mitochondria, *Nu* nucleus, *PM* plasma membrane, *V* vesicles. Reproduced from Pinart et al. (2001b) with permission

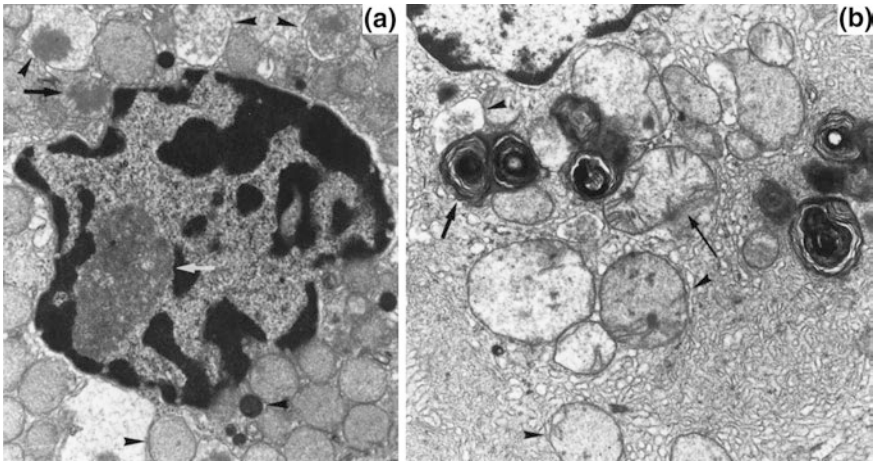


Fig. 4.6 Ultrastructure of mature Leydig cells in mid degeneration stage in abdominal testes of post-pubertal boars with spontaneous unilateral and bilateral abdominal cryptorchidism. ×10,080. **a** Appearance of the nucleus (×12,800). **b** Appearance of the cytoplasm (×20,000). *ER* endoplasmic reticulum, *He* heterochromatin, *Ly* lysosomes, *M* mitochondria, *MF* myelin figures, *N* nucleus, *Nu* nucleus, V vesicles. Reproduced from Pinart et al. (2001b) with permission

whereas tubular degeneration at post-pubertal age may be due to the adverse effect generated by high temperature (Pinart et al. 2000).

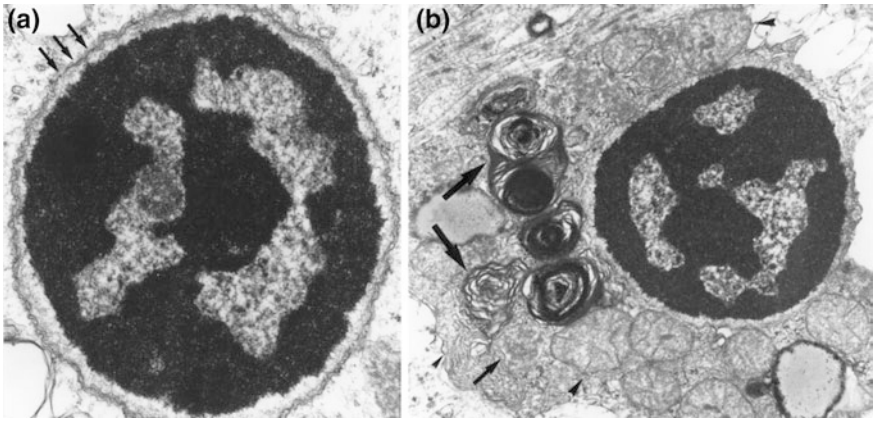


Fig. 4.7 Ultrastructure of mature Leydig cells in advanced degeneration stage in abdominal testes of post-pubertal boars with spontaneous unilateral and bilateral abdominal cryptorchidism. ×10,080. **a** Appearance of the nucleus (×20,000). **b** Appearance of the cytoplasm (×25,000). *En* nuclear envelope, *He* heterochromatin, *M* mitochondria, *MF* myelin figures, *PM* plasma membrane, *V* vesicles. Reproduced from Pinart et al. (2001b) with permission

Ultrastructure of the Interstitial Tissue

In abdominal testes of unilateral and bilateral cryptorchid boars, mature Leydig cells are located in association with blood capillaries and show degenerative signs (Figs. 4.5, 4.6, 4.7) (Pinart et al. 2001d). In an initial degeneration stage, these cells are characterized by a decreased cellular size (averaging 10 × 12 μm) as compared to mature Leydig cells of scrotal testes, and the presence of a round nucleus (averaging 5.5–6.5 μm in diameter) enclosing coarse euchromatin, heterochromatic masses and a prominent nucleolus (Fig. 4.5). The cytoplasm contains poorly-developed Golgi complex and smooth endoplasmic reticulum, myelin figures, few lysosomes and spherical mitochondria (averaging 0.6–0.8 μm in diameter) with tubular cristae. Mid degeneration involves a reduction in nuclear size (averaging 4–4.5 μm in diameter) and a greater development of heterochromatic areas (Fig. 4.6a); the nucleus can exhibit either a regular or an irregular profile (Pinart et al. 2001d). The decrease in cytoplasmic size (averaging 8 × 10.5 μm) results in a disorganization of smooth endoplasmic reticulum and increased numbers of myelin figures. Mitochondria maintain their size and profile but show lower numbers of cristae (Fig. 4.6b). Advanced degeneration leads to a notable decrease in cellular diameter (averaging 6 μm) and nuclear diameter (averaging 3.5 μm), total heterochromatinization of the nucleus, and swelling of the envelope (Fig. 4.7a). In the cytoplasm, myelin figures show great development whereas mitochondria (averaging 0.6 μm in diameter) display swelling, scarce cristae and low electrodensity (Fig. 4.7b).

Non-degenerating immature Leydig cells of abdominal testes from unilateral and bilateral cryptorchid boars lie in contact with seminiferous tubules. They

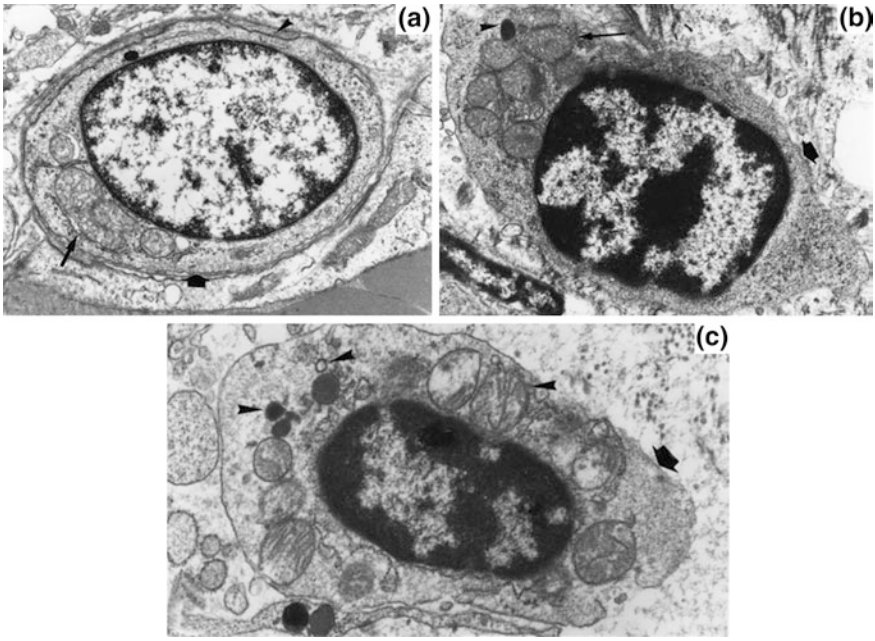


Fig. 4.8 Ultrastructure of immature Leydig cells in abdominal testes of post-pubertal boars with spontaneous unilateral and bilateral abdominal cryptorchidism. **a** Appearance of non-degenerating immature leydig cells in the *right* (×8,000. **b** Appearance of immature leydig cells in initial degeneration (×16,000). **c** Appearance of immature Leydig cells in advanced degeneration the nucleus (×20,000). *ER* endoplasmic reticulum, *He* heterochromatin, *Ly* lysosomes, *M* mitochondria, *N* nucleus, *PM* plasma membrane, *V* vesicles. Reproduced from Pinart et al. (2001b) with permission

show an oval profile (averaging 7.5 × 12 μm) and a rounded nucleus (averaging 3.5–4.5 μm in diameter) constituted by fine euchromatin and one small nucleolus (Pinart et al. 2001d). The cytoplasm is poorly electrodense and encloses a few large, oval mitochondria (averaging 0.8 × 1.2 μm), usually arranged in aggregates and exhibiting lamellar cristae, poorly developed rough endoplasmic reticulum, and some vesicles (Fig. 4.8a). In bilateral cryptorchid boars, picnosis of immature Leydig cells is initially manifested in a decrease in cellular size (averaging 4.5 × 8.5 μm) and a greater development of heterochromatic areas (Fig. 4.8b) (Pinart et al. 2001d). The cytoplasm contains abundant polysomes and round mitochondria (averaging 0.5 × 0.6 μm). Advanced degeneration results in decreased size (averaging 2 × 2.5 μm), heterochromatinization and swelling of the nucleus, with a cytoplasm characterized by polysomes, few lysosomes, and swollen mitochondria (averaging 0.7 × 0.8 μm) (Fig. 4.8c).

Differences in the maturation degree between intersticial and intertubular Leydig cells are also found in men with unilateral and bilateral cryptorchidism (Sirvent et al. 1989; Regadera et al. 1991), in goats with unilateral abdominal cryptorchidism (Ezeasor 1985), and in patients with primary testicular disorders

(Regadera et al. 1991). It has been reported that in both immature and mature testes of healthy males, interstitial, and intertubular Leydig cells differ in number, type, and activity of LH and hCG receptors, so that they respond differently to the same hormonal stimulus (Sasagawa et al. 1994; Antich et al. 1995; Setchell et al. 1995); besides, the morphology and function of intertubular Leydig cells are under the control of neighboring Sertoli cells by means of paracrine factors (Antich et al. 1995). In cryptorchid testes, the presence of immature Leydig cells in peritubular localization correlates mainly with the immaturity of Sertoli cells (Pinart et al. 1999a, 2000), which results in an abnormal paracrine stimulation of neighboring Leydig cells (Antich et al. 1995).

Immature Leydig cells are characterized by lack of smooth endoplasmic reticulum and Golgi complex, and by low mitochondrial content; degenerating mature Leydig cells display regressive changes in smooth endoplasmic reticulum, Golgi complex, and mitochondria (Pinart et al. 2001d). In Leydig cells, the degree of development of these organelles is used as a marker of their capacity to synthesize steroids (Haider and Servos 1998). Therefore, the ultrastructural study indicates that both unilateral and bilateral abdominal cryptorchidism inhibit the steroidogenesis in the abdominal testes at post-pubertal age (Pinart et al. 2001d). Lack of steroidogenesis in abdominal testes results in low testosterone levels in bilateral cryptorchid males (Kawakami et al. 1995; Pathirana et al. 2011). In contrast, no differences arise in plasma testosterone concentrations between healthy and unilateral cryptorchid dogs (Pathirana et al. 2011).

The degeneration of Leydig cells of abdominal testes from post-pubertal boars is more severe in bilateral than in unilateral cryptorchidism. Similar differences are found between unilateral and bilateral cryptorchidism in the picnotic degree of immature Sertoli cells of post-pubertal abdominal testes (Pinart et al. 1999a, 2000). These results indicate that the degeneration of abdominal testes is initiated earlier in bilateral cryptorchidism than in unilateral cryptorchidism (Pinart et al. 2000) due to more severe endocrine, paracrine, and vascular alterations in the former disease (Mieusset et al. 1995). As has been reported in bilateral cryptorchid men (Sheth et al. 1996), the anomalies of bilateral cryptorchid boars are more severe in the right testis than in the left testis (Pinart et al. 2001d); nevertheless, the causes of these differences between the right and left side are still unknown.

In the interstitial tissue of abdominal testes of unilateral and bilateral cryptorchid boars, fibroblasts appear as large immature cells (averaging 4.5 $\times$ 15 μm) containing a fused nucleus (averaging 3.5 $\times$ 13 μm) of either regular or irregular profile (Pinart et al. 2001d). The nucleoplasm has granular euchromatin, small heterochromatic areas, and one prominent nucleolus. The organelle content is composed of aggregates of rough endoplasmic reticulum cisternae, mitochondria, vesicles and lysosomes.

In scrotal testes of healthy adult males, fibroblasts are characterized by a low rate of synthesis and turnover of extracellular matrix components, their main function being the regulation of Sertoli cell activities (Schteingart et al. 1999). Immature fibroblasts with well-developed rough endoplasmic reticulum and prominent nucleolus have high secretory activity, which correlates with the

collagenization and impaired substrate diffusion of the interstitial tissue in abdominal testes of post-pubertal boars (Pinart et al. 2001d, 2002). In healthy adults, mature Sertoli cells secrete paracrine factors implicated in the maintenance of the cytologic features of the fibroblast population (Schteingart et al. 1999). In post-pubertal cryptorchid testes, the alterations in fibroblast number and metabolism correlate with Sertoli cell immaturity (Antich et al. 1995; Pinart et al. 2000), mast cell proliferation (Jezek et al. 1996; Pinart et al. 2001d), and defective testicular perfusion (Setchell et al. 1995; Pinart et al. 2001a).

Unilateral cryptorchidism does not induce abnormalities in the mast cell population of either scrotal or abdominal testes (Pinart et al. 2001d). Instead, the interstitial tissue of the left testis of bilateral abdominal cryptorchid boars has been reported to increase the number of mast cells, as well as that of lymphocytes and, to a lesser degree, the number of plasma cells; the right testis also exhibits frequent mast cells (Pinart et al. 2001d). Abundance of lymphocytes has been found in rats with bilateral abdominal cryptorchidism (Kort et al. 1991); this anomaly is usually associated with inflammatory and autoimmune diseases (Itoh et al. 1998). In this species, defective Leydig cell differentiation and Leydig cell depletion result in mast cell proliferation (Meinhardt et al. 1998). In men, idiopathic infertility (Yamamoto et al. 1994), seminiferous tubule atrophy (Jezek et al. 1996), malignant transformation of germ cells (Arber et al. 1998), and cryptorchidism (Montella and Pirino 1990) lead to testicular mastocytosis. In severe disturbances, mast cells are implicated in testicular fibrosis by enhancing proliferation and collagen production of fibroblasts, and in chronic inflammation by promoting capillary proliferation and lymphocyte infiltration; the presence of abundant mast cells and plasma cells is also considered as a sign of testicular regression (Antón et al. 1998; Ohtsuka 2000). In abdominal testes of bilateral cryptorchid boars, the mastocytosis correlates with the degeneration of Sertoli cells (Pinart et al. 2000) and of Leydig cells (Pinart et al. 2001d).

Despite the great fibrosis of the interstitial tissue (Pinart et al. 1999a, 2001d), abdominal testes from unilateral and bilateral cryptorchid boars also exhibit increased number of blood capillaries interspersed among interstitial cells (Pinart et al. 2001a); at ultrastructural level, interstitial capillaries can display either a mature or an angiogenic appearance. Both capillary angiogenesis and capillary degeneration are greater in bilateral cryptorchidism than in unilateral cryptorchidism. Increased number and altered permeability of capillaries, resulting in a reduction of blood flow, are also reported in ectopic testes of children and men with unilateral and bilateral cryptorchidism (Setchell et al. 1995). Besides, early correction of cryptorchidism at pre-pubertal age does not always improve the testicular blood flow (Mieusset and Bujan 1995). Abnormal vascular supply results in impaired oxygen and nutrient exchange that is manifested in increased fibrosis of the interstitial tissue (Setchell et al. 1995). The increased content of erythrocytes in the right (abdominal) testis of unilateral cryptorchid boars, and of erythrocytes and lymphocytes in the left testis of bilateral abdominal cryptorchid boars is also indicative of abnormalities in vascular permeability (Collin and Bergh 1996; Haider and Servos 1998).

As compared with capillaries of scrotal testes, angiogenic capillaries show an increased number but a decreased size of endothelial cells, lack of pericytes, and decreased thickness and collagen content of the basal lamina (Pinart et al. 2001a). These capillaries are usually associated with small migrating cells (Fig. 4.9a_1–a_2). The morphological events in angiogenesis were first described by Korpelainen et al. (1998) and Pettersson et al. (2000). New blood capillaries develop from preexisting ones by means of degradation of the basal lamina components by endothelial cells and detachment of pericytes; the pericyte loss results in proliferation of endothelial cells, which can further migrate to the surrounding tissue, and finally, associate to form new capillaries (Pettersson et al. 2000). At postpuberty, the proliferation of testicular capillaries can be due to the malignancy of endothelial cells, but also to abnormal stimulation from neighboring tissue as a

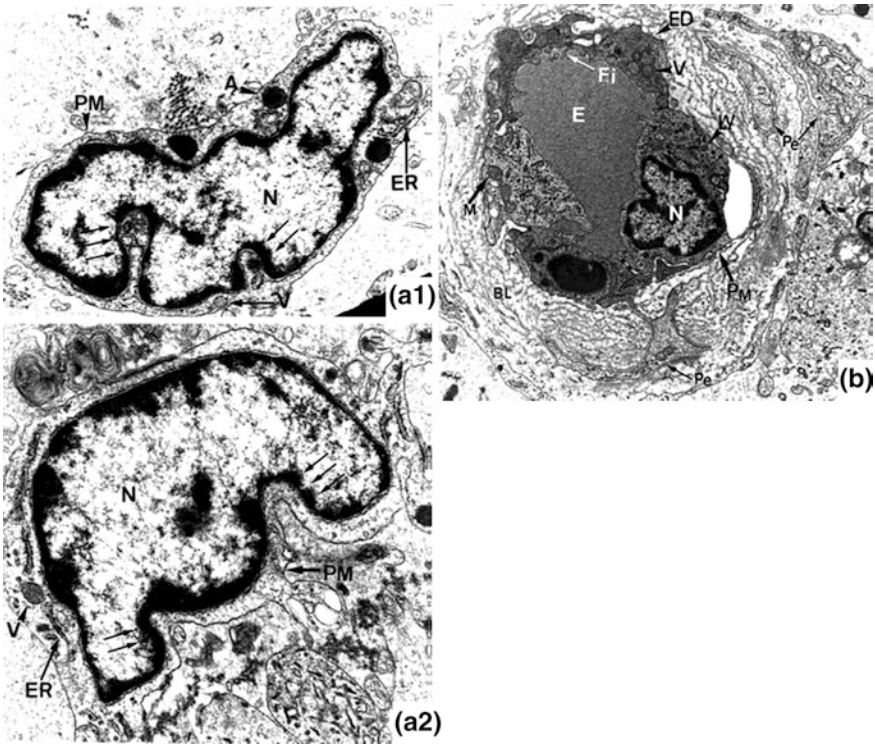


Fig. 4.9 Ultrastructure of blood capillaries in abdominal testes of post-pubertal boars with spontaneous unilateral and bilateral abdominal cryptorchidism. **a** Appearance of a migrating endothelial cell in the right testis of unilateral cryptorchid boars (**a_1**) and in the *left* testis of bilateral cryptorchid boars (**a_2**) (×15,000). Note the presence of deep invaginations (*arrows*) on the nucleus (*N*). **b** Cross section of a degenerative capillary of the left testis of bilateral cryptorchid boars (×8,960). *A* electrodense aggregates, *BL* basal lamina, *E* erythrocyte, *ED* endothelial cell, *ER* endoplasmic reticulum, *Fi* finger-like processes, *Ly* lysosomes, *M* mitochondria, *Pe* pericytes, *PM* plasma membrane, *V* vesicles. Reproduced from Pinart et al. (2001a) with permission

consequence of tumor development (Korpelainen et al. 1998; Feng et al. 2000), severe Leydig cell dysfunctions (Collin and Bergh 1996; Ergün et al. 1998), and stress conditions generated by hypoxia (Kerbel 2000), oxidative damage (Markey et al. 1994), or high temperature (Korpelainen et al. 1998). Tumoral cells (Feng et al. 2000; Kerbel 2000) or altered Leydig cells (Collin and Bergh 1996) secrete high amounts of vascular endothelial growth factor (VEGF), which stimulate several stages of blood vessel formation including endothelial cell proliferation and migration, basal lamina degeneration, and tube formation (Korpelainen et al. 1998). VEGF also acts as a potent pro-survival factor of endothelial cells in newly formed vessels (Kerbel 2000; Otani et al. 2000).

Degenerative blood capillaries of abdominal testes exhibit decreased diameter, narrowing of the lumen, pyknotic transformation of the endothelium, thickening and collagenization of the basal lamina and a great increase in pericytes (Fig. 4.9b) (Pinart et al. 2001a). Similar alterations are also reported in degenerative capillaries of men, rams, bulls and mice with testicular involution caused by age, ischemia, varicocele, cryptorchidism and idiopathic infertility (Markey et al. 1994; Jezek et al. 1996). The degeneration of intertubular capillaries is activated by the thickening and collagenization of the lamina propria of the seminiferous tubules (Jezek et al. 1996; Pinart et al. 1999a, 2001c). In advanced testicular involution, capillary pyknosis extends towards the interstitial vessels (Setchell et al. 1995).

4.2.2.4 Main Semen Abnormalities of Cryptorchid Boars

Fertility is impaired in adult males with unilateral cryptorchidism, although the degree of alteration of semen parameters remains controversial (Mieusset et al. 1995). Divergent results are due to the different conditions in which the studies of cryptorchidism are performed. Thus, the effect of unilateral cryptorchidism in semen quality is different depending on whether it is spontaneous or artificially induced, abdominal or inguinal, and right- or left-sided (Mieusset et al. 1995; Pinart et al. 1999c). Moreover, the sperm quality of unilaterally cryptorchid males is different depending on age since significant differences have been reported between young adults and elderly males (Lee 1993). The effects of bilateral cryptorchidism is different when it is inguinal than when it is abdominal; post-pubertal boars with bilateral abdominal cryptorchidism are sterile due to lack of semen production (Pinart et al. 1998, 1999a, c, 2000).

An extensive study of seminal quality has been performed with post-pubertal boars characterized by unilateral abdominal cryptorchidism on the right side (Pinart et al. 1999c). The ejaculate volume of unilateral abdominal cryptorchid boars is reported to be between 30 and 60 % lower than in healthy boars, whereas the volume of the cell-rich fraction does not differ between both groups of males (Pinart et al. 1999c). In humans, Puri and O'Donnell (1990) state that 50 % of subfertile men with a history of cryptorchidism have decreased semen volume; in these patients, the ejaculate volume can be up to 80 % lower than in healthy men.

The decrease in semen volume in unilateral cryptorchid males is due to an abnormal production of seminal fluid by the accessory glands (Pinart et al. 1999c).

Unilateral abdominal cryptorchidism significantly affects sperm concentration and sperm production per gram of testicular parenchyma, which are 80 and 75 % lower than in healthy boars, respectively (Pinart et al. 1999c). In pubertal dogs with unilateral cryptorchidism, sperm concentration is 50 % lower than in normal dogs (Kawakami et al. 1995). Oligospermia has also been observed in all subfertile men with a history of unilateral cryptorchidism, with a decrease in sperm concentration ranging from 65 to 85 % (Puri and O'Donnell 1990; Mieusset et al. 1995; Rozanski and Bloom 1995; Foresta et al. 1996). In post-pubertal boars with unilateral abdominal cryptorchidism on the right side, the decrease in sperm concentration is due to the lack of sperm production of the abdominal testis (Pinart et al. 1999a, 2000), and the impaired sperm production of the scrotal testis as a result of the partial arrest of spermatogenesis at the meiosis stage (Pinart et al. 1999b) and at the spermatid stage (Pinart et al. 1998, 1999a).

