

Factors influencing semen quality in pigs

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Introduction

Optimal semen production is of great importance for pig breeding programmes, especially when artificial insemination (AI) is used. Production of ejaculates of constant and high quality enables the AI organization to use a relatively small number of boars. Thus selection of males in the breeding stock can be more intense and only the best boars will be used to ensure a high quality offspring.

Unfortunately sperm production does not occur at a constant rate: many factors, known and unknown, influence semen quality and quantity. Fluctuations in sperm production can easily amount to 25–30% (Figs 1 & 2), sometimes without one distinct factor causing a reduction in production. This forces AI centres to keep additional boars not only to cope with fluctuations in the number of insemination doses required but also to compensate for any unforeseen reduction in sperm production.

This paper describes the factors that are known to influence semen quality. Eventually this might help us to find methods for optimizing semen production.

Assessment of semen quality

The ultimate assessment of semen quality is by inseminating a large number of 'standard females' and monitoring the number of pregnancies and piglets born per litter. In such an experiment one would ideally inseminate a suboptimal quantity of sperm cells. Increasing the number of sperm cells in the insemination dose would result in higher pregnancy rates until an optimal level is reached. No further increase in pregnancy rate and/or litter size will be obtained by increasing the number of spermatozoa thereafter. This method is, however, too time-consuming and too expensive. In addition, the 'standard female' does not exist and the expected results of insemination are markedly influenced by factors affecting the female, such as animal housing, climate, infectious diseases and age. Less accurate methods are used to estimate semen quality and quantity. At AI centres the following factors are routinely studied directly when the ejaculates have been obtained:

- volume of the ejaculate;
- concentration of spermatozoa (number/ml);
- motility of the spermatozoa;
- morphology of the spermatozoa.

Sperm number

The concentration of spermatozoa in the ejaculate is generally estimated with a spectrophotometric procedure although other methods are used, e.g. haemocytometric or electronic cell

counting. Excellent correlations between these methods can be found when semen samples from certain species, e.g. cattle, are properly analysed. In practice, however, variations in the number of spermatozoa in samples processed may exceed 30% (Pace, 1980). From the concentration of spermatozoa and the volume of the ejaculate the sperm output is calculated by multiplication.

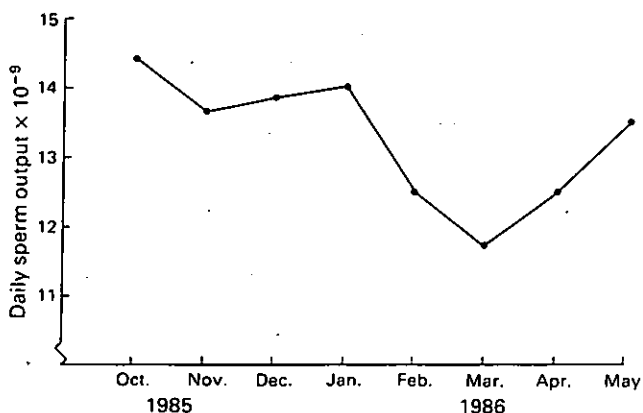


Fig. 1. Mean daily sperm output by the boar ($n = \sim 350$) over time.

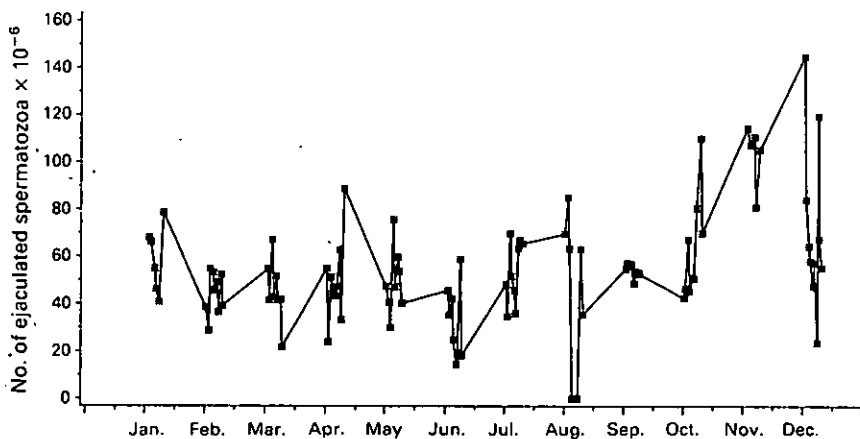


Fig. 2. Variation in number of sperm cells per ejaculate for an individual boar over time.

