Acta vet. scand. 1978, *19*, 229—242.

From the Department of Reproductive Physiology and Pathology, Veterinary College of Norway, Oslo.

FINE STRUCTURE OF SPERMATOGONIA AND SPERMATOCYTES IN THE BLUE FOX (ALOPEX LAGOPUS)

By

Kjell Andersen

ANDERSEN, KJELL: *Fine structure of spermatogonia and spermatocytes in the blue fox (Alopex lagopus).* Acta vet. scand. 1978, *19*, 229—242. — The ultrastructural features, characterizing the different types of spermatogonia and spermatocytes in the blue fox, have been studied within and near the reproductive season, and also in the summer and autumn.

Two distinct types of spermatogonia — A and B — are described. The A-spermatogonia often have a prominent nucleolus and numerous cytoplasmic organelles including characteristic whorls of AER. Large vacuoles containing electron dense particles are sometimes observed. In the B-spermatogonia the chromatin forms condensed areas of varying size, and the nucleolus is usually absent. The number of cytoplasmic organelles is generally small.

Ultrastructural characteristics are further used to distinguish between the different stages in the prophase of the primary spermatocytes. In leptotene the nucleus contains a thread-like chromatin with electron dense peripheral areas. Towards the end of the stage the mitochondria display dilated cristae, and aggregations of a granular material can be observed in the intermitochondrial matrix. Zytogene is characterized by the appearance of synaptinemal complexes in the nucleus, and of the chromatoid body and piles of annulate lamellae in the juxtanuclear cytoplasm. In pachytene the chromosomes become apparent as aggregations of condensed chromatin associated with the synaptinemal complexes. The Golgi complex is more prominent than in the previous stages, and the number of the other cytoplasmic organelles is increasing. In the last stages of the prophase (diplotene and diakenesis) the chromosomes become still more electron dense, the nucleolus appears as a very prominent structure, and there is a marked vesiculation of the cytoplasm.

The secondary spermatocytes have a characteristic nucleus with a somewhat irregular outline and larger peripheral areas of condensed chromatin. In the cytoplasm a double Golgi complex is frequently observed.

In the summer and autumn spermatocytes in zygotene seem to represent the most advanced form of spermatogenic cells.

spermatogonia; spermatocytes; fine structure; blue fox.

The spermatogonia and spermatocytes, which together with the spermatids and the Sertoli cells make up the male germinal epithelium, have been the subject of comprehensive light microscopical studies in a number of mammalian species (*Roosen-Runge* 1962, *Clermont* 1966, *Ortavant et al.* 1969). Detailed descriptions based on electron microscopy of the spermatogenetic epithelium have been given of spermatogonia and spermatocytes in the mouse (*Gardner & Holyoke* 1964) and rabbit (*Nicander & Plöen* 1969).

In the blue fox the fine structure of epididymal spermatozoa has been studied earlier (*Andersen* 1974), and an ultrastructural study of the developing spermatids of this species is also in progress (*Andersen* 1978). The aim of the present work is primarily to give an account of the most important ultrastructural features of the remaining types of the spermatogenetic cells in this species. Secondly, as the blue fox is a seasonal breeder, its reproductive activity mainly being restricted to the months of March and April, it would furthermore be of interest to compare the ultrastructural manifestations of spermatogenesis represented by the different types of cells within the season with those found at other times of the year.

MATERIAL AND METHODS

The material was obtained by castration of 19 foxes, all anaesthetized by an injection of 100–120 mg pentobarbital sodium i.v. In two of the animals this was performed in the middle of January, in nine during the actual breeding season, in two at the end of May, in two in the middle of June, in two in the middle of August and in the last two in the middle of October. In three of the foxes the testis was prefixed by perfusion with 3 % glutaraldehyde in Millonig's phosphate buffer. This was accomplished by insertion of a cannula into the aorta immediately cranial to the posterior mesenteric artery after laparotomy. The animals were castrated after perfusion for about 10 min., whereupon small slices of the testicular tissue were immersed in 3 % glutaraldehyde for further fixation for 1—2 hrs. and subsequently transferred to a solution of OsO_4 for a further 1½ hrs. (*Millonig* 1961). After dehydration in acetone, the material was embedded in Araldite.

From all the remaining animals the specimens were immersed into 3 % glutaraldehyde in 1—2 hrs. for fixation without pre-

ceding perfusion. The preparation was otherwise performed as described above. Ultrathin sections cut on an LKB ultrotome were collected on polyvinyl formal coated copper grids and stained with uranyl acetate and lead citrate (*Reynolds* 1963). The electron microscopy was performed with a Siemens Elmiscop I.A.

OBSERVATIONS

The topography of the different cells within the spermatogenic epithelium is indicated in Figs. 1 and 2. The spermatogonia, of which two distinct types — A and B — can be found, are situated close to the basal lamina, usually surrounded by the basal cytoplasm of the adjacent Sertoli cells. Between the spermatogonia and the spermatids which line the lumen of the seminiferous tubule, one or two generations of primary spermatocytes can be observed. Secondary spermatocytes, when present, are also found in this part of the epithelium.

Spermatogonia

The spermatogonia of type A (Figs. 3 and 4) usually have a spherical or slightly elongated nucleus. The chromatin often has a somewhat loose structure, but may also be found in smaller condensed aggregations. The nucleolus is generally large and may be homogenous, but more often it consists of a distinct nucleolonema and pars amorpha. In some cases more than one nucleolus can be observed, and each of these is then frequently associated with accumulations of granular and rather electron dense material. If the nucleolus is located peripherally, this material makes contact with the nuclear envelope, which usually has numerous pores. The cytoplasm, which is rich in free ribosomes, often contains a high number of slightly elongated mitochondria with distinct and regular cristae. The endoplasmic reticulum is mainly agranular (AER) and may be found in loose whorls. In some of the cells large vesicles containing clusters of electron dense particles are observed. These vesicles may also be seen in the intercellular space between the spermatogonium and the adjacent Sertoli cell.