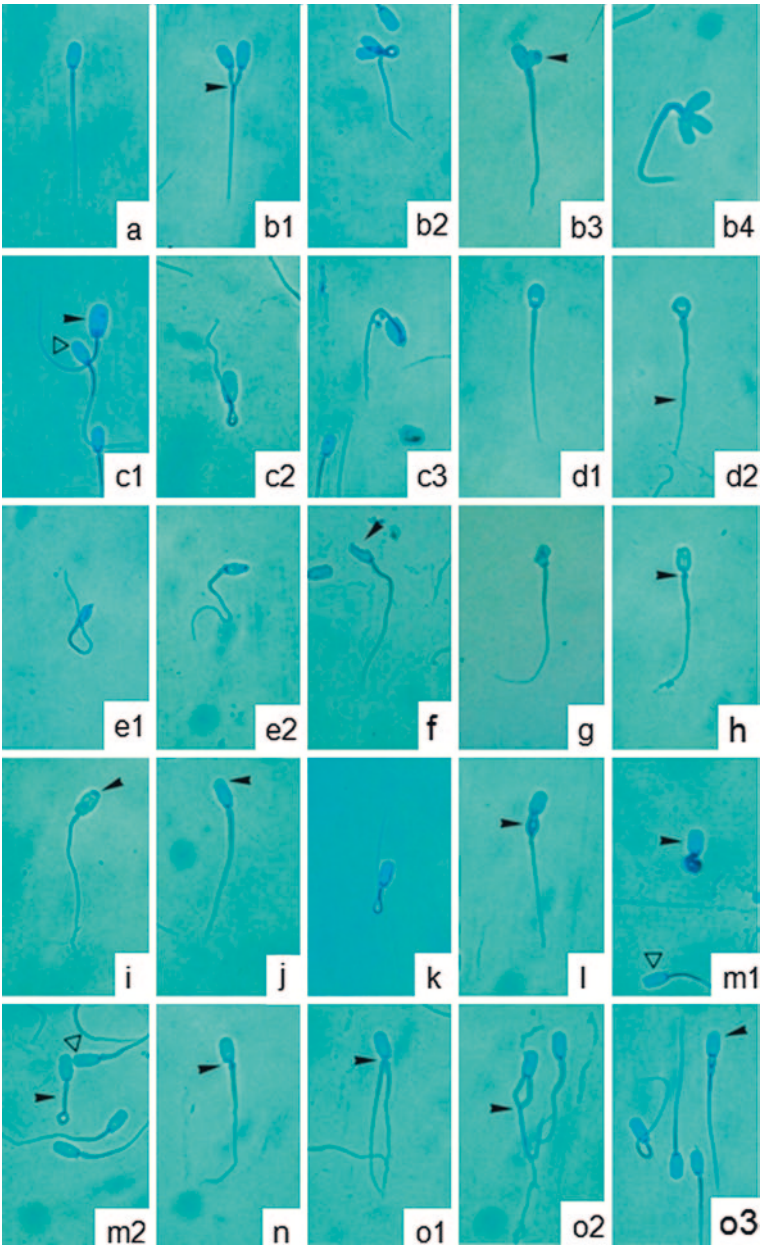
Few data exist about the effect of cryptorchidism on DNA integrity of spermatozoa and the studies performed are quite dated. In a study using the acridine orange test (AO test), it was concluded that this parameter is not significantly affected in post-pubertal boars with unilateral abdominal cryptorchidism (Pinart et al. 1999c). The AO test is based on determining the denaturalization degree of DNA (Evenson and Wixon 2006; Lamb 2010). DNA denaturalization can be originated by an abnormal protaminization during spermatogenesis and/or by anomalies in the epididymal maturation which result in an impaired formation of S–S cross-links between the cysteine-rich protamines (O'Brien and Zini 2005; Evenson and Wixon 2006). In a recent review, Lamb (2010) stated that in both fertile and subfertile patients DNA damage increases progressively during epididymal maturation, although the molecular basis of this damage is completely unknown. According to all these findings, results of Pinart et al. (1999c) seem to indicate that unilateral abdominal cryptorchidism on the right side does not result in an impairment of DNA integrity in boar spermatozoa, either at the testicular level or at the epididymal level. Nevertheless, more studies are required using more robust tests of chromatin integrity and DNA fragmentation in order to better establish the effect of cryptorchidism on the nuclear damage of spermatozoa.

The sperm motility of post-pubertal boars with unilateral abdominal cryptorchidism can be up to 22 % lower than in healthy boars and have non-progressive trajectories (Pinart et al. 1999c). Alterations in the progressive movement of spermatozoa have been reported in unilateral cryptorchid men (Urry et al. 1994) and boars (Bonet et al. 1998), as well as in subfertile men with a history of unilateral cryptorchidism (Puri and O'Donnell 1990; Urry et al. 1994; Mieusset et al. 1995). The most frequent causes of decreased sperm motility are: (1) abnormal spermiogenesis that leads to the formation of spermatozoa with intrinsic defects in the tail ultrastructure (Gopalkrishnan et al. 1995); (2) anomalies in the activity of the epididymal epithelium, which result in imbalance in the ionic and proteic components of epididymal fluid that interferes with the normal development of sperm motility along the duct (Pruneda et al. 2005); or (3) impaired activity of the

accessory glands, which does not only produce alterations in the ejaculate volume but also changes in the osmolality of semen, with further effects on sperm motility (Smital et al. 2005). Impaired activity of the epididymides and accessory glands is attributed to an underlying endocrinopathy generated by the cryptorchid testis (Pinart et al. 1999c).

Sperm morphology, expressed as the percentage of mature, immature and aberrant spermatozoa, and the cephalic stability of spermatozoa, expressed as the frequency of detached heads, do not differ between unilateral abdominal cryptorchid boars and healthy boars (Pinart et al. 1999c). In contrast, oligospermic men with a history of unilateral cryptorchidism usually show a significant decrease in the frequency of mature spermatozoa (Urry et al. 1994; Mieusset et al. 1995). Aberrant spermatozoa of the ejaculate may be of primary origin if they develop in the testis as a result of abnormalities in spermatogenesis and spermiogenesis (i.e. testicular origin), or of secondary origin if they develop along the epididymal duct as a result of an abnormal maturation of spermatozoa (i.e. epididymal origin) (Briz et al. 1995, 1996). The detailed study of sperm abnormalities in unilateral abdominal cryptorchid boars shows that although the frequency of aberrant spermatozoa is normal, a significant increase in the frequency of primary abnormalities occurs, whereas the frequency of secondary abnormalities maintains low values; moreover, the types of primary and secondary abnormalities differ significantly between unilateral abdominal cryptorchid boars and healthy boars (Fig. 4.10a–o) (Pinart et al. 1998).

Among the primary abnormalities, unilateral abdominal cryptorchid boars have a lower percentage of spermatozoa with anomalies in head size (Fig. 4.10c_1–c_3) and in head shape than healthy boars (Fig. 4.10d_1–d_2, e_1–e_2), but a higher proportion of spermatozoa with folded tail (Fig. 4.10 c_2–c_3, k) and with tail length and/or thickness anomalies (Pinart et al. 1998). The percentage of spermatozoa with one head and multiple tails is similar to healthy boars (Fig. 4.10o_1–o_3). Moreover, some primary sperm abnormalities are specific to unilateral abdominal cryptorchid boars: (1) spermatozoa with a nuclear crest, which appears as a transversal ridge on the surface of the spermatozoon head; (2) spermatozoa with crater defect, which is commonly manifested at the apex of the sperm head (Fig. 4.10h), (3) spermatozoa with knobbed acrosome defect, characterized by the flattening of the apex of the acrosome (Fig. 4.10i), (4) spermatozoa with abaxial tail, in which the tail attaches eccentrically to the base of the spermatozoon head (Fig. 4.10l, n), and (5) spermatozoa with mitochondrial swelling (Fig. 4.10l) (Pinart et al. 1998). Spermatozoa with deformed (Fig. 4.10f) or folded (Fig. 4.10g) heads as well as spermatozoa with thin tails have only been observed in unilateral cryptorchid boars (Pinart et al. 1998). The percentage of spermatozoa with primary abnormalities by tail folding is significantly higher in unilateral cryptorchid boars. Moreover, healthy boars and cryptorchid boars also differ in the localization of tail folding; unilateral cryptorchid boars have a significantly higher frequency of spermatozoa with folded tail at the connecting piece (Fig. 4.10c_3) and at the midpiece (Fig. 4.10c_2), and a significantly lower frequency of spermatozoa with folded tail at the principal piece (Pinart et al. 1998).



Among the secondary abnormalities, unilateral abdominal cryptorchid boars have a lower percentage of spermatozoa with folded tails at the Jensen's ring (Fig. 4.10k), whereas the proportion of spermatozoa with multiple heads and spermatozoa with coiled tails does not differ from healthy boars (Pinart et al. 1998). In

◀ **Fig. 4.10** Sperm malformations of the ejaculate of post-pubertal boars with spontaneous unilateral abdominal cryptorchidism on the right side (Quick Panoptic Method of QCA). **a** Mature spermatozoon. **b_1** Bicephalic spermatozoon with two tails fused from the distal end of the midpiece (*arrow*). **b_2** Spermatozoon with two elongated heads, and two tails totally fused and folded at the midpiece. **b_3** Spermatozoon with two heads, one of them aberrant (*arrow*), and two totally fused tails. **b_4** Tricephalic spermatozoon with three totally fused tails. **c_1** Macrocephalic spermatozoon (*arrow*); note the presence of an elongated head spermatozoon (*triangle*). **c_2** Macrocephalic spermatozoon with folded tail at the midpiece. **c_3** Macrocephalic spermatozoon with folded tail at the connecting piece. **d_1** Roundish head spermatozoon. **d_2** Round head spermatozoon; note the corksrew defect affecting the principal piece (*arrow*). **e_1**, **e_2** Pointed head spermatozoon. **f** Deformed head spermatozoon due to a cephalic break (*arrow*). **g** Spermatozoon with a transversely folded head. **h** Spermatozoon with crater defect and proximal droplet (*arrow*). **i** Macrocephalic spermatozoon with knobbed acrosome (*arrow*). **j** Spermatozoon with acrosomal vacuolization (arrow). **k** Spermatozoon with folded tail at the Jensen's ring. **l** Macrocephalic spermatozoon with mitochondrial swelling (*arrow*). **m_1** Macrocephalic spermatozoon with folded tail; note the presence of a spermatozoon with crater defect and abaxial tail (*triangle*). **m_2** Spermatozoon with coiled tail at the terminal and principal pieces (*arrow*); note the presence of a spermatozoon with acrosomal vacuolization (*triangle*). **n** Immature spermatozoon with proximal droplet and abaxial tail (*arrow*). **o_1** Spermatozoon with two unfused tails abaxially attached (*arrow*). **o_2** Spermatozoon with two tails fused from the principal piece (*arrow*). **o_3** Macrocephalic spermatozoon with vacuolated acrosome (*arrow*) and two fused tails. ×500. Reproduced from Pinart et al. (1998) with permission

contrast, spermatozoa with vacuolated acrosome (Fig. 4.10j), which show swelling of this vesicle, disruption of the plasma and acrosomal membranes in the apical region and loss of the acrosomal content, and spermatozoa with tail-to-tail agglutination (Figs. 4.10b_1–b_4) have only been observed in cryptorchid boars (Pinart et al. 1998). Cryptorchid and healthy males do not differ in the percentage of spermatozoa with coiled tails, but they differ in the proportion of spermatozoa with totally coiled or partially coiled tails (Fig. 4.10m_1–m_2). Unilateral cryptorchid boars have a significantly lower percentage of spermatozoa with totally coiled tails, and a significantly higher proportion of spermatozoa with partially coiled tails at the midpiece, at the principal piece and at the terminal piece (Pinart et al. 1998).

At ultrastructural level, sperm abnormalities of the ejaculate of unilateral abdominal cryptorchid boars can be classified as (Pinart et al. 1998): (1) nuclear inclusions, mainly located in the postacrosomal region and manifested as large areas without chromatin or with non-condensed chromatin (nuclear vacuoles), and with membrane aggregates inside them (see Fig. 1.15j, k); (2) myelin figures, which appear between the postacrosomal dense lamina and the perinuclear fibrous material and cause a slight deformation of the cephalic surface (see Fig.1.15g, i); (3) membrane folding, which appears in the equatorial region between the perinuclear fibrous material and the acrosomal vesicle and causes a slight deformation of the cephalic surface; (4) nuclear crests, originated by the presence of three unequal nuclear arms inclined to each other, with membranous material placed between these arms (see Fig.1.15o); (5) acrosomal inclusions, characterized by the presence of large numbers of vesicles and granules of different sizes immersed in a low electrodense acrosomal matrix, leading to an alteration of the acrosome shape (see Fig.1.15e); (6) knobbed acrosome defect, the prominence being originated in some cases by apical and lateral

protrusions (see Fig.1.15c–d), which manifest themselves in association with other defects like the presence of small vesicles and inclusions in the protrusions (see Fig.1.15a) and the lower electrodensity of the acrosome (see Fig.1.15b); in other cases, the acrosomal prominence is due to a big vacuole, with variable electrodensity, that results in a large deformation of the organelle and the non-uniform thickness of the spermatozoon head; (7) festooned acrosome, with an irregular outline, lateral, and apical protrusions and a slightly electrodense matrix (see Fig.1.15f); (8) tail folding, which can be located at the connecting piece, the midpiece, the Jensen's ring (see Fig. 1.14a) or the principal piece; in tail foldings at the principal piece the absence of peripheral microtubular doublets has also been observed; (9) tail coiling, exhibiting axonemal disarrangements (see Fig. 1.14b); (10) two fused tails, with two axonemal structures correctly formed, (11) additional mitochondria, with extra mitochondria placed at the proximal region of the midpiece between the mitochondrial sheath and the plasma membrane and causing a local thickening of the midpiece; and (12) mitochondrial swelling, characterized by the presence of mitochondria without cristae and an electrolucent matrix on the mitochondrial sheath.

The types of sperm malformations observed at both structural and ultrastructural level indicate that unilateral abdominal cryptorchidism affects the spermiogenesis of the scrotal testes by inducing disturbances in the late stages of nuclear and acrosomal differentiation, as well as in the late stages of flagellum remodeling (Pinart et al. 1998). The low percentage of immature spermatozoa suggests that unilateral abdominal cryptorchidism does not alter the migration of the cytoplasmic droplet along the epididymal duct (Briz et al. 1995) and its detachment during ejaculation (Pruneda et al. 2005).

4.3 Extrinsic Environmental Factors

Sperm quality characteristics show clear seasonal changes. The causes of seasonal fluctuations in semen quality are not fully understood but may be mediated by hormonal mechanisms controlled by the photoperiod (Ciereszko et al. 2000). In temperate climates wild pigs are seasonal breeders and do not mate during the summer and fall months (Almeida et al. 2006). Similarly, the lowest reproductive performance of commercial swine is observed in summer. Ancient seasonal mechanisms still influence semen characteristics of boars, especially the semen volume and the number of spermatozoa per ejaculate, the levels of both parameters being the highest during fall and winter (Ciereszko et al. 2000; Huang et al. 2010). On the other hand, many factors may modify semen characteristics of the boar, including high ambient temperature leading to heat stress, frequency of semen collection, age, social environment, and nutrition.

4.3.1 Temperature

Ambient temperature is considered the most important environmental parameter affecting semen quality. Low ambient temperature does not seem to influence

semen composition whereas excessive warm (up to 29 °C) does not only decrease testicular function, leading to a fall of up to 35 % in sperm concentration, but also induces disturbances in the spermatogenic process (Sonderman and Luebbe 2008; Wolf and Smital 2009) that manifests itself in an impairment of sperm motility along with a rise in the frequency of abnormal spermatozoa in the ejaculate (Joseph et al. 2005). The decrease in steroidogenesis results in low testosterone levels and, therefore, in reduced libido (Hemsworth and Tilbrook 2007; Huang et al. 2010); nevertheless, the adverse effect of high temperatures on sexual behavior is generally only temporary (Hemsworth and Tilbrook 2007). On the other hand, pregnancy rates and embryo survival are dramatically reduced in gilts inseminated with semen from heat-stressed boars (Trudeau and Sanford 1986). To ensure constant and high semen quality, boars must be kept at a temperature below 22 ° C (Huang et al. 2010).

Despite the importance of this factor few reliable data correlating high temperature values with specific alterations on semen parameters are available. Most studies on the effects of temperature are focused on the comparative analysis of semen quality among seasons. Semen quality of reproductive boars differs throughout the year but significant breed-by-season interactions have been reported, especially in sperm production. For instance, North European breeds have lower tolerance to heat stress than breeds developed along the Equator (Sonderman and Luebbe 2008).

Comparisons among breeds of semen quality throughout the year show that while the total number of spermatozoa in Meishan boars is the highest in winter, in Duroc boars it is higher during autumn and spring than in the other seasons; in contrast, in Fengjing and Min zhu breeds the total number of spermatozoa is not affected by the season (Borg et al. 1993). In temperate climates, decreased sperm production during summer months occurs concomitantly with a decrease in testicular size (Trudeau and Sanford 1986). Differences also exist among genetic lines in their sensitivity to ambient temperatures; thus some lines experience a modest decrease in sperm production during summer months whereas others recover more quickly, thus indicating that a genetic component may exist governing the homeostatic mechanism of heat stress adaptation (Flowers 2008). In some genetic lines, sperm production is not impacted significantly after exposure to elevated ambient temperatures; exploitation of these genetic lines could conceivably lead to the development of “heat tolerant” AI boars (Flowers 2008). In cattle, genetics definitely affects heat tolerance (Hammond et al. 1996), so it is reasonable to assume the same mechanism in swine (Flowers 2008).

Seasonality has no effect on sperm concentration of Yorkshire and Duroc breeds (Park and Yi 2002); in contrast, in Large White and Pietrain breeds sperm concentration peaks in spring and maintains consistently lower values during autumn and winter (Ciereszko et al. 2000).

Differences in sperm morphology throughout the year have been reported by some authors but not by others. Thus, in temperate climates minimum and maximum percentages of aberrant spermatozoa have been noted in September and March, respectively, and correlate with changes in the rate of spermatozoa with folded tails (Trudeau and Sanford 1986). In contrast, Wolf and Smital (2009) have

reported that seasonal differences in the percentage of abnormal spermatozoa are less than 1 %, and Park and Yi (2002) state that the effects of seasons on sperm morphological traits cannot be considered biologically important. Despite the lack of significant differences in sperm morphology throughout the year, some authors have reported changes in sperm functionality due to disturbances in acrosin activity (Ciereszko et al. 2000) and in membrane integrity (Trudeau and Sanford 1986). Acrosin activity peaks in summer and falls in autumn, probably as a result of disturbances in spermatogenesis caused by high temperatures during summer, which could provoke either or both decreased acrosin concentration and/or poor quality of acrosomal membranes (Ciereszko et al. 2000). According to the former Trudeau and Sanford (1986) have found that membrane integrity of boar spermatozoa is affected by seasonality, with a significant decrease in summer and autumn. Other reports describe an increased frequency of spermatozoa with nuclear vacuoles (Malmgrem and Larsson 1984) and of spermatozoa with knobbed acrosome defect (Sánchez et al. 1989) as a result of heat stress.

The effect of seasonality on sperm membrane integrity has scarcely been studied; in Yorkshire and Duroc breeds the maximum percentages of membrane intact spermatozoa have been recorded in fall (Park and Yi 2002). The effects of season on sperm motility are controversial. Sperm motility is not affected by season in Duroc (Borg et al. 1993; Park and Yi 2002), Min zhu (Borg et al. 1993), and Yorkshire (Park and Yi 2002) breeds, whereas Meishan and Fengjing breeds have lower sperm motility in the autumn than in the subsequent seasons (Borg et al. 1993). Other authors have noticed that despite this apparent lack of seasonality effect on sperm motility the type of sperm movement varies considerably throughout the year, with a greater frequency of progressive motile spermatozoa from October to March (Trudeau and Sanford 1986). This variability throughout the year in the type of sperm movement has been attributed to qualitative changes in sperm metabolism and/or in flagellum activity (Trudeau and Sandford 1986).

Semen volume is not affected by season in Meishan (Borg et al. 1993), Duroc (Borg et al. 1993; Park and Yi 2002), and Yorkshire breeds (Park and Yi 2002). In contrast, in Large White and Pietrain breeds, as well as in Pietrain × Duroc crossbreds, ejaculate volume peaks in autumn and falls in spring (Ciereszko et al. 2000); in Fengjing and Min zhu breeds, the ejaculate volume is the lowest in autumn (Borg et al. 1993). Semen pH varies with the season, the values tending to be lower in winter and higher in spring and early summer (Trudeau and Sanford 1986). Seasonal changes in pH correlate not only with temperature but also with daylight (Trudeau and Sandford 1986). Protein content (Murase et al. 2007, 2010) and acid citric content (Trudeau and Sanford 1986) in seminal plasma exhibit similar seasonal variations, being lower in summer months; conversely, alkaline phosphatase activity does not change appreciably throughout the year (Trudeau and Sanford 1986). These results reflect variations in the behavior of vesicular glands according to the season (Trudeau and Sanford 1986). Since in boars the vesicular gland function is supported by the synergistic action of testosterone and estrogens, seasonal changes in seminal plasma components probably

reflect alterations in testicular steroid levels (Trudeau and Sanford 1986) that ultimately result in sperm DNA instability (Strzezek et al. 1995).

In Duroc and Yorkshire boars, testosterone concentration in serum is significantly higher in spring (3.06 and 5.11 ng/ml, respectively) (Park and Yi 2002), whereas in Meishan, Fengjing, and Min zhu boars, testosterone levels are markedly elevated in autumn (2.8, 5.0 and 4.0 ng/ml, respectively) (Borg et al. 1993). In Yorkshire boars, testosterone levels maintain constant throughout summer, autumn, and winter (2.57 ng/ml) (Park and Yi 2002), as well as in Fengjing (1.0–1.2 ng/ml) (Borg et al. 1993), Meishan (1.2–1.4 ng/ml) (Borg et al. 1993) and Min zhu (0.8–1.6 ng/ml) (Borg et al. 1993), whereas in Duroc boars a dramatic fall in testosterone levels occurs in summer (0.73 ng/ml), followed by a moderate increase in autumn and winter (1.31–1.36 ng/ml) (Park and Yi 2002).

Ambient temperature also affects refrigerability (Murase et al. 2010) and freezability (Park and Yi 2002) of seminal doses, which are significantly lower in summer. During summer, the significantly reduced farrowing rate of refrigerated doses is associated to a higher rate of acrosome reactions as compared to other periods (Murase et al. 2007, 2010). This fast induction of the acrosome reaction in refrigerated doses correlates with the low protein content of seminal plasma during summer (Murase et al. 2007, 2010), as well as with alterations in sperm and acrosomal membrane integrity (Trudeau and Sanford 1986; Ciereszko et al. 2000). In frozen-thawed doses of Yorkshire and Duroc boars, Park and Yi (2002) detected a higher number of motile and aberrant spermatozoa in the spring season than in summer, autumn and winter. Nevertheless, more studies are needed in order to better correlate semen freezability and seasonality.