An accurate method for estimating sperm production by a specific boar would be the measurement of the daily sperm output: the number of spermatozoa produced can be quantified during a certain time span and be divided by the number of days involved. Daily sperm output is correlated with daily sperm production in the boar and usually represents between 80 and 90% of daily sperm production (Swierstra, 1968b, 1973, 1974).

The frequency of ejaculation by itself has a significant effect on semen characteristics, such as volume of the ejaculate, and concentration of spermatozoa. An intensive frequency of ejaculation (3–4 times in 1 day) results in a considerable decrease of volume of the ejaculate but an even more remarkable decrease in the total number of spermatozoa per ejaculate (Cameron, 1985b). Daily sperm output can be estimated by collecting spermatozoa from a boar 3 times per week (Monday, Wednesday, Friday) and calculating the output during the 5th and 6th consecutive week of collection. More frequent collections do not significantly increase the number of spermatozoa that can be

harvested. A more practical method of estimating sperm producing capacity is by an intensive frequency of semen collection, i.e. 4 times in 8 h followed by collections once daily for 4 days.

Sperm motility

The most commonly used laboratory test for evaluating sperm quality is visual estimation of the percentage of motile sperm cells. This is, however, a test with poor potential for predicting fertility (Linford *et al.*, 1976). A less subjective method of evaluating sperm motility, computerized image analysis of sperm migration, has been developed during the past decade. This method also provides more detailed information on the quality of movement of individual spermatozoa, e.g. velocity and percentage of spermatozoa with circular direction of movement (Rath *et al.*, 1988). Characteristics of sperm motion as determined by computer-assisted image analysis can give some indication of fertility (Budworth *et al.*, 1988). There is also a positive correlation between sperm motion and competitive fertility (Amann, 1988). It is possible to use this method for rapid measurement of a range of motility parameters in a large number of individual spermatozoa, and this provides the opportunity to observe alterations that occur, e.g. when spermatozoa capacitate. A change then occurs to less forward and less linear progression, hyperactive motility, and marked lateral head displacement (this applies to spermatozoa from many mammalian species) (Robertson *et al.*, 1988). Motility can also be analysed with the swim-up method in which the percentage of spermatozoa that swim up from seminal plasma into culture medium is determined. Differences in such motility ratings show a good correlation with differences in fertility (Parrish & Foote, 1987). Another method of analysing motility is estimating the migration distance of sperm cells into polyacrylamide gels or Percoll gradients (Parrish & Foote, 1987; de Vries & Colenbrander, 1988).

Sperm morphology

Morphological evaluation of sperm cells by light microscopy is considered an important contribution to prediction of male fertility. A high incidence of random or specific morphological defects of spermatozoa has been associated with impaired fertility. Abnormalities concerned include deviations of the acrosome head, and/or midpiece morphology, persistent proximal cytoplasmic droplets, and coiled tails (Koh *et al.*, 1976; Bach *et al.*, 1982; Malmgren & Larsson, 1984). The condition of the acrosome is especially important (Pursel *et al.*, 1972). Acrosome morphology can be categorized into normal apical ridge, damaged apical ridge, missing apical ridge, and loose acrosomal cap. For morphological and cell physiological evaluation, spermatozoa are stained with supravital or fluorescent staining techniques. Discrimination is therefore done on the ability of membranes to exclude chromophores or fluorophores or on the ability to convert those dyes enzymically (Elliot, 1978; Garner *et al.*, 1986; Woelders, 1988). Using differential interference-contrast microscopy, unstained wet mount preparations can also be studied for the evaluation of sperm morphology. Electron microscopy may reveal ultrastructural changes, such as changes in the organization of the plasma membrane (de Leeuw *et al.*, 1990). Cooling of spermatozoa induces aggregation of intramembranous particles, as a result of lateral phase separation.

