Chapter 16
Reproduction

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This chapter deals with:

- Attainment of puberty in sows and boars
- The sow's oestrous cycle
- Influence of environmental cues on reproductive performance in sows and boars
- Effect of nutrition and metabolic status on male and female reproductive performance
- Interactions between nutrition during gestation and lactation
- Quantitative energy and nutrient supply for gestating sows

1. Introduction

Reproduction is an extremely important trait in pig production. To maintain a high reproductive performance breeding animals should reach puberty at an appropriate age or weight, that they are bred successfully, that gilts and sows give birth to large litters with well developed and uniform piglets with good survivability, that they produce enough milk to support rapid piglet growth during the suckling period and then, when the piglets are weaned, that the sow returns into oestrus within a short time-period, i.e. that the weaning-to-oestrus interval is short. Furthermore, breeding animals should have a long productive life-span, i.e. they should produce several litters before they are culled. Reproduction is a very demanding life stage, and as illustrated in Figure 4.3, Chapter 4, litter size in Danish pig production over the last 20 years has increased profoundly and therefore, breeding of modern prolific pigs is a challenging task for the farmer. This chapter aims to describe the reproductive processes in males and females, and factors that may affect the reproductive performance. The main focus will be on how nutrition can influence female reproduction, because nutrition strongly determines reproductive outcome, and female reproduction is more quantitatively demanding than male reproduction. Furthermore, there are strong interactions between nutrition during gestation and lactation, and therefore the impact of nutrition during different parts of the reproductive cycle will be addressed.

2. Male reproduction

2.1. Breeding characteristics

Breeding boars make up a relatively small part of the pig population, but their importance for herd fertility is evident. A successful breeding boar is characterised by its libido, the number of sperm cells it is capable of producing in a given time, and the quality of these sperm cells in terms of their fertilizing capacity. The libido is a measure of how willing and successful a boar is to mate, and it can be expressed as the proportion of successful mountings in relation to the total number of mounting efforts. For a mounting to be successful it must produce an ejaculate either in a sow or a dummy. The number of sperm cells produced can be determined by counting sperm cells in an ejaculate under a microscope, or after slaughter by quantitative histology of testicles and epididymis. Assessment of the sperm motility in a single ejaculate has suggested an average of 70%, and the occurrence of morphological abnormalities varied between 5 and 13%. The outcome of sperm count studies can, however, be affected by the mating frequency that has preceded the determination. Therefore, comparisons between boars should only be made if the animals have had the same, sufficiently high, mating frequency. Semen quality can be evaluated by microscopic procedures in which the motility of the sperm cells and their morphology are assessed, but most importantly it can be evaluated in terms of fertilization rate and litter size [18], [32].

The boar has four accessory glands: the seminal vesicles, the prostrate gland, the bulbourethral gland and the ampulla. These glands add secretes to the spermatozoa in connection with ejaculation. The secretes contain a variety of substances that can protect semen against oxidation or may facilitate the passage of spermatozoa in the female genital tract or have other, more or less well-known functions. Boar semen is characterised by a large volume (average 225 ml gel-free portion, but ranging from 150 to 500 ml) and low sperm concentration. The ejaculation is comparably time consuming (10 – 20 minutes) and the semen is deposited in the uterus of the sow [57].

2.2. Spermatogenesis and its hormonal control

Spermatogenesis: The process of formation of sperm cells is called spermatogenesis, and it takes place in the seminiferous tubules in the testicles. In this process a stem cell, a spermatogonium, is transformed into a spermatozoon. The wall of the seminiferous tubule is lined with spermatogonia, and it is here the process starts. The final step includes the release of mature spermatozoa in the lumen of the seminiferous tubule. The spermatogenesis starts with mitotic cell division, then follows meiotic division, and the final step is differentiation of the spermatid, which, following the meiotic step, is haploid. Four classes of spermatogonia have been classified: undifferentiated Type A, differentiated type A, intermediate spermatogonia (In) and type B spermatogonia (B). The mitotic cell proliferation starts with an initial division of a spermatogonium. One of the resulting cells does not divide or differentiate any further, but remains in a basal state and replaces the original spermatogonium, whereas the other type A spermatogonium continues to divide and forms other generations of type A spermatogonia. In this way, the mitotic cell division produces a large number of germ cells. Contrary to in the female where the number of germ cells decline throughout the reproductive life, because mitosis ceases at birth, the process in male animals is maintained and the supply of germ cells is continuously replenished. The number of mitotic cell divisions is species-specific, forming a specific number of generations of type A differentiated spermatogonia. The boar has 6 divisions (A_1 – A_4, In, B), producing 68 spermatides per A_1 type spermatogonium. Type A spermatogonia then divide to form intermediate spermatogonia and those divide into Type B spermatogonia. Type B spermatogonia divide in the last mitotic cell division to form primary spermatocytes. When the primary spermatocytes have been formed, their development is stopped for a period while they enter the first prophase of meiosis. In the pig, about ten generations of spermatogonia (undifferentiated and differentiated) are necessary to form this stage of primary spermatocytes from one spermatogonia stem cell.

In the meiosis, which is the second important step in spermatogenesis, the chromosome number is reduced to the haploid state, and secondary spermatocytes are formed. The meiotic division is concluded by a second meiotic division, which results in formation of spermatids. Up to this step the differentiation process is called spermacytogenesis. However, a final third step is required for spermatogenesis to be completed. In this step, which is called spermiogenesis, spermatids mature into spermatozoa. During this maturation process a tail is formed, which helps to increase the movement of spermatozoa in the female genital tract. This movement will consume energy, and to provide the spermatozoa with the necessary fuel mitochondria are developed. The third important maturation process is the development of the acrosome, which is an organelle that allows penetration of the oocyte. Spermiogenesis, which commences in the seminiferous tubules, is completed in the epididymis. The epididymis is important both for maturation and storage of spermatozoa. Only when spermatozoa have passed through the epididymis they will be able to move and to penetrate an oocyte. The concentration of spermatozoa is very high in the tail of the epididymis, which is the storing site of about 80% of the mature spermatozoa. If ejaculation does not occur, no resorption is possible and the fate of the spermatozoa is to be discharged with the urine.

Spermatogenesis occurs in cycles, so-called spermatogenic cycles, which are initiated at regular intervals, which for the boar is 8 days. The duration of spermatogenic cycles is related to that of the complete spermatogenesis with a factor around 4 in most species, with values of 25 or 34 days being reported for pigs. A phenomenon called spermatogenic oordinates spermatogenesis to certain areas of the seminiferous tubules, so that all spermatogonia in a limited longitudinal area are programmed to begin dividing at the same time. By these spermatogenic waves spermatogenesis occurs as a continuous process, always providing mature spermatozoa.

Sertoli cells make up the other major cell type in the seminiferous tubule. They have important regulatory functions in the control of germ cell development, including nutritive function. From spermatogonia, which are located in the basal compartment of the tubule, maturating spermatides move through tight junctions that provide a protected and specialised environment between Sertoli cells, and their development is completed in the central compartment of the tubule. When the maturation process is completed the Sertoli cells control the release of spermatozoa into the lumen of the seminiferous tubule. The Sertoli cells also secrete fluid to the lumen of the tubule as well as proteins and growth factors. Another role of the Sertoli cell is phagocytosis of degenerating germ cells and excess cytoplasm remaining from released sperm. This entire section was based on Stabenfeldt & Edqvist [57] and Hess and Renato de Franca [29].

Hormonal control of spermatogenesis: Two cell types, namely the Leydig cells and the Sertoli cells, are involved in the hormone production in the testis. The main function of the Leydig cells, which are located in the interstitium (interstitial cells) outside the seminiferous tubule, is the production of testosterone. Development and maintenance of spermatogenesis is dependent on testosterone production, and it also plays a similar role for male sex characteristics. Testosterone production in the Leydig cells is controlled by the gonadotrophin luteinizing hormone (LH) that is released from the anterior pituitary in a pulsatile manner. Another effect of LH on the Leydig cells is that it stimulates them to undergo hypertrophy, but, on the other hand, if LH is removed testosterone production ceases and the Leydig cells decrease in size. Testosterone secretion is regulated by a sensitive feedback mechanism between testosterone and LH: Increased secretion of LH is followed by increasing secretion of testosterone, which occurs 30 to 60 minutes after the rise in LH, and these elevated levels of testosterone can last from 1 and up to several hours. Elevated levels of testosterone inhibit LH by a negative feedback response, and as a consequence testosterone levels decline. Testosterone moves from the Leydig cells to the seminiferous tubules, and to the bloodstream. High concentrations of testosterone in the testis are a prerequisite for maintained spermatogenesis, whereas the circulating concentrations in the blood vascular system contribute to development and maintenance of libido, to stimulate production of secretes from the male accessory organs, and for development of male body characteristics [57].

The Sertoli cells also have secretory activity, and this is controlled by follicle-stimulating-hormone (FSH). The Sertoli cells possess membrane receptors for FSH and cytoplasmatic and nuclear receptors for androgens. In the Sertoli cell, testosterone produced by the Leydig cells is converted to oestrogens. Boars have high concentrations of oestrogen in plasma and testes, but it is presently not completely clear how this hormone is involved in feedback regulation of the gonadotrophins and local testicular hormone production, but it has been concluded that oestrogen does not appear to play a regulatory role in gonadotrophin secretion in developing boars, unlike in many other species.

Another function of the Sertoli cells is to produce inhibin, a protein hormone that suppresses FSH secretion. FSH concentrations therefore tend to be inversely related to spermatogenic and Sertoli cell activity, because when Sertoli cell activity is high inhibin is produced. A high plasma FSH value in a male may therefore indicate that Sertoli cell activity, and possibly also spermatogenesis, are depressed.

As appeared above, spermatogenesis cannot be maintained by testosterone alone, but requires the gonadotrophins LH and FSH. LH is necessary because of its role in testosterone production, and FSH has an important function in Sertoli cell activity, which influences the completion of meiosis of germ cells. Unless otherwise stated, this entire section was based on Stabenfeldt & Edqvist [57].

2.3. Puberty

Puberty announces the onset of sexual activity, and in male animals puberty is commonly associated with the first wave of spermatogenesis being completed. A prerequisite for puberty to occur is that the hypothalamus – pituitary – gonadal axis undergoes a maturation process, decreasing the sensitivity to negative feedback by oestrogen. In young boars of most domestic pig breeds, puberty occurs between 5 and 8 months of age, and when the body weight is 80 – 120 kg, but mounting and ejaculation have been reported to occur in boars considerably younger and lighter than that. Fertility has been reported to be low in very young boars, but increasing and reaching its maximum when the boars are 15 – 18 months old. There are differences in the age at onset of puberty between crossbred and purebred pigs, and it has been reported that crossbred pigs may attain puberty as much as 40 days earlier than purebred. It has been stated that age is more determining for attainment of puberty than weight, but many studies have demonstrated a clear impact of energy and protein supply on age and weight at puberty.

Most studies on the influence of energy supply on age and weight at puberty are relatively old, but they show clear trends that still are relevant. In the late 1950s, a study where young boars were fed 100, 70 or 50% of National Research Council (NRC) requirements from weaning onwards reported that onset of puberty was at 203 days for the animals fed to the requirement and was delayed until 212 days when 50% of the NRC requirement was fed. The body weights at puberty, though, were more affected being 101, 78 and 61 kg on the three different levels of energy supply, respectively. Similar results were reported about 20 years later from a study in which purebred and crossbred pigs were fed according to the NRC requirement or 30% below that. Again, the reduced feed intake resulted in a delay of puberty, and animals that were restrictedly fed reached puberty at a lower weight than those fed according to the requirement. Purebred and crossbred animals reacted somewhat differently, puberty in the purebred boars being delayed by 47 days to be compared with only 30 days in the crossbred ones. In both these studies, animals on the lowest energy supply had a 30% lower semen volume at puberty than other groups, but the semen quality and fertilizing capacity was similar among treatments. Collectively, these studies demonstrate that onset of puberty in young boars is dependent on energy intake, and that restricted energy supply delays puberty, but that restrictedly fed animals reach puberty at a lower weight than animals fed to the requirement, and that a restricted intake during rearing delays puberty less in crossbred than in purebred boars.

Also the level of protein supply is determining for the age at onset of puberty. The relatively few studies available suggest that protein restriction delays puberty (193, 182 and 177 days), but that body weight at puberty was similar among treatments (88 kg) when the protein supply was 12.0, 18.0 and 23.0%, respectively. The semen quality was similar among treatments, although boars with the lowest protein supply produced 50% fewer sperm cells per ejaculate than boars on the other treatments. Other studies have compared the sexual development in boars fed diets containing 10.0, 15.0 and 20.0% crude protein, and findings suggest that the sexual development is finished at 230 days of age, and that after this age the 10% crude protein diet sustained normal development of reproductive organs and gonadotrophin levels. Another study with the same levels of protein supply found that the diets with the highest protein supplies resulted in higher pituitary contents of FSH and LH at 230 days but not in one-year-old animals. The findings of these studies suggest that low protein supply can retard puberty, but that the effects do not last or have an impact on reproductive performance later in life. This section was based on review by Close & Cole [18] and Kemp & Soede [32].

2.4. Environmental cues

Environmental cues may exert a positive or negative influence on reproduction. Examples of environmental cues that might be of importance for the breeding boar are environmental temperature, photoperiod, housing conditions and the “female effect”, i.e. the presence of sows. In this context, only the effects of environmental temperature and photoperiod will be addressed, because they are considered to be most within the scope of this text.