Results obtained by different authors regarding seasonal effects on sperm quality are somewhat discrepant, and the divergences cannot only be attributed to the breed but also to latitude, age of the animals, and frequency of semen collection. Moreover, most studies are performed without taking into account that the seasons do not only differ in temperature but also in photoperiod; besides, these studies do not provide detailed information about changes in temperature during the experimental period. All the above-mentioned trials have been carried out including few individuals, which leads to unreliable results, and/or throughout only 1 year, so it is not possible to conclude whether the changes in semen quality are specific of a particular year or are general across years. Therefore, to fully characterize the repeatability of seasonal changes in semen quality traits, further trials should include a large number of samples and should be carried out for multiple years.

In order to correlate the behavior of sperm parameters with the ambient temperature, a comprehensive study has been performed on the effect of mean temperature on semen quality in Pietrain, Large-White and Duroc breeds over a 20-week period comprised between February and July (Yeste et al. 2010). Throughout this period, mean temperature ranged from 8 to 15° C during the first 10 weeks, but it increased suddenly to 18 °C degrees at week 11 and progressively to 21 °C from week 12 to 20 (Yeste et al. 2010).

Data obtained by Yeste et al. (2010) show that semen volume is affected by mean temperature, being inversely correlated in all three breeds. From this study,

Pietrain boars, but not Large-White and Duroc boars, suffer a progressive reduction of sperm concentration from May (week 12) to July (week 20) as a result of the increased temperature. Mean temperature affects sperm viability, measured as the percentage of spermatozoa with intact plasma membrane, intact acrosome and intact mitochondrial sheath, in Pietrain and Large-White breeds, but not in the Duroc breed (91 %); in Pietrain boars sperm viability decreases from 92 % in February to 86 % in July, whereas in Large-White boars the decrease is from 90 to 85 %. Sperm motility is also affected by ambient temperature in Pietrain (88 % at week 0 and 82 % at week 20) and Large-White boars (87 % at week 0 and 81 % at week 20), but not in Duroc boars (89 %). The study of kinematic parameters of sperm movement shows that the percentage of fast spermatozoa is inversely correlated with temperature in Pietrain and Duroc breeds. Therefore, in Pietrain boars the frequency of fast spermatozoa falls from 59 % at week 0 to 53 % at week 20, and in Duroc boars from 64 to 59 %. In Large-White boars the frequency of fast spermatozoa (53 %) is not affected by mean temperature, although a significant decrease in the rate of progressive motile spermatozoa has been observed from week 0 (58 %) to week 20 (52 %). Increased temperature also results in a decrease in the percentage of mature spermatozoa in Large-White boars and in Pietrain boars from week 12 (May), but not in Duroc boars; in Pietrain boars the decrease in the proportion of mature spermatozoa is manifested in association with an increase in the percentage of immature spermatozoa, whereas in Large-White boars it is linked to an increase in the frequency of aberrant spermatozoa. In Pietrain, Large-White and Duroc breeds the osmotic resistance of spermatozoa does not correlate with mean temperature (Yeste et al. 2010).

In a comprehensive 3-year study, Sonderman and Luebbe (2008) compared the percentage of discarded (trashed) ejaculates among three purebred maternal lines and two crossbred terminal lines. They concluded that during cold months trash rates are higher in purebreds Yorshire (14 %), Landrace (12 %) and Duroc (10 %) than in both crossbreds Duroc × Hampshire (F1) and ¾ Duroc × ¼ Hampshire (5 %). Moreover, the three maternal purebred lines appear to be more sensitive to seasonal infertility than the crossbred terminal lines, which results in an 18 % increase in trash rate during warm months in Yorkshire and Landrace breeds, and a 16 % increase in Duroc breeds; in crossbreds the frequency of discarded ejaculates averages 7–9 % (Sonderman and Luebbe 2008).

There is an interaction of season with age that has been reported to involve changes in the thermoregulatory ability of boars during aging (Huang et al. 2010). Age influences the susceptibility of pigs to heat stress through either physical or physiological functions and, in consequence, affects semen quality (Huang et al. 2010). A better ability in thermoregulation of boars aged 10 months than those of full maturity (36 months) correlates with higher body surface area (cm^2) and lesser subcutaneous fat content, which facilitate heat loss (Huang et al. 2010). A comparative study of the semen quality of purebred Duroc boars from 10 to 80 months of age during cold and hot seasons shows that the decrease in semen quality during the hot season is less significant in young boars (from 10 to 30 months) than

in adult boars (>35 months) (Huang et al. 2010). The average semen volume in 10 month old boars is similar during hot and cold seasons (about 185 ml). From this starting point, a progressive increase in semen volume is found with age; in the cold season semen volume reaches a maximal point of 242 ml at 46.2 months of age, while in the hot season the maximum semen volume is of 218 ml at 36.0 months of age. Afterwards, semen volume declines in both hot and cold seasons but at different rates, thus resulting in different reproductive longevities; therefore, in the cold season the semen volume drops to 180 ml at 81.0 months, whereas in the hot season it occurs at 62.8 months of age. The correlation between semen volume and age is $r = 0.45$ in the cold season and $r = 0.49$ in the hot season (Huang et al. 2010).

A similar pattern of variation has also been found for the total number of spermatozoa per ejaculate and for the number of motile spermatozoa per ejaculate. Despite the lack of differences in the total number of spermatozoa per ejaculate in 10 month old boars between hot and cold seasons, the highest total number of spermatozoa per ejaculate (84.3×10^9) during the cold season is reached at 38.7 months of age, whereas in the hot season the highest value (70.0×10^9 spermatozoa per ejaculate) is reached at 34.5 months (Huang et al. 2010). Again, a progressive decline in the total number of spermatozoa per ejaculate occurs from this maximum; in this study, the lower threshold for this parameter has been established in 56.0×10^9 spermatozoa per ejaculate and it has been reached at 69.6 months in the cold season and at 60.5 months of age in the hot season (Huang et al. 2010). A significant correlation exists between total number of spermatozoa per ejaculate and age in the cold season ($r = 0.66$) and in the hot season ($r = 0.76$) (Huang et al. 2010).

Similarly, the total number of motile spermatozoa per ejaculate reaches its maximum at 37.8 months in the cold season and at 32.1 months of age in the hot season, the values being 66.0×10^9 and 54.8×10^9 spermatozoa, respectively (Huang et al. 2010). From this highest point, the number of motile spermatozoa per ejaculate starts to decrease; the lowest threshold for this parameter has been established in 44.9×10^9 spermatozoa and it has been recorded at 72.3 months of age in the cold season and at 55.7 months in the hot season. Correlation between the number of motile spermatozoa and age is 0.60 in the cold season and 0.77 in the hot season (Huang et al. 2010).

Taken together, these results provide compelling evidence that the hot season does not only decrease the sperm quality of ejaculates but also accelerates aging in reproductive biology (Huang et al. 2010). Therefore, Duroc boars submitted to heat stress have reproductive longevity extended until 51 months of age, but if summer heat stress is avoided it may extend to 70 months (Huang et al. 2010).

Decrease of sperm production associated with high temperatures is an universal problem for the swine industry (Flowers 2008). Development of genetic lines of "heat tolerant" boars based on the phenotypic variation that seems to be present in modern terminal sire lines would substantially enhance reproductive efficiency (Flowers 2008).

4.3.2 Photoperiod

Wild boars are short-day breeders whose entire breeding activity takes place in winter and early spring (Almeida et al. 2006). In contrast, the domestic boar has traditionally been considered a non-seasonal breeder even though sperm quality and libido are not constant throughout the year (Andersson et al. 1998a). Controversies exist on the effects of photoperiod on the sperm quality of boars. Autumn photoperiod with short or decreasing day length stimulates the reproductive capacity of boars, which manifests itself in an increase of seminal volume and sperm concentration. It is not clear if photoperiods with long or increasing day length alter the reproductive capacity of boars; the decrease in sperm quality during summer is due to temperature increase and not to hours of light (Huang et al. 2010).

The effects of photoperiod in semen quality and fertility of selected AI boars have been extensively studied in different field experiences. Landrace boars housed under controlled temperature and humidity have been tested for different natural (Sancho et al. 2004) or artificial (Sancho et al. 2006) regime light conditions during 2.5 and 3 months, respectively. The effects of increasing daylight and of long daylight natural photoperiods have been assayed as well on Pietrain, Large-White and Duroc boars (Yeste et al. 2010).

The seminal volume of Landrace boars does not differ between increasing and decreasing natural photoperiods (Sancho et al. 2004). In Pietrain, Large-White and Duroc boars semen volume is neither affected by natural increasing of daylength, but it decreases significantly during a long-day natural photoperiod (Yeste et al. 2010). The sperm concentration is significantly higher in Landrace boars exposed to increasing day length as compared with those exposed to decreasing daylength (Sancho et al. 2004). In Large-White and Duroc, sperm concentration does not differ between increasing day length and long-day natural photoperiods; nevertheless, in Pietrain boars a progressive decrease in sperm concentration has been reported throughout the long-day photoperiod (Yeste et al. 2010). These observations indicate that the effect of photoperiod on sperm concentration is dependent on the breed. In a previous experiment, Claus and Weiler (1985) showed that penning of boars in an artificial photoperiod of long days results in increased sperm production. This increased sperm production has been attributed to a rise in the blood levels of testosterone (Minton et al. 1985) and follicle-stimulating hormone (FSH) (Lee et al. 1987) when boars receive supplemented light.

Increasing and decreasing natural photoperiods have no effect on sperm motility, sperm viability (estimated as the frequency of membrane intact spermatozoa), and sperm morphology of the Landrace breed (Sancho et al. 2004) or those of the crossbreds Large White × Pietrain and Duroc × Landrace × Large White (Rivera et al. 2005). The detailed analysis of sperm morphology indicates that the frequency of immature spermatozoa with proximal droplet is higher in Landrace boars exposed to decreasing photoperiods, and that the frequency of immature spermatozoa with distal droplet is higher in boars exposed to increasing photoperiods (Sancho et al. 2004). These results lead to conclude that in Landrace boars

decreasing photoperiod promotes epididymal deficiencies that alter the migration process of the cytoplasmic droplet and that the increasing photoperiod results in anomalies in the shedding process of the distal droplet during ejaculation (Sancho et al. 2004). In the Duroc breed, sperm motility, sperm viability (estimated as the frequency of spermatozoa with intact plasma membrane, intact acrosome and mitochondrial sheath integrity), and sperm morphology do not differ between increasing photoperiod length and long-day photoperiod (Yeste et al. 2010). In contrast, in Pietrain and Large White breeds, sperm motility, viability and morphology are significantly lower in long-day than in increasing day length (Yeste et al. 2010). The osmotic resistance of spermatozoa is affected by the natural photoperiod in Pietrain, Large White and Duroc boars, being significantly lower in long-day than in increasing day-length (Yeste et al. 2010).

In crossbred boars (Large White × Pietrain and Duroc × Landrace × Large White), no differences have been found between natural increasing and decreasing photoperiods on the sperm quality of both fresh semen and refrigerated doses for 72 h (Rivera et al. 2005). Taken together, these results indicate that significant differences exist among breeds in their response to natural light regimes.

Trudeau and Sanford (1986) reported a negative correlation between testis length and width and daylength ($r = -0.62$). Seasonal variations in testicular size may be mediated in part by photoperiod-induced endocrine mechanisms since testosterone concentrations are also negatively correlated with daylength (Trudeau and Sanford 1986; Andersson et al. 1998b). Thus, testosterone is necessary to maintain spermatogenesis, especially by regulating meiosis, and although FSH is necessary to initiate meiosis, further development of spermatocytes is only reached through maintenance of appropriate testosterone signaling (Zirkin 1998). Likewise, testosterone seems to be an important survival factor that protects germ cells from apoptosis during spermatogenesis; in this sense, a positive correlation between the number of spermatozoa in the testes and testosterone levels of the seminiferous tubule fluid has been reported (Zirkin 1998). Furthermore, testosterone plays an important role in sperm maturation throughout the epididymal duct (Lan et al. 1998). Therefore, it seems that light-induced fluctuations in testosterone levels may promote changes in both the spermatogenic process and the epididymal maturation (Andersson et al. 1998b). Drastic alterations in photoperiod can induce an unparallel synthesis of androgens and estrogens in Leydig cells of both peripubertal (Claus and Weiler 1985) and adult (Andersson et al. 1998a) boars.

Extreme artificial photoperiods affect the semen quality of healthy boars selected for AI, the response being more severe in the boar group kept in absolute darkness (0 h of artificial light per day) than in continuous light (24 h of artificial light per day) (Sancho et al. 2006). As compared with boars exposed to 12 h of artificial light per day, boars kept in continuous light exhibit lower sperm concentration, whereas sperm membrane integrity and sperm motility maintain normal values. Boars kept in absolute darkness show reduced sperm motility and membrane integrity as well as decreased percentages of spermatozoa with intact acrosomes, but they do not differ in sperm concentration from boars exposed to 12 h of artificial light per day (Sancho et al. 2006).

Continuing with the previous approach, semen volume has been observed to decrease in boars housed in continuous light conditions (24 h light), whereas it is not affected in boars housed in absolute darkness (0 h light). Seminal plasma composition determined from glucose, fructose, and sorbitol contents, as well as from total protein levels and their pattern, does not differ among the three artificial light regimens, although glucose contents are lower in both 24 and 0 h artificial light groups (Sancho et al. 2006). The lack of effect of darkness on semen volume is supported by observations by Strzezek et al. (1999). Brandt and Diekman (1985) hold that photophase length does not affect the total semen volume of the ejaculate; in the study by Sancho et al. (2006) artificial daylength is described as affecting seminal plasma in a quantitative, more than in a qualitative way. Lack of qualitative alterations is an interesting finding since the change in the protein composition of seminal plasma is frequently associated with reduced sperm viability and fertilizing ability due to anomalies in the capacitating process (Pérez-Pe et al. 2001). Seminal plasma sugars are the main energy source of ejaculated spermatozoa and necessary for sperm movement (Marin et al. 2003). In both extreme artificial light conditions sperm motility maintains high values, thus indicating that despite the decrease in glucose levels the overall sugar content is not dramatically affected by photoperiod (Sancho et al. 2006).

Results described by Sancho et al. (2006) are in disagreement with a previous study by Trudeau and Sanford (1986), who reported that protein and citric acid content of seminal plasma are highly correlated ($r = 0.98$) and inversely correlated with changes in daylength ($r = -0.80$). Protein and citric acid content are both indicators of the seminal gland function, which is under control of testosterone and estrogens; therefore, photoperiodic changes in glandular activity reflect changes in circulating testicular steroid levels (Trudeau and Sanford 1986). Total protein content in seminal plasma may influence transmembrane cation movement, whereas variations in citric acid levels could indicate changes in the osmotic pressure of seminal plasma (Trudeau and Sanford 1986). Seasonal changes in pH are also correlated with changes in daylength ($r = 0.64$) and total protein content of seminal plasma ($r = 0.64$) (Trudeau and Sanford 1986).

Acrosome integrity of spermatozoa diminishes in boars exposed to extreme artificial photoperiods, especially in those of absolute darkness (Sancho et al. 2006). Trudeau and Sandford (1986) reinforce the previous finding by reporting that the functional status and composition of sperm membranes vary with photoperiod. Indeed, the stability of plasma and acrosome membranes of spermatozoa mainly depends on the rate at which proteins and lipids become incorporated during spermiogenesis and epididymal maturation, as well as on the adsorption of proteins secreted by seminal vesicles during ejaculation (Ciereszko et al. 2000). Since no changes are detected in the total protein content of seminal plasma, anomalies in the acrosome membrane probably develop during spermiogenesis and/or epididymal maturation (Sancho et al. 2006).

An artificial light regime also affects reproductive performance. In boars exposed to continuous light (24 h light) or to absolute darkness (0 h light) both farrowing rate and libido scores are reduced by 30 %; however, the total number of piglets born is

unaffected (Sancho et al. 2006). In boars exposed to 12 h of artificial light, the farrowing rate also decreases at the end of the treatment, whereas the number of piglets born and the boar's libido remain constant (Sancho et al. 2006). To our knowledge, this is the only report correlating artificial photoperiod with boar libido, fertility, and prolificacy. Lack of clear correlation between semen traits and male fertility is in agreement with other reports stating that indicators of semen quality are not useful for predicting boar fertility (Gadea 2005; Sancho et al. 2006; Wolf 2010; Yeste et al. 2011). Extreme artificial photoperiods of constant light and absolute darkness may induce subtle defects on spermatozoa that cannot be detected using classical sperm parameters, but that result in decreased fertility and prolificacy rates.

In boars, increased blood and seminal plasma steroid levels are reported during artificial photoperiods of short daylength (Claus and Weiler 1985), whereas FSH blood levels increase when boars have supplemental lighting (Lee et al. 1987). Other studies have suggested that artificial photoperiods can influence prolactin levels in domestic pigs (Andersson et al. 1998a). Nevertheless, more studies are needed in order to better understand the effect of photoperiod on the hormone levels and reproductive performance of boars.

Results obtained in the three trials mentioned above (Sancho et al. 2004, 2006; Yeste et al. 2010) also suggest that the effect of photoperiod differ when boars are exposed to natural or artificial light, or to constant or variable daylength. Nevertheless, further research is needed to better determine the consequences of light exposition regimes on the reproductive performance of boars.

4.4 Extrinsic Husbandry Factors

Management of AI boars plays an important role in efficient semen production (Colenbrander et al. 1993; Flowers 1997). Several husbandry factors, such as nutrition, herd health, routine animal care, semen collection rhythm, and physical environment (in terms of housing, ventilation systems and collection pens) are reported to be essential for the maintenance of high semen quality and high field fertility (Kunavongkrit et al. 2005; Flowers 2008). A goal of large swine companies is to standardize management conditions across farms so that production is uniform throughout the entire system (Flowers 2008).

4.4.1 Frequency of Semen Collection

Frequency of semen collection is considered as one of the main husbandry factors significantly affecting both semen quality and fertility of boars (Pruneda et al. 2005; Smital 2009). Studies on the effect of collection frequency on boar semen quality have yielded variable results. While few authors have determined that boars submitted to a high collection rhythm produce semen with similar

progressive motility and sperm morphology (Audet et al. 2009), other authors have reported that boars under a high semen collection frequency have poor semen quality as a result of the forced passage of sperm cells from caput to cauda of the epididymis (Bonet et al. 1991; Strzezek et al. 1995; Pruneda et al. 2005). These divergent results indicate that consequences of the semen collection rhythm on sperm quality depend not only on the intensity of collection, but also on breed and age, as well as on ambient factors (Pruneda et al. 2005).

Regarding the intensity of semen collection, after a single extraction 60 % of the sperm cells stored in the epididymal cauda is emptied, and when three to four ejaculates have been collected at intervals of 12 h the epididymal cauda is almost completely depleted (Pruneda et al. 2005). Moreover, this high semen collection rhythm forces the passage of sperm cells through the epididymis and leads to defective sperm maturation (Briz et al. 1995, 1996) that results in a decreased percentage of motile spermatozoa and a rise in immature spermatozoa with proximal droplet (Bonet et al. 1991; Pruneda et al. 2005). Different semen parameters such as semen volume and sperm concentration are also altered (Bonet et al. 1991; Pruneda et al. 2005).

Other results demonstrate that the breed also modulates the effects of the collection rhythm. When submitting one-year-old boars characterized by high seminal quality to a rhythm of three collections per week during 2 months, sperm concentration in the Landrace breed is reduced by 43 % and sperm motility drops to only 35 % (Bonet et al. 1991), whereas in Landrace-Large White boars, sperm motility remains at 72 % (Huang and Johnson 1996). The analysis of sperm morphology of stressed Landrace boars also shows a significant decrease in the percentage of mature spermatozoa as a result of a significant increase in the proportion of immature spermatozoa with proximal droplet, which reaches 61 %, and in the percentage of aberrant spermatozoa, of about 6.5 % (Bonet et al. 1991). Aberrant spermatozoa are characterized by disturbances in the acrosome and/or the mitochondrial sheath (Bonet et al. 1991). In Duroc boars submitted to a collection of three ejaculates per week, Audet et al. (2009) found a significant decrease in sperm concentration and total sperm production, of 24 and 30 %, respectively, and a decrease of 11 % in semen volume; however, the percentages of total motile spermatozoa, progressive motile spermatozoa and normal spermatozoa were not affected.

Another approach was used on Pietrain boars submitted to two daily semen collections during 2 days (Pruneda et al. 2005). In these animals, the decrease in semen volume and sperm concentration was of 50 and 95 %, respectively, as compared with control males; the total number of spermatozoa per ejaculate was calculated to be $33.30 \pm 1.16 \times 10^9$ in control boars, whereas in stressed boars it was only $1.40 \pm 0.35 \times 10^9$ spermatozoa per ejaculate (Pruneda et al. 2005). Although sperm membrane integrity was not affected (85 % of spermatozoa with intact membrane) sperm motility dropped to 20 %. Other studies, including boars submitted to daily ejaculations during 10 days, corroborate that sperm membrane integrity decreases (Strzezek et al. 1995). The analysis of sperm morphology in the study by Pruneda et al. (2005) reveals increased percentages of immature

spermatozoa with proximal droplet (around 50 %) and of aberrant spermatozoa (around 6.5 %). Stressed boars, as compared with control boars, show an increased percentage of spermatozoa with abnormal head and tail shape and of spermatozoa with head and tail number abnormalities. In a similar trial performed on Duroc boars submitted to daily collections during 14 days, Audet et al. (2009) found a decrease of 19 % in semen volume, of 50 % in sperm concentration, and of 55 % in sperm production; nevertheless, these authors do not report alterations in sperm motility and sperm morphology.

The study performed by Pruneda et al. (2005) was not restricted to the analysis of semen quality on stressed boars, but it also focused on the effects of semen collection rhythm in the pattern of absorption and secretion of the epididymal epithelium, and in the maturation process of spermatozoa throughout the epididymal duct. To our knowledge this is the only study assessing the effects of increased semen collection on epididymal cell function and sperm maturation. Results obtained highlight that intensive sexual exploitation of Pietrain boars has a significant effect on the pattern of absorption and secretion of the epididymal fluid, decreasing the resorption in the proximal caput and altering the dynamics of resorption and secretion from the distal caput to the distal cauda (Pruneda et al. 2005). This change in the pattern of epididymal fluid resorption and secretion also results in significant alterations in intraluminal sperm concentration throughout the epididymal duct. An increase in sperm concentration associated to epididymal fluid resorption is observed from the proximal to distal caput of stressed boars, as in control boars; nevertheless, both intraluminal sperm concentration and percentage of fluid resorbed are significantly lower than in control boars. In stressed boars, intraluminal sperm concentration and percentage of fluid resorbed in the caput are 99 and 80 % lower respectively than in control boars (Pruneda et al. 2005). In control males a significant decrease in luminal sperm concentration is found throughout the proximal corpus due to intense fluid secretion; in stressed boars, intraluminal sperm concentration is maintained due to lack of fluid secretion (Pruneda et al. 2005). Intraluminal sperm concentration increases progressively from distal corpus to distal cauda of control boars as a result of fluid resorption; the distal corpus of stressed boars also exhibits a clear resorption activity, but throughout the proximal and distal cauda there is a decrease in intraluminal sperm concentration as a result of fluid secretion (Pruneda et al. 2005).

