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HISTOLOGICAL STUDIES ON THE KINETICS OF THE SPERMATOGENESIS IN THE MINK (*MUSTELA VISON*)*1 VII CELLULAR ASSOCIATION IN THE SEMINIFEROUS EPITHELIUM IN THE PRE-BREEDING SEASON (16 MONTHS OLD)

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The cellular association of the seminiferous epithelium in five minks, 16 months old, was quantitatively observed. Two different types of cellular associations were found. One was quite similar to the cellular associations observed in the breeding season and could be divided into eight steps. The other did not correspond to any step. Significant differences were noted among the relative frequency of the eight steps from six sites of both testes. The decrease in the number of each generation of the spermatogenic cells was related to the degenerations of the primary spermatocytes at the pachytene phase in steps 6~8 and of the intermediate-type spermatogonia in step 6.

INTRODUCTION

In previous studies, TIBA[9,10] found that most cellular associations of the seminiferous epithelium observed in the pre-breeding season in the mink were divided into nine "stages" of the cycle of the seminiferous epithelium in the breeding season. There was another feature discovered in the kinetics of the spermatogenesis in the mink pre-breeding season.

Generally, the corresponding periods of the seminiferous epithelium cycle with their definite cellular associations are referred to as "stages", or as "stages of the cycle" in adult animals (LEBLOND & CLERMONT); therefore, the conception of "stage" in the mink pre-breeding season (TIBA)[9,10] seems to be different from that of the "stage" in the breeding season. For these reasons, the term "step", rather than "stage", was used in this study.

The present study was carried out in order to determine quantitatively the relative frequency of the nine steps in three sites of the testis following the

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methods described by TIBA[9], and to define the relationship between the degenerations of the spermatogenic cells and the kinetics of the spermatogenesis in the mink pre-breeding season.

MATERIALS AND METHODS

Five adult, male Pastel minks, ranging from 2,030 g to 2,440 g body weight, were used in this study. All animals were 16 months old before the commencement of the second breeding season.

The testes of all animals were removed and fixed in Allen's PFA-3 fixative fluid for 7 hours. Each testis was cut serially to 7 μ thickness from head to tail. The sections were divided into three sites: the capital pole; the equatorial zone; and the caudal pole, respectively. The three sections were obtained from each site every 700 μ and stained by the periodic acid-schiff-haematoxylin technique (PAS-haem).

The cellular associations of the seminiferous epithelium in the cross-section of the seminiferous tubule were divided into nine steps following the method described by TIBA[9]. The relative frequency of these steps was determined by classifying the round or nearly rounded seminiferous tubules, at each of six sites in both testes microscopically.

In each of the nine steps, the tubular diameters were determined by measuring at least 30 round seminiferous tubules, and the nuclear diameters were determined by measuring at least 50 whole nuclei of each of the seminiferous epithelium cells. Measurements were done with the eye-micrometer. Each of the tubular and nuclear diameters were measured at angles, and the two values were averaged, respectively.

All of the whole nuclei and fragments of the spermatogenic and Sertoli cells for each of the nine steps were counted in the same seminiferous tubules as used for the measurements of the nuclear diameters of these cells. All of the 'raw' counts of the spermatogenic cells, except the Sertoli cells, were transformed to nuclear points (ABERCROMBIE) by an adaptation of Abercrombie's formula (SWIERSTRA & FOOTE).

The mitotic index of each type of the spermatogonia and pyknotic index of each generation of the spermatogenic cells for each of nine steps were determined by calculating the percentage of these cells undergoing mitosis or pyknosis. The seminiferous tubules for the determination of the mitotic and pyknotic indexes were used for the determination of the mean number of spermatogenic cell's nuclei.

A χ^2-test was made on the six sites of both testes of the four minks following the method described by TIBA & ISHIKAWA, and SUNG.

Results

The gonocyte-like cells, spermatogonia, primary spermatocytes and Sertoli cells were observed, but the remainders of the spermatogenic cells, i. e., the secondary spermatocytes, spermatids and spermatozoa, did not appear. Morphologically, it was difficult to distinguish the type-A spermatogonia (type-A) from

TABLE 1 *Relative frequency (%) of eight steps with normal cellular associations*