The spermatogonia of type B (Fig. 5) generally have a somewhat more ovoid nucleus than those of type A. The chromatin forms condensed aggregations of varying size, some of these being situated in close apposition to the nuclear envelope which

has a regular appearance, with rather few pores. The nucleolus is absent or may be found in rare cases as a small irregular structure slightly less granular than the aggregations of condensed chromatin. The cytoplasm is sparse, and the number of organelles usually much smaller than in the spermatogonia of type A. The centrioles are found in the vicinity of a comparatively simple Golgi complex, close to the nucleus which in this region has a concave outline, forming a kind of shallow recess (Figs. 6 and 7).

Spermatocytes

The primary spermatocytes in interphase can only be distinguished from the spermatogonia of type B by their location within the spermatogenetic epithelium. More often, however, they are found in different stages of meiotic prophase, where the cells can be identified on the basis of a number of distinct ultrastructural characteristics.

In leptotene (Fig. 8) the nucleus is comparatively small and contains thread-like configurations of chromatin, which sometimes may form larger electron dense aggregations especially peripherally, close to the irregular nuclear envelope. The cytoplasm is not very abundant, but contains a relatively large number of mitochondria and free ribosomes. Towards the end of the stage, the mitochondria, which now very often display dilated cristae, are mostly found in clusters. In the intermitochondrial matrix within these clusters, accumulations of a granular, electron dense material are frequently observed.

In zygotene (Figs. 9 and 10) the nucleus undergoes a pronounced enlargement, and the characteristic synaptinemal complexes become apparent. The chromatin is still thread-like, but the peripheral electron dense aggregations are not so frequently observed as in the previous stage. In the cytoplasm, which is more voluminous than in leptotene, there is a marked increase of organelles. The Golgi complex has become more prominent, and the centrioles are now always found between this structure and the nucleus. Several piles of annulate lamellae are seen, predominantly in the juxtanuclear part of the cytoplasm. Structures of similar appearance are sometimes found near or even within the nuclear pores. In the vicinity of some of the annulate lamellae a spherical structure, presumably representing the so-called chromatoid body, can be observed close to the nuclear envelope. The endoplasmic reticulum is tubular and mostly

of the agranular type, and a comparatively large number of free ribosomes as well as some slightly larger particles can be seen.

In pachytene, which is the stage most frequently registered the chromatin aggregates in condensed areas around the synaptinemal complexes. The cytoplasm becomes more voluminous containing an increased number of the different organelles. The Golgi complex in particular reaches a high degree of development, and occasionally one or two of the larger vesicles may contain some homogenous material with a relatively high electron density. The annulate lamellae are not only found in the vicinity of the nucleus, but also in more peripheral parts of the cytoplasm.

In the later stages of the prophase (Fig. 12), there is a further increase in the electron density of chromatin aggregations adjoining the synaptinemal complexes, and a large nucleolus including a conspicuous pars amorpha and a prominent nucleolonema appears, usually somewhat peripherally in the nucleus. The nuclear envelope is often strikingly irregular, and there is a marked vesiculation of the cytoplasm. Occasionally, incipient formation of proacrosome granules can be observed.

In contrast to the spermatogonia, the primary spermatocytes are very rarely observed in the later phases of cell division.

The secondary spermatocytes (Fig. 13) are less frequently seen that the other spermatogenic cells, but may be found in a few sections together with primary spermatocytes in late leptotene. The nucleus is rather small with a characteristic irregular outline. The condensed chromatin forms numerous aggregations of varying size, the largest usually being found at the periphery of the nucleus. Centrally, a granular mass of a somewhat lower electron density than that of the heterochromatin may be observed, probably consisting of nucleolar material. In the juxtanuclear cytoplasm a very prominent Golgi complex appears which often includes larger vesicles containing proacrosome granules. In some cells a double Golgi complex can be seen. Otherwise, the cytoplasm includes all the other organelles found in the late primary spermatocytes except for the annulate lamellae which no longer seem to be present.

All types of the cells described above as well as spermatids in different stages of development have been observed, not only in the material collected during the actual breeding season, but also in that obtained from animals as early as in the middle of

January, and as late as the end of May. In the material obtained in the middle of June and later, most of the spermatogenic cells were identified as A-spermatogonia. These were as usual found close to the basal lamina of the epithelium together with a few B-spermatogonia. More centrally, surrounded by the apical cytoplasm of the Sertoli cells, some primary spermatocytes could also be observed, but these were never found in stages later than zygotene.

No clear difference in quality was found between the sections processed from the material prefixed by the in vivo perfusion technique and those from the material fixed only by immersion.

DISCUSSION

The ultrastructural characteristics used to define the different types of spermatogonia and various stages of premeiotic spermatocytes, are mainly the same as those described in rabbit (*Nicander & Plöen* 1969).

The prominent nucleoli, the numerous nuclear pores, cytoplasmic organelles and free ribosomes of the A-spermatogonia seem to indicate a high degree of cellular activity. The electron dense material found in the large intra- and extracellular vacuoles may be aggregations of glycogen particles. The significance of these vacuoles is not easily interpreted. The functional role of the whorls of AER is also rather obscure. It is possible, however, that both structures are involved in specific metabolic activities, especially since they are found predominantly in cells from testicular tissue obtained during the breeding season.

The comparatively large amount of coarse heterochromatin usually observed in the spermatogonia of type B, together with the absence of a prominent nucleolus, seems to indicate that these cells do not have the same capacity of protein synthesis as those of type A. This is also in agreement with observations in the rabbit (*Nicander & Plöen*) and mouse (*Monesi* 1965). Moreover, the spermatogonia of type B seem to divide further without the pronounced increase in the number of cytoplasmic organelles found in type A.