Therefore, high semen collection frequency brings about an altered resorption and secretion pattern of the epididymal fluid, which results in defective sperm maturation and abnormal development of sperm motility (Pruneda et al. 2005). Corroborating these outcomes, Strzezek et al. (1995) demonstrated that increased semen collection frequency induces changes in the biochemical properties of the sperm cells.

The comparative analysis of sperm quality throughout the epididymal duct between stressed and control boars also reveals significant differences. Sperm membrane integrity, used as an estimation of sperm viability, has been set at about 85 % along the entire epididymal duct in control boars; in stressed boars sperm membrane integrity has been of 65 % in the proximal caput, and of 85 % from

distal caput to distal cauda (Pruneda et al. 2005). This decrease in sperm membrane integrity only in the proximal caput has been correlated with the short duration of the stress period, of only 4 days; bearing in mind that stress caused by the semen collection rhythm results in an altered activity of epididymal cells, it is reasonable to suppose that longer stress situations may result in a progressive decrease in sperm membrane integrity throughout the epididymal duct. In control boars, the percentage of total motile spermatozoa increases progressively from 12 % in the proximal caput to 82 % in the distal cauda, achieving maximum values in the ejaculate (90 %); in contrast, in stressed boars the percentage of total motile spermatozoa increases steadily along the epididymis, and reaches only 20 % in the ejaculate. These results indicate that the decrease in sperm motility observed in boars submitted to stress by semen collection frequency is due to the shortened time that spermatozoa spend in the epididymal duct, in addition to the alteration in secretion-absorption flows at the epididymal epithelium (Pruneda et al. 2005).

Moreover, it has been concluded that the incidence of spermatozoa with proximal, intermediate or distal cytoplasmic droplet in control boars varies along the length of the epididymis. Most of the spermatozoa found in the proximal caput retain a proximal droplet (90 %) and only 0.5–1 % have intermediate droplets; spermatozoa with distal droplets have not been found. The percentage of spermatozoa with proximal droplet decreases to 18 % in the distal caput, whereas for spermatozoa with intermediate and distal droplet the percentages increase to 50 and 20 %, respectively. In the proximal corpus of control boars there is no presence of spermatozoa with proximal and intermediate droplets, while the frequency of spermatozoa with distal droplets reaches 75 %. The rate of spermatozoa with distal droplets does not change throughout the distal corpus and the proximal and distal cauda, but it decreases to 5 % in the ejaculate. In stressed boars, the pattern of migration of the cytoplasmic droplet throughout the epididymal duct is similar to that of control boars; nevertheless, the percentage of spermatozoa with proximal droplets is significantly higher than in control boars (Pruneda et al. 2005). Therefore, it can be concluded that a high semen collection rhythm results in anomalies in the migration of proximal droplets. As stated above for sperm motility, anomalies in cytoplasmic droplet migration correlate to both the shortened time spermatozoa spend in the epididymal duct and to the altered absorption-secretion pattern of the epididymal fluid (Pruneda et al. 2005). These latter changes probably cause alterations in the balance of proteins and ions of the epididymal fluid that interfere with the sperm maturation process (Briz et al. 1996).

The detailed study of sperm abnormalities shows in healthy boars that the percentages of spermatozoa with head and tail shape anomalies remain constant at around 10 % along the epididymal duct, while the proportion of spermatozoa with abnormalities in the head size, tail shape, and head and tail number are close to 0 % (Pruneda et al. 2005). In all the epididymal regions of stressed boars, the percentage of aberrant spermatozoa with abnormal head and tail shape is greater than in control boars; moreover, in the stressed boars the frequency of both anomalies

is significantly higher in the caput than in the cauda of epididymis. The frequency of spermatozoa with abnormalities in head size does not differ significantly between healthy and stressed boars throughout the caput and corpus, but it is significantly higher in the epididymal cauda of stressed boars (Pruneda et al. 2005). As stressed boars in this study were submitted to high semen collection frequency for only 4 days, and taking into account that spermatogenesis lasts 30 days, all the anomalies observed in the epididymis and ejaculate of these stressed boars are of epididymal origin. Throughout epididymal maturation, abnormalities in the shape and the size of the sperm head develop as a result of anomalies in the maturation of the nucleus and acrosome, whereas spermatozoa with abnormal tail shape are the result of tail coiling or bending along the epididymal duct (Briz et al. 1996). Anomalies in the number of heads and tails are the result of sperm agglutination throughout the epididymis (Briz et al. 1996).

The interval between semen collections has also a great effect on sperm concentration. Therefore, a progressive boost in sperm concentration occurs when dilating the interval between collections, reaching a plateau at 10 days (Wolf and Smital 2009). A slight gain in semen volume is observed when the elapsed time between collection intervals goes from two to 7 days; for longer intervals, the values do not change markedly (Wolf and Smital 2009). Motility tends to slow down, and the percentage of aberrant spermatozoa tends to increase when lengthening the interval between collections, but these changes are not significant (Wolf and Smital 2009). The optimum collection interval to magnify the number of sperm cells per ejaculate and the total sperm output ranges from 2 to 5 days (Smital 2009).

4.4.2 Nutrition

A positive correlation also exists between nutrition and semen quality. Supplemented diets with vitamins, antioxidants, or PUFA have been developed as a potential strategy for enhancing boar fertility (Audet et al. 2004; Strzezek et al. 2004; Castellano et al. 2010a, b; Yeste et al. 2010, 2011). Despite the large number of studies performed, several controversies still arise about the benefits generated by a specific substance.

To illustrate this controversy we will analyze the effects of the addition of PUFA to the diet of reproductive boars of different breeds on sperm quality. PUFA have more than one double bond within their molecular structure; depending on the position of the first double bond regarding the methyl end of the molecule we obtain omega-3 (ω-3), omega-6 (ω-6) or omega-9 (ω-9) PUFA (Howe et al. 2002). Two long chain ω-3 PUFA, eicosapentaenoic (EPA; 20:5, ω-3) and docosahexaenoic (DHA; 22:6, ω-3) acids, are especially abundant in natural sources as fish oils (Howe et al. 2002). PUFA influence the physical nature of cell membranes and are involved in membrane protein-mediated responses, lipid-mediator generation, cell signaling, and gene expression in many different cell types (Calder and Yaqoob

2009). High content in ω-3 PUFA is involved in plasma membrane fluidity and integrity, both being crucial for sperm fertility (Conquer et al. 2000). DHA has been detected as the main PUFA in the spermatozoa of human and other mammalian species (Tavilani et al. 2006) and it binds to phospholipids, which play a major role in the fluidity of the sperm plasma membrane (Ollero et al. 2000).

Supplementing the boar diet with PUFA has been used up to now as a strategy to improve the quality of seminal doses (Strzezek et al. 2004), and could also be used to improve the freezability of sperm samples (Safranski 2008). The effects of feeding boars with ω-3 PUFA differ depending on the breed; in Yorkshire × Landrace boars, supplementing the feed with ω-3 PUFA results in an increased number of sperm cells in the ejaculate but has an impact on their sexual behavior (Estienne et al. 2008); it improves sperm motility in Pietrain boars (Mitre et al. 2004). Different effects have been reported on sperm in the ejaculate of Large-White boars: increased percentage of progressive motility (Rooke et al. 2001; Mitre et al. 2004), increased percentage of intact plasma membranes and osmotic resistance (Strzezek et al. 2004), and decreased frequency of aberrant spermatozoa (Rooke et al. 2001). In contrast, Castellano et al. (2010a) reported that in Duroc boars, although supplementing their diet with ω-3 PUFA modifies the fatty acid composition of the sperm plasma membrane, it does not affect sperm production and quality. Divergences are probably related to the low number of animals used in most of these different approaches.

In order to obtain robust and reliable data about the effects of dietary supplementing with ω-3 PUFA on boar sperm quality, our group performed a comparative study among three different breeds including large numbers of animals: 48 Duroc boars, 48 Large-White boars, and 48 Pietrain boars (Yeste et al. 2011). The evaluation period lasted 26 weeks; at the beginning of the experiment, the age of the boars ranged between 12 and 14 months. Within each breed, boars were randomly separated into two groups; one group was fed with a diet supplemented with ω-3, and the other group was fed with a control diet. Throughout the experiment, the boars were housed in climate-controlled conditions and submitted to a semen collection rhythm of once per week (Yeste et al. 2011).

The results obtained indicate that supplementing boars' diet with ω-3 PUFA does not affect semen volume, sperm concentration, sperm functionality (estimated from the analysis of the integrity of the plasma membrane, the acrosome and the mitochondrial sheath), and sperm motility of Duroc, Large White, and Pietrain boars. Nevertheless, a significant increase in the percentage of mature spermatozoa is observed in Large-White and Pietrain breeds, but not in Duroc breeds, from week 10 until the end of the treatment (Yeste et al. 2011). In Large-White boars the increase in the percentage of mature spermatozoa manifests itself in association with a decrease in the frequency of immature spermatozoa from week nine until the end of the experimental period; in Pietrain boars, the increased proportion of mature spermatozoa is associated with a decrease in both the percentage of immature spermatozoa from week nine and of aberrant spermatozoa from week 12 (Yeste et al. 2011). The osmotic resistance of spermatozoa is not affected by

ω-3 PUFA supplementing in Large-White and Duroc boars, but it is significantly increased in Pietrain boars from week 11 to the end of the trial. Improved sperm morphology has also been reported in Large-White boars fed with a ω-3 PUFA supplemented diet (Rooke et al. 2001), but not in Yorkshire × Landrace boars (Estienne et al. 2008). In agreement with the results obtained by Yeste et al. (2011) concerning the osmotic resistance of spermatozoa, Rooke et al. (2001) reported an increase in the percentage of spermatozoa with intact acrosomes. Despite lack of abnormalities in osmotic resistance, Wathes et al. (2007) notice that ω-3 PUFA make spermatozoa vulnerable to reactive oxygen species.

The effects of ω-3 PUFA supplementing on sperm morphology and osmotic resistance of spermatozoa in both Pietrain and Large-White boars became statistically significant by weeks 9–12 (Yeste et al. 2011); in other studies, the effects of ω-3 PUFA (Estienne et al. 2008), as well as other diet supplements based on herbal preparations (Frydrychová et al. 2011), were also manifested 8 weeks after the experiment began. This may be related to spermatogenesis and sperm transit within the epididymis, which lasts 49–52 days (França et al. 2005). Differences among breeds regarding the effects of ω-3 PUFA supplementing could be related to the different composition of sperm plasma membrane, and to different influences of these fatty acids on spermatogenesis (Yeste et al. 2011). Further research is needed to determine how plasma membrane structure influences sperm morphology parameters.

Feeding ω-3 PUFA has also been considered a potential strategy to improve the storage of semen both in refrigeration and in cryopreservation (Castellano et al. 2010b), but no success has been achieved in cryopreservation and inconsistent responses have been obtained after long-term liquid storage. The results of PUFA supplementing on boar fertility in terms of farrowing rate and litter size have not yet been investigated.

Such disparity in outcomes indicates that the addition of a dietary supplement does not have a direct effect on sperm quality in itself, and that the effects depend on other physiological, genetic, and ambiental factors. Another study was set-up in order to show this interrelation among factors. It consisted of the evaluation of L-carnitine as a dietary supplement in boars of different breeds housed in variable ambient conditions (Yeste et al. 2010). L-carnitine (β-hydroxy-γ-trimethyl ammonium butyrate) is a vitamin-like compound synthesized in the liver, kidney, and brain through the conversion of two essential amino acids, lysine, and methionine (Krajcovicova-Kudlackova et al. 2000; Hoppel 2003). It plays an essential role in the cellular energetic metabolism because it functions as a carrier of fatty acids across the inner mitochondrial membrane, thereby facilitating β-oxidation and enhancing cell energy production (Hoppel 2003). L-carnitine is also known to fulfill important roles in mammalian sperm maturation and metabolism throughout the epididymis (Yakushiji et al. 2006), although its exact mechanism of action is unknown. An antiapoptotic effect of L-carnitine on different cells, including neurons, myocytes, hepatocytes, and lymphocytes has also been reported (Ng et al. 2004).

The first contact of spermatozoa with L-carnitine occurs within the lumen of the epididymis. In boars, the levels of L-carnitine in epididymal plasma vary from 24.0 ± 5.7 nmol/mg of protein in the proximal caput to 442.9 ± 39.1 nmol/mg of protein in the distal cauda (Pruneda et al. 2007). In humans, L-carnitine supplementation results in increased sperm concentration (Vitali et al. 1995). In boars, the addition of 500 mg/day of L-carnitine in the diet increases the semen volume and sperm concentration, thereby augmenting the number of AI doses produced per ejaculate (Baumgartner 1998). Nevertheless, Kozink et al. (2004) pointed out that the addition of 500 mg/day L-carnitine is only worthwhile when boars are submitted to an intensive collection period.

In the study of L-carnitine, Yeste et al. (2010) evaluated a total of 120 boars of Pietrain, Duroc, and Large-White breeds, with 40 boars equally sampled per breed. Boars in each breed were separated into two experimental groups, one group being fed with a control diet and the other group with a supplemented diet (625 mg/day L-carnitine) over a 20-week period to account for fluctuations in temperature and photoperiod (from February to July). All boars were aged between 10 and 15-months old at the beginning of the experiment.

Results obtained indicate no effect of L-carnitine supplementation on semen volume, sperm concentration, sperm viability, sperm motility, and osmotic resistance of spermatozoa in Pietrain, Duroc, and Large White boars, but a significant effect on sperm morphology in Pietrain boars (Yeste et al. 2010). Lack of effect of L-carnitine supplementation on semen volume and sperm concentration supports the hypothesis that L-carnitine has a key role in the epididymis by influencing sperm survivability, but it does not act directly upon spermatogenesis and accessory glands (Kozink et al. 2004; Jacyno et al. 2007). In asthenozoospermic patients, treatment with L-carnitine results in increased sperm motility (Ng et al. 2004); these results lead to speculate that high concentration of L-carnitine in the epididymal fluid, or even within the spermatozoa, produces energy for sperm motility (Yeste et al. 2010). Such an effect has not been observed, although this sperm parameter is negatively affected by high temperature and long-day photoperiod in Pietrain and Large White breeds (Yeste et al. 2010).

The main effects of feeding L-carnitine are observed in the sperm morphology of Pietrain boars after 13 weeks of treatment, whereas no remarkable effects on Duroc and Large-White breeds in terms of sperm morphology have been found (Yeste et al. 2010). In Pietrain boars, sperm morphology is negatively affected by high temperature and long-day photoperiods, resulting in a decrease in the percentage of mature spermatozoa associated to an increase in the frequency of immature spermatozoa with proximal and medial cytoplasmic droplet (Yeste et al. 2010). However, feeding L-carnitine may have a compensatory effect in terms of percentage of mature spermatozoa during summer weeks, because it prevents the increase in the frequency of immature spermatozoa (Yeste et al. 2010). These results are in agreement with the potential role of L-carnitine in sperm maturation. Jacyno et al. (2007) also describe a positive effect of L-carnitine on the sperm morphology of Pietrain boars after 1 week of administration.

It has been reported that epididymal spermatozoa accumulate both free L-carnitine and acetylated L-carnitine; L-carnitine acts as a transporter of fatty acids to the mitochondria, which are the main energy source for epididymal spermatozoa (Jeulin and Lewin 1996). Moreover, L-carnitine possesses antioxidant properties, plays an important role in cellular detoxification, and protects cell membranes against oxidative damage (Kalaiselvi and Panneerselvam 1998). L-carnitine enters spermatozoa by passive diffusion in the proximal cauda (Jeulin and Lewin 1996). According to this fact, Yeste et al. (2010) hypothesize that in Pietrain boars the levels of L-carnitine in the epididymal fluid are reduced during long-day photoperiods and high temperatures, and that feeding them with a dietary L-carnitine supplement may compensate for this reduction.

The effects of other supplements on the diet are poorly studied and the results are somewhat controversial. Prolonged selenium (Se) deficiency in boars manifests itself in low sperm concentration and sperm motility, and increased frequency of immature spermatozoa (Liu et al. 1982); however, the addition of inorganic Se to the diet of non-deficient boars does not improve sperm quality (Henson et al. 1983) or their reproductive performance (Marin-Guzman et al. 1997). Dietary Se in growing boars results in increased numbers of Sertoli cells and germ cells in the immature testes, thus leading to increased sperm production in adulthood (Marin-Guzman et al. 2000).

Dietary supplementation with fat-soluble and water-soluble vitamins also leads to increased semen production (Yousef et al. 2003) even in boars submitted to intensive semen collection (Audet et al. 2004). In Duroc boars, fat-soluble and water-soluble vitamin supplementation does not influence sperm production or sperm quality regardless of collection frequency (Audet et al. 2009). Vitamin E deficiencies cause testicular degeneration which results in a decreased number of germ cells and, therefore, in low sperm production (Cooper et al. 1987). Marin-Guzman et al. (1997) found a positive effect of vitamin E supplementation on sperm motility and the percentage of mature spermatozoa, which manifests itself in an increased fertility rate. Nevertheless, their results overlapped with environmental temperature and semen collection rhythm, so they could not infer a clear effect of vitamin E supplementation on boar reproductive performance. In a previous report, Brzezinska-Slebodzinska et al. (1995) found increased sperm concentration in the semen of boars supplemented with vitamin E.

The combined results indicate that the effects of supplemented diets are variable depending on the breed but also on ambient factors (Yeste et al. 2010, 2011). Others studies have also highlighted the importance of taking into account physiological factors such age or illness, genetic factors such as breed, and husbandry factors such as semen collection rhythm, when using dietary supplements (Marin-Guzman et al. 1997; Audet et al. 2004, 2009). It is also important to note that inadequate dietary supplementing could result in a lack of effect (Marin-Guzman et al. 1997) or even in an adverse effect (Frydrychová et al. 2011) on the reproductive performance of boars.

4.4.3 Social Factors

Social factors on the sexual behavior of boars can be profound since they can exert a long-term, and perhaps even permanent, effect. Nevertheless, few studies have been performed to determine the effects of social contact during rearing. Boars reared from three to 30 weeks of age without visual or physical contact with pigs achieve fewer copulations and display less courting behavior than boars reared in either all-male or mixed sex groups (Hemsworth and Tilbrook 2007). Since mating dexterity of socially restricted boars appears satisfactory, it is likely that sexual motivation is affected by social restriction during rearing. Subsequent research indicates that the lack of physical contact *per se* is predominantly responsible for depression in sexual behavior caused by rearing in social restriction (Hemsworth and Tilbrook 2007). Furthermore, the age at which social restriction is imposed affects the extent of depression in sexual behavior: boars reared in social restriction from 3 weeks of age have lower testicular size (Trudeau and Sanford 1986) and lower levels of sexual activity (Hemsworth and Tilbrook 2007) than boars reared in social restriction from 12 weeks. Moreover, boars reared in groups display a fully coordinated mating response at an earlier age than boars reared individually (Thomas et al. 1979).

The social environment around puberty and after puberty may also affect the sexual behavior of boars. Isolation of mature boars from sows depresses their sexual behavior; however, this effect is not permanent since rehousing isolated boars near females restores their sexual behavior within 4 weeks (Hemsworth and Tilbrook 2007). Housing in isolation of females does not affect testosterone concentrations. Interestingly, the oestrus status does not influence the effectiveness of females in maintaining the sexual behavior of mature boars (Hemsworth and Tilbrook 2007). Isolation from sows of young post-pubertal boars from 6 to 9 months of age reduces their subsequent sexual behavior at 26–40 months of age, even though they are housed near females when tested; in adult boars, short-term isolation from females does not affect semen quality, but it is associated with a reversible depression in copulatory performance (Hemsworth and Tilbrook 2007). The effects on sexual behavior of young boars of housing near mature boars or near a semen collection area, in which olfactory, visual, and auditory stimulations are provided, are unknown (Hemsworth and Tilbrook 2007). However, the interest in using exogenous hormones such as $PGF_{2\alpha}$ to expedite the training of young, sexually inexperienced boars for semen collection suggests the need to improve understanding in this area (Kozink et al. 2002). The benefits of social contact have clear implications for boars used in natural mating but might also have implications for boars used for semen collection, although sexual motivation may be less critical because of the moderate collection frequency generally required (Hemsworth and Tilbrook 2007).

Sexual stimulation, like observation of other animals mating, can increase short-term levels of sexual behavior in males but the presence of dominant males, even when physically separated, is proven to inhibit sexual behavior in rams and

bulls (Price 1987). Allowing boars to observe other pigs mating immediately prior to semen collection has been shown to increase the number of spermatozoa in the ejaculate (Thiengtham 1992). This form of sexual stimulation has been demonstrated in a number of studies to produce acute increases in plasma cortisol and testosterone concentration in boars (Jongman 1993). Other attempts, such as sexually stimulating boars by briefly delaying collection after mounting, have been unsuccessful in increasing sperm output (Arkins et al. 1988). Kuciel et al. (1983) reported a significant correlation between duration of ejaculation and volume of the ejaculate ($r = 0.58$), number of spermatozoa per ejaculation ($r = 0.30$) and sperm motility ($r = 0.29$).

In a study performed in Landrace boars from a temperate climate, Trudeau and Sanford (1986) demonstrated that semen volume for socially non-restricted boars (boars penned adjacent to each other and allowed minimal physical contact with gilts) is maximal during autumn and winter, declines in spring, and gradually increases again during late spring and summer. In contrast, socially-restricted boars have consistently lower volumes than socially non-restricted boars, and the seasonal variation is less pronounced (Trudeau and Sanford 1986). Differences in semen quality between socially non-restricted and restricted boars are more obvious in autumn and winter, although the increase in semen volume during spring and summer is also noted in socially-restricted boars (Trudeau and Sanford 1986). The social environment does not appear to have a major effect on seminal pH, sperm concentration, total number of spermatozoa per ejaculate, sperm motility or sperm morphology; the protein content per ejaculate is significantly lower in socially-restricted than in non-restricted boars (Trudeau and Sanford 1986). The reasons for the dramatic differences in semen volume between socially-restricted and non-restricted boars are unclear, but they could be related to differences in ejaculatory capability (Hemsworth and Tilbrook 2007) and in gonadal steroid production (Joshi and Raeside 1973).

Flowers (2006) performed a study in which boars were fostered at birth to be reared in litters of six or more than eight individuals. When trained for semen collection and mated with sows under controlled conditions, boars reared in litters of six were found to mount the dummy earlier, have higher sperm output between 39 and 54 weeks of age, and sire more piglets in inseminations with pooled semen from different males than boars in litters of more than eight. Therefore, adequate handling of individuals at early stages in life can have a profound influence on their productivity, although more research is needed in order to better establish the effects of social environment on boar fertility.

4.4.4 Sperm Handling

During semen processing (see Parts 3 and 4), spermatozoa are exposed to light and oxygen and subjected to several diluents, temperatures, pH gradients, and mechanical forces that can potentially alter the structure and function of the plasma

membrane (Leahy and Gadella 2011a). Prolonged exposure of cells to light and oxygen can create an oxidative environment that will trigger detrimental peroxidative processes (Browners et al. 2005).