Other in-vitro parameters

A number of other tests can be used in evaluating semen quality and predicting male fertility. Some of these tests are more reliable than others, but none of them is routinely used at most pig AI centres. Such methods are: osmotic stress resistance (Schilling & Vengust, 1985), enzyme release (Pace *et al.*, 1981; Wood *et al.*, 1986), mucus penetration test (Mortimer *et al.*, 1986), metabolic activity assay (Rogers & Bentwood, 1982), sperm-chromatin structure assay (Ballachey *et al.*, 1987), and in-vitro fertilizing capacity (Clarke & Johnson, 1987). Some of these tests are better correlated with fertility than are others (Wood *et al.*, 1986; Foote, 1988).

Conditions affecting sperm production

Several factors that may influence sperm production of boars, will be discussed separately, e.g. season, social environment, nutrition, breed, age, and testis size.

Season

Seasonal influences on sperm production have been well established. In temperate regions increased semen volume and/or sperm concentration can be observed from September until February compared with March until August (Peter *et al.*, 1981; Grabner *et al.*, 1986) (Fig. 1). Season-dependent fluctuations in number of morphologically abnormal spermatozoa were, however, not observed (Claus *et al.*, 1985b). Seasonal influences on reproduction can be exerted through different factors such as light, temperature, nutrition, and their interaction. Under experimental conditions some of the seasonal factors have been studied independently and therefore the effect of light and temperature will be discussed separately.

Light

In wild boar the mating period is in late autumn and early winter (Mauget, 1982). In the domestic boar this seasonal influence is less obvious. The effects of supplementary lighting on endocrine and gametogenic function in the developing and adult boar have been studied. Supplementary lighting may accelerate the onset of mating behaviour and decrease the age at first successful semen collection (Mahone *et al.*, 1979; Berger *et al.*, 1980; Hoagland & Diekman, 1982). However, supplementary lighting did not influence puberty in boars by altering GnRH content in the hypothalamus or pituitary content of gonadotrophic hormones (Lee *et al.*, 1987b). Plasma testosterone concentration during development tends to be higher in young boars raised during a decrease in photoperiod relative to boars raised during an increase in photoperiod (Claus & Weiler, 1985), although serum concentrations of gonadotrophic hormones and testosterone were not affected by supplementary lighting (Hoagland *et al.*, 1981; Hoagland & Diekman, 1982; Brandt & Diekman, 1985). Moreover, no positive influence of supplementary lighting was found on testicular volume, volume percentage of seminiferous tubules, tubular diameter (Minton & Wetteman, 1987), hormone concentrations or semen quality (Greenberg & Mahone, 1981; Lee *et al.*, 1987a, b). It can therefore be concluded that pubertal development hardly seems to be influenced by supplementary lighting. The effect of increasing or decreasing photoperiod during pubertal development on testicular and endocrine function in pigs is of great interest.

Data on the effect of supplementary lighting on reproductive function in adult boars are also controversial. Photoperiod changes semen production while short daylength increased total sperm output in control boars compared with those exposed to long daylength (Mazzari *et al.*, 1968). Other investigators (Claus & Weiler, 1985; Claus *et al.*, 1985a) have also observed an increase in total number of spermatozoa in ejaculates, libido, hormone production (e.g. testosterone and oestrogens) in boars with a reversed lighting regimen, i.e. an increase of light in the fall and a decrease of light in the spring. The nadir in the summer period, observed in boars under natural photoperiod, was removed. Trudeau & Sanford (1986) observed that seasonal decreases in semen quality preceded the summer increase in ambient temperature and therefore might be mediated via photoperiod-induced endocrine changes. A decrease in photoperiod (natural or artificial) therefore stimulates sexual function in the boar. In addition, Brandt & Diekman (1985) could not demonstrate a positive effect of supplementary lighting on total motile spermatozoa produced. In addition, no significant differences in serum LH or testosterone concentrations were observed when comparing treated and control boars. Brandt & Diekman (1985) thus concluded that supplemental lighting does not change semen quality of post-pubertal boars.