Environmental temperature: Boars may be exposed to varying environmental temperatures, and while within their thermoneutral zone no influence on reproductive performance can be expected. Boars are, however, often kept under conditions of low stocking density, and may be kept in buildings that are poorly or not insulated at all. This will affect their lower critical temperature (LCT), and when environmental temperature decreases below the LCT, the energy supply must be increased in order to compensate for the heat loss caused by thermoregulation; otherwise the boar will lose body condition, and in extreme situations that might influence the reproductive performance. The LCT decreases with increasing body weight and feeding intensity. For a 250 kg boar kept at maintenance energy intake and in draught-free conditions and on a well insulated floor, the LCT is approximately 20 °C. When temperature falls below LCT, the heat loss will increase by 16 kJ/kg $BW^{0.75}$ · °C a day, and the boar must compensate for all thermal losses, but when doing so, environmental temperatures below LCT do not affect reproduction [18].

Heat stress, on the other hand, may have a detrimental effect on boar sperm production and semen quality. Normally, the temperature of the testes and epididymis is kept about 2 °C below that of the body by means of a system where venous blood leaving the testes is cooling arterial blood entering the testes. Furthermore, testes can be pulled close to the body for warmth, but in a heat stress situation they can be lowered for cooling. If boars are exposed to heat stress, however, these mechanisms are not sufficient, and it seems that the critical temperature, when semen quality is negatively influenced is 29 °C, but heat stress conditions must prevail for at least two or possibly even six weeks before the negative effects become manifest resulting in impaired semen production and decreased fertilizing capacity. After ended exposure to heat stress, another five weeks are required for semen quantity and quality to return to normal values [18].

Photoperiod: The European wild boar is a seasonal breeder with its breeding season in early winter, i.e. it is a short-day breeder. Piglets are born in late spring, and the reproductive season is followed by a period of reproductive quiescence, mainly dictated by photoperiod [38]. Effects of photoperiod have also been shown to affect sexual maturation of young crossbred domestic boars: animals reared under an autumn-winter light regimen (short-day conditions) had higher plasma concentrations of testosterone, heavier bulbourethral glands and a considerably higher frequency of animals with a mature spermatogenesis at slaughter at 150 days of age than animals reared

under a spring-summer photoperiod (long-day conditions) [1]. These findings suggest that despite the domestic boar breeds throughout the year photoperiod may affect fertility, and reminiscences of seasonal reproduction still remain.

2.5. Nutrition of the working boar

Because of the relatively small numbers of working boars in relation to the total pig population not much attention has been paid to their nutritional demands and to their optimal feeding. Therefore, the knowledge on the nutritional requirements of breeding boars is rather scanty. Taking into account that the working boar is expected be a healthy individual able to perform a large number of fertile mountings and to have good general longevity, feeding must support these aspects. Given the prevailing selection criteria for pigs, it is fair to assume that boars selected for breeding have a high capacity for lean tissue growth. Then when considering that ad libitum feed intake and maximum growth rate may predispose the animal to leg weakness, it seems reasonable that optimal feeding of growing future breeding boars must be different from that of growing-finishing pigs.

Studies on effects of nutrition on semen production and quality and its fertilization capacity must take into account that spermatogenesis is a time-consuming process, and, in addition to the 25-34 days estimated to be required to complete spermatogenesis, the passage of spermatozoa through the epididymis takes another 10 to 14 days. Therefore, any effects of nutritional manipulation cannot be expected to appear earlier than about 6 weeks after the start of the experimental treatment. Furthermore, the frequency of semen collection may affect the results, so a proper experimental protocol must make sure that semen is collected at similar intervals in all treatment groups, and that collection frequency is sufficiently high.

Over the years, it seems that the supply of protein, and specifically the amino acids lysine and methionine plus cystin, has attracted most attention when evaluating the nutritional demands of working boars, but also effects of level of energy supply have been investigated. Studies have also concerned effects of supply of some minerals and vitamins. Here a brief review of the most evident trends found in available research will be presented. Highly varying dietary crude protein (CP) contents have been evaluated, from about 7% and up to slightly above 28%. In a single study, the high (28.3%) CP diet supplemented with either L-lysine (12 g/boar · day) or DL-methionine (16 g/boar · day) resulted in higher sperm output than a diet with lower protein content (19.3% CP) or the unsupplemented high CP diet when boars were kept at a high semen collection frequency. The semen quality, however, was not different among dietary treatments. Other studies have not corroborated these results: when dietary CP ranged from 15.8% to 18.4% and different levels of lysine and methionine supply were practised no effects on sperm output or semen quality were found, but in these studies the boars were used at a lower frequency than in the study described above. A low-protein diet (7.3% CP, 0.31% total lysine) compared with a diet containing 16.2% CP and 0.83% total lysine resulted in impaired reproductive performance in terms of significantly reduced libido, lower semen volume per ejaculate and lower testis volume, but semen quality was not affected. The low-protein diet did not cause any changes in LH (before and after GnRH challenge) or testosterone, but lower plasma concentrations of oestrogen. These and other results have suggested that low oestrogen concentrations may be causative for impaired libido and reduced semen volumes. The lowest documented level of protein in a working boar diet that did not cause negative effects was 14.5% CP, 0.68% total lysine and 0.44% total methionine plus cystine at a dietary energy concentration of 12.56 MJ ME/kg. Collectively, these investigations suggest that diets with a protein content lower than 14.5% may cause negative effects on boar libido and semen volume, but semen quality is not likely to be negatively affected. Meanwhile, there seems to be no beneficial effects of feeding diets with very high protein content.

Energy supply seems to have little influence on semen output and characteristics unless the energy provision is very poor. For instance, if boars were fed from 1.2 to 1.9 times the maintenance energy requirement (MEm) no differences among treatments were detected. In a study where boars were fed on low, medium and high (ad libitum) energy supply, corresponding to 1.0, 1.8 and 2.9 times MEm, the number of sperm cells ejaculated per week started to decline in boars on the low energy supply after 8 weeks on the experimental treatment, but the semen quality was not affected. After completion of the first 12 weeks of the experiment, the low group was transferred to medium energy supply, while the group on medium supply was kept on the same energy supply for the rest of the experiment. After 7 weeks on the medium energy supply, sperm output started to increase in the group given low energy supply in the first part of the experiment, and by week 8 the differences between this and the medium group were alleviated. Based on available experimental evidence it seems that adult boars are at risk of experiencing detrimental effects on semen output and/or libido if fed levels below 1.4 times MEm. Feeding on a high level of energy supply may, however, have negative effects in terms of boars getting too heavy, which increases the risk of leg weakness problems.

The energy costs of reproduction are limited: mating activity (mounting a dummy sow) has been estimated to increase the heat production by 18 kJ/kg$^{0.75}$, which corresponds to less than 3% of MEm for a 200 kg boar with a daily ME requirement for maintenance and growth of 35.18 MJ ME. The energy contained in an ejaculate is likewise almost negligible: assuming one service per day and an ejaculate volume of 250 ml with an energy and protein content of 1.04 MJ and 37 g/kg, respectively. The major energy yielding substrates in the ejaculate are fructose and citric acid, and the efficiency of utilization of carbohydrates 0.6, which would give an energy cost of 0.43 MJ ME for semen production, hence slightly more than 1% of MEm.

Regarding mineral and vitamin supply, some specific precautions can be taken. A strong skeleton is a necessity for a working boar, and available experimental data suggest that bone mineralisation and leg strength can be improved if Ca and P are supplied above the minimum requirements recommended by NRC for growing pigs, and NRC [40] recommends that boars over 50 kg live weight and breeding boars be given 7.5 g Ca and 6.0 g P per kg diet, assuming a feed intake of 2.0 kg per day. Zinc plays an important role in spermatogenesis, and in zinc-deficient animals Leydig cell development may be retarded and the response to LH as well as testicular steroidogenesis reduced. The recommended dietary supply is 100 mg/kg diet [40]. Selenium has important antioxidative effects and works in concert with vitamin E that protects cell membranes from oxidation. Both serve as antioxidants in boar semen, and they can affect both semen volume and quality characteristics such as motility and fertilization capacity. For all these traits, the positive effect of supplementation is stronger for Se than for vitamin E, and a deficiency in Se exerts stronger effects than a vitamin E deficiency. NRC [40] suggests an allowance of 0.3 mg Se/kg diet to breeding boars. Furthermore, without being based on experimental information from pigs, NRC [40] recommends an allowance of 200 ppb Chromium per kg diet. The recommendation is based on the effect of Cr for improving glucose tolerance and its stimulating action on insulin, and the observation that it has enhanced sperm count and fertility in male rats. The above minerals are the only where the requirements of the working boar may be different from those of sows.

As mentioned above, vitamin E may influence boar reproductive performance positively because of its antioxidative properties, but it cannot replace Se and its effects are generally weaker. Supplementation of boar diets with biotin has been speculated to reduce the incidence of foot lesions, and some positive effects have been documented in sows. The precise mode of action of biotin in prevention of foot lesions is not known, and despite lack of experimental support NRC [39] recommends an allowance of 0.3 mg per kg diet to be raised to 1.0 mg if foot problems are persistent. As appeared in this paragraph, recommendations of additional supplementation of vitamins to working boars are not based on strong experimental evidence. This view was corroborated in a recent study [4] in which boars were supplemented with fat soluble vitamins (3 to 5 times the content in the control diet) and water soluble vitamins (10 times the content in the control diet) for a prolonged period. Semen collection frequency was varied systematically from moderate to very frequent (daily collection). Although extra vitamin supplementation resulted in increased levels of

most water soluble vitamins and vitamin E in plasma, and although most water soluble vitamins were transferred to seminal plasma, and direct transfer of vitamin E to spermatozoa occurred, the vitamin supplementation did not result in any effects on sperm production or semen quality, regardless of semen collection frequency. This being an experiment with a relatively large number of animals, and partly performed under commercial conditions would support the view that additional vitamin supplementation to working boars is not very likely to have beneficial effects on reproductive traits. Unless otherwise stated, this section was based on Close & Cole [18] and Kemp & Soede [32].

3. Female reproduction

3.1. The oestrous cycle and its hormonal regulation

The sow is a polyoestrous breeder and the normal length of an oestrous cycle is 21 days. The oestruous cycle can be divided into a follicular phase, during which follicles grow and mature in the ovary, and a luteal phase, following ovulation and characterised by development of corpora lutea (CL). The different phases of the oestrous cycle can be described in more detail: prooestrous is the period of rapid follicle growth that precedes the onset of oestrous. In the pig this period occurs after the CL have regressed. During oestrous, the sow is sexually receptive, i.e. it shows swelling of the vulva, its behaviour may change so that it is restless and has decreased appetite. The sow also shows standing reflex and allows the boar to mount. Tools in oestrous detection include visual observation of the swelling of the vulva, and the back pressure test, to which a sow in heat responds with rigidity. Ovulation usually occurs late in oestrous, and in pigs spontaneous ovulation takes place some 36 to 42 hours after the onset of oestrous. Ovulation usually occurs in both ovaries, and the ovulation rate exceeds the uterine capacity. Because of high fertilisation rate early embryonic losses can be high. At the end of oestrous, the metoestrous starts and this period is characterised by the early development of the CL. Dioestrous is a period with mature luteal activity and it ends with the regression of the CL. The luteal activity in the sow lasts about 14 days, and the timeframe from regression of CL to a new oestrous is 5 – 6 days. Hence, the duration of each of the phases of the oestrous cycle in pigs is approx. 3 days for prooestrous, 2 days for oestrous, 4-5 days for metoestrous and 10-12 days for dioestrous. If the sow does not conceive, a new 21 days cycle starts, but if the sow is fertilised the fertilised ova will enter the uterus about 3 – 4 days after conception. Implantation of the embryos in the uterus occurs 14 – 20 days after conception and an epitheliochorial placenta is formed. The length of gestation is 113 – 115 days. This paragraph is based on Stabenfeldt & Edqvist [57].

3.1.1. Hormonal control of the oestrous cycle

The oestrous cycle is controlled by the interplay between steroid hormones produced by the ovary and protein hormones synthesised in and released from the anterior pituitary gland. The main ovarian hormones are oestrogens, the primary ones being oestradiol-17β and oestrone, and progesterone. Oestrogen production occurs in the granulosa cells of the ovarian follicles, in the foetoplacental unit and in the adrenal cortex. Progesterone is produced by the CL, by the placenta and by the adrenal cortex. Both oestrogen and progesterone synthesis is controlled by hormones from the anterior pituitary gland: ovarian oestrogen synthesis is controlled by FSH and LH, and progesterone synthesis by the CL is controlled by LH in non-gestating animals and also by prolactin (PRL). The steroid hormones are lipid-soluble, and therefore they are transported bound to plasma proteins, and only a minor amount of the hormones (less than 5%) occur in free form.