Sperm handling procedures such as centrifugation and resuspension (Matás et al. 2011), density gradients (Berger and Parker 1989; Matás et al. 2003; Suzuki and Nagai 2003; Morrell et al. 2009), filtration (Bussalleu et al. 2009; Ramió-Lluch et al. 2009), refrigeration (Waterhouse et al. 2004; Gogol et al. 2009) and cryopreservation (Leahy and Gadella 2011a, b) induce plasma membrane (Bailey et al. 2008; Kim et al. 2011) and DNA alterations (Love et al. 2002; Fraser and Strzezek 2004), increase the susceptibility to oxidative damage (Neild et al. 2005; Leahy et al. 2010a), and/or activate capacitation-like processes and acrosome reaction (Leahy and Gadella 2011b). Maternal line breeds are reported to be more sensitive to sub-optimal semen handling than terminal line breeds (Sonderman and Luebbe 2008).

The majority of procedures which involve washing and pelleting cause a partial stripping of loosely associated extracellular coating material in ejaculated spermatozoa (Caballero et al. 2009), and also remove decapacitation factors and other proteins from seminal plasma (Leahy and Gadella 2011a). Density gradient washings, as well as filtration procedures (see Chap. 10), lead to the separation of immotile spermatozoa and debris from progressively motile spermatozoa, thus providing a sub-population of spermatozoa with signs of superior maturation, higher chromatin condensation and removal of cytoplasmic droplets (Bussalleu et al. 2009; Leahy and Gadella 2011a). These selection mechanisms are an analogy of sperm surface decoating during transport in the female tract and of competitive selection of mature spermatozoa mediated by the cervix and the oviductal isthmus (Holt and Fazeli 2010), and are also common practice for in vitro fertilization (Mortimer 2000). Selected spermatozoa are very sensitive to additives in the extenders, especially to the presence of capacitative agents such as bicarbonate, bovine serum albumin (BSA), and calcium ions (Leahy and Gadella 2011a, b; Puigmulé et al. 2011).

On the other hand, most handling procedures are performed below the physiological temperature of 37 °C. It must be taken into account that boar spermatozoa are very susceptible to cooling below 15 °C, the sperm plasma membrane being the primary site of cold-induced damage (Watson 2000; Bailey et al. 2008). It is likely that this damage is related to lipidic phase changes and the altered functional status of the plasma membrane (Watson 2000). The major phase change occurs during cooling from 15 to 5 °C (Drobnis et al. 1993), this being the most sensitive temperature range for temperature-dependent injury (Kim et al. 2011). Therefore, as temperature declines there is an inevitable reduction in the proportion of spermatozoa that maintains membrane integrity, ultrastructure and biochemical components, which is manifested in decreased sperm viability and sperm motility (Johnson et al. 2000; Casas et al. 2010), and increased acrosome alterations (Flores et al. 2008).

Capacitation-like processes have been extensively studied throughout the cryopreservation procedure (see Chap. 11). Boar spermatozoa undergo “cryo

capacitation" or capacitation-like changes when submitted to frozen-thawed protocols (Watson 2000; Bravo et al. 2005). In physiological or real capacitation, increased Ca^{2+} uptake due to cholesterol removal in the oviducts promotes a cascade of phosphorylations, which lead to changes in enzymatic activity and to hyperactivation of boar sperm motility (Guthrie and Welch 2005; Fàbrega et al. 2011; Puigmulé et al. 2011). Similar modifications have also been observed during cryo capacitation, which result in diminished lifespan of spermatozoa (Green and Watson 2001) and activation of apoptosis (Yoshimoto et al. 2008). Thus, cryo capacitation could be described as a premature capacitation in which most of the features of the physiological process are only partially accomplished (Green and Watson 2001).

Synthetic freezing diluents commonly contain tris(hydroxymethyl)aminomethane (TRIS) or citrate to provide an isotonic environment and pH buffering, glucose or fructose as an energy source, and non-penetrating, sand penetrating factors to protect spermatozoa from freezing-thawing damage, such as egg yolk and glycerol. It is accepted that egg yolk minimizes cold shock damage but its mechanism of action is still debated (Leahy and Gadella 2011b). Direct associations between lipids of egg yolk and lipids of the sperm membrane have been reported, minimizing lateral phase separations involved in cold shock deterioration (Ricker et al. 2006). Glycerol and other penetrating cryoprotectants are used to protect the cell content and prevent intracellular ice formation; however, inclusion of such agents must be carefully managed to reduce potentially detrimental osmotic effects (Kim et al. 2011; Leahy and Gadella 2011b).

In spite of considerable improvement in the last decades, cryopreservation remains highly damaging, resulting in significant lethal and sub-lethal effects on boar spermatozoa (Sancho et al. 2007; Casas et al. 2009, 2010; Kim et al. 2011). Regarding sperm surface, cryopreservation has three major effects: (1) decoating of extracellular matrix components and concomitant coating of proteins and lipids from the cryoprotective diluents (milk, albumin or egg yolk) (Ricker et al. 2006), (2) lateral phase separation of lipids and thus a lateral reordering of membrane components (Drobnis et al. 1993) and (3) the permeability of the sperm surface to water, ions, and cryoprotectants is altered (Leahy and Gadella 2011a). The extent of these changes determines whether or not spermatozoa from certain ejaculates (good freezers vs. poor freezers) can be used for cryopreservation (Hagiwara et al. 2009; Casas et al. 2009, 2010; Oldenhof et al. 2010). Compared to bovine and human spermatozoa, cryopreservation of boar spermatozoa is more critical due to the relatively low levels of cholesterol (Rath et al. 2009) and to the high content of PUFA (Cerolini et al. 2000) in the plasma membrane.

Temperature fluctuations and cell dehydration during cooling and freezing procedures induce lateral phase separation of lipids and, therefore, reordering of membrane components (Drobnis et al. 1993) and loss of PUFA and cholesterol (Chakrabarty et al. 2007). This alters the permeability of the sperm surface to water, ions and cryoprotectants (Oldenhof et al. 2010), and leads to cell weakening, reducing its ability to withstand future stresses (Leahy and Gadella 2011b). Proteins and lipids of cryoprotective diluents provide a partial protection from

these deleterious effects of freezing (Ricker et al. 2006). Rewarming of cryopreserved spermatozoa evidences the capacitating-like changes occurred during freezing, which follow a different pathway than true capacitation (Green and Watson 2001). These capacitating-like changes are activated by both temperature changes and osmotic stress, and they correlate with the poor fertility of frozen-thawed boar spermatozoa following AI (Guthrie and Welch 2005; Leahy and Gadella 2011b). Lateral phase separation of lipids in frozen-thawed spermatozoa is not completely restored upon thawing, inhabilitating the specific lateral rearrangements of the sperm surface lipids and proteins that spermatozoa undergo during true capacitation (Leahy and Gadella 2011a), which result in alterations to semen quality and fertility (Flores et al. 2008; Casas et al. 2009, 2010).

Due to the decapacitation effects of seminal plasma outlined above and its partial or complete removal during freezing, several researchers have investigated the protective effect of the addition of seminal plasma during sperm cryopreservation. Incubation of frozen-thawed boar spermatozoa with 50 % (v/v) crude seminal plasma improved motility and viability (Garcia et al. 2010), although the effect of seminal plasma varies according to the boar (Hernández et al. 2007), the ejaculate portion (Garcia et al. 2009) and the seasonality (Leahy et al. 2010b), as well as the previous processing of seminal plasma (i.e. fractionation) (Ollero et al. 1997) or sperm samples (i.e. washing) (Pérez-Pe et al. 2001). Finally, protocol variations such as time of addition (i.e. pre-freeze vs. post-freeze) and protein concentration also affect the response of spermatozoa to seminal plasma (Leahy et al. 2010b). These variants contribute to the controversial effects of seminal plasma on field fertility (Leahy and Gadella 2011b).

Besides stabilization of the sperm plasma membrane, inclusion of the entire seminal plasma during the freezing process holds the sperm sample in a non-capacitated state (Leahy and Gadella 2011b). Other studies report that supplementation with 10 % of crude seminal plasma to a capacitation medium prevents induced capacitation-like changes during liquid storage, but it is unable to prevent them during freezing (Vadnais and Roberts 2010). On the other hand, supplementation of 10 % seminal plasma to frozen-thawed spermatozoa is reported to have no effect on the farrowing rate (Abad et al. 2007a) but in other cases it is observed to improve it (Okazaki et al. 2009). Likewise, thawing boar spermatozoa in 50 % of seminal plasma is reported to increase litter size (Garcia et al. 2010), but post-thaw supplementation with 10 % of seminal plasma has no effect on this parameter (Abad et al. 2007a) or on the establishment of an oviductal sperm reservoir (Abad et al. 2007b).

Despite being poorly studied, freeze–thawing procedures also induce an impairment of DNA integrity, which could explain the low fertilizing ability of thawed samples despite showing reasonably good results in other sperm quality parameters, such as sperm viability, sperm motility, and acrosome integrity (Flores et al. 2008). Both the cooling phase and thawing process do not seem to result in DNA fragmentation in spermatozoa, but they do induce significant alterations in protamine-1-DNA complexes (Flores et al. 2008) and disrupt the disulfide bonds between cysteine residues in sperm head proteins (Flores et al. 2011). Alterations

in these complexes lead to the loss of DNA compactness, which manifests itself in a slight increase in the nuclear volume of spermatozoa. Moreover, boar ejaculates with poor survival after freeze–thawing show less homogeneous sperm chromatin than those with good freezing characteristics (Flores et al. 2008). Both the alteration in protamine-1-DNA structure and the disruption of disulfide bonds between cysteines could be induced by the oxidative damage of spermatozoa during the cooling process and by osmotic and mechanical stress during the thawing process (Flores et al. 2008).

Significant differences among animals have been reported in the ability of their spermatozoa to withstand freeze-thawing damage (Roca et al. 2006; Casas et al. 2009, 2010). In swine, this is the main factor affecting cryosurvival (Roca et al. 2006). The reasons for this significant male-to-male variation are not well understood but they could be related to differences among boars in the regulation of sperm volume (Chaveiro et al. 2006), to genetic factors (Thurston et al. 2002a, b), to the expression of housekeeping proteins (Casas et al. 2009, 2010), and to the content of seminal plasma proteins (Jobim et al. 2004).

In refrigerated AI seminal doses, oxidative damage of spermatozoa has been widely reported (Guthrie and Welch 2006; Awda et al. 2009). An extense range of commercial diluents have been developed for optimal preservation of boar semen at 15–17 °C during three or more days post-collection (Gadea et al. 2004); according to their conservative capacities, commercial extenders are classified as short-term (3–4 days), medium-term (5–6 days), long-term (7–8 days), and extra-long term (9 or more days) extenders (see Chap. 10). Not only do long term extenders enhance the longevity of sperm cells but they also preserve sperm motility and the structural integrity of the plasma membrane and the acrosome throughout the claimed period (Waterhouse et al. 2004; Estienne et al. 2007). The exact composition of commercial extenders is unknown but they contain an energy source (usually glucose) that allows the spermatozoa to maintain basal function, protecting substrates against thermal shock (usually BSA), buffer salts to avoid the negative effects of pH fluctuations [sodium bicarbonate, sodium citrate, TRIS, or N-2-hydroxyethylpiperzaine-N'-2-ethanesulphone acid (HEPES)], basic salts to ensure a proper osmotic balance (NaCl, KCl, and/or sodium citrate), and antibiotics to inhibit bacterial growth (Gadea et al. 2004; Gogol et al. 2009).

Semen dilution with an extender reduces the concentration of proteins present in seminal plasma, thus increasing the risk of functional and structural damage during storage, even for a short period (Waterhouse et al. 2004; Estienne et al. 2007; Gogol et al. 2009; Pérez-Llano et al. 2009). The plasma membrane is a dynamic structure that plays a crucial role in maintaining sperm viability by keeping the intracellular ambient intact, but also in the control of sperm function prior and during the fertilization process (Eddy and O'Brian 1994; Flesch and Gadella 2000). Therefore, the preservation of the ultrastructural characteristics of the sperm membrane is important for maintaining the fertilizing ability of spermatozoa during prolonged storage (Waterhouse et al. 2004). Semen dilution results in decreased fluidity of sperm membranes when using short-term extenders, but in increased fluidity when using long-term extenders (Dubé et al. 2004; Waterhouse et al. 2004; Gogol et al.

2009). This different effect correlates with the specific composition of short- and long-term extenders; therefore, several long-term extenders contain BSA, which favours the efflux of membrane cholesterol (Dubé et al. 2004; Matás et al. 2010), and HEPES, a zwitterionic organic buffer that is known to capture heavy metals and to control the pH (Johnson et al. 2000; Dubé et al. 2004). Increased membrane permeability leads to a rise in the intracellular calcium content that induces a capacitation-like destabilization in the membrane during storage, which results in a premature acrosome reaction and, subsequently, in the loss of the fertilizing ability of refrigerated doses (Waterhosue et al. 2004; Oh et al. 2010).

A subtle decrease in membrane fluidity in stored spermatozoa with short-term extenders is due to peroxidative damage; peroxidative damage occurs as a result of aging in stored spermatozoa with long-term extenders (Awda et al. 2009; Leahy et al. 2010a; Pérez-Llano et al. 2010). Peroxidative damage develops as a result of the reduced activity of antioxidant enzymes in spermatozoa (Neild et al. 2005; Leahy et al. 2010a; Pérez-Llano et al. 2010), and because of metabolic changes, which lead to an enhanced production of free oxygen radicals during storage (Hammerstedt 1993). PUFA of the plasma membrane are the preferred substrates of free radicals, resulting in the generation of reactive oxygen species (ROS) (Cerolini et al. 2000; Browners et al. 2005) and the irreversible reduction of the fluidity of the plasma membrane (Gogol et al. 2009). Decreased membrane permeability leads to anomalies in the activity of several proteins and enzymes, such as membrane ATPases, which finally results in altered levels of intracellular ions (Gogol et al. 2009). One intracellular source of ROS may be the leakage of electrons from the mitochondrial transport chain and the impairment of enzymes such as the NADPH-oxidase, synthesizing the superoxide anion (Baker and Aitken 2005; Guthrie and Welch 2006). The presence of ROS also leads to the formation of substances having cytotoxic properties (Aitken et al. 1998), which inhibit numerous cellular enzymes and metabolic processes including anaerobic glycolysis, further impairing ATP generation (Gogol et al. 2009) and protein phosphorylation (De Lamirande and Gagnon 1992). Altered content of ATP and intracellular ions (Gogol et al. 2009) and decreased phosphorylation of axonemal proteins (De Lamirande and Gagnon 1992) manifest themselves in reduced sperm motility.

Sperm motility has been used as an indicator of active metabolism and membrane integrity, and of the fertilizing capacity of spermatozoa diluted in commercial extenders (Estienne et al. 2007; Oh et al. 2010). Some authors have reported a significant boar effect with regard to the preservation of sperm motility (Gogol et al. 2009) and sperm viability (Pérez-Llano et al. 2009) during the conservation period, whereas others have pointed out the importance of identifying the most successful extenders in maintaining sperm quality during storage (Estienne et al. 2007). The addition of bicarbonate (Holt and Harrison (2002) and caffeine (Yeste et al. 2008) in short-term extenders has been correlated with increased sperm motility of semen doses, but also with an increased percentage of capacitated spermatozoa and acrosome-reacted spermatozoa.

Regardless of the type of extender, prolonged liquid storage results in the aging of spermatozoa due to increased DNA instability, mainly as a result of DNA

fragmentation (Love et al. 2002; Fraser and Strzezek 2004; Pérez-Llano et al. 2009, 2010; Kim et al. 2011). The increase in DNA fragmentation has been reported to be coincident with the progressive decrease in sperm motility during liquid storage (Pérez-Llano et al. 2010). Significant differences exist among boars in the percentage of spermatozoa with altered DNA during liquid storage (Fraser and Strzezek 2004), each boar showing a different critical time for DNA fragmentation (Pérez-Llano et al. 2010). Individual variation in the rate of DNA damage could be related to the inherent chromatin packaging of the spermatozoa (Love et al. 2002), and the time of storage before insemination is also a key factor to reduce the incidence of this alteration (Pérez-Llano et al. 2010). Storage temperature has a significant effect on the rate of DNA fragmentation, being significantly higher and earlier as temperature increases from 15 to 37 °C (Pérez-Llano et al. 2010). Therefore, DNA fragmentation begins at day two or three during sperm storage at 37 °C for 10 days, depending on the boar; it is detected in over 80 % of spermatozoa at day seven and nearly in 100 % of the sperm population at day 10; in contrast, during sperm storage at 15° for 15 days, DNA fragmentation begins at day seven or eight, and at day 15 it ranges between 5 and 43 % depending on the male (Pérez-Llano et al. 2010). Not only the temperature but also the extender type affects DNA status (Love et al. 2002); thus, extenders containing lipoprotein fractions result in lower levels of DNA damage, indicating that these fractions help to slow down sperm aging changes associated with genome alterations during storage (Fraser and Strzezek 2004).

DNA damage as a result of the aging of refrigerated spermatozoa is associated with oxidative damage (Aitken et al. 1998; Zini and Libman 2006; Kim et al. 2011), which produces single-stranded DNA breaks (Sundaram and Panneerselvam 2006); ROS are particularly efficient at producing 8-oxyguanosine residues ubiquitously distributed along single DNA strands (Oger et al. 2003). DNA damage during liquid storage can also be related to the increased activity of acrosomal enzymes released to the extracellular medium from non-viable spermatozoa; these enzymes could trigger a cascade of detrimental effects on viable spermatozoa, as a consequence of side-by-side cell death (Pérez-Llano et al. 2010). This hypothesis agrees with the decline in sperm quality parameters before observing any defects on DNA.

Antioxidant supplementation has been shown to increase the oxidative resistance of liquid-stored spermatozoa (Johnson et al. 2000; Aurich 2008) and of frozen-thawed spermatozoa (Kalthur et al. 2011). Such optimization techniques have resulted in increased fertility rates, particularly in liquid-stored semen (Leahy and Gadella 2011b).

4.5 Conclusions

Purebreds differ in sperm production, but not in sperm quality. The coefficient of variation among breeds is estimated at 30–40 % for semen volume, total number of spermatozoa and sperm concentration, whereas the coefficient of variation for sperm motility, sperm viability and sperm morphology is below 10 %. The

heritability of semen traits ranges depending on the breed from 0.14 to 0.28 for semen volume, from 0.13 to 0.20 for sperm concentration, and from 0.10 to 0.18 for total number of spermatozoa. Sperm motility, sperm viability, and sperm morphology have a low heritability, usually below 0.10; for this reason, they are frequently considered as variable parameters, not heritable seminal traits.

The selection of boars consistently producing high quality ejaculates which are resistant to refrigeration and cryopreservation procedures are key events in AI. In order to ensure high fertility rates, threshold values for seminal parameters have been established. However, the poor relationship between seminal parameters and fertility limits the sensitivity and specificity of such a cut-off. In order to improve the efficiency of AI procedures in terms of fertility and prolificacy, boar selection must be performed according to testis size at pre-pubertal age. In boars, testis size is positively correlated with daily sperm production, testosterone levels, libido, and reproductive longevity. Genetic defects in testicular size and structure, such as in cases of cryptorchidism, result in partial or total arrest of spermatogenesis at post-pubertal age.

The reproductive performance of AI boars in terms of sperm quality, fertility, and sexual behavior show clear seasonal changes. High ambient temperatures do not only decrease testicular function, but also induce transient disturbances in spermatogenesis and a decrease in steroidogenesis. Differences exist among breeds in their tolerance to heat stress, breeds from northern Europe being less tolerant than those from along the Equator. Divergences exist regarding the effect of photoperiod in the reproductive efficiency of AI boars. While some authors state that autumn photoperiods with decreasing day length stimulate the reproductive capacity of boars as compared with photoperiods of increasing daylength, others report significant differences among breeds in their response to light regimes.

Management of AI boars plays an important role in efficient semen production. One of the main husbandry factors affecting sperm quality and fertility of males is the frequency of semen collection. The optimal collection interval ranges between 2 and 5 days, and the reduction in this interval manifests itself in a decrease in semen volume, sperm concentration, and sperm motility, and in an increase in the frequency of immature spermatozoa with proximal droplet. Such disturbances in sperm quality are the result of both the forced passage of spermatozoa throughout the epididymis and the altered pattern of absorption-secretion of epididymal cells. The alteration degree of sperm quality is dependent on the breed, semen collection rhythm, and extent of stress.

A positive correlation exists between nutrition and semen quality. In order to enhance the reproductive performance of AI boars, different supplemented diets with vitamins, antioxidants, or PUFA have been tested. Nevertheless, controversies still arise about the benefits generated by a specific substrate, due to the fact that effects of supplementation are variable depending on the breed and the physiological status of males, as well as on environmental and husbandry factors. Moreover, inadequate dietary supplementing could result in lack of effect or even in an adverse effect on the reproductive performance of boars.

Social factors can exert profound and long-term effects on the reproductive efficiency of AI boars, and an adequate handling of boars during early stages of life can have great influence on their productivity. In this sense, social restriction results in reduced levels of sexual activity, the extent of depression inversely correlated with the age at which social restriction is imposed. Despite these results, further research is needed in order to better establish the effects of social environment on boar reproductive efficiency.

In AI practice, semen processing results in the exposition of spermatozoa to light and oxygen, and to several diluents, temperatures, pH gradients, and mechanical forces, which alter the structure and function of the plasma membrane and trigger detrimental peroxidative processes. Procedures which involve washing and pelleting cause the stripping of loosely associated extracellular coating material in ejaculated spermatozoa, and also remove decapacitating factors. Therefore, these washed and pelleted spermatozoa become very sensitive to certain additives in the extenders, especially to capacitative agents. Changes in temperature alter both membrane integrity and composition, which is manifested in decreased sperm viability and motility. Freeze-thawing protocols also induce both the "cryo capacitation" or capacitation-like changes in spermatozoa, as a result of lateral phase separation of membrane lipids, and the impairment of DNA integrity due to the loss of DNA compactness. Nevertheless, significant differences exist among boars in the ability of their spermatozoa to withstand freeze-thawing damage. Refrigeration of seminal doses results in the oxidative damage of spermatozoa and altered membrane permeability; prolonged liquid storage leads to the aging of spermatozoa which manifests itself in increased DNA fragmentation. Again differences exist among boars in the rate of DNA damage during liquid storage.