Temperature

Low ambient temperature *per se* does not seem to influence sperm production. Boars housed at low ambient temperature ($< -10^{\circ}\text{C}$) produced larger semen volumes than did control boars housed at $+17^{\circ}\text{C}$. However, sperm concentrations were lower and total sperm yield per ejaculate did not differ between groups. During these experiments boars housed at low ambient temperatures were fed *ad libitum* (Swierstra, 1970). When feeding levels appropriate for temperate conditions are used, they may lead to undernutrition in the cold, due to thermoregulatory processes necessary to maintain normal body temperature (Kemp *et al.*, 1988b). High ambient temperature decreases sperm motility and sperm production for a longer period as sperm production takes approximately 2 months. The critical air temperature above which sperm output in the boar is impaired is 29°C (Stone, 1982). Decreased sperm motility and an increased number of morphologically abnormal spermatozoa are observed when boars are exposed to heat stress, for example when they were kept at 35°C for 100 h. The time lapse from heat stress to the occurrence of sperm deviations indicates that spermiogenesis but not the early stages of spermatogenesis were negatively affected. Pronounced semen alterations were observed 2–6 weeks after heat stress (McNitt & First, 1970; Larsson & Einarsson, 1984; Heitman & Cockrell, 1984; Wetteman & Bazer, 1985). Ejaculates with poor fertility were characterized by reduced number of spermatozoa per ejaculate, decreased sperm motility and increased numbers of morphologically abnormal spermatozoa. Heat-stressed boars have a greater proportion of basic albumin-like proteins in the seminal plasma which render their spermatozoa more susceptible to cooling than those of control boars. As a consequence their spermatozoa will be less suitable for preservation (Egbunike & Jeyakumar, 1980).

Social environment

Social environment exerts an important influence on reproduction of the boar both during puberty and adulthood. In young prepubertal boars social restriction, i.e. lack of physical contact with males and/or females, caused a depression in development of sexual behaviour. When adult, these males achieved fewer copulations and displayed less courting behaviour than control boars. No effect on semen quality or testis size was observed (Hemsworth *et al.*, 1977, 1978; Nelssen *et al.*, 1982). Boars kept on earth lots and reared in groups reached puberty earlier and demonstrated greater sexual aggression than otherwise similar controls reared singly or on concrete floors (Thomas *et al.*, 1979). In another study, confinement-rearing did not affect sexual development of young boars (Esbenshade *et al.*, 1979). Isolation of adult boars from sows caused a reversible depression in the level of sexual behaviour. The presence of sows, in or out of oestrus, restored the sexual behaviour. Olfactory and auditory stimuli appeared to be most important (Hemsworth, 1982). Ejaculate volume also was consistently less in socially restricted boars compared with non-restricted boars (Trudeau & Sanford, 1986). This might be related to differences in ejaculatory ability (Hemsworth *et al.*, 1983b). Total sperm production did not differ significantly between the two groups (Trudeau & Sanford, 1986).

Nutrition

Few investigations dealing with the influence of nutritional factors on reproductive performance of the boar have been published. Kemp & den Hartog (1989) have reviewed the literature on the influence of dietary energy and protein supply on libido and sperm quantity and quality. Libido is usually not affected by dietary protein or energy intake (Stevemer *et al.*, 1961; Yu *et al.*, 1985). Only in extreme circumstances, such as prolonged undernutrition resulting in generalized loss of condition of the animals, is libido affected in boars (Stevemer *et al.*, 1961).

Dietary energy intake equal to or less than defined maintenance requirement is accompanied by a reduction of the number of sperm cells produced by rams and bulls. Studies on the effect of energy

intake on pig semen production were done in a very small number of animals (Stevemer *et al.*, 1961) or at feeding levels well above maintenance requirements (Yen & Yu, 1985) and did not show any effect of dietary energy intake.