Both oestrogens and progesterone have several important roles in the organism. Oestrogen stimulates growth of endometrial glands, which are necessary for maintenance of the zygote before implantation. It also stimulates growth of ducts in the mammary gland, and it causes secretory activity in the oviduct, a function that improves the survival of oocytes and spermatozoons. In relation to oestrous, it is oestrogen that initiates the sexual receptivity, and it also has a role in ovulation by regulating gonadotrophin secretion, including the pre-ovulatory LH surge that triggers ovulation. It may also be involved in the release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the non-gestating uterus and from the gestating uterus at parturition, in both cases causing regression of the CL. Other functions not directly related to reproduction include initiating closure of the epiphyseal growth plate that causes cessation of growth of the long bones, stimulation of protein anabolism and being epitheliotrophic. Also progesterone causes several important tissue responses: it promotes endometrial gland growth, and lobuloalveolar growth in the mammary gland; it affects the secretory activity of the oviduct and endometrial glands so that nutrients to support the zygote before implantation are produced. Another important function of progesterone is to prevent the gestating uterus from contracting, and it is also involved in the regulation of secretion of gonadotrophins. Progesterone often acts in synergy with oestrogen, and oestrogen priming is often needed. The steroid hormone synthesis rate may vary: the mature CL produces progesterone at a stable rate, whereas there is a steady decline during the 36 hours following the initiation of luteolysis. Oestrogen production shows a steady increase in the developing follicle, but oestrogen production in the granulosa cells declines to basal concentrations within 24 hours in concert with the pre-ovulatory LH surge. Steroid hormones have a rapid metabolic clearance with turnover rates of 20-30 minutes.

Female reproductive processes depend on three hormones produced by the anterior pituitary gland: FSH, LH and PRL. FSH and LH act in a synergistic way, but their main functions are different. The main action of FSH is to stimulate follicle growth. LH has an important function in the ovulatory process, and it is also involved in the luteinisation of the granulosa, resulting in the formation of the CL. The main action of PRL is in the development of sectretory tissue in the mammary gland and the maintenance of lactation. The protein hormones have receptors located on the surface of their target cells which they bind to. When the protein hormone binds to its receptor, it activates the enzyme adenyl cyclase, which converts ATP to cyclic AMP, which in turn activates intracellular protein kinases that activate the enzyme systems that allow for achieving the physiological effect of the hormone. Secretion of the anterior pituitary protein hormones FSH and LH is controlled by the hypothalamic 10 – amino acids peptide, gonadotrophin-releasing hormone (GnRH). However, both synthesis and release of LH are much more responsive to GnRH than those of FSH. GnRH is released in a pulsatile manner, and it is clear that this pulsatile secretion is necessary for the maintenance of LH and FSH secretion, and in response to one GnRH pulse one pulse of LH is released. Therefore, changes of the pulse frequency or amplitude of GnRH will be mirrored by changes in the LH pulse frequency and amplitude. The secretion of FSH and LH can also be affected by changing the sensitivity of the anterior pituitary to GnRH pulses through modifying effects oestrogens and progesterone. Progesterone generally has an inhibitory effect, whereas the general effect of oestrogens is stimulatory. However, steroids, and particularly oestrogens, can affect the release of gonadotrophins both positively and negatively, caused by phenomena called positive and negative feedback, respectively. Oestradiol - 17β can exert negative feedback control of gonadotrophin release, i.e. it suppresses the release of LH and FSH. The negative feedback control occurs in response to exposure to basal or very low concentrations of oestrogen for a short period. In contrast, under the influence of exposure to gradually increasing high concentrations of oestrogen for a prolonged period a positive feedback response occurs, and it results in the release of a surge of gonadotrophins, and the pre-ovulatory LH surge occurs in response to such positive feedback (see Figure 16.1).

Also PRL is released in a pulsatile fashion, but the control of its release is dependent on PRL inhibitory factors, one such being dopamine and another being γ – aminobutyric acid (GABA). Therefore, when the secretion of dopamine or GABA, decreases, the release of PRL increases. This section is based on Stabenfeldt & Edqvist [57].

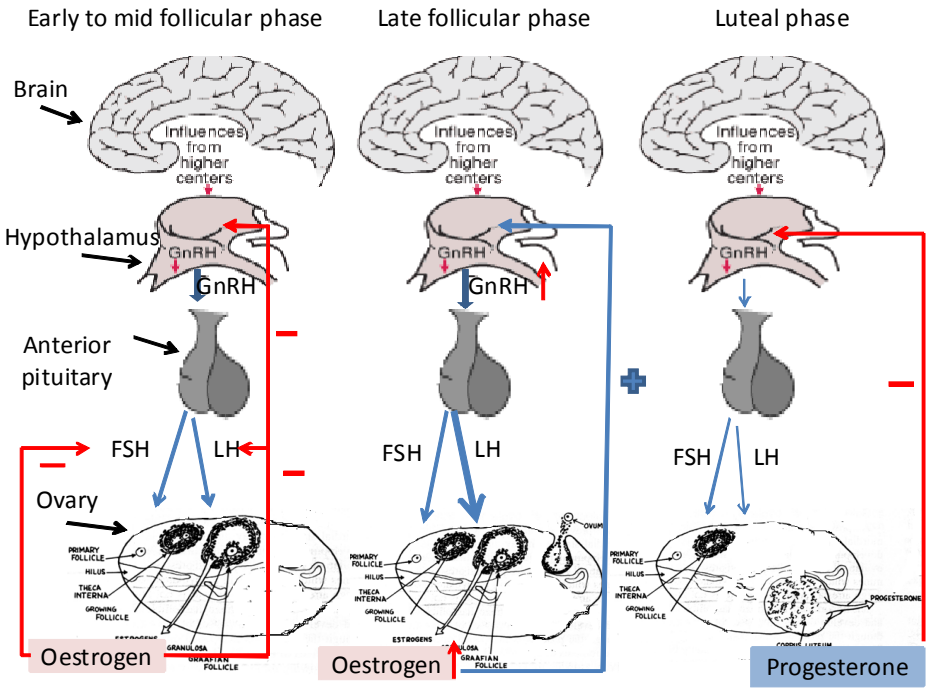

Figure 16.1. The hypothalamus-pituitary-ovarian axis, and its hormonal regulation during different phases of the oestrous cycle. Red arrows with minus sign depict negative feedback and blue arrow with plus sign positive feedback.

3.1.2. Folliculogenesis

The embryonic ovary produces gametes through mitotic division of primordial germ cells. This process ceases at birth, so at that point in time the neonatal female has the maximal number of oocytes it will possess in its entire life. Meiosis in these oocytes soon starts, but it only progresses to the dictyotene (resting stage), and further meiotic division is only resumed at the onset of puberty.

The resumption of meiotic division is without influence of gonadotrophins, and the initial follicle development is hormone independent. During this early development the follicle increases in size and activity, including RNA and ribosome production. The oocyte was initially surrounded by follicular cells, but at this stage they start to grow and divide and are transformed to granulosa cells. The granulosa cells then form the zona pellucida, which is a glycoprotein layer immediately around the oocyte. Theca cells are vascularised and they supply the granulosa and oocyte with nutrients. Now, at the end of the hormone-independent stage, the follicle is still pre-antral.

For the follicle to enter the hormone – dependent stage, synthesis of receptors for FSH and oestrogen in the granulosa and for LH in the theca is necessary. The theca, influenced by LH, produces androgens, which under the influence of FSH, are converted to oestrogen in the granulosa. The follicle's responsiveness to steady amounts of FSH in plasma increases, because FSH induces its own receptors. The mitogenic capacity of oestrogens causes further growth and division of the granulose, and the granulosa starts to synthesise substances that cause cell separation, resulting in the formation of a space called an antrum. The follicle has now developed to an antral follicle which consists of the oocyte and surrounding supporting cells. The granulosa produces a protein hormone, inhibin, which inhibits follicle growth. During the late part of follicle development and just before ovulation, inhibin exerts a progressive negative feedback inhibition of FSH synthesis and release. In the late follicular phase, FSH induces receptors for LH in the granulosa whereas LH, mainly during the pre-ovulatory LH surge, decreases the number of FSH receptors in the granulosa. These changes in receptors are important for the change of granulosa from oestrogen

secretion in the follicular phase to progesterone secretion in the luteal phase. So far, this section is based on Stabenfeldt & Edqvist [57].

Follicle development is a process that goes on continuously in all parts of the oestrous cycle, even during the luteal phase, and every day a few follicles leave the primordial stage and start to develop. When development has started, the follicles can either continue to develop to ovulatory follicles or undergo atresia, which means regression and eventually destruction of the follicle. Prior to puberty and also up into sexual maturity most follicles are primordial, remaining dormant in a resting pool until a number of follicles are stimulated to grow each day. The follicles grow to reach primary follicle stage (~ 0.12 mm) and now the oocyte is full-sized and surrounded by one to three layers of granulosa cells. Then the follicle grows to a diameter of 0.14 – 0.40 mm and with 3 – 20 layers of granulosa cells, and has then entered the secondary stage. Up to this stage, the follicle has been hormone-independent. When the follicle grows beyond a size of 0.40 mm its size allows for formation of an antrum, and then it enters the hormone-dependent stage [34].

A crucial question regarding follicle development is: which follicles are going to ovulate and which are destined for atresia? Two terms apply to this: recruitment and selection. The population of small and medium-sized follicles that are present on the surface of the ovary make up the recruited follicles, and out of these some may be selected. The follicles that are selected are those that are going to ovulate. The recruited pool is made up by some 50 1 – 6 mm follicles. This group of follicles may be asynchronous in their stage of development, but they can be available for selection 5 – 7 days before oestrous, but very few of them will mature and ovulate. In the mature, cyclic gilt selection of follicles occurs while CL are present, and the follicle population is dramatically different before and after CL formation as well as before and after luteolysis. After ovulation, small and medium follicles appear rapidly and increase in size during the luteal phase, but large follicles are absent. After luteolysis, the small and medium follicles disappear, but as oestrous approaches, large follicles (>6.5 mm) appear and increase in number. The selected follicles derive from the recruited pool, and when selection occurs the follicle hormone dependence shifts from FSH to LH, and both small and medium follicles loose FSH receptors during the first 3 days of the follicular phase, whereas LH receptors increase in all follicle classes. During the first part of the follicular phase, most follicles are healthy, but on day 5 all small follicles have undergone atresia and the rate of atresia among medium follicles is high [34].

3.1.3. Ovulation

The pre-ovulatory LH surge induces important changes in the antral follicles during the 24 hours preceding ovulation: first, the pre-ovulatory LH surge contributes to the maturation of the oocyte by blocking for oocyte inhibiting factors. Thereby meiosis, which has been kept in check at prophase stage, can resume about 24 hours before ovulation. Secondly, with the surge release of LH, the granulosa becomes responsive to LH and starts to secrete progesterone, initiating the conversion of the granulosa to a luteal structure even before ovulation. The third event caused by the pre-ovulatory LH surge is that it contributes to induce ovulation by stimulating the production of intrafollicular substances that support the rupture of the follicle.

The ovary, hypothalamus and anterior pituitary are all involved in the occurrence of ovulation. The ovarian signal that the follicles are ready for ovulation is the increasing amounts of oestrogen produced by the maturing follicles. The hypothalamus responds to this by positive feedback and increases the frequency of the release of gonadotrophins, resulting in the pre-ovulatory gonadotrophin surge (Figure 16.1). Both high concentrations of oestrogen and a sufficient time of exposure are necessary for inducing the positive feedback response leading to the pre-ovulatory release of gonadotrophins. The large surge release of LH triggers the initiation of ovulation, but the release of LH occurs together with a small surge of FSH, which may be synergistic to the LH surge. The duration of the LH surge is about 24 hours in pigs, and the interval from the start of the LH surge

release to ovulation is about 36 hours. In each cycle, a large number of follicles are ovulated, the rate being in the order of 14-20, which often exceeds the capacity of the uterus.

3.1.4. Corpus luteum

The pre-ovulatory LH surge starts the conversion of the granulosa from oestrogen to progesterone secretion, which is called luteinisation. This is the starting point of CL formation. The CL life span is supported by progesterone secretion, and a sufficient progesterone secretion is necessary for maintaining gestation in pigs. In non-gestating pigs, the uterus is important in controlling the life span of the CL. The regression of the CL is initiated by the action of $PGF_{2\alpha}$, which is synthesised by the uterus and released in a pulsatile manner, starting about 14 days after ovulation. In the sow, increased $PGF_{2\alpha}$ secretion occurs several days before actual luteolysis starts, and it appears that the CL is unresponsive to $PGF_{2\alpha}$ until very late in the luteal phase. Declining concentrations of progesterone towards the end of the luteal phase seem to facilitate the release of $PGF_{2\alpha}$, and this enhanced release of $PGF_{2\alpha}$ contributes to complete the regression of the CL.

The $PGF_{2\alpha}$ synthesised by the uterus can either reach the ovary through the general circulation (general transfer) or be transferred from the utero-ovarian vein to the ovarian artery by local transfer mechanisms. In pigs, general transfer of $PGF_{2\alpha}$ occurs, and it means that each uterine horn can affect the life span of the CL on the opposite side ovary [57].

3.2. Puberty

The reproductive life of an individual is initiated with the attainment of puberty, and in female animals this means onset of cyclic ovarian activity and the first appearance of oestrous. Onset of puberty is a quite complex, centrally-regulated process, and all details of the mechanisms involved are still not completely known. As in males, the onset of puberty in females is preceded by maturation of the hypothalamus resulting in a decreased sensitivity to negative feedback inhibition of hypothalamic gonadotrophin release by oestrogen. Under conditions of negative feedback inhibition, very small amounts of oestrogen are sufficient to suppress gonadotrophin (LH and FSH) synthesis and release, and therefore follicles are unable to grow and develop to any significant extent. When puberty approaches, two main events occur: first, the sensitivity of the hypothalamus to oestrogen feedback decreases. This leads to an increased frequency of GnRH pulsatile release from hypothalamic neurons. The GnRH is transported through the hypophyseal portal veins to the anterior pituitary gland, where it stimulates synthesis and secretion of LH and FSH, which results in the start of secretion of gonadotrophin in significant amounts. Follicle development is stimulated by the higher frequency of GnRH, and it reaches antrum formation and finally maturation. Secondly, when oestrogen secretion increases it induces a positive feedback stimulation of the hypothalamus and the anterior pituitary, resulting in discharge of gonadotrophins. This first surge of gonadotrophins does, however, not lead to ovulation, but it causes a short-lived phase of progesterone secretion, probably from luteinised follicles. This short progesterone phase is followed by a new LH surge, which results in ovulation [57], and by that the gilt has attained puberty.