ANIMAL NUMBER		STEP	1	2	3	4	5	6	7	8	TOTAL NO. OF TUBULES
		C.A.*	GA	GAI	GAB	GABR GAR	GAL GAZ	GAIP GABR	GABRP GARP	GALP GAZP	
B_{11}	R	I	64.3	1.3	1.6	10.6	20.0	1.3	0.3	0	310
		II	71.6	0.4	1.8	9.1	14.0	0.9	1.8	0.4	451
		III	72.4	2.1	4.5	7.8	11.5	0	1.2	0.4	243
	L	I	71.2	3.0	1.9	6.0	13.5	3.0	0.5	0.8	364
		II	74.8	2.2	3.1	6.7	11.4	1.1	0.2	0.4	448
		III	85.8	3.2	2.0	4.0	4.0	0	0.8	0	247
B_{12}	R	I	88.3	1.5	2.8	3.6	3.8	0	0	0	394
		II	88.1	1.9	3.4	2.3	3.9	0.3	0	0.2	622
		III	87.3	2.1	3.8	1.9	4.2	0.2	0.2	0.2	520
	L	I	89.0	4.3	2.8	0.6	2.8	0.6	0	0	326
		II	89.4	2.5	3.4	2.8	1.4	0.5	0	0	435
		III	87.1	3.7	3.5	3.5	1.2	0.7	0	0.2	402
B_{13}	R	I	91.9	5.5	1.6	0.9	1.4	0.7	0	0	434
		II	92.8	3.5	1.8	0.6	1.0	0.3	0	0	684
		III	93.4	3.0	0.7	0.2	2.3	0.3	0.2	0	603
	L	I	91.3	5.0	1.0	0.8	1.2	0.6	0	0	483
		II	87.2	6.5	3.2	0.9	1.5	0.7	0	0	537
		III	91.1	5.8	1.0	0.2	1.2	0.6	0.2	0	503
B_{14}	R	I	56.0	1.0	4.0	29.5	4.5	0.5	3.5	1.0	200
		II	50.5	1.5	7.0	30.0	5.5	1.5	3.5	1.0	200
		III	54.5	1.5	3.5	32.0	4.0	0	3.0	1.5	200
	L	I	53.5	1.0	3.0	36.5	1.0	0	3.5	1.0	200
		II	55.5	0.5	3.5	34.5	1.0	0	5.0	0	200
		III	52.0	0	3.0	38.0	1.0	0	5.0	1.0	200
B_{15}	R	I	100.0	0	0	0	0	0	0	0	483
		II	100.0	0	0	0	0	0	0	0	565
		III	100.0	0	0	0	0	0	0	0	397
	L	I	99.6	0.2	0.2	0	0	0	0	0	504
		II	99.2	0.3	0.5	0	0	0	0	0	643
		III	99.8	0	0.2	0	0	0	0	0	460

A total of 12,258 seminiferous tubules in the cross-section were classified.
R: right testis, L: left testis, I: capital pole of a testis, II: equatorial zone and III: caudal pole.
GA, I, B, R, L, Z and P: refer to the text for definitions.
* C.A.: cellular association

the gonocyte-like cells described by TIBA[11]; therefore, in this study, the spermatogonia were divided into three types of spermatogonium: 1) type-A involving a gonocyte-like cell (type-GA); 2) an intermediate-type spermatogonium (type-I); and 3) type-B spermatogonium (type-B).

The cellular association indicating step 9 did not appear in any seminiferous tubule in any of the minks. Type-A occurred in steps 1~8; type-I were formed from type-A in steps 2 and 6; type-B were formed from type-I in steps 3 and 6; and the primary spermatocytes could be classified according to the phases of these cells: pre-leptotene (R); leptotene (L); zygotene (Z); and pachytene (P).

The results of the relative frequency of the eight steps are shown in table 1. The frequency in step 1 was the highest, and the frequencies in steps 6~8 were lower than those in steps 1~5. The cellular association of step 1 was characterized only by the presence of type-GA and Sertoli cells.

The results of the relative frequency of the steps (tab. 1) were analyzed and tested using the discriminatory analysis and χ^2-test. A significant difference was noted in the relative frequency of each step among the six sites in total in both testes [$\chi^2_s = 51.09 > \chi^2_{35}(0.05) = 50.05$].

The numbers of seminiferous tubules which did not correspond to any step in the seminiferous epithelium are shown in table 2. The occurrence of cellular asssociation GAP (type-GA, primary spermatocytes (P) and Sertoli cells) was the highest.

TABLE 2 *The numbers of tubules do not correspond to any step or relative frequency of GAP*[*1]

ANIMAL NUMBER		NO. OF TUBULES					TOTAL	GAP[*1] (%)
		C.A.[*2] GAP	GAIB	GAIBR	GAIR	GAIL		
B_{11}	R	106	1	1	0	1	109	9.5
	L	90	1	0	0	1	92	7.5
B_{12}	R	35	1	0	1	0	37	2.2
	L	27	0	0	0	0	27	2.3
B_{13}	R	31	3	0	0	0	34	1.8
	L	29	0	0	0	0	29	1.9
B_{14}	R	23	0	2	2	0	27	3.7
	L	26	1	5	4	0	36	4.2
B_{15}	R	0	0	0	0	0	0	0
	L	6	0	0	0	0	6	0.4