Studies on the influence of dietary protein intake are to some extent contradictory. Some authors found that a daily intake of 280 g crude protein, 11.6 g lysine and 7.2 g methionine plus cystine combined is sufficient when 30 MJ/day metabolizable energy is provided (Yen & Yu, 1985), while other investigators found a daily requirement of 743 g crude protein, 54 g lysine and 37 g methionine plus cystine (Poppe *et al.*, 1974). The effect of dietary energy and protein intake on semen quality is apparently not very critical.

Our recent investigations were designed to see whether commercial sow diets contain sufficient protein for breeding boars and to determine the effects of feeding level on male reproductive performance.

Studies on the effect of high protein intake were performed using 97 boars kept at AI centres. The animals were fed either a control diet (commercial sow feed) containing, per kg, 12.56 MJ metabolizable energy (ME), 145 g crude protein, 6.8 g lysine and 4.4 g methionine + cystine, or an iso-energetic high protein diet, containing 222 g crude protein, 12 g lysine and 8.1 g methionine + cystine. Semen was collected 3–4 or 6 times per week. Libido was estimated from the number of mountings refused and the ejaculates were evaluated by estimation of the total number of spermatozoa, the percentage of motile spermatozoa and the pattern of movement. None of the parameters measured was influenced by dietary amino acid supply and therefore it was concluded that the commercial sow feed provided sufficient protein for optimal male reproductive performance (Kemp *et al.*, 1988a). This conclusion is in full agreement with that of Yen & Yu (1985) and contradicts that of Poppe *et al.* (1974) who found that protein supply might be insufficient.

To study the effect of level of feed intake, 3 groups of 14 Yorkshire boars were fed *ad libitum* (74 MJ ME/day), a medium level of (47 MJ ME/day), or a low level (25 MJ ME/day) of a commercial sow diet for 12 weeks. Sperm number and motility were evaluated, as well as the non-return percentage at 56 days. Any differences were not anticipated to occur during the initial 6 weeks of the experiment, since the development from an A-type spermatogonium to spermatozoa, and their passage through the epididymis, takes about 39–45 days (Singh, 1962; Swierstra, 1968a; Colenbrander *et al.*, 1982). After 8 weeks statistically significant differences in total sperm output were detected and the differences increased thereafter (Fig. 3). During the last 2 weeks of the experiment the high intake group produced 18% more spermatozoa than did the medium feeding level group. Boars kept at the low feeding level produced 26% fewer spermatozoa than did the medium level group. No differences with respect to sperm motility parameters or non-return percentage were found (Kemp *et al.*, 1988b).

From the results in both experiments it was concluded that a commercial sow diet contains sufficient protein (and amino acids) for optimal male reproductive performance. Total sperm production is dependent on the level of food intake and insufficient dietary energy will result in reduction of reproductive performance.

Breed

In some breeds, sexual maturity is attained at an earlier age than in others (Bazer *et al.*, 1988). Significant differences in semen characteristics exist between breeds. For comparison of sperm production by different breeds the calculation of the daily sperm output or potential number of insemination doses per ejaculate seems appropriate. Kennedy & Wilkins (1984) compared 6 different breeds. For each parameter investigated one of the breeds excelled, i.e. volume (Hampshire), concentration (Duroc), percentage of live cells (Duroc) and motility (Yorkshire), while Lacombe boars performed least well for all parameters measured. Several other authors have also reported significant between-breed differences in semen production (Swierstra, 1973; Koh *et al.*, 1976; Conlon & Kennedy, 1978; Johnson *et al.*, 1980).

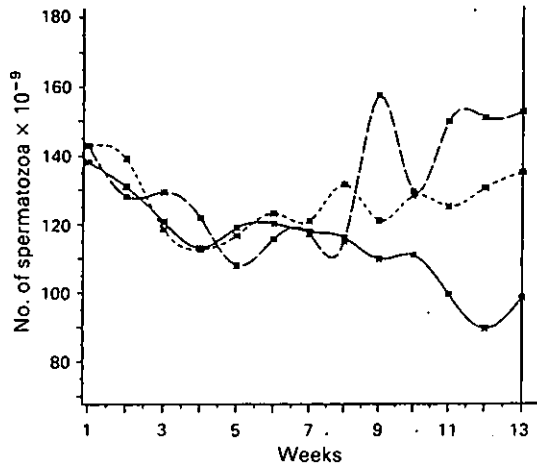


Fig. 3. Number of sperm cells produced per week for boars fed a high (---), a medium (---) or a low (—) level of commercial sow diet.