The transition from prepubertal anoestrous to puberty includes increase in the activity of the GnRH pulse generator and then in pulsatile secretions of FSH and LH, which will promote development of the gonads and gametes. GnRH is synthesised in the pre-optic area of the hypothalamus, and it is released in a pulsatile fashion at intervals of about 60 minutes in adult animals. In young animals, intervals between pulses are considerably longer - about 90 to 120 minutes. The onset of the pubertal processes seems to be activated by an increase in pulse frequency and amplitude, and total concentration of GnRH, which will result in activation of transduction signals to promote the synthesis and release of LH and FSH.The neuroendocrine control of puberty is genetically regulated, and it is presently suggested that three candidate genes are the main controllers of

pubertal processes. These genes are the OCT-2, TTF-1 and EAP-1 genes. Common for all these three genes is that their hypothalamic expression increases during pre-puberty or puberty, and that elimination of their expression leads to delay of puberty and other reproductive dysfunction [20], [39].

Gilts often reach puberty at an age of 200 to 220 days, but the individual variation may be considerable and extremes of 102 to 350 days have been reported. The onset of puberty can be affected by several factors among which age, genetic origin, social environment, boar exposure, season, nutritional status and body composition can be mentioned. However, the interactions between factors that affect age at attainment of puberty are not completely understood. Breed effects can be dramatic: among the Chinese Meishan, which is an unimproved highly prolific breed with high capacity for fat accretion, gilts reach puberty at about half the age compared to modern breeds [28]. Modern breeds with a large capacity for lean tissue growth have less appetite (see Chapter 19) and young animals have lower body energy reserves than breeds with less capacity for lean tissue growth. Therefore modern breeds can be anticipated to be more vulnerable if their nutritional demands are not adequately met.

3.3. Environmental cues

Boar effect: Follicular development and expression of oestrous behaviour in sows can be stimulated by boar contact. The stimulating effects of presence of a boar on sow oestrous behaviour include olfactory (the odour of the boar), visual (the sight of the boar), auditory (hearing the boar grunting) and tactile (the touch of the boar, e.g. rubbing of back and flank). The presence of a boar can stimulate gilts in reaching puberty earlier than gilts without boar contact. The “boar effect” may reduce the age at first oestrus with several weeks. [28].

Also mimicking of one or more of the boar stimuli may elicit responsive behaviour in the sow, and that is a strategy often used when artificial insemination is practised. When stimuli mimicking a boar are used, the most positive response has, in most cases, been achieved when a combination of stimuli has been used, and among the different stimuli tactile stimulation seems essential. However, a live boar is more potent for eliciting a response than mimicked stimuli, but when using boars the habituation aspect must be considered so that habituation of sows to a certain boar is prevented [35].

Boars excrete androgens that can act as pheromones, but it seems that these substances alone cannot exert a stimulatory boar effect. Olfactory stimuli from the boar probably involve a complex of pheromones, arising from different sources, and they do probably exert their effect in concert with other sensory inputs, such as auditory, visual and tactile. Tactile stimuli seem necessary for the olfactory cues to come into action [28]. Also visual stimulation, i.e. when the gilt or sow sees a boar, might have an effect on standing heat. Tactile stimulation, i.e. when the boar manipulates the sow’s abdominal, inguinal, vulva and pelvic areas, can elicit the standing response, and this cue has been considered as important in stimulating the gilt/sow [45].

Seasonal infertility: Reduced fertility during the summer months (“summer infertility”) is a frequently reported problem in pig production. Puberty in gilts may be delayed, and the weaning-to-oestrous interval may be increased causing farrowing rate to decrease. The present view is that affected sows first conceive and have embryos present for a short period of time, but that the embryos die and the whole litter is lost within a week of implantation. A cascade of events (see 4.6.1) leads to retarded embryos that are unable to produce enough embryonic signals required for the sow to recognise and maintain gestation [46].

Environmental temperature: High ambient temperatures have often been blamed as the cause of summer infertility, but it is very difficult to clearly ascribe the reproductive disorders to a single env-

ironmental cue, because concomitant with high ambient temperatures long-day conditions prevail. The European wild boar is a short-day breeder and reminiscences of photoperiodic effects may also influence reproductive performance in domestic pigs (see below). There are, however, several reports that claim that ambient temperature alone has a significant influence on reproductive performance. When gilts were heat stressed by being kept at 33.3 °C puberty was delayed from 204 days in the control group to 230 days in the heat stressed group [24]. Heat stressed females had, compared to controls, suppressed plasma concentrations of FSH and LH, which was ascribed to a diminished ability of the hypothalamus – pituitary axis to secrete FSH and LH. This in turn had physiological consequences for follicular growth, which was impaired in the heat stressed gilts [24].

A prolonged interval between weaning and return to oestrous during summer months has commonly been reported. Although the return rate was found to be higher for sows kept under short-day conditions at high ambient temperature than for those kept at long days, it has been concluded that ambient temperature is more strongly determining for the weaning-to-oestrus interval than daylight conditions [49]. Underlying endocrine changes that may contribute to the prolonged weaning-to-oestrous interval in heat stressed sows may be a reduced LH pulse frequency as shown in sows kept at 30 °C compared to sows maintained at 22 °C. The heat stressed sows had, furthermore, higher plasma concentrations of GH, but lower concentrations of cortisol than the controls [5]. Prolonged weaning-to-oestrous intervals, occurring when ambient temperature is high, are largely caused by a decreased feed intake. A decreased food intake can also explain the endocrine changes that were found [5], [51], which are changes likely to occur in animals with impaired reproductive function. Indeed, Peltoniemi et al. [46] suggest that seasonally suppressed LH caused by low feed intake, together with restricted feeding early in gestation, further suppresses LH, which in turn leads to suppression of progesterone secretion. The lower progesterone secretion negatively influences the histotrophic secretions from the endometrium. This impairs the viability of the embryos and their capability to produce the second embryonic oestrogen signal, which is necessary for gestation to be maintained beyond day 30. The sow then is unable to respond to these inadequate embryonic signals and as a result CL regresses and gestation is terminated.

Photoperiod: The role of daylight conditions on reproductive function in the female pig was long obscure, partly because of confounding factors such as environmental temperature and contact with boars. Also, there was controversy over whether the domesticated pig expressed a nocturnal rise in melatonin, which could generate a photoperiodic rhythm. Studies performed under light-controlled and cool conditions have, however, clearly shown that the pig responds to photoperiodic conditions, and that the attainment of puberty can be delayed under long-day conditions, i.e. long days are inhibitory to attainment of puberty, and photoperiod is an important cue for reproductive function in domestic pigs [44].

The controversy regarding whether or not the domestic pig has a circadian rhythm of melatonin secretion was caused by assays not being sensitive or specific enough, and it has later been shown that the domestic pig expresses a nocturnal rise in plasma melatonin concentrations, which clearly reflects seasonal changes in photoperiod with concentrations being high during the dark phase for a longer time during the winter than during the summer. Furthermore, domestic gilts (kept under conventional piggery conditions) and European wild boars (kept outdoors) showed similar patterns of melatonin secretion, suggesting that despite of changes in genotype during domestication and keeping of pigs indoors under reduced light environment, domesticated pigs are able to respond to changes in natural photoperiod. These data support that the domestic pig still has a vestige of seasonality, and that photoperiod is likely to be part of the occurrence of seasonal infertility [61].

3.4. Gestation

The length of gestation in pigs is 113 – 115 days. When ovulation occurs, the oocytes enter the infundibulum, which is the funnel-shaped opening of the oviduct. Fertilisation occurs in the

oviducts. The viability of the oocytes is approx. 18 hours after ovulation, and swine spermatozoa retain their fertilising capacity for 24 to 48 hours. When fertilisation occurs, a zygote is formed. The zygote then starts its cell division, and when it reaches the 16 to 32 cell stage, a morula is formed. The next step in the development is that a cavitation is formed within the embryo, resulting in the formation of a blastocyst. This occurs when the zygote is 6 to 8 days old, and from the blastocyst stage and until the differentiation of organs and formation of a placenta, the conceptus is termed embryo. After this stage it is termed foetus. The embryos pass to the uterus after 3 – 4 days, but before this progesterone from the developing CL contributes to prepare the uterus to retain and nurture the embryos. Progesterone decreases the muscular activity of the uterus, and it enhances the development of the glandular epithelium, which produces uterine milk. Until the embryos are implanted and a placenta is formed, they rely on yolk material, secretions from the oviduct and uterine milk. Maternal recognition of gestation is essential for gestation to be established and the life span of the CL to be prolonged and continued secretion of progesterone ensured. The gestation can only be maintained if the pulsatile release of $PGF_{2\alpha}$ is inhibited. As implantation occurs later than would be the normal life span of the CL, the implantation as such cannot serve as a gestation signal. What seems to be important is that embryos interact with a large area of the endometrium, and in pigs spacing of embryos between both uterine horns allows embryos to have contact throughout the endometrium in both uterine horns, and this seems to prevent regression of the CL. When the pig blastocyst is 12-13 days, it starts to synthesise oestrogens, mainly oestrone. This is before implantation has started, and it results in elevated concentrations of oestrone sulphate in the maternal circulation already on day 16 of gestation. This oestrogen synthesis occurs during a period when the embryo undergoes rapid growth and development. From being about 2 mm in diameter day 10 it has changed form to a 5 mm diameter loose sac on day 11, and on day 14 it has been elongated to about 1 m. Along with the elongation of the blastocyst, and its ability to produce oestrogens, the ability of the uterus to produce $PGF_{2\alpha}$ in a pulsatile manner decreases and that prevents luteolysis, and the CL can continue to be functional and gestation can be established.

Implantation in the pig occurs 14 – 20 days after conception. The implantation occurs gradually, and during this process the embryo becomes fixed in position and establishes both physical and functional contact with the uterus. Implantation in pigs is non-invasive, and throughout gestation an intimate contact is maintained between the trophectoderm and the luminal epithelium of the endometrium.

Complex hormonal interactions between the conceptus trophectoderm and the uterine endometrium regulate implantation during “the window of receptivity” to implantation. This receptivity requires progesterone and/or oestrogen to act on the uterus to regulate local production of a number of factors, e.g. cytokines and growth factors. Loss of expression of the progesterone receptor occurs already prior to implantation, and it appears to be a prerequisite for implantation to occur. In pigs, the hormones necessary for gestation recognition and CL maintenance are interferon τ (INFT) that silences the oestradiol receptor α, and oestradiol that in concert with PRL exerts its antiluteolytic effect on the uterine epithelia to prevent endocrine release of luteolytic $PGF_{2\alpha}$ (review by [9]).

The pig placenta is diffuse epiteliochorial. This type of placenta does not allow for passage of macromolecules such as immunoglobulins and therefore the newborn piglet is totally dependent on intake of colostrum for acquiring immunity. Uterine survival in all species, and especially in the pig with epitheliochorial-type placentation, is completely dependent on establishment of nutrient passage to the placenta through capillary blood flow within the endometrium. The vascular networks of the maternal uterus and foetal placenta are complementary to one another and become more complex as gestation proceeds. With this type of placenta, the surface area where there is attachment between the placenta and endometrium is a limiting factor for foetal growth. The capacity of the placenta to transport oxygen and nutrients and to produce nitric oxide directly affects foetal growth. Placenta blood flow is generally positively correlated with foetal growth in pigs. In gestating sows with large litters, there is a considerable competition for space in the uterus, and certain uterine sites are most likely to produce small placentas and small foetuses: foetuses localized close to the ovary mostly are large and vital, whereas those in proximity to the cervix tend to be small and less viable.

Endocrinology of gestation: Progesterone is essential for maintaining gestation in the sow where it derives mainly from the CL, because placental progesterone production is low. During the last third of gestation, plasma concentrations of progesterone, and oestrogen, produced by the foeto-placental units, are high and this leads to inhibition of follicular development and LH secretion. Relaxin is a protein hormone that in most species is derived from the placenta, but is produced by the CL in the pig. Its function is suggested to be to maintain the uterus quiescent, which occurs in conjunction with progesterone. Progesterone also maintains the cervix contracted. Prolactin is important for the development of the mammary gland, the initiation and, after parturition, maintenance of lactation. A sequence of endocrine events contribute to induce parturition: In late gestation, there is a changeover from production of progesterone to production of oestrogen, starting about a week before parturition in the sow. This changeover is brought about by the maturation of the foetus and particularly the adrenal cortex of the foetus. The increasing concentrations of oestrogen probably trigger the increased synthesis and release of $PGF_{2\alpha}$, which are factors that contribute to initiate the final phase of the parturition process. $PGF_{2\alpha}$ will contribute to the regression of the CL, and it will contribute to increase the contractile state of the uterine musculature. PGF2α also makes the uterine musculature more responsiveness to oxytocin. Relaxin probably plays a role in parturition by softening the tissues surrounding the pelvic canal, and by relaxation of the cervix. Oxytocin release is initiated by the foetus in the pelvic canal, and it, together with $PGF_{2\alpha}$, enhances the rhythmic contraction of the uterine musculature during expulsion of the foetus [57].