The possibility that some of the cells registered as spermatogonia of type B actually represent a generation of intermediate cells can not be completely excluded. The total number of spermatogonia distinctly different from that of type A is, however, comparatively low in all of the sections studied, thus indicating

that spermatocytogenesis from each stem cell does not involve a high number of mitoses. An exact determination of this number would, however, demand extensive light microscopical examinations of series of sections.

The change in nuclear fine structure of the primary spermatocytes constitutes the main basis for defining the different stages of the first meiotic prophase. The increase of the nuclear volume would seem consistent with an active synthesis of DNA, prerequisite for the doubling of each chromosome in pachytene (*Bishop & Walton* 1960). According to *Ortavant et al.* (1969), however, this synthesis occurs already in preleptotene (interphase). The synaptinemal complexes appearing in zygotene and throughout the rest of the prophase were first described in spermatocytes from crayfish (*Moses* 1956). In the blue fox, these complexes have been observed exclusively in the primary spermatocytes, and it would therefore seem reasonable to consider them as elements possibly associated with the process of close pairing off of homologous chromosomes taking place in the prophase of the first meiotic division. In other species, however, synaptinemal complexes have also been observed in spermatids (*Zamboni* 1971).

The appearance of a large nucleolus at the transition from late pachytene to early diplotene is in accordance with observations made in mouse spermatocytes (*Kierszenbaum & Tres* 1974) and in quail oocytes (*Mirre & Stahl* 1976) where the nucleolar development seems to be associated with definite chromosal structures in this stage of the prophase.

The fine structure of the cytoplasmic organelles does not deviate in principle from that observed in the primary spermatocytes of other mammals. The interstitial accumulation of granular material in the mitochondrial clusters of spermatocytes in leptotene has previously been observed in rabbits (*Nicander & Plöen*) and in other rodents (*Fawcett et al.* 1970). These accumulations may be the origin of the granular material later found in spermatids of the blue fox (*Andersen* 1978) or the so-called satellite of the chromatoid body described in other species (*Fawcett* 1972). The chromatoid body itself, appearing in the following stage of the prophase, could also be derived from the intermitochondrial accumulations as suggested by *Fawcett et al.* From other observations pertaining to morphology and staining reactions, it is, on the other hand, concluded that the chromatoid

body may be of nucleolar origin (*Comings & Okada* 1972). In blue fox spermatocytes, its close topographical relation to the nucleus provides some support to the last theory, even if no conclusive evidence can be obtained from a purely morphological study. As a whole, the origin of the chromatoid body still seems to be somewhat controversial, and several questions as to its chemical composition and its function remain to be answered. Results of autoradiographic experiments in rats seem, however, to imply that it contains RNA (*Söderström & Parvinen* 1976), thus probably being an organelle where stable messenger RNA can be stored until later in spermatogenesis. Also in the blue fox it reappears in the spermatids where it can be observed almost throughout the entire spermateleosis (*Andersen* 1978) possibly playing a role in the regulation of this complex process of cytodifferentiation.

The stacks of flattened cisternae referred to as the cytoplasmic annulate lamellae, which in leptotene are found in immediate vicinity to the nuclear envelope and later widely distributed also in the peripheral parts of the cell, have likewise been observed in primary spermatocytes of rabbits (*Nicander & Plöen*), and also in human primary oocytes (*Zamboni*) and primary oocytes in pig (*Norberg* 1972). Moreover, in the latter species these structures were seen in the pronuclear stage of fertilized tubal ova where also intranuclear annulate lamellae could be demonstrated (*Norberg* 1973). In addition, intranuclear annulate lamellae have been found in fertilized ova collected from rabbits (*Zamboni & Mastroianni* 1966) and human oviducts (*Zamboni et al.* 1966). According to a rather widely accepted theory, the annulate lamellae would arise from vesicles produced by blebbing of the two membranes constituting the nuclear envelope (*Zamboni*). The presence of single flattened cisternae within the nuclear pores in the primary spermatocytes of the blue fox lends some support to this hypothesis. Another possibility is that the annulate lamellae are formed as intranuclear structures, whereupon they are extruded through the nuclear pores. The functional significance of the lamellae, which at least in the blue fox spermatocytes seem to disappear during diplotene, is not easily interpreted. As they are also found in embryonal (*Merkow & Leighton* 1966) and neoplastic cells (*Schulz* 1957) it would, however, seem that their presence is in some way associated with a high rate of cellular growth and differentiation.

The change in the internal structure of the mitochondria, probably taking place during leptotene, gives these organelles an "empty" appearance. This seems to be a characteristic feature of the mitochondria in spermatocytes when the material is prefixed in glutaraldehyde, whereas normal narrow cristae are seen after potassium permanganate fixation (*Nicander & Plöen*). This structural lability may indicate a high rate of metabolic activity.

The gradual increase in size and complexity of the Golgi apparatus observed in the primary spermatocytes during the prophase is a clear manifestation of developmental events preparatory to the role of this entity in the formation of the acrosome within the spermatid in the later part of spermatogenesis. The accumulation of dense material in a few of the larger Golgi vesicles sometimes observed in the late prophase, is obviously identical with the proacrosome granules found in the primary spermatocytes of rabbits (*Nicander & Plöen*).

According to the very few observations of actively dividing primary spermatocytes, the later phases of the first meiotic division must be of a very short duration.

The identification of the secondary spermatocytes is based on their location within the epithelium, and on their rather characteristic morphology. Apart from being more irregular in outline, the nucleus mainly displays the same ultrastructural features as in the corresponding cells of the mouse (*Gardner & Holyoke* 1964). The occurrence of a double Golgi complex shows that this structure may devide before the entire cell enters the second meiotic division.

The scarcity of secondary spermatocytes within the sections examined indicates that these cells represent a very short phase of spermatogenesis. This is in agreement with the conclusions from light microscopical studies in other species (*Ortavant et al.*).