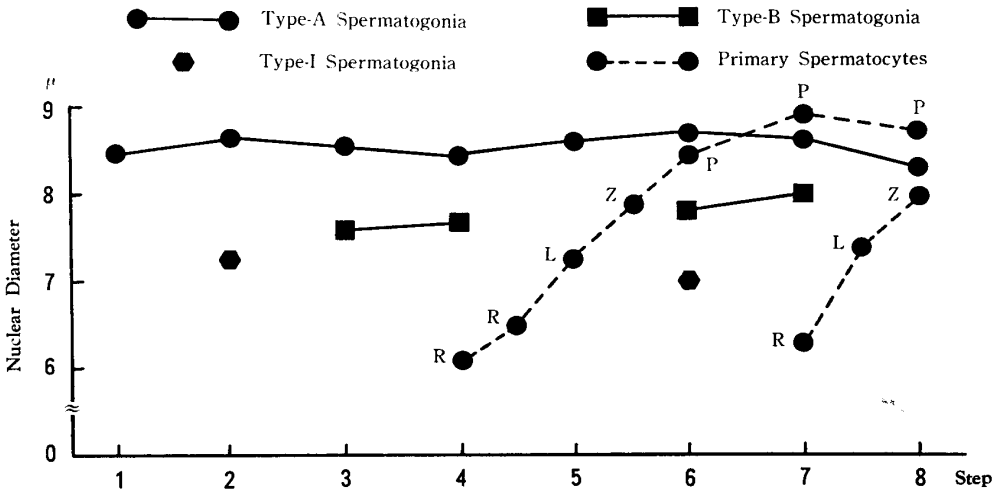
R: right testis L: left testis

G, A, I, B, R, L and P: refer to the text for definitions.

*1 Relative frequency of GAP: total numbers of tubules of cellular association GAP/(No. of tubules of GAP+No. of tubules from step 1 to step 8)×100 (%)

*2 C.A.: cellular association

TEXT-FIGURE 1 *Nuclear diameters of spermatogenic cells in eight steps* (Mean ± 0.02~0.23 μ)



R : Pre-leptotene (primary spermatocyte) Z : Zygotene
L : Leptotene P : Pachytene
A total of 1,986 whole nuclei was measured.

The tubular diameters for steps 1~8 were 83.5±0.21, 83.2±0.19, 85.9±0.21, 90.2±0.20, 88.9±0.18, 96.3±0.21, 90.0±0.23 and 94.0±0.22 μ (mean±SE), respectively.

The nuclear diameters of the different spermatogenic cell types of the eight steps are shown in text-figure 1. By an adaptation of Abercrombie's formula, the mean numbers of nuclear points for the three types of spermatogonia and for each phase of the primary spermatocytes, as well as the mean nuclear numbers of the Sertoli cells at the eight steps, are shown in table 3.

The mitotic indexes of type-A, type-I and type-B, and the pyknotic indexes of these spermatogonia and each phase of the primary spermatocytes at the eight steps are shown in table 4.

The mitoses were observed both in the gonocyte-like cells and in three different types of the spermatogonia, i. e., the type-A, type-I and type-B spermatogonia. A high degenerative index was noted mainly in type-I and in the primary spermatocytes (P). The decreases in the mean numbers of type-I and type-B spermatogonia and of the primary spermatocytes may be attributed to the degenerations of type-I spermatogonia in step 6, and to the degeneration of primary spermatocytes in steps 6~8 at the pachytene phase.

DISCUSSION

Two different types of cellular associations of the seminiferous epithelium in cross-section of the seminiferous tubules in 16 month old minks were observed.

TABLE 3 *Mean numbers of nuclear points for spermatogonia and spermatocytes per cross-section of seminiferous tubules for eight steps and GAP*[*1]

TYPE OF CELLS	STEP	1	2	3	4		5		6		7		8		GAP
	C.A.[*2]	GA	GAI	GAB	GABR	GAR	GAL	GAZ	GAIP	GABP	GABRP	GARP	GALP	GAZP	
Spermatogonia															
Type-GA (GA)		0.47	0.90	0.35	0.31	0.51	0.47	0.50	0.91	0.41	0.29		0.22		0.47
Type-I (I)			2.71						1.51						
Type-B (B)				4.58	2.59					2.69	1.89				
Spermatocytes															
Pre-leptotene (R)					5.79	7.31					1.04	3.85			
Leptotene (L)							5.82						4.32		
Zygotene (Z)								6.27						3.86	
Pachytene (P)									5.98	4.08	2.32	3.57			
Sertoli cells	Small	9.2	4.3	6.2	3.3	6.6	8.1	6.6	2.4	6.4	6.1		7.0		7.8
	Large	40.6	45.5	46.3	47.9	44.2	45.1	46.5	47.4	50.6	48.1		47.0		51.3

←— : divisions of spermatogonia
←---- : growth of spermatocytes

*1 A total of 21, 318 whole and fragments of nuclei was classified in 368 tubular cross-sections. Section thickness was 7 μ. All tubules were corrected to a standard diameter of 89μ.