3.5. Lactation; effect on follicular development

Parturition induces changes in the hormonal environment of the sow, and nursing and milk production subsequently bring about complicated hormonal and metabolic events, where the role of nursing cannot be distinguished from that of milk production because the two processes occur concomitantly. LH secretion, which had been inhibited in late gestation, increases immediately after parturition in response to the fall in the circulating concentrations of progesterone and oestrogens. However, the secretion of LH is inhibited again after two to three days of suckling, probably because of inhibition of the GnRH pulse generator, and altered responsiveness of the pituitary to GnRH. This low mean concentration of LH and low number of LH pulses remain during the first two lactation weeks, and then the LH secretion partially resumes again, possibly caused by increased responsiveness of the pituitary to GnRH, or partly by decreased suckling intensity. Simultaneously, FSH secretion is suppressed by inhibin secretion from the growing follicles. Because follicle maturation and growth to ovulatory size (6 – 10 mm) is dependent on high frequency LH release, only small and medium-sized follicles are present in the ovary during the second week of lactation. Then, when the LH pulse frequency increases, follicular growth increases again, but during lactation follicles seldom develop beyond 5 mm in diameter. Also the positive feedback of oestradiol on LH release is kept in check during lactation, and is only partially restored during the third and fourth lactation weeks.

The effects of nursing and milk production include release of neurotransmitters and neuropeptides which stimulate secretion of the pituitary hormones PRL, growth hormone (GH), adrenocorticotropin (ACTH), thyroid-stimulating hormone (TSH) and oxytocin. These hormones will contribute to stimulate secretion of IGF-1, cortisol and thyroid hormones. These hormones, in turn, are involved in milk production, but also have metabolic effects: oxytocin is essential for milk ejection at nursing, but could also facilitate mobilisation of body reserves. High concentrations of GH will exert anti-lipogenic effects resulting in glucose and lipids being preferentially directed to the mammary gland. In combination with high IGF-1, this will contribute to minimize catabolism of endogenous protein and hence, spare lean tissue mass. Cortisol release leads to mobilization of energy substrates from body stores, and thyroid hormones contribute to stimulate protein synthesis by the mammary gland (review by [52]).

4. Nutrition and reproduction

4.1. General aspects

Nutrition of sows throughout the life cycle must aim to optimize reproductive performance and maintain sow longevity. This is a difficult task, because the different stages of the reproductive cycle are closely interrelated, and feeding of the sow must aim at long-term beneficial effects on sow productivity and health. For instance, feeding during gestation affects the feed intake during lactation, and feeding must therefore allow for maximum fertilization rate and survival and development of a maximum number of foetuses, but also allow for a maximum feed intake during lactation in order to support milk yield, piglet growth and maintaining sow body reserves so that the interval between weaning and return to oestrous can be as short as possible.

Nutritional status has a clear impact on reproductive performance, and metabolites and metabolic hormones can function as nutritional signals, signals that can be detected by the central nervous system (CNS), and report metabolic status to the hypothalamus-pituitary axis. In response to signalling of metabolic status, pulsatile LH release may be modified: in response to food deprivation LH pulse frequency generally decreases, and in response to chronic feed restriction this may also lead to cessation of oestrous cycles. Metabolic signals that are considered important for relaying nutritional status to the CNS in pigs are glucose, insulin, IGF-1, leptin and free fatty acids (FFA) (review by [6]). Nutrition can also exert an effect directly at ovarian level, and in this context under-nutrition will lead to decreased circulating concentrations of insulin, IGF-1, and possibly of its binding protein, and leptin and increased concentrations of circulating GH. However, systemic glycemia is tightly controlled and therefore maintained even under conditions of nutrient deficiency. The decrease in plasma IGF-1 that occurs during undernutrition is caused by “uncoupling” of systemic IGF-1 and GH secretions. When IGF-1 is low in plasma and/or in follicular fluid, folliculogenesis is commonly impaired and ovulation rate reduced. Insulin is known to have a stimulating role in folliculogenesis, probably due to its ability to decrease atresia in small and medium-sized follicles. Results regarding effects of GH manipulation are, however, contradictory. It has been suggested that stimulatory effects of GH on ovulation rate are achieved when IGF-1 is high, but that its inhibitory effect is related to insulin resistance at the ovarian level. The role of leptin on the ovarian level is as yet unclear, but it does not seem likely that it is directly involved, although it has been reported that it had a synergistic effect with IGF-1 in vitro on oestradiol secretion by pig follicles. Also steroid hormones can be involved in nutritional effects on the ovarian level. Progesterone concentrations respond to feed supply, such that when feed supply is reduced the hepatic blood flow and metabolic clearance rate of progesterone are decreased, resulting in high plasma concentrations. High progesterone concentrations may impair folliculogenesis by inhibiting LH pulsatility (reviews by [48], [52]).

With the increasing awareness of adipose tissue as an important endocrine organ, the role of adipokines in signalling metabolic status and size of the body energy stores has become even more intriguing, and leptin is obviously playing a central role in coordinating energy homeostasis and neuroendocrine functions. Recent evidence supports the view that there is a direct link between hypothalamic neurons in the regulation of fat metabolism and reproduction. When adipose tissue mass and circulating leptin concentrations increase, catabolic pathways within the hypothalamus are stimulated, but conversely when adipose mass and blood leptin concentrations are reduced, anabolic pathways are stimulated and they inhibit catabolic pathways. These two pathways comprise a number of genes that, besides regulating appetite and energy balance, also exert an impact on the reproductive axis. For example, α-melanocyte stimulating hormone (α-MSH), which is associated to hypothalamic NPY and proopiomelanocortin (POMC) (see Chapter 19) can affect hypothalamic GnRH release and subsequent feeding behaviour. Peripheral effects of adipocyte leptin expression and secretion include suppression of appetite, stimulation of hypothalamic GnRH release and reversion of the inhibitory effect of energy deprivation on LH secretion. Co-localization of leptin receptor mRNA with NPY gene expression provides evidence that hypothalamic NPY is the primary potential target for leptin in the pig, but NPY alone may not mediate the effects of leptin: metabolic signals may be communicated to the GnRH neurons by other neuropeptides, among

them kisspeptin. Thus, as previously stated, reproductive function is metabolically gated, but mechanisms interfacing energy stores or metabolic cues and fertility are not yet completely understood. In conclusion, the evidence from above would suggest that leptin may serve as the primary metabolic signal interacting with neuropeptides such as kisspeptin and NPY that link energy status with the neuroendocrine axis and subsequent reproduction (review by [8]). The general principles for interaction between energy supply and reproductive function are described in Figure 16.2.

4.2. Nutrition, metabolic status and timing of puberty

In pigs, attainment of puberty is most often considered to be more dependent on age than on nutritional status or body composition. The reason for this is probably that when living in the wild, and seasonal reproduction was clearly expressed, young gilts reached puberty in the late autumn at an age of approximately 8 months, and then gave birth in the early spring when feed availability was favourable for piglet survival. However, timing of puberty in domestic pigs may be affected by several factors including nutritional status and body composition as pointed out below.

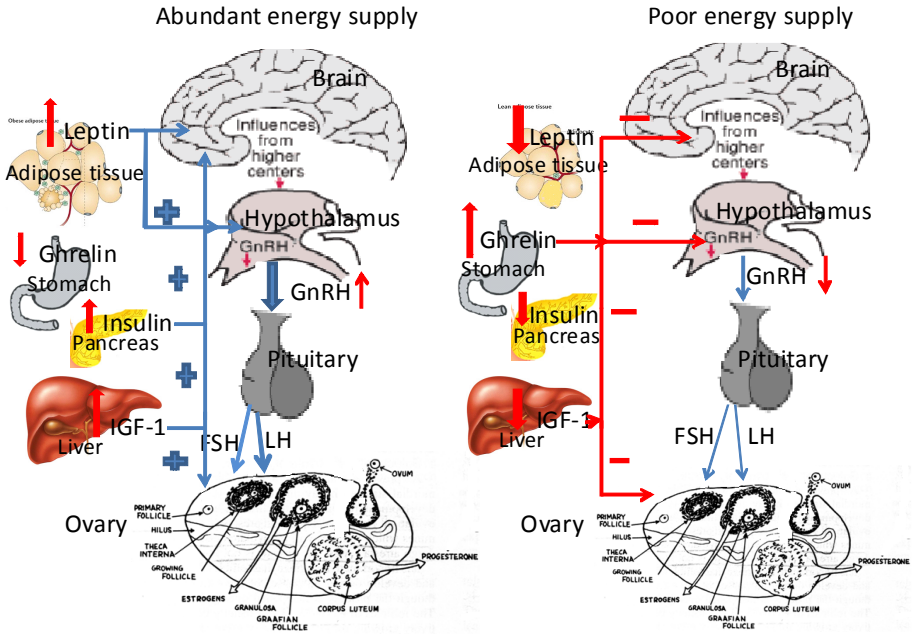

Figure 16.2. Overview of the influence of energy supply and metabolic status on reproductive processes.

The understanding of how metabolic status affects the onset of puberty is still incomplete, but there are some general theories of how metabolic cues are involved in inducing or inhibiting attainment of puberty: one simply states that an animal must have reached a critical minimum body weight before it can reach puberty. This theory was later refined to claim that the animal must have a minimum body fat content, or a minimum body fat-to-lean tissue ratio, i.e. puberty cannot be attained unless a certain threshold value for body composition is reached. Such a scenario would make sense when taking into account that the animal needs a certain amount of body reserves to be able to perform the energetically challenging process of supporting foetal growth, and then supply a sufficient amount of milk to its offspring. If body reserves were insufficient, the individual would jeopardise not only the survival of its offspring, but also its own life, and therefore it would be advantageous to abstain from conceiving.

Another theory states that a minimum amount of oxidisable metabolic fuels must be available for puberty to occur and cyclicity to be maintained [64]. This means that if the animal does not have access to nutrients in such an amount that it exceeds the immediate needs for maintenance (cellular maintenance, thermoregulation and a minimum locomotor activity), life processes like body growth, fat accretion and reproduction will be abolished. The metabolic fuel that seems to be most crucial is glucose, and glucodeprivation has been shown efficient in stopping cyclicity in hamsters, whereas the animals responded less to a block of fat oxidation [56].

With the discovery of leptin [69], the protein product of the obesity gene, new aspects on the onset of puberty were revealed. It was soon shown that leptin administration restored the reproductive capacity of ob/ob mice, which are characterised by infertility [15].

Later, Chehab et al. [16] claimed that puberty onset was advanced in normal female mice that were treated with leptin injections. However, studies in rats did not show an advancement of puberty by leptin treatment, but rather that it could over-ride the delay in puberty caused by feed restriction, and it was concluded that leptin acted as a metabolic gate with permissive function ([17]. The current concept of the role of leptin in the attainment of puberty is that it is one of several important metabolic signals. Foster & Nagatani [27] reviewed leptin's role in onset of puberty and they suggested that leptin may have a role by having a triggering effect on IGF-1 secretion and by increasing glucose availability.

4.2.1. Body weight and composition

In pigs, body fatness has been proposed to be determining for attainment of puberty [19], [5], but also body fat-to-lean tissue ratio has been suggested to be the main determining factor [33]. However, for gilts fed ad libitum during rearing, Rozeboom et al. [55] showed that there was a considerable variation in age, body weight, body fat content and body composition when puberty was attained, and they concluded that body reserves are not directly determining for the timing of onset of puberty, but merely permissive. That body composition is not the main factor determining onset of oestrous was also proposed by Armstrong & Britt [2] who fed gilts restrictedly until they became anoestrous and then refed them until they resumed cyclicity and found that body weight was higher in gilts when they resumed cyclicity than when they became anoestrous, but backfat thickness remained the same. It was therefore concluded that body composition was different when cyclicity ceased and was resumed. Also Rozeboom et al. [54] found that when feeding cyclic gilts restrictedly they reached anoestrous at highly variable body composition. From these data it can be concluded that body composition may be permissive for gilts to attain puberty, but it is not the main determining factor.

4.2.2. Availability of oxidisable metabolic fuels and metabolic status

The theory that the availability of oxidisable metabolic fuels is important for attaining puberty can be given indirect support by several studies in pigs: When glucose was administered to gilts fed restricted, so that the circulating concentrations of insulin increased, there was a concomitant rapid increase in LH pulse frequency, similar to the response achieved after refeeding [12]. Conversely, when glucose deprivation was experimentally induced by blocking for glucose oxidation, LH pulse frequency was reduced [7]. Another study used prepubertal littermate gilts that were either raised on ad libitum feeding from 75 to 85 kg live weight and then fed at maintenance level until slaughter, or first kept at maintenance level at 75 kg live weight and then fed ad libitum until reaching 85 kg. There were no differences in backfat thickness or longissimus muscle area, but those who had been first restricted and then refed had significantly increased follicle development compared with their littermates that had been fed restrictedly after reaching 85 kg live weight, and plasma LH and FSH release differed between the groups in response to a LHRH challenge. The animals also dif-

fered in metabolic status with restricted animals having lower pre-prandial plasma glucose, post-prandial cortisol and insulin, total plasma IGF-I, and basal and postprandial free triiodothyronine than the refed littermates. These results suggested that short-term manipulation of energy supply caused responses that were likely to affect timing of puberty [13]. Other indirect evidence for the importance of available amounts of oxidisable metabolic fuel was achieved when littermate pre-pubertal gilts were fed at maintenance level for 7 days after which one littermate in each pair was given feed ad libitum during days 8 to 14. As soon as 6 hours after feeding to appetite had started, the LH pulse frequency was increased, and both plasma insulin and glucose rose in response to repletion whereas plasma IGF-1 increased gradually. When the gilts were slaughtered at day 15 those that had been fed to appetite had greater numbers of ovarian follicles, follicular volume and uterine weights than those that were fed restrictedly. This rapid enhancement of LH secretion in response to dietary repletion was suggested to have been mediated by the changes recorded in glucose and insulin status. The increases in plasma glucose, insulin, and IGF-1 were proposed to have potentiated the ovarian responses to the repletion [14]. Taken together, these investigations show that a sufficient amount of oxidisable metabolic fuels affects metabolic status in a direction that favours developing reproductive function in prepubertal individuals (review by [8]).