References

Abad M, Garcia JC, Sprecher DJ, Cassar G, Friendship RM, Buhr MM, Kirkwood RN (2007a) Effect of insemination-ovulation interval and addition of seminal plasma on sow fertility to insemination of cryopreserved sperm. Reprod Dom Anim 42:418–422

Abad M, Sprecher DJ, Ross P, Friendship RM, Kirkwood RN (2007b) Effect of sperm cryopreservation and supplementing semen doses with seminal plasma on the establishment of a sperm reservoir in gilts. Reprod Dom Anim 42:149–142

Ahmed EA, Barten-van Rijbroek AD, Kal HB, Sadri-Ardekani H, Mizrak SC, van Pelt AMM, de Rooij DG (2009) Proliferative activity in vitro and DNA repair indicate that adult mouse and human sertoli cells are not terminally differentiated quiescent cells. Biol Reprod 80:1084–1090

Aitken JR, Gordon E, Harkiss D, Twigg JP, Milne P, Zo, Irvine DS (1998) Relative impact of oxidation stress on the functional competence and genomic integrity of human spermatozoa. Biol Reprod 59:1037–1046

Almeida FL, Leal MC, França LR (2006) Testis morphometry, duration of spermatogenesis, and spermatogenic efficiency in the wild boar (*Sus scrofa scrofa*). Biol Reprod 75:792–799

Andersson H, Rydhmer L, Wallgren M, Lundström K, Andersson K, Forsberg M (1998a) Influence of artificial light regimens on sexual maturity and boar taint in entire male boars. Anim Reprod Sci 51:31–43

Andersson H, Wallgren M, Rydhmer L, Lundström K, Andersson K, Forsberg M (1998b) Photoperiodic effects on pubertal maturation of spermatogenesis, pituitary responsiveness to exogenous GnRH, and expression of boar taint in crossbred boars. Anim. Reprod Sci 54:121–137

Antich M, Fabian E, Sarquella J, Bassas L (1995) Effect of testicular damage induced by cryptorchidism on insulin-like growth factor I receptor in rat Sertoli cells. J Reprod Fertil 104:267–275

Antón F, Morales C, Aguilar R, Bellido C, Aguilar E, Gaytan F (1998) A comparative study of mast cells and eosinophil leukocytes in the mammalian testis. Zentralbl Veterinarmed A 45:209–218

Arber DA, Tamayo R, Weiss LM (1998) Paraffin section detection of the c-kit gene product (CD 117) in human tissues: value in the diagnosis of mast cell disorders. Hum Pathol 29:498–504

Arenas MI, Madrid JF, Betherncourt FR, Fraile B, Paniagua R (1998) Lectin histochemistry of the human testes. Int J Androl 21:332–342

Arkins S, Thompson LH, Giles JR, Camacho T, Hosmon BD (1988) The effect of collection procedure on the sperm output and copulatory behaviour of AI stud boars. Anim Reprod Sci 16:277–283

Audet I, Bérubé N, Bailey JL, Laforest JP, Matte LL (2009) Effects of dietary vitamin supplementation and semen collection frequency on reproductive performance and semen quality in boars. J Anim Sci 87:1960–1970

Audet I, Laforest JP, Martineau GP, Matte JJ (2004) Effect of vitamin supplements on some aspects of performance, vitamin status, and semen quality in boars. J Anim Sci 82:626–633

Aurich C (2008) Recent advances in cooled-semen technology. Anim Reprod Sci 107:268–275

Awda BJ, Mackenzie-Bell M, Buhr MM (2009) Reactive oxygen species and boar sperm function. Biol Reprod 81:553–561

Badia E, Briz MD, Pinart E, Sancho S, Garcia-Gil N, Bassols J, Pruneda A, Bussalleu E, Yeste M, Casas I, Bonet S (2006) Structural and ultrastructural features of boar seminal vesicles. Tissue Cell 38:79–91

Bailey JL, Lessard C, Jacques J, Breque C, Dobrinski I, Zeng W, Galantino-Homer HL (2008) Cryopreservation of boar semen and its future importance to th industry. Theriogenology 70:1251–1259

Baker MA, Aitken RJ (2005) Reactive oxygen species in spermatozoa: methods for monitoring and significance for the origins of genetic disease and infertility. Reprod Biol Endocrinol 3:67

Basso B, Fritz S, Druet T, Guillaume F, Rossignol MN, Amigues Y, Gabriel R, Sellm E, Salas-Cortes L, Humblot P, Druart X (2005) Estimation de parameters génétiques et detection de QTL liés à des caractères de fertilité male, de production de semence et de qualité de la semence chez le taureou laitier. Renc Rech Rumiants 12:145–148

Baumgartner M (1998) Boars react positively to L-carnitine supplements. Int Pig Top 13:32

Berger T, Parker K (1989) Modification of the zona-free hamster ova bioassay of boar sperm fertility and correlation with in vivo fertility. Gamete Res 22:385–397

Bernal-Mañas CM, Morales E, Pastor LM, Pinart E, Bonet S, de la Rosa P, Briz MD, Zuasti A, Ferrer C, Canteras M (2005) Proliferation and apoptosis of spermatogonia in postpuberal boar (*Sus domesticus*) testes with spontaneous unilateral and bilateral abdominal cryptorchidism. Acta Histochem 107:365–372

Berndtson WE, Igboeli G, Parker WG (1987) The numbers of Sertoli cells in mature Holstein bulls and their relationship to quantitative aspects of spermatogenesis. Biol Reprod 37:60–67

Bolet G, Bidanel JP, Ollivier L (2001) Selection for litter size in pigs. II. Efficiency of closed and open selection lines. Genet Sel Evol 33:515–528

Bonet S, Briz M, Fradera A (1991) The sperm quality and fertility of boars after two different ejaculation frequencies. Scientia Gerundensis 17:77–84

Bonet S, Briz M, Pinart E, Sancho S, Garcia-Gil N, Badia E (2000) Morphology of boar spermatozoa. Institut d'Estudis Catalans, Barcelona

Bonet S, Pinart E, Briz M, Sancho S, Escuder M (1998) Aportación al conocimiento de la criptorquidia espontánea abdominal y unilateral en porcino: análisis microscópico del eyaculado. Revista de la Asociación Nacional de Porcinocultura Científica (ANAPORC) 178:91–112

Borg KE, Lunstra DD, Christenson RK (1993) Semen characteristics, testicular size, and reproductive hormone concentrations in mature Duroc, Meishan, Fengjing, and Minshu boars. Biol Reprod 49:515–521

Brandt KE, Diekman MA (1985) Influence of supplemental lighting on serum LH, testosterone and semen quality in prepubertal and postpubertal boars. Anim Reprod Sci 8:287–294

Bravo MM, Aparicio IM, Garcia-Herreros M, Gil MC, Peña FJ, Garcia-Marin LJ (2005) Changes in tyrosine phosphorylation associated with true capacitation and capacitation-like state in boar spermatozoa. Mol Reprod Dev 71:88–96

Briz M, Bonet S, Pinart E, Camps R (1996) Sperm malformations throughout the boar epididymal duct. Anim Reprod Sci 43:221–239

Briz M, Bonet S, Pinart E, Egozcue J, Camps R (1995) Comparative study of boar sperm coming from the caput, corpus and cauda regions of the epididymis. J Androl 16:175–188

Browners J, Silva PF, Gadella BM (2005) New assays for detection and localization of endogenous lipid peroxidation products in living boar sperm after BTS dilution or after free-thawing. Theriogenology 63:458–469

Bruning G, Dierichs R, Stumpel C, Bergmann M (1993) Sertoli cell nuclear changes in human testicular biopsies as revealed by three dimensional reconstruction. Andrologia 25:311–316

Brzezinska-Slebodzinska E, Slebodzinski AB, Pietras B, Wieczorek G (1995) Antioxidant effect of vitamin E and glutathione on lipid peroxidationn in boar semen plasma. Biol Trace Elem Res 47:69–74

Bussalleu E, Pinart E, Rivera MM, Briz M, Sancho S, Yeste M, Casas I, Fàbrega A, Rigau T, Rodríguez-Gil JE, Bonet S (2009) Effects of matrix filtration of low-quality boar semen doses on sperm quality. Reprod Dom Anim 44:499–503

Bussalleu E, Pinart E, Yeste M, Briz M, Sancho S, Garcia-Gil N, Badia E, Bassols J, Pruneda A, Casas I, Bonet S (2005) Development of a protocol for multiple staining with flurochromes to assess the functional status of boar spermatozoa. Microsc Tes Tech 63:277–283

Buzzard JJ, Wreford NG, Morrson JR (2003) Thyroid hormone, retinoic acid, and testosterone suppress proliferation and induce markers of differentiation in cultured rat Sertoli cells. Endocrinology 144:3722–3731

Caballero I, Vázquez JM, Mayor GM, Alminana C, Calvete JJ, Sanz L, Roca J, Martínez EA (2009) PSP-I/PSP-II spermadhesin exert a decapacitation effect on highly extended boar spermatozoa. J Androll 32:505–513

Caires KC, Schmidt JA, Oliver AP, de Avila J, McLean DJ (2008) Endocrine regulation of the establishment of spermatogenesis in pigs. Reprod Dom Anim 43(Suppl 2):280–287

Calder PC, Yaqoob P (2009) Understanding omega-3 polyunsaturated fatty acids. Postgrad Med 1216:148–157

Casas I, Sancho S, Ballester J, Briz M, Pinart E, Bussalleu E, Yeste M, Fàbrega A, Rodríguez-Gi JE, Bonet S (2010) The HSP90AA1 sperm content and the prediction of the boar ejaculate freezability

Casas I, Sancho S, Briz M, Pinart E, Bussalleu E, Yeste M, Bonet S (2009) Freezability prediction of boar ejaculates assessed by functional sperm parameters and sperm proteins. Theriogenology 74:940−950

Castellano CA, Audet I, Bailey JL, Chouinard PY, Laforest JP, Matte JJ (2010a) Effect of dietary n-3 fatty acids (fish oils) on boa reproduction and semen quality. J Anim Sci 88:2346–2355

Castellano CA, Audet I, Bailey JL, Laforest JP, Matte JJ (2010b) Dietary omega-3 fatty acids (fish oils) have limited effects on semen stored at 17o C or cryopreserved. Theriogenology 74:1482–1490

Cerolini S, Maldjian A, Surai P, Noble R (2000) Viability, susceptibility to peroxidation and fatty acid composition of boar semen during liquid storage. Anim Reprod Sci 58:99–111

Chakrabarty J, Banerjee D, Pal D, De Ghosh A, Majumder GC (2007) Shedding of specific lipid constituents from sperm cell membrane during cryopreservation. Cryobiology 54:27–35

Chaveiro A, Liu J, Engel B, Critser JK, Woelders H (2006) Significant variability among bulls in the sperm membrane permeability for water and glycerol: possible implications for semen freezing protocols for individual males. Cryobiology 53:349–359

Chemes HE, Rey RA, Nistal M, Regadera J, Musse M, González-Peramato P, Serrano A (2008) Physiological androgen insensivity of the fetal, neonatal, and early infantile testis is explained by the ontogeny of the androgen receptor expression in Sertoli cells. J Clin Endocrinol Metab 93:4408–4412

Ciereszko A, Ottobre JS, Glogowski J (2000) Effects of season and breed on sperm acrosin activity and semen quality of boars. Anim Reprod Sci 64:89–96

Cinti S, Barbatelli G, Pierleoni C, Caucci M (1993) The normal, cryptorchid and retractile prepubertal human testis: a comparative morphometric ultrastructural study of 101 cases. Scan Microsc 7:351–358

Clark SG, Schaeffer DJ, Althouse GC (2003) B-mode ultrasonic evaluation of paired testicular diameter of mature boars in relation to average total sperm numbers. Theriogenology 60:1011–1023

Claus R, Weiler U (1985) Influence of light and photoperiodicity on pig prolificacy. J Reprod Fertil 33(Suppl):725–729

Codesal J, Paniagua R, Quiezan A, Santamaría L, Nistal M (1993) Cytophotometric DNA quantification in human spermatogonia of cryptorchid testes. J Urol 149:382–385

Colenbrander B, Feitsma H, Grooten HJ (1993) Optimizing semen production for artificial insemination in swine. J Reprod Fertil (Suppl) 48:207–215

Collin O, Bergh A (1996) Leydig cells secrete factors which increase vascular permeability and endothelial cell proliferation. Int J Androl 19:221–228

Conquer JA, Martin JB, Tummon I, Watson L, Tekpetey F (2000) Effect of DHA supplementation on DHA status and sperm motility in asthenozoospermic males. Lipids 35:149–154

Cooper DR, King OR, Carpenter MP (1987) Effect of vitamin E deficiency on serum concentrations of follicle-stimulating hormone and testosterone during testicular maturation and degeneration. Endocrinology 120:83–90

Courtens JL, Ploen L (1999) Improvement of spermatogenesis in adult cryptorchid rat testis by intratesticular infusion of lactate. Biol Reprod 61:154–161

Dadoune JP, Demoulin A (1993) Structure and functions of the testis. In: Thibault C, Levasseur MC, Hunter RHF (eds) Reproduction in mammals and man. Ellipses, Paris, pp 27–255

Dalton JC, Deragon L, Vasconcelos JLM, Lopes CN, PeresRFG, Ahmadzadeh A (2012) Fertility-associated antigen in Nelore bull sperm and reproductive outcomes following first-service fixed-time AI of Nelore cows and heifers. Theriongenology 77:389–394

David I, Bodin L, Lagriffoul G, Manfredi E, Robert-Granié C (2006) Genetic parameters of ram semen traits using an animal model accounting for serial correlations. In: 8th World congress on genetics applied to livestock production, Belo Horizonte (CD-ROM). Communication 25-02

De Lamirande E, Gagnon C (1992) Reactive oxygen species and human spermatozoa. II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. J Androl 13:379–386

De Rooij DJ (2000) Russell LD (2000) All you wanted to know about spermatogonia by were afraid to ask. J Androl 21:776–798

Dekkers JCM (2003) Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. J Anim Sci (Suppl 1) 81:5

Demircan M, Akinci A, Mutus M (2006) The effects of orchiopexy on serum ant-Müllerian hormone levels in unilateral cryptorchid infants. Pediatr Surg Int 22:271–273

Drobnis EZ, Crowe LM, Berger T, Anchordoguy TJ, Overstreet JW, Crowe JH (1993) Cold shock damage is due to lipid phase transitions in cell membranes: a demonstration using sperm as a model. J Exp Zool 265:432–437

Dubé C, Beaulieu M, Reyes-Moreno C, Guillemette C, Bailey JL (2004) Boar sperm storage capacity of BTS and Androhep plus: viability, motility, capacitation, and tyrosine phosphorylation. Theriogenology 62:874–886

Dyck MK, Foxcroft GR, Novak S, Ruiz-Sánchez A, Patterson J, Dixon WY (2011) Biological markers of fertility. Reprod Dom Anim 46(Suppl. 2):55–58
Eddy EM, O'Brian DA (1994) The spermatozoon. In: Knobil E, Neill JD (eds) The physiology of reproduction, vol 1. Raven Press, New York, pp 29–77
Egbunike GN, Holtz W, Endell W, Smidt D (1975) Reproductive capacity of German Landrace boars. I. Gonadal and extragonadal sperm reserves. Zuchthygiene 10:184–187
Ergün S, Harnet S, Paust HHJ, Mukhopadhyay AK, Holstein AF (1998) Endothelin and endothelin receptors A and BB in the human testis. Anat Embryol (Berl) 199:207–214
Estienne MJ, Harper AF (2004) Semen characteristics and libido in boars treated repeatedly with PGF2∝. J Anim Sci 82:1494–1498
Estienne MJ, Harper AF, Day JL (2007) Characteristics of sperm motility in boar semen diluted in different extenders and stored for seven days at 18º C. Reprod Biol 7:221–231
Estienne MJ, Harper AF, Crawford RJ (2008) Dietary supplementation with a source of omega-3 fatty acids increases sperm number and the duration of the ejaculation in boars. Theriogenology 70:70–76
Evenson DP, Wixon R (2006) Clinical aspects of sperm DNA fragmentation detection and male infertility. Theriogenology 65:979–991
Ezeasor DN (1985) Light and electron microscopical observations on the leydig cells of the scrotal and abdominal testes of naturally unilateral cryptorchid West African dwarf goats. J Anat 141:27–40
Fàbrega A, Puigmulé M, Yeste M, Casas I, Bonet S, Pinart E (2011) Impact of epididymal maturation, ejaculation and in vitro capacitation on tyrosine phosphorylation patterns exhibited of boar (Sus domesticus) spermatozoa. Theriogenology 76:1356–1366
Feng D, Nagy JA, Dvorak AM, Dvorak HF (2000) Different pathways of macromolecule extravasation from hyperpermeable tumor vessels. Microvasc Res 59:24–37
Flesch FM, Gadella BM (2000) Dynamics of the mammalian sperm plasma membrane in the process of fertilization. Biochim Biophys Acta 1469:197–235
Flores E, Cifuentes D, Fernández-Novell JM, Medrano A, Bonet S, Briz MD, Pinart E, Peña A, Rigau T, Rodríguez-Gil JE (2008) Freeze-thawing induces alterations in the protamine-1/DNA overall structure in boar sperm. Theriogenology 69:1083–1094
Flores E, Ramió-Lluch L, Bucci D, Fernández-Novell JM, Peña A, Rodríguez-Gil JE (2011) Freezing-thawing induces alterations in histone H1-DNA binding and the breaking of protein-DNA disulphide bonds in boar sperm. Theriogenology 76:1450–1464
Flowers WL (1997) Management of boars for efficient semen production. J Reprod Fertil (Suppl) 52:67–78
Flowers WL (2002) Increasing fertilization rate of boars: influence of number and quality of spermatozoa inseminated. J Anim Sci (Suppl 1) 80:47–53
Flowers WL (2006) Increasing sperm production in mature boars via manipulation of their neonatal environment. In: Proceedings of the national swine improvement federation
Flowers WL (2008) Genetic and phenotypic variation in reproductive traits of AI boars. Theriogenology 70:1297–1303
Flowers WL, Turner ZA (1997) Relationships among motility, morphology, and fertility estimates for boar semen. J Anim Sci 75 (Suppl 1):223 (abstract)
Ford JJ, Wise TH (2009) Sertoli cell differentiation in pubertal boars. J Anim Sci 87:2536–2543
Ford JJ, Wise TH (2011) Assessment of pubertal development of boars derived from ultrasonographic determination of testicular diameter. Theriogenology 75:241–247
Ford JJ, Wise TH, Lunstra DD, Rohrer GA (2001) Interrelationships of porcine X and Y chromosomes with pituitary gonadotropins and testicular size. Biol Reprod 65:906–912
Foresta C, Ferlin A, Garolla A, Milani C, Oliva G, Rossato M (1996) Functional and cytologic features of the contralateral testis in cryptorchidism. Fertil Steril 66:624–629
Foxcroft GR, Dyck MK, Ruiz-Sánchez A, Novak S, Dixon WT (2008) Identifying useable semen. Theriogenology 70:1324–1336
França LR (1992) Daily sperm production in Piau boars estimated from Sertoli cell population and Sertoli cell index. In: Proceedings of the 12th international congress on animal reproduction and artificial insemination (The Hague) 4:1716–1718

França LR, Avelar GF, Almeida FFL (2005) Spermatogenesis and sperm transit through the epididymis in mammals with emphasis in pigs. Theriogenology 63:300–318
Fraser L, Strzezek J (2004) The use of comet assay to assess DNA integrity of boar spermatozoa following liquid preservation at 5° C and 16° C. Fol Histochem Cytobiol 42:49–55
Frydrychová S, Opletal L, Macáková K, Lustyková A, Rozkot M, Lipenský J (2011) Effects of herbal preparation on libido and semen quality in boars. Reprod Dom Anim 46:573–578
Fuji J, Otsu K, Zorzato F, DeLeon S, Khanna VK, Weiler JE, O'Brien PJ, McLennan DH (1991) Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253:448–451
Gadea J (2005) Sperm factors related to in vitro and in vivo porcine fertility. Theriogenology 63:341–444
Gadea J, Sellés E, Marco MA (2004) The predictive value of porcine seminal parameters on fertility outcome under commercial conditions. Reprod Dom Anim 39:303–308
Garcia EM, Calvete JJ, Sanz L, Roca J, Martinez EA, Vazquez JM (2009) Distinct effects of boar seminal plasma fractions exhibiting different protein profiles on the functionality of highly diluted boar spermatozoa. Reprod Dom Anim 44:200–205
Garcia JC, Dominguez JC, Pena FJ, Alegre B, Gonzalez R, Castro MJ, Habing GG, Kirkwood RN (2010) Thawing boar semen in presence of seminal plasma: effects on sperm quality and fertility. Anim Reprod Sci 119:160–165
Gargiulo AM, Monaci M, Pedinii V, Polisca A, Veccarelli P (1991) Testicular ultrastructure in azoospermic bulls. Acta Med Vet 37:139–146
Gogol P, Szczesniak-Fabianczyk B, Wierzchos-Hilczer A (2009) The photon emission, ATP level and motility of boar spermatozoa during liquid storage. Reprod Biol 9:39–49
Gomedio M, Roldan ERS (1991) Sperm competition influences sperm size in mammals. Proc Royal Soc London, Ser B-Biol Sci 243:181–185
Gopalkrishnan K, Padwal V, D'Souza S, Shah R (1995) Severe asthenozoospermia: a structural and functional study. Int J Androl 18(Suppl 1):67–74
Gotoh M, Miyake K, Mitsus H (1987) Elastic fibers in tunica propria of undescended and contralateral scrotal testes from cryptorchid patients. Urology 30:359–363
Gracia J, Navarro E, Guirado F, Pueyo C, Fernández A (1997) Spontaneous ascent of the testis. Br J Urol 79:113–115
Green CE, Watson PF (2001) Comparison of the capacitation-like state of cooled boar spermatozoa with true capacitation. Reproduction 122:889–898
Guraya SS (1987) Biology of Spermatogenesis and Spermatozoa in Mammals. Springer, Berlin, p 430
Guthrie HD, Welch GR (2005) Effects of hypothermic liquid storage and cryopreservation on basal and induced plasma membrane phospholipid disorder and acrosome exocytosis in boar spermatozoa. Reprod Fertil Dev 17:467–477
Guthrie HD, Welch GR (2006) Determination of intracellular reactive oxygen species and high mitochondrial membrane potential in Percoll-treated viable boar sperm using fluorescence-activated flow cytometry. J Anim Sci 84:2089–2100
Hagiwara M, Choi JH, Devireddy RV, Roberts KP, Wolkers WF, Makhlouf A, Bischof JC (2009) Cellular biophysics during freezing of rat and mouse sperm predicts post-thaw motility. Biol Reprod 81:700–706
Haider SG, Servos G (1998) Ultracytochemistry of 3β-hydroxysteroid dehydrogenase in Leydig cell precursors and vascular endothelial cells of the postnatal rat testis. Anat Embryol 198:101–110
Hammerstedt RH (1993) Maintenance of bioenergetic balance in sperm and prevention of lipid peroxidation: review of the effect on design of storage preservation systems. Reprod Fertil Dev 5:675–690
Hammond AC, Olson TA, Chase CC, Bowers EJ, Randel RD, Murphy CN (1996) Heat tolerance in two tropically adapted *Bos taurus* breeds, Senepol and Romosinuano, compared with Brahman, Angus, and Hereford cattle in Florida. J Anim Sci 74:295–303
Hanrahan JP, Quirke JF (1977) Testis size and plasma luteinizing hormone as aids to selection for fecundity in sheep. Anim Prod 24:148 (abstract)

Harder RR, Lunstra DD, Johnson RK (1995) Growth of testes and testicular morphology after eight generation of selection for increased predicted weight of testes at 150 days of age in boars. J Anim Sci 73:2186–2195

Harrison RAP (1997) Sperm plasma membrane characteristics and boar semen fertility. J Reprod Fertil (Suppl) 52:271–283

Hemsworth PH, Tilbrook AJ (2007) Sexual behaviour of male pigs. Horm Behav 52:39–44

Henson MC, Kattesh HG, Hitchoock JP, Kincaid SA (1983) The effects of dietary selenium on growth and selected reproductive parameters in young boars. Anim Prod 37:401–407

Hernández M, Roca J, Calvete JJ, Sanz L, Muiño-Blanco, Cebrian-Perez JA, Vázquez JM, Martínez EA (2007) Cryosurvival and in vitro fertilizing capacity postthaw is improved when boar spermatozoa are frozen in the presence of seminal plasma from good freezer boars. J Androl 28:689–697

Heyns CF, Hutson JM (1995) Historical review of theories on testicular descent. J Urol 153:754–767