In contrast to the findings reported in various other species (*Nicander & Plöen, Dym & Fawcett* 1971) the mitotic divisions in blue fox spermatogenesis must generally result in a complete cytokinesis, since very few intercellular bridges can be found between either the spermatogonia both of type A and B, or between the primary spermatocytes. As to the first meiotic division, no definite conclusion can be made, because of the low number of secondary spermatocytes observed.

Testicular regression following the cessation of the breeding

Figures 1—13. Electron micrographs of spermatogenic cells in the blue fox.

Figure 1. Spermatogenic epithelium near the basal lamina (Bl). S = Sertoli cell enclosing a part of the cytoplasm of a spermatogenic cell (arrow). Sn = Sertoli cell nucleus. SgA = Spermatogonium of type A. SgB = Spermatogonium of type B. ScI = Primary spermatocyte. 4600 ×.

Figure 2. Spermatogenic epithelium near the lumen of the tubule. Sn = Sertoli cell nucleus. ScI = Primary spermatocytes (early prophase). ScII = Secondary spermatocyte. St = Late spermatids. 4600 ×.

Figure 3. Spermatogonium of type A showing a high number of mitochondria (M) and some tubular AER which may form loose whorls (W). The nucleus (N) has a prominent nucleolus (Nl) and numerous small aggregations of condensed chromatin. 12,000 ×.

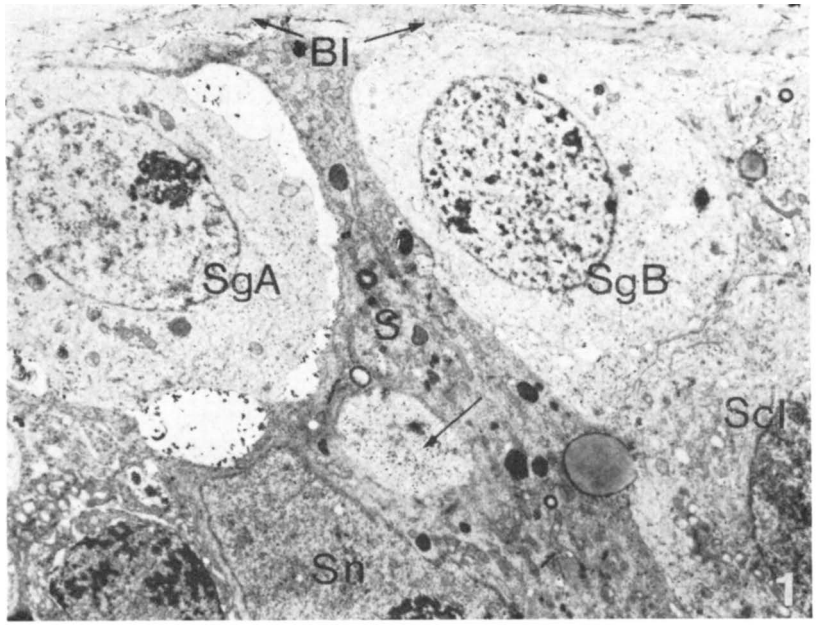
Figure 4. Spermatogonium of type A with a large vacuole (V) containing some clusters of granular material. In the nuclear envelope (Ne) numerous pores can be seen. N = Nucleus, Nl = Nucleolus, M = Mitochondria. 12,000 ×.

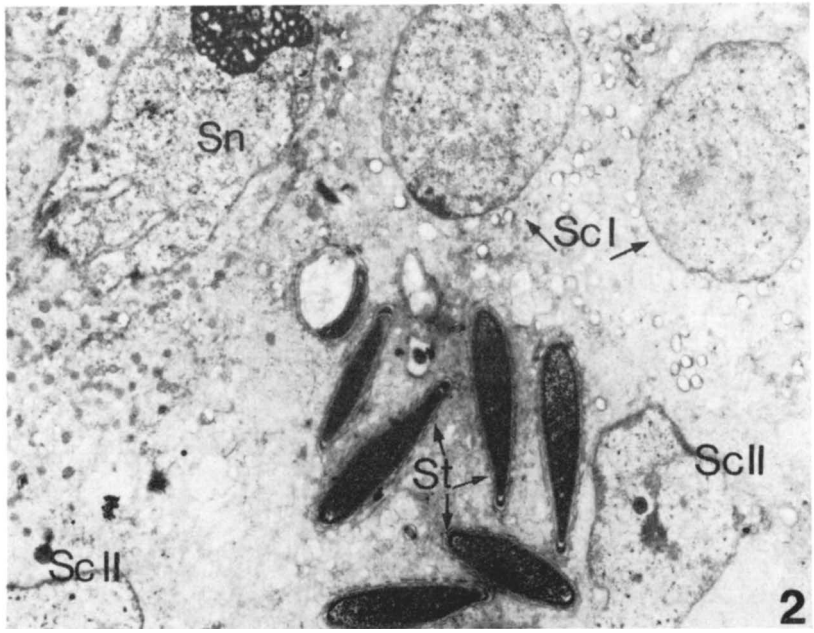
Figure 5. Spermatogonium of type B. In the nucleus (N) areas of condensed chromatin can be seen, some of these are found close to the nuclear envelope (arrows). In the cytoplasm, which contains some free ribosomes, a few round or ovoid mitochondria (M) can be observed together with tubules of AER. Cm = membrane. Bl = Basal lamina. 9000 ×. Inset: Centrioles (C) found in a juxtanuclear position. G = Golgi complex. 18,000 ×.