4.2.3. Leptin

Leptin has been suggested to be a metabolic gateway with permissive function in relation to timing of puberty. The understanding of the mechanisms for its action is, however, not yet complete. For a metabolic signal to play a role in timing of puberty it must increase, or decrease, substantially in the period preceding puberty. Indeed, expression of the biological form of the leptin receptor in hypothalamus, expression of leptin in adipose tissue and serum leptin concentrations all increased in pigs by 3.5 months of age, which makes leptin a potential metabolic signal with importance for regulation of puberty. It seems that leptin effects are mediated through the hypothalamic NPY expression. In vitro, leptin has increased LH secretion from pig pituitary cells and GnRH release from hypothalamic tissue, and this evidence suggests that leptin acts through the hypothalamus. Co-localization of leptin receptor mRNA and NPY gene expression gives support for the view that hypothalamic NPY is the primary target for leptin in the pig, but probably NPY alone may not mediate the action of leptin.

4.2.4. Aspects on energy and nutrient supply, feeding strategies

Rearing of gilts must aim to optimize gilt productivity and longevity, and accomplishing both these goals may be contradictory. Gilts of modern fast-growing pig breeds will enter their reproductive life with lower body fat reserves than pigs less intensely selected for lean tissue growth. Indeed, culling of young sows is often caused by anoestrous or failure to breed, problems often related to low body fat stores. It has been proposed that selection for reduced backfat thickness may have negative effects on long-term reproductive performance while lifetime productivity of sows has been positively related to backfat thickness at mating.

Most studies into the effects of energy supply to prepubertal gilts suggest that the age at puberty is delayed in animals that were exposed to feed restriction during rearing. Compilation of investigations concerning a range of energy supplies of 1-1.5 times MEm to 2.5-3 times MEm most often showed that age at first oestrous or first ovulation, or the percentage of gilts that were cyclic at a fixed age were significantly affected by the plane of rearing, and that a low plane of energy supply delayed attainment of puberty. However, the ovulation rate at first oestrous was unaffected by plane of rearing [48].

Experiences from experiments into effect of rearing intensity on long-term reproductive performance, however, seem to show relatively little effect of rearing intensity; a few positive effects of

slight feed restriction have been found and conversely, high feeding levels corresponding to ad libitum feeding or 2.5 times maintenance or higher resulted in impaired reproductive performance. Likewise, when modern genotype gilts were allocated to different feeding regimes at 120 days of age, the gilts that were fed a high-energy and high-protein diet at a restricted scale were those that were the most likely to farrow, whereas those given the same diet ad libitum had the poorest performance over four parities. High rearing intensity may increase the risk of leg disorders, which were significantly increased when gilts were reared at levels that were higher than allowing for maintenance and a moderate growth. Additional supply of minerals did not decrease the incidence of leg disorders [63].

An example of the limited influence of rearing intensity of gilts is a four-parity study performed under Danish conditions. Gilts were fed differently from 6 weeks of age until mating: one group according to standards (C), one group 75% of standard (75C) and one group was fed ad libitum until 10 weeks of age and then semi-ad libitum by free access to feed for 30 minutes at two daily feedings (Semi ad lib). Feed intake differed significantly between treatment groups during the period of differentiated feed supply, being highest in the Semi ad lib group, resulting in body weights ranging from 134 kg (Semi ad lib) to 110 kg (75C) at mating, which occurred at a similar age for all groups. Litter size tended to be poorest in the 75C group (one piglet per litter below the other groups), but no significant differences in reproductive performance, piglet weight or sow milk yield were recorded [58].

Restriction of protein supply may delay the onset of oestrous, but information still is limited regarding the importance of protein level for puberty development. However, it appears that protein/lysine restriction to 14.4 % CP and 0.7 % total lysine compared to 18.7 % CP and 1.0 % total lysine in the prepubertal period had no influence on mammary gland development, whereas energy restriction by 20% decreased the mass of mammary tissue [22].

Replacement gilts reared in practice in Denmark are usually fed restrictedly from 50 kg live weight. Growth rate between 30 kg live weight and first service at an age of 230 – 240 days, a live weight of 140 kg and a backfat thickness of 12 – 18 mm is maintained at a daily weight gain of 700 g. The first service usually occurs in the gilts' second oestrous. In the weight interval 30-50 kg, a grower diet is fed ad libitum, but during restriction a diet with lower energy concentration, e.g. a sow lactation diet rich in fibre is recommended, and the feed supply is restricted to a maximum of 2.5 feed units (FUgP). When the gilts reach 90 - 100 kg live weight, the protein content of the diet is decreased, which will allow for the gilts to increase their body fat accretion, and thereby they will gain body fat stores, which will be needed during their future reproductive life. Feed is supplied at 2 -3 FUgp depending on body condition of the gilts.

4.3. Maintaining of ovarian cyclicity

Once a gilt has reached puberty, the feeding strategy should aim to maintain ovarian cyclicity so that the animal produces a large number follicles of good quality, and that oocytes with a high degree of maturation can be produced. This implies that the animals should be given a sufficient feed supply to support pulsatile GnRH release of high frequency and subsequently release of LH and FSH. As described previously, feed restriction causes suppression of the pulsatile GnRH and LH release and chronic feed restriction can eventually cause the GnRH pulse generator to stop and animals become anoestrous. This was demonstrated by restricting cyclic gilts to a daily intake of 2.0 MJ ME (<0.2 FUsow) of a diet that supported the requirements for protein, minerals and vitamins. Anoestrous occurred after averagely 46 days, but the range was quite broad (14-63 days). By then body weight had decreased by 15% and backfat thickness by 25%. The gilts fed restricted had lower postprandial insulin and higher concentrations of FFA than the well-fed gilts, and the plasma concentration of LH and its pulse frequency were reduced to close to the detection limit of the assay and 0, respectively. However, the anterior pituitary remained normally responsive to GnRH, because infusion of GnRH resulted in increased plasma concentrations of LH. Furthermore,

administration of GnRH or LH at hourly intervals increased basal concentrations of LH, induced follicular growth and increased the secretion of oestradiol. Therefore, it was concluded that the effect of the feed restriction was found at the hypothalamic level, causing the release of GnRH to decline. Resumption of ovarian cyclic activity of the restricted gilts occurred after slightly more than a month during which body weight had increased, so that the body weights of the animals were 1.5% below the initial weight, but backfat thickness had not increased significantly and remained more than 30% below the initial level. These results demonstrate the importance of a sufficient energy supply for maintaining cyclic activity, and the role of the hypothalamic GnRH pulse generator for the pulsatile LH release, but this study was performed before the discovery of leptin, so its possible role in the cessation and induction of ovarian cyclic activity could not be deduced [2].

4.4. Nutrition and quality of oocytes

Several factors such as age of the animal, breed or genotype, environment and nutrition affect the quality of the oocytes. It appears that the progression of folliculogenesis is, at least partly, determining for the oocyte quality and embryogenesis. Nutrition is an important factor for the quality of the oocytes and their ability to be fertilized and develop to a surviving embryo. One nutritional factor is the energy supply, and generally a restricted energy supply results in impaired oocyte quality whereas an ample energy supply most often influences oocyte quality positively. Similarly, also a reduced protein intake may cause poor oocyte quality. Effects of nutrition have been demonstrated in several studies with pigs, for instance with primiparous lactating sows that were fed either to appetite for the first 21 days of lactation and then at an energy supply that was restricted to 50% from day 22 through 28 of lactation (restricted) or restricted for the first 21 days of lactation and then fed to appetite from day 22 until day 28 (refed). The refed sows had superior embryo survival (85% vs. 64%), and a group of sows that were slaughtered shortly before expected ovulation had more large preovulatory follicles and more follicles that had matured to metaphase II of meiosis than sows that were restricted in late lactation despite the fact that there were no differences in plasma oestradiol concentrations. Further evidence for a nutritional effect was achieved when a large number of follicles derived from abbatoirs were incubated in vitro with follicular fluid from restricted or refed sows, respectively: follicular fluid from refed sows was better able to support the maturation of the oocytes than follicular fluid from restricted sows [67], [68]. Similar results were found in a study investigating the effect of feed supply in the oestrous cycle preceding mating. Gilts receiving a high plane of energy supply (3 x MEm) had more (22.7 vs. 19.0) and heavier CL and better embryo survival (95.5% vs. 74.8%) than gilts that were fed at maintenance level [3].

The impact of lysine supply was demonstrated when primiparous lactating sows were exposed to different supplies of lysine over an 18-day lactation period and the effect of lysine supply was evaluated in the subsequent reproductive cycle. Total lysine was supplied at low (0.4%), medium (1.0%) or high (1.6%) level, and diets were iso-energetic. Ovarian data were evaluated for sows that were slaughtered in the first prooestrous period after weaning. Sows given the low lysine supply tended to have lower follicular fluid volume and follicular fluid oestradiol concentration than the other groups, whereas follicular fluid IFG-1 concentrations were similar. Sows on the low lysine supply also had fewer large follicles, more medium sized follicles, but the same number of small follicles as those given the medium and high lysine supplies. Also the follicular fluid from sows on the low lysine supply was less able to support maturation of follicles aspirated from follicles of prepubertal gilts than such derived from sows on medium and high lysine supplies. However, there was no advantage of giving the high compared to the medium lysine supply on the reproductive traits that were studied [66].

Not only the energy and amino acid/protein supply influence oocyte maturation; evidence also suggests that the fibre content of the diet may exert an influence. A diet with high fibre content has been demonstrated to improve pig oocyte maturity and embryo survival rate in gilts, when providing the same net energy supply as a control diet (23.4 MJ NE daily; ≈ 3.0 FUsow) with moderate fibre content. Despite having fewer large follicles (9.3% vs. 38.3%) and lower follicular fluid vo-

lumes (62.9 µl vs. 108.7 µl), more oocytes from the high fibre sows reached metaphase II after culture (75.7% vs. 65.7%). The high-fibre diet had caused some changes in the preovulatory endocrinology that were proposed to mediate the improved oocyte quality: the number of LH pulses was higher in the high-fibre diet group, and on day 19 in the oestrous cycle plasma oestradiol was lower among gilts fed the high-fibre diet. The increase in LH pulsatility was ascribed to lower circulating steroid concentrations causing decreased negative feedback to the hypothalamus [23].

The effects of nutrition on oocyte quality can be mediated by providing an appropriate preovulatory development. Nutrition can modify concentrations of circulatory gonadotrophins, steroids and metabolic hormones, and also the environment within the ovarian follicle. Oocyte maturity is proposed to be advanced in a hormonal milieu that favours LH pulse frequency by decreased circulating steroids to exert negative feedback on the hypothalamus. The influence of nutritional background on the ability of follicular fluid to support oocyte maturation indicates that e.g. IGF-1 and other factors in the follicular fluid are involved in mediating these effects.

4.5. Flushing – a tool for improved litter size?

Flushing is a feeding strategy that aims at increasing the ovulation rate. It applies to animals in a low body condition or fed at a low plane of nutrition. Flushing is a short-term nutritional modulation of the reproductive axis, and as such it induces changes in the metabolic status of the animal without causing any major changes in body weight or body fat content. Flush feeding was first applied, and investigated, in sheep, but also in pigs this feeding strategy can be useful, especially in gilts. Flushing is performed by increasing the energy intake of the animals for at period of about 10 – 14 days before breeding. The effect is mainly induced by the increased energy supply, because increase in protein supply has not resulted in similar positive effects. The length of the flushing period should be limited, because if prolonged flushing periods are practised the response in ovulation rate wanes. Flushing is not a tool to achieve super-ovulation, but it is a way of restoring ovulation rate in individuals that are in poor body condition, or have been fed restrictedly, to the level of well-fed animals. Therefore, gilts that are fed restrictedly prior to puberty can be expected to respond positively to flushing. Indeed, ovulation rate may be increased by 6 – 7 ovulations in response to flush feeding from day 8 in the oestrous cycle they were bred in. Flushing induces changes in reproductive and metabolic hormones affecting the hypothalamus – pituitary-ovarian axis. Increased plasma concentrations of FSH and an increased number of LH pulses per time unit have been reported, as well as increased plasma concentrations of insulin and IGF-1. The changes in gonadotrophin secretion are probably mediated via insulin, because it has been shown that insulin alone is able to increase ovulation rate. Insulin may directly increase the GnRH release, but it may also increase the pituitary sensitivity to GnRH, thus increasing the release of gonadotrophins. Insulin also seems to act directly on the ovarian level by decreasing the rate of atresia in the growing follicles, hence maintaining a larger number of healthy pre-ovulatory follicles [9], [25], [37].