Hill MM, Clark SF, Tucker DF, Birnbaum MJ, James DE, Macaulay SL (1999) A role of protein kinase Bbeta/Akt2 in insulin-estimulated GLUT4 translocation in adipocytes. Mol Cell Biol 19:7771–7781

Hirai M, Boersma A, Hofflich A, Wolf E, Foll J, Aumuller R, Braun AJ (2001) Objectively measured sperm motility and sperm head morphometry in boars (Sus scrofa): relation to fertility and seminal plasma growth factors. J Androl 22:104–110

Hochereau-de Reviers MT (1981) Control of spermatogonial multiplication. In: McKerns KW (ed) Reproductive processes and Contraception. Plenum Press, New York, pp 307–331

Hofmann N, Fervers B, Hilscher B (1992) Correlation between multi-headed spermatozoa in the ejaculate and histological findings in the testis. Andrologia 24:227–230

Holt WV, Fazeli A (2010) The oviduct as a complex mediator of mammalian sperm function and selection. Mol Reprod Dev 77:934–943

Holt WV, Harrison RAP (2002) Bicarbonate stimulation of boar sperm motility via a protein kinase A-dependent pathway between cell and between ejaculate differences are not due to deficiencies in protein kinase A activation. J Androl 23:557–565

Hoppel C (2003) The role of carnitine in normal and altered fatty acid metabolism. Am J Kidney Dis 41:4–12

Howe PR, Downing JA, Grenyer BF, Grigonis-Deane EM, Bryden WL (2002) Tuna fishmeal as a source of DHA for ω-3 PUFA enrichment of pork, chicken, and eggs. Lipids 37:1067–1076

Huang SY, Lin JH, Teng SH, Sun HS, Chen YH, Chen HH, Liao JY, Chung MT, Chen MY, Chuang CK, Lin EC, Huang MC (2011) Differential expression of porcine testis proteins during postnatal development. Anim Reprod Sci 123:221–233

Huang YH, Lo LL, Liu SH, Yang TS (2010) Age-related changes in semen quality characteristics and expectations of reproductive longevity in Duroc boars. Anim Sci J 81:432–437

Huang YT, Johnson RK (1996) Effect of selection for size of testes in boars on semen and testis traits. J Anim Sci 74:750–760

Huhtaniemi I, Pelliniemi LJ (1992) Fetal Leydig cells: cellular origin, morphology, life span, and special functional features. PSEPM 201:10–14

Huisman AE, Veerkamp RF, Vand Arendonk JAM (2002) Genetic parameters for various random regression models to describe the weight data of pigs. J Anim Sci 80:575–582

Hutson JM, Balic A, Nation T, Southwell B (2010) Cryptorchidism. Sem Pediatr Surg 19:215–224

Islam ABM, Hill WG, Land RB (1976) Ovulation rate of lines of mice selected for testis weight. Genet Res 27:23

Itoh M, Moriyama H, Yano A, Li XQ, Takeuchi Y (1998) Mode of migration of normal lymphocytes inside murine testis. Anat Rec 251:152–160

Jacyno E, Kolodzeij A, Kamyczek M, Kawecka K, Dziadek K, Pietruszka A (2007) Effect of L-carnitine supplementation on boar semen quality. Acta Vet Brno 62:595–600

James FV, Kumar A, Jayaprakash PG (2009) Metachronous bilateral testicular germ cell tumours: report of two cases. J Cancer Res Ther 5:206–207

Jannes P, Spiessens C, Van der Auwera I, Hooghe TD, Verhoeven G, Vanderschueren D (1998) Male subfertility induced by acute heating affects embryo quality in adult female mice. Hum Reprod 13:372–375

Jansz GF, Pomerantz DK (1986) A comparison of Leydig cell function after unilateral and bilateral abdominal cryptorchidism and efferent-duct-ligation. Biol Reprod 34:316–321

Jégou B, Sharpe RM (1993) Paracrine mechanisms in testicular control. In: de Kretser DM (ed) Molecular biology of the male reproduction system. Academic, New York, pp 271–310

Jeulin C, Lewin LM (1996) Role of free L-carnitine and acetyl-L-carnitine in post-gonadal maturation of mammalian spermatozoa. Hum Reprod 2:87–102

Jezek D, Schulze W, Rogatsch H, Hittmair A (1996) Structure of small blood vessels in the testes of infertile men. Int J Androl 19:299–306

Jobim MIM, Oberst ER, Salbego CG, Souza DO, Wald VB, Tramontina F, Mattos RC (2004) Two-dimensional polyacrylamide gel electrophoresis of bovine seminal plasma proteins and their relation with semen freezability. Theriogenology 61:255–266

Johnson L (1986) Spermatogenesis and aging in the human. J Androl 7:331–354

Johnson LA, Albers JG, Arts JAM (1982) Use of boar spermatozoa for artificial insemination. Part II. Fertilizing capacity of fresh and frozen spermatozoa in gilts inseminated either at a fixed time or according to Walsmeta readings. J Anim Sci 54:126–131

Johnson LA, Albers JG, Willems CMT, Subesma W (1981) Use of boar spermatozoa for artificial insemination. Part I. Fertilizing capacity of fresh and frozen spermatozoa in sows of 36 farms. J Anim Sci 52:1130–1136

Johnson LA, Weitze KF, Fiser P, Maxwell WMC (2000) Storage of boar semen. Anim Reprod Sci 62:143–172

Johnson RK, Eckardt GR, Rathje TA, Drudik DK (1994) Ten generations of selection for predicted weight of testes in swine: direct response and correlated response in body weight, backfat, age at puberty, and ovulation rate. J Anim Sci 72:1978–1988

Jongman EC (1993) The effects of conditions around the time of mating on reproductive efficiency in pigs. PhD Thesis, University of Melbourne, Australia

Joseph A, Okere C, Ezekwe M (2005) Seasonal and genotype variations in libido, semen production and quality in artificial insemination boars. J Anim Vet Adv 4:885–888

Joshi HS, Raeside JI (1973) Synergistic effects of testosterone and oestrogens on accessory sex glands and sexual behaviour of the boar. J Reprod Fertil 33:411–423

Kalaiselvi T, Panneerselvam C (1998) Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats. J Nutr Biochem 9:575–581

Kalthur G, Raj S, Thiyagarajan A, Kumar S, Kumar P, Adiga SK (2011) Vitamin E supplementation in semen-freezing medium improves the motility and protects sperm from freeze-thaw-induced DNA damage. Fertil Steril 95:1149–1151

Kanematsu N, Jin W, Watanabe G, Taya K (2006) Age-related changes of reproductive hormones in young Meishan boars. J Reprod Dev 52:651–656

Kawakami E, Hirayama S, Tsutsui T, Ogasa A (1993) Pituitary response of cryptorchid dogs to LH-RH-analogue before and after sexual maturation. J Vet Med Sci 55:147–148

Kawakami E, Tsutsui T, Saito S, Kakimoto T, Ogasa A (1995) Changes in peripheral plasma luteining hormone and testosterone concentrations and semen quality in normal and cryptorchid dogs during sexual maturation. Lab Anim Sci 45:258–263

Kennedy BW, Wilkins JN (1984) Boar, breed and environmental factors influencing semen characteristics of boars used in artificial insemination. Can J Anim Sci 64:833–843

Kerbel RS (2000) Tumor angiogenesis: past, present and near future. Carcinogenesis 21:505–515

Kim S, Lee YJ, Kim YJ (2011) Changes in sperm membrane and ROS following cryopreservation of liquid boar semen stored at 15°C. Anim Reprod Sci 124:118–124

Klobucar I, Kosec M, Cebulj-Kadunc N, Majdic G (2003) Postnatal hypotiroidism does not affect prepubertal testis testis development in boars. Reprod Dom Anim 38:193–198

Kluin PM, Kramer MF, de RooijDG (1984) Proliferation of spermatogonia an Sertoli cells in maturing mice. Anat Embryol 169:73–78

Kohler C, Riesenbeck A, Hoffmann B (2007) Age-dependent expression and localization of the progesterone receptor in the boar testis. Reprod Dom Anim 42:1–5

Kojima Y (1991) Ultrastructure of abnormal boar spermatid: acrosomic fragmentation and vacuolization. J Vet Med Sci 53:593–600

Kondracki S, Banaszewska D, Mielnicka C (2005) The effect of age on the morphometric sperm traits of domestic pigs (Sus scrofa domestica). Cell Molec Biol Lett 10:3–13

Korpelainen EI, Karllainen MJ, Tenhunen A, Lakso M, Rauvala H, Vierula M, Parvinen M, Alitalo K (1998) Overexpression of VEGF in testis and epididymis causes infertility in transgenic mice: evidence for nonendothelial targets of VEGF. J Cell Biol 143:1705–1712

Kort JW, Hekking-Weijma I, Vermeij M (1991) Temporary intraabdominal cryptorchidism in the wealing rat leads to irreversible azoospermia. J Surg Res 51:138–142

Kozink DM, Estienne MJ, Harper AF, Knight JW (2002) The effect of lutalyse on the training of sexually inexperienced boars for semen collection. Theriogenology 58:1039–1045

Kozink DM, Estienne MJ, Harper AF, Knight JW (2004) Effects of dietary L-carnitine supplementation on semen characteristics in boars. Theriogenology 61:1247–1258

Krajcovicova-Kudlackova M, Simoncic R, Bederova A, Babinska K, Beder I (2000) Correlation of carnitine levels to methionine and lysine intake. Physiol Res 49:399–402

Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF (1998) Generation and reproductive phenotypes of mice lacking estrogen receptor beta. Proc Natl Acad Sci USA 95:15677–15682

Kuciel J, Masek N, Kovarna Z (1983) Sexual behaviour of boars in relation to their fertility. Pigs New Info 4:1

Kunavongkrit A, Suriyasomboon A, Lundeheim N, Heard T, Einarsson S (2005) Management and sperm production of boars under differing environmental conditions. Theriogenology 63:657–667

Kvist K, Thorup J, Byskov AG, Hoyer PE, Mollgard K, Andersen CY (2006) Cryopreservation of intact testicular tissue from boys with cryptorchidism. Hum Reprod 21:484–491

Lamb DJ (2010) Semen analysis in 21st century medicine: the need for sperm function testing. Asian J Androl 12:64–70

Lan ZJ, Labus JC, Hinton BT (1998) Regulation of gammaglutamyl transpeptidase catalytic activity and protein level in the initial segment of the rat epididymis by testicular factors: role of basic fibroblast growth factor. Biol Reprod 58:197–206

Leahy T, Celi P, Bathgate R, Evans G, Maxwell WNC, Marti JI (2010a) Flow-sorted ram spermatozoa are highly susceptible to hydrogen peroxide damage but are protected by seminal plasma and catalase. Reprod Fertil Develop 22:1131–1140

Leahy T, Gadella BM (2011a) Capacitation and capacitation-like sperm surface changes induced by handling boar semen. Reprod Dom Anim 46(Suppl 2):7–13

Leahy T, Gadella BM (2011b) Sperm surface changes and physiological consequences induced by sperm handling and storage. Reproduction 142:759–778

Leahy T, Marti JI, Evans G, Maxwell WMC (2010b) Seasonal variation in the protective effect of seminal plasma on frozen-thawed ram spermatozoa. Anim Reprod Sci 119:147–153

Lee KH, Diekman MA, Brandt KE, Grieger DM, Allrich RD (1987) Hormonal patterns of boars exposed to natural or supplemental lighting during pubertal development. J Anim Sci 64:1110–1116

Lee PA (1993) Fertility in cryptorchidism: does treatment make a difference? Adolesc Endocrinol 22:479–490

Lee PA, Coughlin MT (2001) Fertility after bilateral cryptorchidism. Evaluation by paternity, hormone and semen data. Horm Res 55:28–32

Levis DG, Reicks DL (2005) Assessment of sexual behaviour and effect of semen collection pen design and sexual stimulation of boars on behaviour and sperm output-a review. Theriogenology 63:630–642

Liu CH, Chen YM, Zhang JZ, Huang MY, Su Q, Lu ZH, Yin RX, Shao GZ, Feng D, Zheng PL (1982) Preliminary studies on influence of selenium deficiency to the developments of genital organs and spermatogenesis of infancy boars. Acta Vet Zootech Sin 13:73–77

Long CR, Dobrinsky JR, Johnson LA (1999) In vitro production of pig embryos: comparisons of culture media and boars. Theriogenology 51:1375–1390

Love CC, Thompson JA, Brinsko SP, Rigby SL, Blanchard TL, Varner DD (2002) Relationship of seminal plasma level and extender type to sperm motility and DNA integrity. Theriogenology 58:221–224

Lovercamp KW, Safranski TJ, Fischer KA, Manandhar G, Sutovsky M, Herring W (2007) Arachidonate 15 lipoxygenase and ubiquitin as fertility markers in boars. Theriogenology 67:704–718

Lubritz D, Johnso B, Robinson OW (1991) Genetic parameters for testosterone production in boars. J Anim Sci 69:3220–3224

Lunstra DD, Wise TH, Ford JJ (2003) Sertoli cells in the boar testis: changes during development and compensatory hyperthrophy after hemicastration at different ages. Biol Reprod 68:140–150

Ma T, Guo J, Yang W, Zhou H, Zhao C, Zhang Y (2011) Testicular cancer in patients after treatment of cryptorchidism. Chinese-German J Clin Oncol 10:40–42

Madjic G, Snoj T, Horvat A, Mrkun J, Kosec M, Cestnik V (1998) Higher thyroid hormone levels in neonatal life result in reduced testis volume in postpubertal bulls. Int J Androl 21:352–357

Maekawa M, Kazan H, Kamimura K, Nagano T (1995) Changes in the arrangement of actin filaments in myoid cells and Sertoli cells of rate testes during postnatal development and after experimental cryptorchidism. Anat Rec 241:59–69

Makinen A, Andersson M, Hakkinen A, Kuosmanen S (1999) A reciprocal translocation between autosomes 8 and 10 in a boar used for artificial insemination service and its effects on litter size. Anim Reprod Sci 56:237–243

Malmgrem L, Larsson K (1984) Semen quality and fertility after heat stress in boars. Acta Vet Scand 25:425–435

Malmgrem L, Rodriguez-Martinez H, Einarsson S (1996) Attainment of spermatogenesis in Swedish crossbred boars. J Vet Med 43:169–179

Marchetti F, Bua J, Tornese G, Piras G, Toffol G, Ronfani L, Isqut IS (2012) Management of cryptorchidism: a survey of clinical practice in Italy. BMC Pediatr 12:accepted

Marin S, Chiang K, Bassilian S, Lee WN, Boros LG, Fernández-Novell JM (2003) Metabolic strategy of boar spermatozoa revealed by a metabolic characterization. FEBS Lett 554:342–346

Marin-Guzman J, Mahan DC, Chung YK, Pate JL, Pope WF (1997) Effect of dietary selenium and vitamin E on boar performance and tissue responses, semen quality, and subsequent fertilization rates in mature gilts. J Anim Sci 75:2994–3003

Marin-Guzman J, Mahan DC, Pate JL (2000) Effect of dietary selenium and vitamin E on spermatogenic development in boars. J Anim Sci 78:1537–1543

Markey CM, Jequier AM, Meyer GT, Martin GB (1994) Testicular morphology and androgen profiles following testicular ischaemia in rams. J Reprod Fertil 101:643–650

Matás C, Coy P, Romar R, Marco M, Gadea J, Ruiz S (2003) Effect of sperm preparation method on in vitro fertilization in pigs. Reproduction 125:133–141

Matás C, Sansegundo M, Ruiz S, García-Vázquez FA, Gadea J, Romar R, Coy P (2010) Sperm treatment affects capacitation parameters and penetration ability of ejaculated and epididymal boar spermatozoa. Theriogenology 74:1327–1340

Matás C, Vieira L, García-Vázquez FJ, Avilés-López K, López-Úbeda R, Carvajal JA, Gadea J (2011) Effects of centrifugation through three different discontinuous Percoll gradients on boar sperm function. Anim Reprod Sci 127:62–72

Matuszczak E, Hermanowicz A, Debek W, Oksiuta M, Dzienis-Koronkiewicz E, Zelazowska-Rutkowska B (2011) Serum AMH concentration as a marker evaluating gonadal function in boys operated on for unilateral cryptorchidism between 1st and 4th year of life. Endocrine 11:1–4

Matzuk M, Lamb DJ (2002) Genetic dissection of mammalian fertility pathways. Nat Cell Biol 4:41–49

McCoard SA, Lunstra DD, Wise TH, Ford JJ (2001) Specific staining of Sertoli cell nuclei and evaluation of Sertoli cell number and proliferative activity in Meishan and White Composite boars during the neonatal period. Biol Reprod 64:689–695
McCoard SA, Wise TH, Ford JJ (2003) Endocrine and molecular influences of testicular development in meishan and white composite boars. J Endocrinol 178:405–416
McMahon DM, Kramer SA, Hussmann DA (1995) Antiandrogen induced cryptorchidism in the pig is associated with failed gubernacular regression and epididymal malformations. J Urol 154:553–557
Meinhardt A, Bacher B, Metz CN, Bucala R, Wreford N, Lan H, Atkins R, Hedger MP (1998) Local regulation of macrophage subsets in the adult rat testis: examination of the roles of the seminiferous tubules, testosterone, and macrophage-migration inhibitory factor. Biol Reprod 59:371–378
Miething A (1995) Multinuclearity of germ cells in the senescent human testis originates from a process of cell–cell fusion. J Submicrosc Cytol Pathol 27:105–113
Mieusset R, Bujan L (1995) Testicular heating and its possible contributions to male infertility: a review. Int J Androl 18:169–184
Mieusset R, Bujan L, Massat G, Mansat A, Pontonnier F (1995) Clinical and biological characteristics of infertile men with a history of cryptorchidism. Biol Reprod 10:613–619
Mieusset R, Bujan L, Massat G, Mansat A, Pontonnier F (1997) Inconstant ascending testis as a potential risk factor for spermatogenesis in infertile men with no history of cryptorchidism. Hum Reprod 12:974–979
Miller D (2000) 25 years of NSIF history. In: Proceedings of national swine improvement federation 2000 annual meeting
Minton JE, Fent RW, Wettemann RP (1985) Influence of the duration of photoperiod on growth, testicular characteristics and endocrine function of boars. Dom Anim Endocrinol 2:53–59
Mitre R, Cheminade C, Allaume P, Legrand P, Legrand AB (2004) Oral intake of shark liver oil modifies lipid composition and improves motility and velocity of boar sperm. Theriogenology 74:1482–1490
Montella A, Pirino A (1990) Morphological findings in cryptorchidism in the adult male. Boll Soc Ital Biol Sper 166:215–222
Morrell JM, Saravia F, van Wienen M, Wallgren M, Rodríguez-Martínez H (2009) Selection of boar spermatozoa using centrifugation on a glycidoxypropylmethoxysialine-coated silica colloid. J Reprod Dev 55:547–552
Mortimer D (2000) Sperm preparation methods. J Androl 21:357–366
Murase T, Imaeda N, Yamada H, Miyazawa K (2007) Seasonal changes in semen characteristics, composition of seminal plasma and frequency of acrosome reaction induced by calcium and calcium ionophore A23187 in Large White boars. J Reprod Dev 53:853–865
Murase T, Imaeda N, Yamada H, Takasu M, Taguchi K, Katoh T (2010) Dilution of boar ejaculates with BTS containing HEPES in place of bicarbonate immediately after ejaculation can reduce the increased inducibility of the acrosome reaction by treatment with calcium and calcium ionophore A23187, which is potentially associated with boar subfertility. J Reprod Dev 56:309–314
Neely JD, Robison OW (1983) Estimates of heterosis for sexual activity in boars. J Anim Sci 56:1033–1043
Neild DM, Brouwers J, Colenbrander B, Aguero A, Gadella BM (2005) Lipid peroxide formation in relation to membrane stability of fresh and frozen thawed stallion spermatozoa. Mol Reprod Dev 72:230–238
Ng CM, Blackman MR, Wang C, Swerdloff RS (2004) The role of carnitine in the male reproductive system. Ann NY Acad Sci 1033:177–188
Nistal M, Jiménez F, Paniagua R (1990) Sertoli cell types in Sertoli cell-only syndrome: relationships between Sertoli cell morphology and aetiology. Histopathology 16:173–180
O'Brien J, Zini A (2005) Sperm DNA integrity and male infertility. Urology 65:16–22
Oger I, Da Cruz C, Panteix G, Menezo YJR (2003) Evaluating human sperm DNA integrity: relationship between 8-hydroxydeoxyguanosine quantification and the sperm chromatin structure assay. Zygote 11:367–371

Oh SA, Park YJ, You YA, Mohamed EA, Pang MG (2010) Capacitation status of stored boar spermatozoa is related to litter size of sows. Anim Reprod Sci 121:131–138

Oh SH, See MT, Long TE, Galvin JM (2006) Genetic parameters for various model regression models to describe total sperm cells per ejaculate over the reproductive lifetime of boars. J Anim Sci 84:538–545

Ohtsuka T (2000) Different interaction of mast cells with human endothelial cells and fibroblasts. Eur J Dermatol 10:115–121

Okazaki T, Abe S, Yoshida S, Shimada M (2009) Seminal plasma damages sperm during cryopreservation, but its presence during thawing improves semen quality and conception rates in boars with poor post-thaw semen quality. Theriogenology 71:491–498

Okwun OE, Igboeli G, Ford JJ, Lunstra DD, Johnson L (1996) Number and function of Sertoli cells, number and yield of spermatogonia, and daily sperm production in three breeds of boar. J Reprod Fertil 107:137–149

Oldenhoff H, Friedel K, Sieme H, Glasmacher B, Wolkers WF (2010) Membrane permeability parameters for freezing of stallion sperm as determined by fourier transform infrared spectroscopy. Cryobiology 61:115–122

Ollero M, Bescos O, Cebrian-Perez JA, Muino-Blanco T (1997) Improvement of cryopreserved ram sperm heterogeneity and viability by addition of seminal plasma. J Androl 18:732–739

Ollero M, Powers RD, Alvarez JG (2000) Variation of docosahexanoic acid content in subsets of human spermatozoa at different stages of maturation: implications for sperm lipoperoxidative damage. Mol Reprod Dev 55:326–334

Orth JM, Gunsalus GL, Lamperti AA (1988) Evidence for sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. Endocrinology 122:787–794

Otani A, Takagi H, Oh H, Suzuma K, Matsumura M, Ikeda E, Honda Y (2000) Angiotensin II-stimulated vascular endothelial growth factor expression in bovine retinal pericytes. Invest Ophtalmol Vis Sci 41:1192−1199

Paniagua R, Martínez.Onsurbe P, Santamaría L, Sáez FJ, Amat P, Nistal M (1990) Quantitative and ultrastructural alterations in the lamina propia and Sertoli cells in human cryptorchid testes. Int J Androl 13:470–487

Park CS, Yi YJ (2002) Comparison of semen characteristics, sperm freezability and testosterone concentration between Duroc and Yorkshire boars during seasons. Anim Reprod Sci 73:53–61

Pathirana IN, Yamasaki H, Kawate N, Tsuji M, Büllesbach EE, Takahashi M, Hatoya S, Inaba T, Tamada H (2011) Plasma insulin-like peptide 3 and testosterone concentrations in male dogs: changes with age and effects of cryptorchidism. Theriogenology (accepted)