Age

Semen production in boars is directly related to age. Ejaculates from adult boars were superior to those from animals aged less than 9 months in terms of volume, sperm concentration and potential number of insemination doses (Kennedy & Wilkins, 1984). Fluid and gel volume did not increase much after 12 months of age. The daily sperm output gradually increased with age, being higher in 18-month-old animals than in younger ones (Swierstra, 1973, 1974; Cameron 1985a). According to Greenberg & Mahone (1981) sperm quality (the percentage of morphologically normal spermatozoa increased during pubertal development. However, in a different study, the percentage of morphologically abnormal spermatozoa gradually increased with age from puberty onwards (Bach *et al.*, 1982). Increasing age resulted in a higher percentage of abnormal spermatozoa, while both pregnancy rate and litter size decreased (Stemmler *et al.*, 1982).

Testis size

Testis size is related to the age of the animal and varies seasonally. In the wild boar the testis weighs significantly more during winter than in the summertime. The autumnal increase is inversely proportional to the change in daylength. This increase paralleled an increase in sexual activity and plasma androgen concentrations (Mauget & Boissin, 1987). In the adult domesticated pig testis size may also vary with season in that testicular length was increased during winter months relative to that during the summer (Trudeau & Sanford, 1986). In young domestic boars testis measures were positively correlated with testis weight, spermatogenic activity, and endocrine characteristics. Testicular size was also influenced by breed or strain (Schinckel *et al.*, 1983, 1984a, b). A significant correlation between testis weight at puberty and the production of spermatogenic cells exists. This was also observed for testis size and sperm output in the adult boar (Hemsworth *et al.*, 1983a). However, in a different study testicular width and length had only a very weak correlation with daily sperm output (Cameron, 1985a). In boars of similar age and weight the differences in testicular width as measured in the live animal will be only slight and have no potential to predict differences in sperm production rates. It remains arguable whether sperm production can be positively correlated with testis size in adult boars kept at commercial AI centres. Such a relationship could not be detected in some other species, e.g. older bulls (Carter *et al.*, 1980).

Unintended or uncontrolled influences

Factors affecting the health status of the boar can cause a decrease in sperm production, both qualitatively and quantitatively, especially when a period of high fever occurs. For example, an infection with pseudorabies virus, either spontaneously or after vaccination, decreased sperm production (Larsen *et al.*, 1980; Hall *et al.*, 1984).

Another influence on sperm production is caused by biotechnical and laboratory handling techniques. Technician and day of the week significantly influence ejaculate volume and sperm concentration (Kennedy & Wilkins, 1984). When the ejaculate was transported to the laboratory, diluted and re-evaluated, the quality of the diluent, the duration of storage, and the temperature greatly influenced the sperm quality (Bamba & Cran, 1985; Johnson *et al.*, 1988).

Conclusions

Many factors influence semen quality and quantity, some of which are specified above. Routinely, the following parameters are studied when an ejaculate is obtained: volume (or weight, as a rapid and accurate estimate of volume), sperm concentration, sperm motility, and sometimes sperm morphology. The ultimate assessment of sperm quality and production is by inseminating sows and monitoring the number of pregnancies and number of piglets born per litter.

Circumstantial and technical factors can markedly influence the ejaculate as it is produced. In addition, handling the ejaculate in the laboratory may influence the measures recorded. Incidental and total sperm production can be quantified by quite reliable methods. Parameters that are measured *in vitro* to predict fertilizing capacity *in vivo*, such as sperm motility, are more markedly influenced by methods of handling and processing the ejaculate. Furthermore, most of the routinely used indicators, as well as others assumed to have predictive value in assessing fertilizing capacity, have not been evaluated *in vivo* to a satisfactory extent.

To develop in-vitro methods to predict biological quality *in vivo*, new techniques are currently under investigation or will have to be developed. Some of them may be available for routine use in the future, while others may possess merits as investigative tools for research purposes only.

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