In Danish practice, flushing is recommended for gilts from 5 – 10 days before expected oestrous and until the gilt is bred. The recommended energy allowance is set at 3.5 FUgp daily, but immediately after service the feed supply should be reduced to 2.0 FUgp.

4.6. Nutrition throughout gestation

Nutrition throughout gestation must aim at supporting survival and growth of a maximum number of foetuses, development of the mammary gland, and in the case of sows up to their fourth to fifth parity, also its own body growth. The voluntary feed intake of the gestating sow usually exceeds its requiremens for maintenance and accretion in uterine and maternal tissues and therefore feed restriction needs to be applied in order to avoid that sows accrete too much body fat. However, demands for energy and nutrients increase very rapidly during the late period of gestation and if sows with large litters are fed too restrictedly they may not be able support their energy and nutrient demands by feed intake only, but start to mobilize body reserves. Feeding must therefore be

balanced so as to avoid that sows become catabolic towards the end of gestation. The aim must be that the sow increases its body weight without becoming too fat. It has been suggested that a weight gain of 22 – 30 kg is required to prevent loss of backfat during gestation. The feed intake during gestation also influences the feed intake during lactation, and if the sow is allowed to consume feed to appetite during gestation, its feed intake during lactation will be too low to support a maximum milk yield, and the result will be that the sow mobilises from its own body reserves, and loses much weight.

4.6.1. Early gestation – embryonic survival

Early embryonic losses may be substantial in the pig, and in the period immediately after breeding and until implantation feeding must aim at minimizing embryonic losses and supporting implantation of a large number of robust foetuses. Embryo survival in the pre-implantation period may be influenced by the feed intake immediately after breeding. If a high plane of nutrition, resembling the feeding level during flushing, is maintained after breeding, embryo survival often is compromised, especially in gilts, but conflicting results exist. By decreasing the feed supply to near-maintenance level the day after mating, embryo survival is promoted. These effects are mediated via progesterone, which is important for developing and maintaining the CL. When the feeding level is high after mating, the hepatic blood flow increases, and more progesterone is cleared from the circulation, resulting in low plasma progesterone concentrations. When feed intake is low, less clearance of progesterone occurs, and high plasma concentrations prevail and enhance embryo survival (see Figure 16.3; [30], [31].

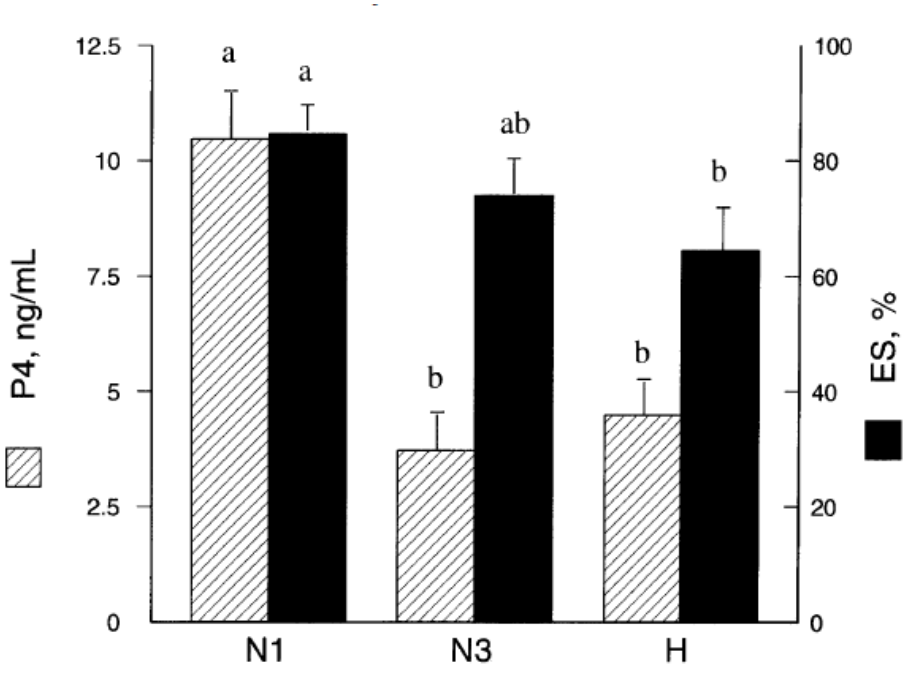

Figure 16.3. Plasma progesterone (P4) and embryo survival rate (ES, %) in gilts fed on a low plane of nutrition from day one (N1) or day 3 (N3) after breeding or staying on a high plane of nutrition (H). From Jindal et al. [30].

4.6.2. Placentation to late gestation

Nutrient supply may affect placenta function, and in pigs both under- and overnutrition has lead to impaired foetal growth. When it concerns undernutrition, the deficit in energy, or protein, must be large to reduce foetal growth: in gilts given protein and vitamins to their requirement, but sup-

plied with only 50% of their energy requirement, birth weight of piglets was unaffected, whereas a restriction in energy intake to about 25% of the requirement resulted in reduced piglet birth weight and lower glycogen stores in the newborn piglets. In gilts fed either 30 or 20 MJ ME (approximately 2.6 vs. 1.7 FUsow) from mating and until they were slaughtered at different stages of gestation differences in placenta and foetus weights were only recorded in late gestation (around day 100), being lower in gilts on the low energy supply.

Gilts fed a protein-free diet throughout gestation gave birth to normally sized litters, but the piglets' birth weight was reduced as was their brain and liver weights. Gilts that were given a 4-day transitory supply of protein to the requirement during the period when implantation occurred, but were fed the protein-free diet before and after this period, produced piglets that were more than 100 g heavier at birth than those of the gilts fed the protein-free diet throughout, but the weights were below those of the control group that was fed protein to the requirement. These results demonstrate that energy or protein deficits or imbalances must be severe to cause growth retardation in pig foetuses. However, inadequate feed intake during the time of placentation may compromise the development of the placenta and its ability to support the foetuses with oxygen and nutrients also after the nutrient deficit has subsided. In a long-term perspective, foetuses exposed to malnutrition may have inferior performance, health and product quality (review by [65]).

4.6.3. Foetal growth, piglet birth weight and survival

Foetal growth rate is moderate during the first two thirds of gestation, but during the last third it increases almost exponentially, which means that the demands for energy and nutrients for foetal growth increase dramatically. As an example, it has been calculated from quantitative measurements that in the period from day 50 to day 110 in gestation the ME requirement for reproduction increased from 3 to 12% of the maternal energy intake. In the same time interval, the requirement of protein for reproductive processes increased from 7 to 41% of the maternal daily protein intake. Prediction equations for the development in the different reproductive tissues throughout gestation were developed according to Gompertz functions. The energy retention in foetuses and different uterine compartments is depicted in Figure 16.4 [41].

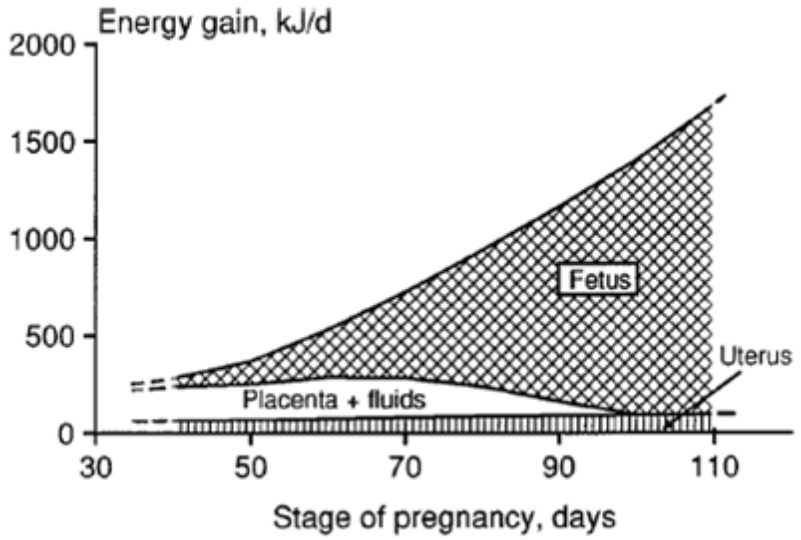

Figure 16.4. Partition of daily uterine energy deposition between different uterine compartments ([43] adapted from [41]).

Foetal growth determines piglet birth weight, which in turn is predictive for piglet survival and performance in later life. Litter size has a strong influence on piglet survival, and in a comprehensive survey it was found that when litter size increased from fewer than 11 to more than 16 piglets, birth weight was concomitantly decreased from 1.59 to 1.26 kg corresponding to a decrease of 35 g per additional piglet born. With increasing litter size, also the proportion of very small piglets (below 1.0 kg) increased from 7 to 23% of total born. Among the smallest piglets with birth weight below 1.0 kg, 11% were stillborn which should be compared to 3% in piglets weighing more than 1.0 kg. Also the perinatal mortality can be expected to be higher in very small piglets: out of those weighing below 1.0 kg at birth, 17% died within 24 hours, whereas mortality among those that weighed more than 1.0 kg only was 4% during the first 24 hours of life. Also the fraction of piglets surviving until weaning was much higher among piglets that were heavy at birth than in the smallest ones [53].

As appeared above, piglet birth weight is not easily manipulated by modifying the energy or protein supply to gestating sows, because the foetal growth has a high priority. Piglets are born with very low body fat content (about 1 - 2%) so they have virtually no possibility to mobilize fat from the body to sustain their energy demands nor is the neonatal piglet capable of protein oxidation. Glycogen would therefore be the only body energy source that could be oxidized to support neonatal survival until intake of colostrum and milk can cover the demands. It was therefore attempted to enforce the glycogen stores of the foetuses through the feeding of gestating sows. Investigations concerned the addition of starch or fat to diets in late gestation, and although some positive results have been reported the overall conclusion must be that foetal glycogen stores are not easily manipulated by sow diet. For instance, a recent Danish study investigated the influence of four different gestation diets with dietary fibre contents ranging from 17 to 40% until day 108 of gestation, after which the sows were allocated to 6 different transition diets with high fat content, and with the fat sources representing long-chain, saturated medium-chain, mono- and polyunsaturated long-chain fatty acids or combinations. Neither gestation nor transition diets affected piglet birth weight nor glycogen pools in liver and muscle tissue, despite the gestation diet affecting liver weight. Glycogen pool size was, however, clearly related to piglet birth weight, the heaviest piglets having the largest glycogen pools. Even though the birth weight of all studied piglets was above 1.0 kg, the smallest ones were at a clear disadvantage concerning amount of glycogen available as metabolic fuel and thereby chance of survival. It was concluded that glycogen retention in foetal tissues is of such a high priority in the gestating sow that dietary manipulation is not likely to affect the amount of glycogen deposited in foetal tissues [62].

4.6.4. Mammary gland development

Mammary gland development in gilts occurs in distinct phases: it is slow until about 90 days of age. Then comes a period when the accretion of mammary tissue and DNA increases 4-6-fold, and during gestation the accumulation continues until term, but the accumulation of mammary tissue and DNA is almost entirely concentrated to the last third of gestation. Mammary development in prepubertal gilts seems to be independent of feeding level up to 3 months of age, but from then on and until about 5.5 months of age, feeding level affected mammary gland development so that gilts that were fed ad libitum in this period developed more mammary tissue, RNA and DNA than gilts that were fed restrictedly (slightly more than 70% of the feed intake among ad libitum fed animals). Reaching puberty seemed to stimulate the development of the mammary gland, mainly by increasing the amount of nucleotides in mammary tissue, while the total amount of mammary tissue remained unaffected. This suggested that there was an extensive growth of ducts into mammary adipose tissue after the animals had reached puberty. In other species, IGF-1 is an important signalling pathway in mammary epithelial growth, but in pigs there seems to be no clear correlation between circulating IGF-1 and its binding proteins and mammary development. Prolactin is essential to support mammary development in gestating gilts. Suppression of endogenous PRL by its antagonist bromocriptin resulted in significantly reduced mammary gland parenchymal tissue, and decreased amount of RNA and DNA, but the bromocriptin treatment did not affect plasma concentrations of progesterone or IGF-1. Taken together, these findings demonstrate that the pig mam-

mary gland development is responsive to feeding level, and that it is dependent on PRL, but that IGF-1 not seems to play any important role. The importance of the mammary gland development for sow life time milk yield might, however, be questioned, because rearing of gilts on a feed supply that was 75% of Danish standards between 6 weeks of age and mating did not impose any difference in milk yield over four lactations compared to gilts that were reared on the standard plane of nutrition [59], [60], [21], [58].