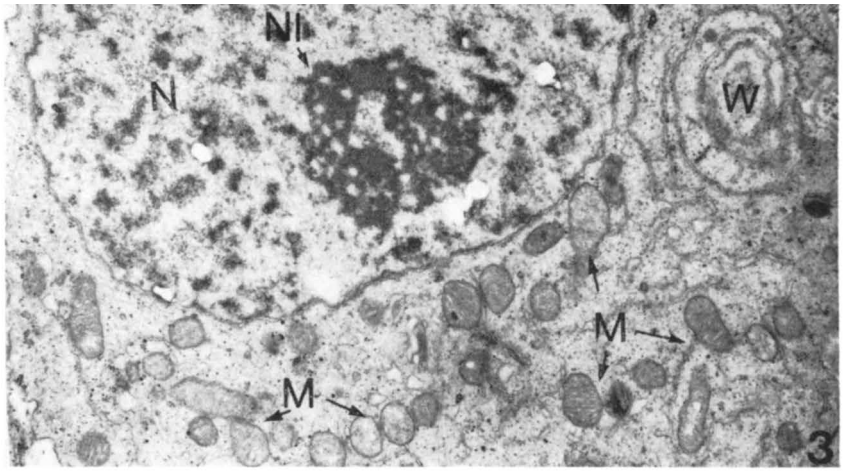
Figure 6. Dividing spermatogonium (Late prophase or early metaphase). The nuclear envelope has disappeared, the chromosome (Ch) lying free in the cytoplasm. Bl = Basal lamina. 3450 ×.

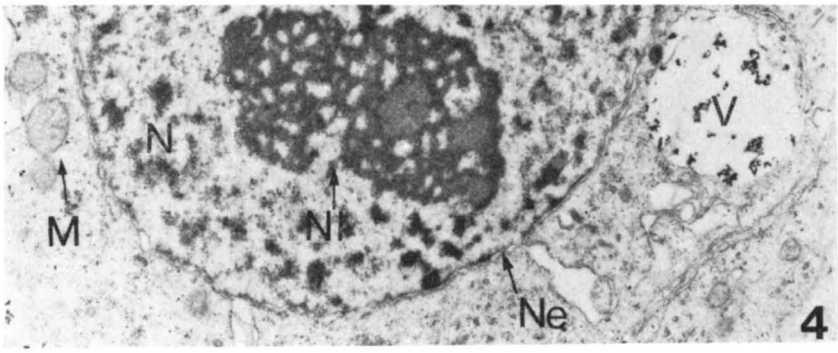
Figure 7. Dividing spermatogonium (anaphase). Cytoplasmic organelles (mitochondria — M) are seen between the two groups of chromosomes (Ch) (The large vacuoles (V) situated between the spermatogonium and surrounding Sertoli cell would indicate that the former is of type A). Bl = Basal lamina. 3450 ×.

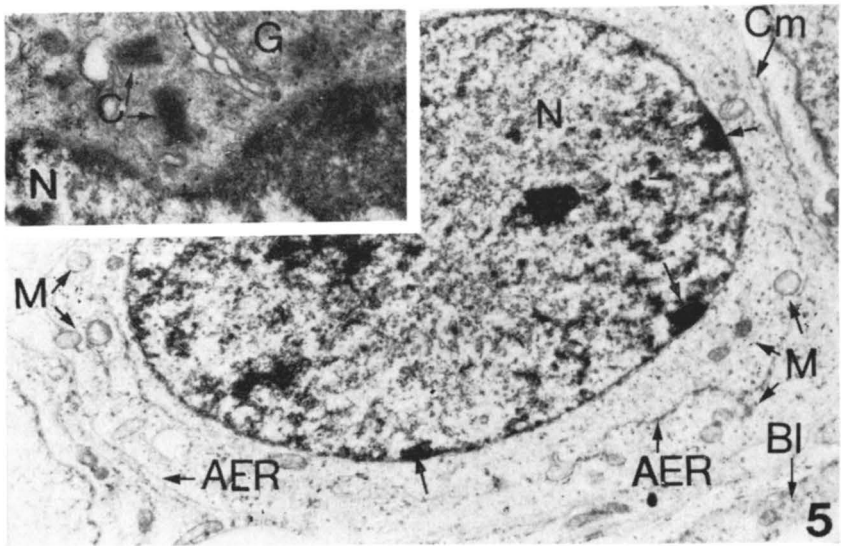
Kjell Andersen: Fine structure of spermatogonia and spermatocytes in the blue fox (Alopex lagopus).