Peña FL, Saravia F, Núñez-Martínez I, Tapia JA, Johanisson A, Wallgren M, Rodríguez-Martínez H (2006) Do different portions of the boar ejaculate vary in their ability to sustain cryopreservation? Anim Reprod Sci 93:101–113

Pérez-Llano B, López-Fernández C, García-Casado P, Arroyo F, Gosalbez A, Sala R, Gosálvez J (2010) Dynamics of sperm DNA fragmentation in the swine: ejaculate and temperature effects. Anim Reprod Sci 119:235–243

Pérez-Llano B, Sala R, Reguera G, García-Casado P (2009) Changes in subpopulations of boar sperm defined according to viability and plasma and acrosome membrane status observed during storage at 15º C. Theriogenology 71:311–317

Pérez-Pe R, Barrios B, Muiño-Blanco T, Cebrián-Pérez JA (2001) Seasonal differences in ram seminal plasma revealed by partition in an aqueous two-phase system. J Chromatogr Biomed Sci Appl 760:113–121

Peter W, Dorner G, Stahl F, Uerckert A (1980) Relationship between testosterone levels in sperm, spermatozoa, and fertility performance in young boars. Arch. Exp. Vetmed 34:629–634

Petrunkina AM, Waberski D, Günzel-Apel AR, Töpfer-Petersen E (2007) Determinants of sperm quality and fertility in domestic species. Reproduction 134:3–17

Pettersson A, Nagy JA, Brown LF, Sundberg C, Morgan E, Carter R, Krieger JE, Manseau EJ, Harvey VS, Eckelhoefer IA, Feng D, Dvorak AM, Mulligan RC, Dvorak HF (2000) Heterogenity of the angiogenic response induced in different normal adult tissues by vascular permeability factor/vascular endothelial growth factor. Lab Invest 80:99–115

Pinart E, Bonet S, Briz M, Pastor LM, Sancho S, García N, Badia E (2001a) Morphologic and histochemical study of blood capillaries in boar testes: effects of abdominal cryptorchidism. Teratology 63:42–51

Pinart E, Bonet S, Briz M, Pastor LM, Sancho S, García N, Badia E, Bassols (2001b) Lectin affinity of the seminiferous epithelium in healthy and cryptorchid post-pubertal boars. Int J Androl 24:153–164

Pinart E, Bonet S, Briz M, Pastor LM, Sancho S, García N, Badia E, Bassols (2001c) Morphological and histochemical characteristics of the lamina propria in scrotal and abdominal testes from postpubertal boars: correlation with the appearance of seminiferous epithelium. J Anat 199:435–448

Pinart E, Briz M, Pastor LM, Sancho S, García N, Badia E (2002) Histochemical study of the interstitial tissue in scrotal and abdominal boar testes. Vet J 163:68–76

Pinart Bonet, Briz M, Sancho S, García N, Badia E (2001d) Cytology of the interstitial tissue in scrotal and abdominal testes of post-puberal boars. Tis Cell 33:8–24

Pinart E, Camps R, Briz M, Bonet S, Egozcue J (1998) Unilateral spontaneous abdominal cryptorchidism: structural and ultrastructural study of sperm morphology. Anim Reprod Sci 49:247–268

Pinart E, Sancho S, Briz M, Bonet S (1999a) Morphologic study of the testes from spontaneous unilateral and bilateral abdominal cryptorchid boars. J Morphol 239:225–243

Pinart E, Sancho S, Briz M, Bonet S, Badia E (1999b) Efficiency of the process of meiosis in scrotal testes of healthy boars and unilateral abdominal cryptorchid boars. Teratology 60:209–214

Pinart E, Sancho S, Briz M, Bonet S, García N (1999c) Characterization of the semen quality of postpuberal boars with spontaneous unilateral abdominal cryptorchidism on the right side. Anim Reprod Sci 55:269–278

Pinart E, Sancho S, Briz M, Bonet S, García N, Badia E (2000) Ultrastructural study of the boar seminiferous epithelium: changes in cryptorchidism. J Morphol 244:190–202

Popwell JM, Flowers WL (2004) Variability in relationships between semen quality and estimates of in vivo and in vitro fertility in boars. Anim Reprod Sci 81:97–113

Price EO (1987) Male sexual behaviour. In: Price EO (ed) The veterinary Clinics of North America. Farm Animal Behav 3:405–422

Pruneda A, Pinart E, Briz MD, Sancho S, Garcia-Gil N, Badia E, Kádár E, Bassols J, Bussalleu E, Yeste M, Bonet S (2005) Effects of high semen-collection frequency on the quality of sperm from ejaculates and from six epididymal regions in boars. Theriogenology 63:2219–2232

Pruneda A, Yeung CH, Bonet S, Pinart E, Cooper TG (2007) Cocentrations of carnitine, glutamate and myo-inositol in epididymal fluid and spermatozoa from boars. Anim Reprod Sci 97:344–355

Puigmulé M, Fàbrega A, Yeste M, Bonet S, Pinart E (2011) Study of the proacrosin—acrosin system in epididymal, ejaculated and in vitro capacitated boar spermatozoa. Reprod Fertil Dev 23:837–845

Puri P, O'Donnell B (1990) Semen analysis of patients operated on for unpalpable testes. Br J Urol 68:646–647

Raeside JI, Renaud RL, Friendship RM (1988) Differences in aromatase activity between leydig cells from the scrotal and abdominal testis in naturally unilateral-cryptorchid boars. Biol Reprod 39:566–572

Ramesh C, Pearl CA, At-Taras E, Roser JF, Berger T (2007) Ontogeny of androgen and estrogen receptor expression in porcine testis: effect of reducing testicular estrogen synthesis. Anim Reprod Sci 102:286–299

Ramió-Lluch L, Balasch S, Bonet S, Briz M, Pinart E, Rodríguez-Gil JE (2009) Effects of filtration through Sephadex columns improve overall quality parameters and "in vivo" fertility of subfertile refrigerated boar-semen. Anim Reprod Sci 115:189–200

Rath D, Bathgate R, Rodríguez-Martínez H, Roca J, Strzezek J, Waberski D (2009) Recent advances in boar sperm cryopreservation. Soc Reprod Fertil Suppl 66:51–66

Rathje TA, Johnson RK, Lunstra DD (1995) Sperm production in boars after nine generations of selection for increased weight of testis. J Anim Sci 73:2177–2185

Rawlings N, Evans ACO, Chandolia RK, Bagu ET (2008) Sexual maturation in the bull. Reprod Dom Anim 43(Suppl 2):295–301

Regadera J, Codesal J, Paniagua R, González-Peramat P, Nistal M (1991) Immunohistochemical and quantitative study of interstitial and intratubular Leydig cells in normal men, cryptorchidism, and Klinefelter's syndrome. J Pathol 164:299–306

Rey R, Lukas-Croisier C, Lasala C, Bedecarrás P (2003) AMH/MIS: what we know already about the gene, the protein and its regulation. Mol Cell Endocrinol 211:21–31

Richardson LL, Kleinman HK, Dym M (1995) The effects of ageing on basement membrane in the testis. J Androl 16:118–126

Ricker JV, Linfor JJ, Delfino WJ, Kysar P, Scholtz EL, Tablin F, Crowe JH, Ball BA, Meyers SA (2006) Equine sperm membrane phase behaviour: the effects of lipid-based cryoprotectants. Biol Reprod 74:359–365

Rivera M, Quintero-Moreno A, Barrera X, Palomo MJ, Rigau T, Rodríguez-Gil JE (2005) Natural mediterranian photoperiod does not affect the main parameters of boar-semen quality analysis. Theriogenology 64:934–946

Robinson JAB, Buhr MM (2005) Impact of genetic selection on management of boar replacement. Theriogenology 63:668–678

Roca J, Hernández M, Carvajal G, Vázquez JM, Martínez EA (2006) Factors influencing boar sperm cryosurvival. J Anim Sci 84:2692–2699

Rodríguez-Martínez H (2003) Laboratory semen assessment and prediction of fertility: still utopia? Reprod Domest Anim 38(4):312–318

Rohrer GA, Ford JJ, Wise T (2000) A preliminary genomic scan for chromosomal regions affecting FSH concentration in pubertal boars. J Anim Sci 78(Suppl 2):27

Rooke JA, Shao CC, Speake BK (2001) Effects of feeding tuna oil on the lipid composition of pig spermatozoa and in vitro characteristics of semen. Reproduction 121:315–322

Rozanski TA, Bloom DA (1995) The undescended testis. Theory and management. Urol Clin North Am 22:107–118

Rune GM, Mayr J, Neugebauer H, Anders C, Sauer H (1992) Pattern of sertoli cell degeneration in cryptorchid prepubertal testes. Int J Androl 15:19–31

Safranski TJ (2008) Genetic selection of boars. Theriogenology 70:1310–1316

Safranski TJ, Ford JJ, Rohrer GA, Guthrie HD (2011) Plenary contribution to international conference on boar semen preservation 2011. Genetic selection for freezability and its controversy with selection for performance. Reprod Domest Anim 46 (Suppl. 2):31–34

Sánchez M, Cereza JM, García M (1989) Influencia de las condiciones ambientales en la fertilidad del verraco. Proceedings X symposium asociación nacional de porcinocultura científica, pp 67–73

Sancho S, Casas I, Ekwall H, Saravia F, Rodriguez-Martínez H, Rodríguez-Gil JE, Flores E, Pinart E, Briz M, Garcia-Gil N, Bassols J, Pruneda A, Bussalleu E, Yeste M, Bonet S (2007) Effects of cryopreservation on semen quality and the expression of sperm membrane hexose transporters in the spermatozoa of Iberian pigs. Reproduction 134:111–121

Sancho S, Pinart E, Briz M, Garcia-Gil N, Badia E, Bassols J, Kadar E, Pruneda A, Bussalleu E, Yeste M, Coll MG, Bonet S (2004) Semen quality of postpubertal boars during increasing and decreasing natural photoperiods. Theriogenology 62:1271–1282

Sancho S, Rodríguez-Gil JE, Pinart E, Briz M, Garcia-Gil N, Badia E, Bassols J, Pruneda A, Bussalleu E, Yeste M, Casas I, Palomo MJ, Ramió L, Bonet S (2006) Effects of exposing boars to different articial light regimens on semen plasma markers and "in vivo" fertilizing capacity. Theriogenology 65:317–331

Santamaria L, Martínez-Onsurbe P, Paniagua R, Nistal M (1990) Laminin, type IV collagen and fibronectin in normal and cryptorchid human testis. An immunocytochemical study. Int J Androl 13:135–146

Santi CM, Santos T, Hernández-Cruz, Darszon A (1998) Properties of a novel pH-dependent Ca^{2+} permeation pathway present in male germ cells with possible roles in spermatogenesis and mature sperm function. J Gen Physiol 112:33–53

Saravia F, Núñez-Martínez I, Morán JM, Soler C, Muriel A, Rodríguez-Martínez H, Peña FJ (2007) Differences in boar sperm head shape and dimensions recorded by computer-assisted sperm morphometry are no related to chromatin integrity. Theriogenology 68:196–203

Sasagawa I, Nakada T, Kubota Y, Sawamura T, Tateno T, Ishigooka M (1994) Gonadal function and testicular histology in Noonan's syndrome with bilateral cryptorchidism. Arch Androl 32:135–140

Sasagawa I, Yanagimachi R (1997) Spermatids from mice after cryptorchid and reversal operations can initiate normal embryo development. J Androl 18:203

Schinckel AP (1991) Use of estimated breeding value microcomputer programs to improve pork production efficiency. Comp Electr Agric 6:63–69

Schinckel A, Johnson RK, Kittok RJ (1984) Testicular development and endocrine characteristics of boars selected for either high or low testis size. J Anim Sci 58:675

Schinckel A, Johnson RK, Pumfrey RA, Zimmerman DR (1983) Testicular growth in boars of different genetic lines and its relationship to reproductive performance. J Anim Sci 56:1065–1076

Schteingart HF, Meroni SB, Canepa DF, Pellizzari EH, Cigorraga SB (1999) Effects of basic fibroblast growth factor and nerve growth factor on lactate production, gamma-glutamyl transpeptidase and aromatase activities in cultured Sertoli cells. Eur J Endocrinol 141:539–545

Selles E, Gadea J, Romar R, Matas C, Ruiz S (2003) Analysis of in vitro fertilizing capacity to evaluate the freezing procedures of boar semen and to predict the subsequent fertility. Reprod Domest Anim 38:66–72

Setchell BP, Bergh A, Widmark A, Damber JE (1995) Effect of testicular temperature on vasomotion and blood flow. Int J Androl 18:120–126

Setchell BP, Ekpe G, Zupp JL, Surani MAH (1998) Transient retardation in embryo growth in normal female mice made pregnant by males whose testes had been heated. Hum Reprod 13:342–347

Sheth FJ, Multani AS, Sheth JJ, Radhakrishna U, Shah VC, Chinoy NJ (1996) Incomplete gonadal dysgenesis. Urol Int 56:57–60

Singh A, Ezeasor D (1989) Ultrastructure of sertoli cells in cryptorchid goats. Arch Androl 23:61–70

Sirvent JJ, Bernat R, Rodríguez-Tora J, Navarro MA, Garcia B, Brunet J (1989) Postpuberal cryptorchidism. Morphofunctional study with special reference to Leydig cells. Eur Urol 16:433–439

Smital J (2009) Effects influencing boar semen. Anim Reprod Sci 110:335–346

Smital J, DeSousa LL, Mohsen A (2004) Differences among breeds and manifestation of heterosis in AI boar sperm output. Anim Reprod Sci 80:121–130

Smital J, Wolf J, DeSousa LL (2005) Estimation of genetic parameters of semen characteristics and reproductive traits in AI boars. Anim Reprod Sci 86:119–130

Sonderman JP, Luebbe JJ (2008) Semen production and fertility issues related to differences in genetic lines of boars. Theriogenology 70:1380–1383

Sprott LR, Harris MD, Forrest DW, Young J, Zhang HM, Oyarzol JN (2000) Artificial insemination outcomes in beef females using bovine sperm with detectable fertility-associated antigen. J Anim Sci 78:795–798

Strzezek J, Fraser L, Kuklinska M, Dziekonska A, Lecewicz M (2004) Effects of dietary supplementation with polyunsaturated fatty acids and antioxidants on biochemical characteristics of boar semen. Biol Reprod 4:271–287

Strzezek J, Kordan W, Glogowski J, Wysocki P, Borkowski K (1995) Influence of semen collection frequency on sperm quality of boars, wih special reference to biochemical markers. Reprod Dom Anim 30:85–94

Strzezek J, Saiz Cidoncha F, Martin Rillo S (1999) Fisiología de la reproducción del verraco. Revista de la Asociación Nacional de Porcinocultura Científica (ANAPORC) 191:54–75

Sundaram K, Panneerselvam KS (2006) Oxidative stress and DNA single strand breaks in skeletal muscle of aged rats: role of carnitine and lipoicacid. Biogerontology 7:111–118

Suriyasomboon A, Lundeheim N, Kunavongkrit A, Einarsson S (2005) Effect of temperatura and humidity on sperm morphology in Duroc boars under different housing systems in Thailand. J Vet Med Sci 67:777–785

Suzuki K, Nagai T (2003) In vitro fertility and motility characteristics of frozen-thawed boar epididymal spermatozoa separated by Percoll. Theriogenology 60:1481–1494

Swierstra EE (1970) The effect of low ambient temperatures on sperm production, epididymal sperm reserves, and semen characteristics of boars. Biol Reprod 2:23–28

Swierstra EE (1973) Influence of breed, age, and ejaculation frequency on boar semen composition. Canadian J Anim Sci 53:43–53

Tan HS, Raeside JI (1980) Developmental patterns of plasma dehydroepiandrosterone sulfate and testosterone in male pigs. Anim Reprod Sci 3:73–81

Tanigawa T, Mimata H, Takeshita M, Ogata J (1990) Membrane fluidity and lipid composition of rat testicles in experimental cryptorchidism. Urol Int 45:302–309

Tavilani H, Doosti M, Abdi K, Vaisiraygani A, Joshaghani HR (2006) Decrease polyunsaturated and increase saturated fatty acid concentration in spermatozoa from asthenozoospermic males as compared with normozoospermic males. Andrologia 38:173–178

Thiengtham J (1992) Some relationships between sexual behavioural parameters and semen characteristics in the boar. Thai J Vet Med 22:237−249

Thomas HR, Kattesh HG, Knight JW, Gwazdauskas FC, Meacham TN, Kornegay ET (1979) Effects of housing and rearing of age of puberty and libido in boars. Anim Prod 28:231–243

Thorup J, MaLachlan R, Cortes D (2010) What is new in cryptorchidism and hypospadias-a critical review on the testicular dysgenesis hypothesis. J Pediatr Surg 45:2074–2086

Thurston LM, Siggins K, Mileham AJ, Watson PF, Holt WV (2002a) Identification and amplified restriction length polymorphism markers linked to genes controlling boar sperm viability following cryopreservation. Biol Reprod 66:545–554

Thurston LM, Siggins K, Mileham AJ, Watson PF, Holt WV (2002b) Semen cryopreservation: a genetic explanation for species and individual variation? Cryo Letters 23:255–262

Thurston LM, Watson PF, Mileham AJ, Holt WV (2001) Morphologically distinct sperm subpopulations defined by Fourier Shape Descriptors in fresh ejaculates correlate with variation in boar semen quality following cryopreservation. J Androl 22:382–394

Töpfer-Petersen E (1999) Carbohydrate-based interactions on the route of a spermatozoon to fertilization. Hum Reprod Up 5:314–329

Toppari J, Virtanen H, Skakkebaek NE, Main KM (2006) Environmental effects on hormonal regulation of testicular descent. Steroid Biochem Mol Biol 102:184–186

Trudeau V, Sanford LM (1986) Effect of season and social environment on testis size and semen quality of the adult Landrace boars. J Anim Sci 63:1211–1219

Ueno H, Nishimune Y, Mori H (1991) Cyclic localization change of Golgi apparatus in Sertoli cell induced by mature spermatids in rats. Biol Reprod 44:656–662

Urry RL, Carrell DT, Starr NY, Snow BW, Middleton RG (1994) The incidence of antisperm antibodies in infertility patients with a history of cryptorchidism. J Urol 151:381–383

Vadnais ML, Roberts KP (2010) Seminal plasma proteins inhibit in vitro- and cooling-induced capacitation in boar spermatozoa. Reprod Fertil Dev 22:893–900

Vanha-Perttula T, Arya M (1985) Lectin staining of rat testis and epididymis after ligation of excurrent ducts at different levels. Biol Reprod 33:477–485
Veeramachaneni DNR, Heath EH, Ott RS, McEntee K, Hixon JE (1987) Changes in basal lamina of seminiferous tubules associated with degenerated spermatogenesis in the bull. Am J Vet Res 48:243–245
Vitali G, Parente R, Melotti C (1995) Carnitine supplementation in human idiopathic asthenospermia: clinical results. Drugs Exp Clin Res 21:157–159
Walker S, Robinson OW, Whisnant CS, Cassady JP (2004) Effect of divergent selection for testosterone production on testicular morphology and daily sperm production in boars. J Anim Sci 82:2259–2264
Waterhouse KE, De Angelis PM, Haugan T, Paulenz H, Hofmo PO, Farstad W (2004) Effects of in vitro storage time and extender on membrane quality of boar sperm assessed by flow cytometry. Theriogenology 62:1638–1651
Waterhouse KE, Hofmo PO, Tverdal A, Miller RR (2006) Within and between breed differences in freezing tolerance and plasma membrane fatty acid composition of boar sperm. Reproduction 131:887–894
Wathes DC, Abayasekara DR, Aitken RJ (2007) Polyunsaturated fatty acids in male and female reproduction. Biol Reprod 77:190–201
Watson PF (2000) The causes of reduced fertility with cryopreserved semen. Anim Reprod Sci 60:481–492
Wolf J (2009) Genetic parameters for semen traits in AI boars estimated from dat on individual ejaculates. Reprod Dom Anim 44:338–344
Wolf J (2010) Heritabilities and genetic correlations for litter size and semen traits in czech large white and landrace pigs. J Anim Sci 88:2893–2903
Wolf J, Smital J (2009) Quantification of factors affecting semen traits in artificial insemination boars from animal model analyses. J Anim Sci 87:1620–1627
Wu N, Murono F (1996) Temperature and germ cell regulation of leydig cell proliferation stimulated by sertoli cell-secreted mitogenic factor: a possible role in cryptorchidism. Andrologia 28:247–257
Wyrobek AJ, Eskenazi B, Young S, Arnhein N, Tiemann-Boege I, Jabs EW, Glaser RL, Pearson FS, Evenson D (2006) Advancing age has differential effects on DNA damage, chromatin integrity, gene mutations, and aneuploidies in sperm. Proc Nat Acad Sci Un St Am 103:9601–9606
Wysokinska A, Kondracki S, Banaszewska D (2009) Morphometrical characteristics of spermatozoa in polish landrace boars with regard to the number of spermatozoa in an ejaculate. Reprod Biol 9:271–282
Xu X, Pommier S, Arbov T, Hutchings B, Sotto W, Foxcroft GR (1998) In vitro maturation and fertilization techniques for assessment of semen quality and boar fertility. J Anim Sci 76:3079–3089
Yakushiji K, Kai S, Yamauchi M, Kawajima M, Osada Y, Toshimori K (2006) Expression and distribution of OCTN2 in mouse epididymis and its association with obstructive azoospermia in juvenile visceral steatosis mice. Int J Urol 13:420–426
Yamamoto M, Hibi H, Miyake K (1994) Appearance of spermatozoon after administration of mast cell blocker to a patient with azoospermia. Hinyokika Kiyo 40:541–543
Yeste M, Barrera X, Coll D, Bonet S (2011) The effects on boar sperm quality of dietary supplementation with omega-3 polyunsaturated fatty acids differ among porcine breeds. Theriogenology 76:184–196
Yeste M, Briz M, Pinart E, Sancho S, Garcia-Gil N, Badia E, Bassols J, Pruneda A, Bussalleu E, Casas I, Bonet S (2008) Hyaluronic acid delays boar sperm capacitation after 3 days of storage at 15º C. Anim Reprod Sci 109:236–250
Yeste M, Sancho S, Briz M, Pinart E, Bussalleu E, Bonet S (2010) A diet supplemented with L-carnitine improves the sperm quality of Piétrain but not of Duroc and Large White boars when photoperiod and temperature increase. Theriogenology 73:577–586

Yoshimoto T, Nakamura S, Yamauchi S, Muto N, Nakada T, Ashizawa K, Tatemoto H (2008) Improvement of the post-thaw qualities of Okinawan native pig spermatozoa frozen in an extender supplemented with ascorbic acid 2-O-alpha-glucoside. Glycobiology 57:30–36

Young LD, Leymaster KA, Lunstra DD (1986) Genetic variation in testicular development and its relationship to female reproductive traits in swine. J Anim Sci 63:17–26

Yousef MI, Abdallah GA, Kamel KI (2003) Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. Anim Reprod Sci 76:99–111

Zakaria O, Shono T, Imajima T, Suita S (1998) Fertility and histological studies in an unilateral cryptorchid rat model during early and late adulthood. Br J Urol 82:404–407

Zini A, Libman J (2006) Sperm DNA damage: clinical significance in the era of assisted reproduction. CMAJ 175:496

Zirkin BR (1998) Hormonal control of spermatogenesis. In: Knobil E, Neill JD (eds) The physiology of reproduction. Raven Press, New York, pp 556–562