4.7. Weaning-to-oestrous interval

In order to keep sow productivity as high as possible, the interval from weaning of a litter until the sow comes into heat again and can be bred and conceive needs to be kept as short as possible. Several factors can influence the length of the weaning-to-oestrous interval (WOI), and among them is the metabolic status of the sow, which in turn reflects its energy and nutrient intake during the preceding lactation, the number of suckling piglets and the weight loss during lactation. Suckling sows usually do not come into heat and ovulate, because LH release is suppressed (see 3.5). Lactation and milk production induce metabolic changes in the sow, and if feed intake during lactation is too limited the sow enters a catabolic state that will contribute to delay the return to oestrous. The mammary gland uses great amounts of glucose and this causes blood glucose concentrations to be lower than during gestation, and they will decline further during the period of maximum milk yield. Concomitantly, high concentrations of GH, or energy deficit, induce mobilization of lipids from adipose tissue, which results in elevated plasma concentrations of FFA. The elevated plasma concentrations of FFA probably contribute to induce the insulin resistance which is an important physiological adaptation of the lactating sow, improving the glucose availability for the mammary gland. The sow is capable of further physiological adaptations to maintain a high milk yield even if energy or protein supplies are limited, and these adaptations will be on the expense of maternal body tissue. In a situation when a sow enters a strongly catabolic state because of lack of feed, one response will be decreasing plasma IGF-1 concentrations, probably a result of uncoupling between GH and IGF-1 secretion. The low IGF-1 concentration will result in mobilization of body protein, which will make amino acids available for milk protein synthesis and for gluconeogenesis.

Because of the low feed intake, plasma concentrations of insulin will be low, and that will lead to mobilization of lipids from adipose tissue. Also postprandial, but not preprandial, concentrations of leptin will be influenced by feed deficit. All these changes in metabolic state exert an influence on the reproductive axis, and therefore a strongly catabolic state will suppress LH secretion and contribute to a prolongation of the time from weaning until return to oestrous. When feed restriction results in body weight loss during lactation, the WOI interval is prolonged especially in primiparous sows, but also to some extent in later parity sows. Prolonged WOI as an effect of low energy or protein intake, loss of body weight/ backfat or even loss of body protein was constantly shown in experiments twenty years ago or more, but in more recent experiments WOI is usually only marginally affected by plane of nutrition. This has been attributed to indirect selection for WOI along with genetic selection for prolificacy. In recent experiments, other consequences of feed restriction for reproductive performance were occasional negative effect on ovulation rate, and a consistently impaired embryo survival rate. These effects will then result in a smaller second litter, and if the embryo survival is severely impaired it may cause a reduction in farrowing rate after first weaning [52].

5. Quantitative energy and nutrient requirements for gestation

5.1. Energy requirement for maintenance, and partition between dam and foetus

The energy and nutrient requirements of a gestating sow consist of the requirement for maintenance, and for sows up to 4th to 5th parity also requirement for body growth, and the requirement

for deposition in uterus, conceptus, placenta, uterine fluids and the mammary gland. As appeared above, the requirement for reproductive processes is small in early gestation and increases strongly in the last third of gestation. Indeed, about 60% of the total energy deposition in foetuses and uterine tissues occur during the last 30 days of gestation. The ME requirement for maintenance has been estimated to range between 400 and 440 kJ/$kg^{0.75}$ with an average of 420 kJ/ $kg^{0.75}$ in gestating gilts at thermoneutral conditions and with a moderate physical activity. The same level applies to multiparous sows as well, and it remains constant throughout gestation. Environmental conditions may, however, influence MEm to a considerable extent: the LCT of the gestating, individually housed sow is approx. 20 °C. This estimate of LCT is not a definite value, but it is affected by housing conditions, and may be higher in an adverse environment with draught or wet concrete floors. Also the body condition of the sow affects LCT, and in very thin sows LCT is higher than in sows in a moderate to high body condition. Conversely, LCT may be lower for sows fed on a higher plane of nutrition or kept on straw bedding or being group-housed. For every degree C below LCT, the MEm requirement increases by approx. 15 kJ/ $kg^{0.75}$, but also this value can vary depending on environmental conditions. An increase of approx.15 kJ/ $kg^{0.75}$ per degree C below LCT corresponds to approx. 4% of MEm/ °C or about 70 g feed for a 200 kg sow. Another factor with significant influence on MEm is the energetic cost of physical activity, which may vary to a large extent depending on housing system, and the possible occurrence of stereotypic behavior. It has been estimated to 27 kJ/kg $BW^{0.75}$/100 min. standing, and this level is four to five times higher than in other species. Translated into energy provided via feed, a difference of daily standing time of 100 minutes corresponds to approximately 110 g feed.

The accretion in foetus, placenta, uterine fluids and uterus during a 114 days gestation has been estimated to 4.8 MJ per kg foetus at farrowing, and of this approx. 72% is retained in foetuses, 12% in placenta, 5% in fluids, and 11% in the empty uterus, respectively (see Table 16.1). The energy deposited in the mammary gland makes up part of the maternal gain, and it has been estimated to increase from 340 kJ/day at day 70 of gestation to 400 and 630 kJ/day at days 90 and 110, respectively. Taken together, the total energy retained in the uterus and mammary gland amounts to about 1.1, 1.6, and 2.2 MJ/day at 70, 90, and 110 days of gestation, respectively, for a litter of 12 foetuses, corresponding to approx. 0.14, 0.21 and 0.29 FUsow, respectively.

Table 16.1. Partition of uterine gain between compartments, calculated for a 110 days gestation and 12 foetuses (adapted after [42].

Compartment	Weight		Dry matter		Protein		Energy	
	kg	%	g	%	g	%	MJ	%
Foetus	13.75	61	2444	73	1368	68	46.54	72
Placenta	4.31	19	387	12	272	13	7.79	12
Fluids	2.09	9	173	5	108	5	3.01	5
Empty uterus[a]	2.25	10	350	10	276	14	6.99	11
Total	22.15	100	3365	100	2153	100	64.33	100

a: Weight and content at mating were subtracted from values found at 110 days of gestation.

The impact of different weight gain during gestation for the ME requirement of gestating sows and its partition on different components is shown in Table 16.2. As appears, the daily ME requirement for a gestating sow weighing 150 kg at mating and kept at thermoneutral conditions ranges from approx. 25 to 30 MJ ME per day. For heavier sows, and sows with more physical activity or kept below LCT, these values can be considerably increased.

Table 16.2. Energy requirements of gestating sows of 150 kg body weight at mating according to their body weight gain during gestation (adapted after [42].				
Requirements for	**Body weight gain, kg**			
	30		40	
	MJ ME/day	%	MJ ME/day	%
Maintenance	21.14	85.7	21.60	75.3
Uterine growth	1.34	5.4	1.34	4.2
Maternal gain	2.18	8.8	5.7	20.0
Total requirement	24.66	100	28.67	100

The efficiency of utilization of ME for maintenance, maternal gain, and uterine gain has been estimated to 77, 75, and 50%, respectively. The values obtained for maternal gain and uterine gain are close to common estimates of efficiency of utilization of ME for fat and protein gain, respectively, and reflect that maternal body gain is mainly made up of fat accretion whereas mainly protein is retained in uterine tissues [41], [42], [43]. If these coefficients are adapted to the values in Table 15.2, and the data are recalculated to FUsow units they would correspond to an approximate daily feed supply of 2.4 and 2.8 FUsow for sows with 30 and 40 kg gain during gestation, respectively. Danish practical recommendations state that gestating sows should be fed according to body condition from service and until week 16 of gestation and the recommended daily feed allowance is 2.0 – 2.7 FUsow which corresponds well with the values calculated above. The feed supply is adjusted according to the body condition of the sow and it is recommended that it is gradually increased as gestation progresses. It is further recommended that the feed supply is increased by 0.2 FUsow per day during the last two weeks of gestation in order to accommodate the increasing needs for foetal growth. The last two to three days before expected parturition are considered as a preparation period, and it is recommended that the feed supply is decreased to 2.5 FUsow per day in order to minimize the occurrence of agalactia.

5.2. Protein and amino acids

Protein deposition in uterine tissues during gestation appears from Table 16.1 and amounts to approx. 2.2 kg for a litter of 12 foetuses until day 110 of gestation. This protein accretion should be supported by dietary protein, but in sows with very large litters and fed restrictedly some may be provided by mobilization of maternal body reserves. As appeared above, sow reproductive performance over a single parity is not very responsive to differentiated protein/amino acid supply.

Danish recommendations for protein and amino acid supply to gestating sows are issued by Pig Research Centre in a publication called "Nutrient Standards", and they state a minimum supply of 90 g digestible crude protein per FUsow. Recommendations for dietary supply of individual essential amino acids in g digestible amino acid per FUsow are provided as well: lysine 3.3, methionine 1.6, methionine + cystin 3.2, threonine 3.0, tryptophan 1.0, isoleucine 3.0, leucine 2.6, histidine 1.2, phenylalanine 1.9, phenylalanine + tyrosine 3.6, valine 3.5.

5.3. Vitamins and minerals

The Danish recommendations for vitamin supply to gestating sows are, according to "Nutrient Standards", stated in added amounts because the natural content in feedstuffs may vary considerably. The recommended supply per FUsow is as follows: vitamin A 8000 IU, vitamin D (D3) 800 IU, vitamin E 35 mg, vitamin K (K_3) 2 mg, thiamine (B_1) 2 mg, riboflavin (B_2) 5 mg, pyridoxine (B_6) 3 mg, niacin 20 mg, biotin 0.2 mg, D-pantothenic acid 15 mg, folic acid 1.5 mg and vitamin B_{12} 20 µg.

The vitamin requirement for reproductive processes is in many cases not completely known, and recommendations are to some extent based on extrapolation to requirements of animals in other life stages. It is also reasonable to assume that the requirement of some vitamins is elevated in reproducing animals, and there might be critical periods during gestation when a proper supply of these specific vitamins is necessary for survival and development of the progeny. Vitamin A is among vitamins for which a high dose supply to reproducing sows was claimed to improve reproductive performance. Vitamin A deficiency may cause weak or absent oestrous, resorption of foetuses and stillbirths, and therefore investigations looked into the effect of extra supply of vitamin A either as a dietary supplement or administered by injections. In some cases, positive effects on reproductive performance were reported, but in others no effect was found. Experiments carried out under Danish conditions did not demonstrate any positive effect of supplementary vitamin A, and it was concluded that with the feeding regimens applied in Denmark, sows are well supplied with vitamin A and no beneficial effect can be expected by increasing the supply further.

Folic acid is another vitamin that plays a vital role in early foetal development, and also for this vitamin it was speculated that extra supply could improve reproductive performance. Some findings indicate that a supply of folic acid of approx. 3 times the Danish recommendations decreased embryonic mortality. However, the experimental evidence is so far too limited to justify any changes of the recommendations.

The knowledge of the vitamin D requirement for pig reproduction is incomplete, and the results of a recent study [36] suggest that increasing the vitamin D supply (in the form of D_3 or 25-hydroxy-cholecalciferol) up to 1400 or 2000 IU/kg diet, which is considerably more than the presently recommended allowance, decreased the rate of stillborn piglets significantly.

The Danish recommendations for mineral supply according to "Nutrient Standards" include a safety margin, the exception being digestible phosphorus, which is a minimum recommendation. The dietary content of digestible phosphorus can be estimated from the total phosphorus content of the diet and the amount of added phytase, and the recommended minimum content of total phosphorus at normal phytase addition is 3.6 g per FUsow, whereas this amount can be reduced to 3.4 g per FUsow if a double amount of phytase is added. The recommended dietary content of essential minerals per FUsow is: calcium (Ca) 7.0 g, Ca when phytase is added 6.5 g, digestible phosphorus (P) 2.0 g, sodium (Na) 1.5 g, chloride (Cl) 2.5 g, potassium (K) 2.5 g, magnesium 0.4 g, iron (Fe) 80 mg, copper (Cu) 6 mg, manganese (Mn) 40 mg, zink (Zn) 100 mg, iodine (I) 0.2 mg and selenium (Se) 0.2 mg. However, Se must not exceed 0.5 mg.

The availability of dietary mineral elements is of critical importance, and the form in which a certain mineral is supplied may affect its availability, which in turn may influence animal performance. There are studies that have reported that sow reproductive performance was improved when trace minerals were supplied in organic form as mineral proteinases compared to inorganic mineral salts. The positive effect was recorded in total number of born and live born piglets [47].

6. Concluding remarks

This chapter has aimed to describe the general principles of the reproductive processes in domestic pigs. The sections concerning basal reproductive physiology have, owing to the scope of this book, been limited to the most important features, and the reader is therefore encouraged to find more detailed information from relevant textbooks. The main focus of the chapter was on the effects of nutrition on reproductive performance, and it can be concluded that nutrition has a substantial impact on the reproductive results, and that the reproductive performance can be manipulated by feeding strategy. For instance, the age at puberty can be delayed by sub-optimal energy and amino acid supply, and low energy supply may abolish cyclicity in animals after puberty.

However, if the gilt or sow conceives, foetal development will have such a high priority that even severe energy and protein restriction will have little effect on litter size, but might decrease piglet birth weight, and thereby piglet survival rate. Possibilities to improve piglet birth weight by manipulating feeding of the gestating sow are limited, though. Flush feeding of restrictedly fed gilts can restore ovulation rate to that of well-fed individuals, and it can in general be expected that gilts and sows in good nutritional state have a good reproductive performance. Feeding early after breeding should aim at avoiding embryo losses, and gilts on a low plane of energy supply have been shown to have low embryo mortality. Because of interactions between feed intake in gestation and lactation, restricted feeding should be practised for gestating sows, in order to allow for a sufficient feed intake during lactation. Owing to the high energetic costs of lactation sows usually remain anoetrous during lactation, and excessive weight loss of the lactating sow should be avoided in order to keep the weaning-to-oestrous interval short. However, in recent years the weaning-to-oestrous interval seems less affected by the metabolic status of the sow that previously. This is probably an effect of sows being selected for prolificacy.

7. References

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