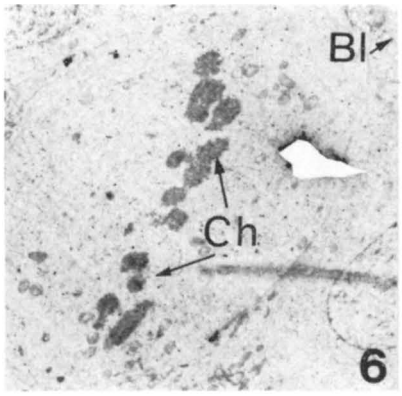


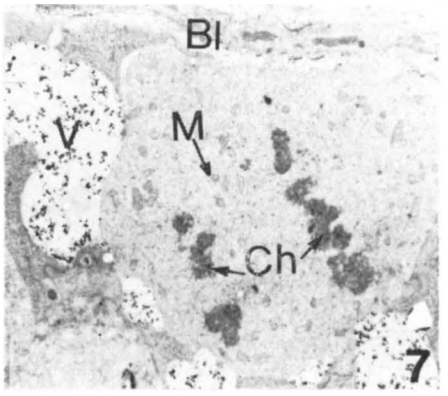


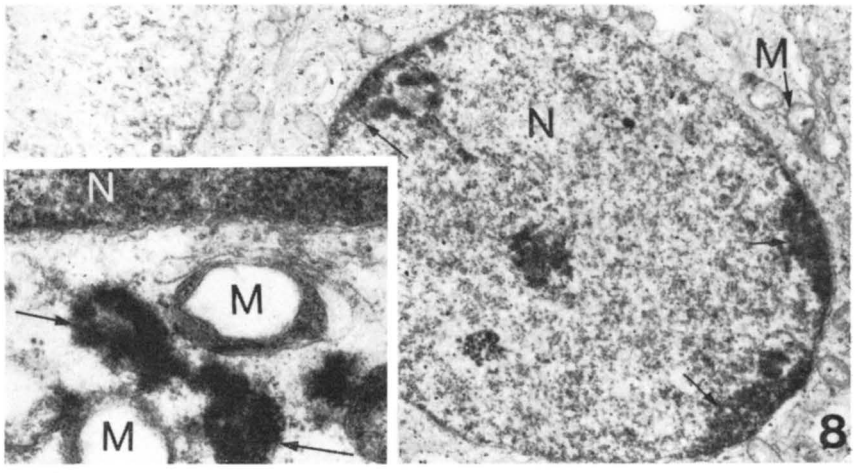

Nl
N
W
M
M
3

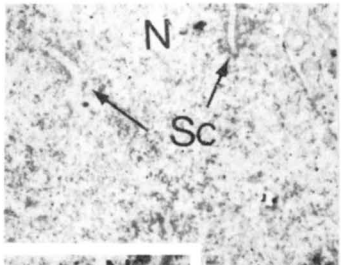

V
N
M
Nl
Ne
4

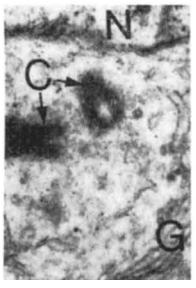

G
C
N
Cm
N
M
M
Bl
AER
AER
5

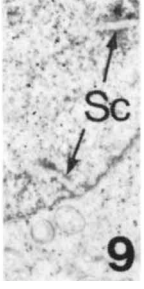

Bl
Ch
6

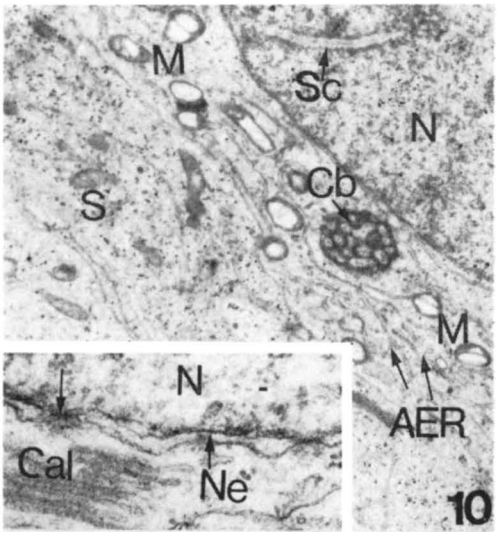

Bl
V
M
Ch
7

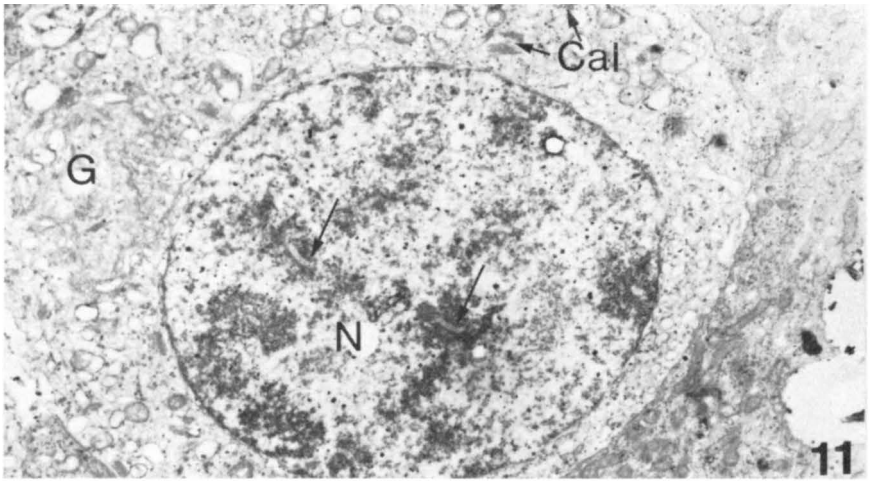

M
N
N
M
M
8

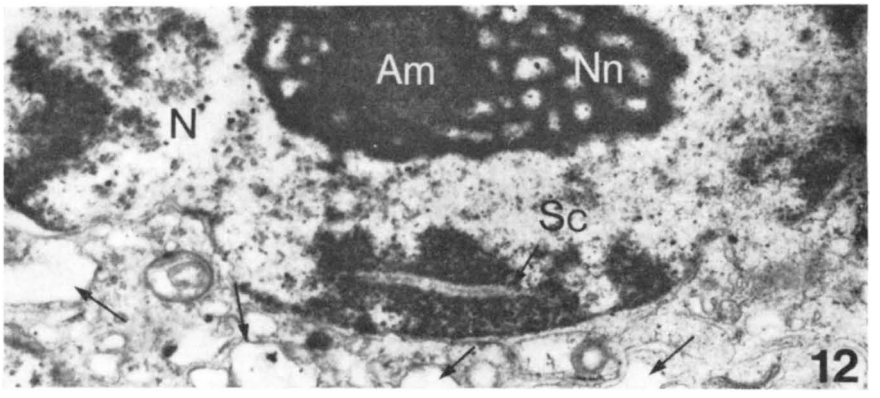

N
Sc


N
C
G


Sc
9


M
Sc
N
S
Cb
M
AER
N
Cal
Ne
10


Cal
G
N
11


Am
Nn
N
Sc
12

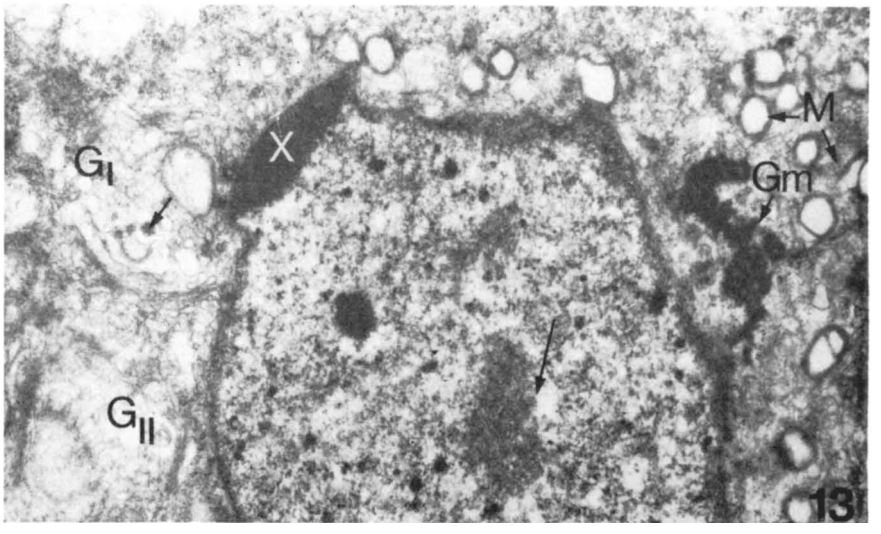

M
X
GI
Gm
GII
13

Figure 8. Primary spermatocyte, early leptotene. In the nucleus (N) the chromatin has a thread-like appearance with some condensed areas especially at the periphery (arrows). In the cytoplasm, which contains numerous dense particles, some mitochondria (M) with slightly dilated cristae can be observed. 9000 ×. Inset: Intermitochondrial accumulations of electron dense material (arrows) in the cytoplasm close to one of the peripheral condensed areas of the nucleus. 39,000 ×.

Figure 9. Primary spermatocyte in zygotene, the nucleus (N) showing thread-like chromatin and several synaptinemal complexes (Sc). 9000 ×. Inset: Centrioles (C) situated between the nucleus and the Golgi complex (G). 18,000 ×.

Figure 10. Primary spermatocyte, zygotene. The chromatoid body (Cb) is found in a juxtanuclear position. Tubules of AER and some mitochondria (M) are indicated. N = Nucleus, Sc = Synaptinemal complex, S = Sertoli cell cytoplasm. 12,000 ×. Inset: Cytoplasmic annulate lamellae (Cal) close to the nuclear envelope (Ne). In one of the nuclear pores a lamellar structure can be discerned (arrow). 45,000 ×.

Figure 11. Primary spermatocyte, pachytene. In the nucleus (N) synaptinemal complexes are seen within some of the pairs of homologous chromosomes (arrows). The prominent Golgi complex (G) and some of the cytoplasmic annulate lamellae (Cal) are indicated. 6900 ×.

Figure 12. Primary spermatocyte in late prophase. In the nucleus (N) a peripherally oriented pair of dense granular chromosomes can be seen in association with a synaptinemal complex (Sc). A prominent nucleolus with a pars amorpha (Am) and a nucleolonema (Nn) is indicated. In the cytoplasm numerous vesicles are seen (arrows). 18,000 ×.

Figure 13. Secondary spermatocyte. In the nucleus a peripheral area of condensed chromatin (X) can be seen in addition to smaller electron dense areas. Some granular material of a somewhat lower electron density is found centrally (long arrow). A double Golgi complex (G_I and G_{II}), with one of the vesicles containing a granule (short arrow), is situated close to the nucleus. On the opposite side accumulations of granular material (Gm) are indicated. The mitochondria (M) show dilated cristae. 17,000 ×.

season seems to take place in late May — early June. The fact that the spermatogonia of type A are the spermatogenic cells most frequently observed at this time of the year, would probably lend some support to the idea of regarding them as the actual stem cells of the seminiferous epithelium. The few other cells, which can be recognized on the ultrastructural criteria generally used for identification of spermatogenic cells, might reflect a certain degree of mitotic activity in the epithelium, the spermatogenesis presumably being completely arrested early in the prophase of the first meiotic division.

REFERENCES

Andersen, K.: Ultrastructural studies of blue fox spermatozoa. Acta vet. scand. 1974, *15*, 620—630.

Andersen, K.: Fine structure of developing spermatids used as a basis for staging of the spermateleosis in the blue fox (Alopex lagopus). Zbl. Vet.-Med. Reihe A 1978. In press.

Bishop, M. W. & A. Walton: Spermatogenesis and the structure of mammalian spermatozoa. *In* Marshall's Physiology of Reproduction, ed. Parkes. Longmans, London 1960, vol. 1:2, 1—129.

Clermont, Y.: Spermatogenesis in man. A study of spermatogonial population. Fertil. and Steril. 1966, *17*, 705—721.

Comings, D. E. & T. A. Okada: The chromatoid body in mouse spermatogenesis: Evidence that it may be formed by extrusion of nucleolar components. J. Ultrastruct. Res. 1972, *39*, 15—23.

Dym, M. & D. W. Fawcett: Further observations on the spermatogonia, spermatocytes and spermatids connected by intercellular bridges in mammalian testis. Biol. Reprod. 1971, *4*, 195.

Fawcett, D. W.: Observation on cell differentiation and organelle continuity in spermatogenesis. Proc. Int. Symp. Spermatozoon, Edinburgh, ed. Beatty and Gluecksohn-Waelsch 1972, p. 37—68.

Fawcett, D. W., E. M. Eddy & D. M. Phillips: Observations on the fine structure and relationship of the chromatoid body in mammalian spermatogenesis. Biol. Reprod. 1970, *2*, 129—153.

Gardner, P. J. & E. Holyoke: Fine structure of the seminiferous tubule of the Swiss mouse. I. The limitting membrane, Sertoli cell, spermatogonia and spermatocytes. Anat. Rec. 1964, *150*, 391—404.

Kierszenbaum, A. L. & L. L. Tres: Nucleolar and perichromosomal RNA synthesis during meiotic prophase in the mouse testis. J. Cell Biol. 1974, *60*, 39—53.

Merkow, L. & J. Leighton: Increased numbers of annulate lamellae in the myocardium of chick embryos incubated at abnormal temperatures. J. Cell Biol. 1966, *28*, 127—137.

Millonig, G.: Advantages of a phosphate buffer for OsO_4 solutions in fixation. J. appl. Phys. 1961, *32*, 1637.

Mirre, C. & A. Stahl: Ultrastructural study of nucleolar organizers in the quail oocyte during meiotic prophase. I. J. Ultrastruct. Res. 1976, *56*, 186—201.

Monesi, V.: Synthetic activity during spermatogenesis in the mouse. Exp. Cell Res. 1965, *39*, 197—224.

Moses, M. J.: Chromosomal structure in crayfish spermatocytes. J. biophys. biochem. Cytol. 1956, *2*, 215—218.

Nicander, L. & P. Plöen: Fine structure of spermatogonia and primary spermatocytes in rabbits. Z. Zellforsch. 1969, *99*, 221—234.

Norberg, H. S.: The follicular oocyte and its granulosa cells in domestic pig. Z. Zellforsch. 1972, *131*, 497—517.

Norberg, H. S.: Ultrastructure of pig tubal ova. The unfertilized and pronuclear stage. Z. Zellforsch. 1973, *141*, 103—122.

Ortavant, R., M. Courot & M. T. Hochereau: Spermatogenesis and morphology of the spermatozoon. *In* Reproduction in Domestic Animals, ed. H. H. Cole and P. T. Cupps. Sec. Ed. Acad. Press, New York and London 1969, 251—276.

Reynolds, E. S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 1963, *17*, 208—212.

Roosen-Runge, E. C.: The process of spermatogenesis in mammals. Biol. Rev. 1962, *37*, 343—377.

Schulz, H.: Elektronenmikroskopische Untersuchungen eines Mammakarzinoms der Ratte. (Electron microscopy of a mammary carcinoma in rat). Oncologia (Basel) 1957, *10*, 307—329.

Söderström, K. O. & M. Parvinen: Incorporation of ^3H uridine by the chromatoid body during rat spermatogenesis. J. Cell Biol. 1976, *70*, 239—246.

Zamboni, L.: Fine Morphology of Mammalian Fertilization. Harper & Row. New York, Evanston, San Francisco, London 1971.

Zamboni, L. & L. Mastroianni: Electron microscopic studies on rabbit ova. II. The penetrated tubal ovum. J. Ultrastruct. Res. 1966, *14*, 118—132.

Zamboni, L., D. R. Mishell, J. Bell & M. Bacca: Fine structure of the human ovum in the pronuclear stage. J. Cell Biol. 1966, *30*, 579—600.

SAMMENDRAG

Ultrastrukturelle undersøkelser av spermatogonier og spermatocytter hos blårev.

De forskjellige typer av spermatogonier og spermatocytter hos blårev er undersøkt ved elektronmikroskopi. Undersøkelsesmaterialet besto av testikkelvev skaffet tilveie ved kastrasjon av kjønnsmodne rever — dels like før, under og like etter avlssesongen (mars — april) — dels utover sommeren og høsten.

Av spermatogoniene, som alltid ligger nær inntil basalmembranen av epitelet, ble det funnet to distinkt forskjellige typer. Type A-spermatogoniene hadde tilnærmet rund kjerne med små partier av fortettet kromatin og en stor velutviklet nucleolus. I cytoplasma, som var relativt rikt på organeller, kunne en av og til iaktta koncentriske dannelser bestående av AER. Videre ble det forholdsvis ofte observert større og mindre vakuoler, dels intracellulært, dels intercellulært mellom spermatogoniet og den tilgrensende Sertoli-celle. Type B-spermatogoniene hadde avlang kjerne av noe varierende størrelse, med større kromatinfortetninger og en lite framtredende nucleolus. Det var relativt sparsomt med cytoplasmatiske organeller.

De primære spermatocytter ble oftest funnet i meiotisk profase, og på grunnlag av en del ultrastrukturelle karakteristika kunne denne også hos blårev inndeles i flere klart definerte stadier. I leptotene var kromatinet trådaktig med fortetninger ut mot kjernemembranen. Mot slutten av stadiet kunne en iaktta dilaterte cristae i mitochondriene. Mellom disse ble det ofte observert ansamlinger av granulær elektron-tett substans. I zygotene opptrådte såkalte synaptinemal-komplekser i kjernen, og i cytoplasma kunne en tett inntil kjernemembranen påvise det s.k. kromatoidlegeme og dessuten tydelige lamellstrukturer (cytoplasmic annulate lamellae). I pachytene kom kromosomene tydelig fram som markante kromatin-fortetninger knyttet til synaptinemal-kompleksene, og i cytoplasma som nå hadde tiltatt sterkt i omfang fant en et velutviklet Golgi-kompleks foruten tallrike andre cytoplasmatiske organeller. I de siste stadier av profasen (diplotene og diakinesis) økte kromosomenes elektrontetthet ytterligere, nucleolus ble vanligvis svært framtredende, og cytoplasma var gjerne sterkt vesiculert.

De sekundære spermatocytter viste en karakteristisk, ofte noe uregelmessig kjerne med større perifere partier av fortettet kromatin. I cytoplasma kunne en ofte se to, tilsynelatende atskilte Golgi-komplekser.

Samtlige av de omtalte celletyper kunne påvises fra midten av januar til slutten av mai. Fra og med midten av juni syntes spermatogenesen å stoppe opp relativt tidlig i profasen av først meiotiske deling, idet en nå ikke kunne registrere mer avanserte celletyper enn primære spermatocytter i zygotene.

(Received December 22, 1977).

Reprints may be requested from: Kjell Andersen, The Department of Reproductive Physiology and Pathology, Veterinary College of Norway, Postboks 8146, Oslo Dep., Oslo 1, Norway.