*2 C.A.: cellular association

TABLE 4 *Mitotic and pyknotic indexes (%) of spermatogenic cells for eight steps and GAP*

STEPS	C.A.*1	MITOTIC AND PYKNOTIC INDEXES (%)
1	GA	GA (0.8)
2	GAI	GA (5.6)→ I (2.0) {2.0}
3	GAB	GA (5.9) B
4	GABR	GA (7.8) B (5.5)→ R
	GAR	R {0.5}
5	GAL	GA (15.6) L {5.0}
	GAZ	Z {1.1}
6	GAIP	GA (9.7)→ I {15.2} P {15.0}
	GABP	B P
7	GABRP	GA B→ R P {40.0}
	GARP	R P
8	GALP	GA Z P {24.0}
	GAZP	Z P
	GAP	GA P {12.0}

GA, I, B, R, L, Z and P: refer to the text for definitions.
( ): mitotic index (%) {_}: pyknotic index (%)
*1 C.A.: cellular association

One was quite similar to the cellular associations observed in the breeding season and could be divided into eight steps. The other did not correspond to any step (tab. 1 & 2).

These results were almost the same as those found in 7 and 19 month old minks (TIBA)[10]. TIBA et al.[15] and TIBA[12] found that the arrangement of segments of the wave of seminiferous epithelium were very irregular in the pre-breeding season, while it was regular in the breeding season. TIBA[12] discussed the possibility that the appearances of cellular associations did not correspond to any step and that the irregular segments were caused by the degeneration or pyknosis of the primary spermatocytes. The degenerating spermatocytes during the pre-breeding season in the mink were observed by BOSTROM et al. and ONSTAD.

In this study, the degeneration or pyknosis of the spermatogenic cells was observed not only in the primary spermatocytes, but also in the type-I spermatogonia. Moreover, it was clear from the results of the mean numbers, the mitotic indexes and the pyknotic indexes of the different types of spermatogonia and each

phase of the primary spermatocytes (tab. 3 & 4), that the decreases in the mean numbers of these cells were related to the degenerations of primary spermatocytes at the pachytene phase in steps 6~8, particularly the type-I spermatogonia in step 6. The relative frequencies in steps 6~8 were lower than those in steps 1~5 because of the higher degenerations of the type-I spermatogonia in step 6 and of the primary spermatocytes in steps 6~8.

These findings suggest that the type-I spermatogonium also plays an important role in the kinetics of the spermatogenesis in the mink pre-breeding season.

DYM & FAWCETT found that the synchrous division and degeneration in each spermatogenic cell throughout spermatogenesis were associated with the occurrence of intercellular bridges in the same generation of these cells.

The relationship between the disappearances of type-I in step 6 and the primary spermatocytes in steps 6~8 from the seminiferous tubules and the expected newly formed cellular associations observed in the cross-section are summarized in table 5. The appearances of cellular association GAP (type-GA, primary spermatocytes (P) and Sertoli cells) in the pre-breeding season are explained more clearly in table 5. Thus, it may not be concluded directly that

TABLE 5 *Expected cellular associations related to the degenerations of intermediate-type spermatogonia in step 6 and primary spermatocytes in the pachytene phase in steps 6~8*

STEPS	C.A.*1	I			II		III		
		1	2	3	1	3	1	2	3
6	GAIP	I		GAP	P	GAI	I, P		GA
	GABP		B	GAP	P	GAB	P	B	GA
7	GABRP		B, R	GAP	P	GABR	P	B, R	GA
	GARP		R	GAP	P	GAR	P	R	GA
8	GALP		L	GAP	P	GAL	P	L	GA
	GAZP		Z	GAP	P	GAZ	P	Z	GA

I: indicates the disappearance of intermediate-type spermatogonia (type-I) in step 6.

II: indicates the disappearance of primary spermatocytes in the pachytene phase (P) in steps 6~8.

III: indicates the occurrence of both I and II.

1: disappeared spermatogenic cells, in which the higher pyknotic indexes were observed (tab. 4)

2: unobserved spermatogenic cells due to degeneration of type-I spermatogonia (I) in step 6

3: expected cellular associations

GA, I, B, R, L, Z and P: refer to the text for definitions.

*1 C.A.: cellular association

there was another feature in the kinetics of the spermatogenesis in the pre-breeding season.

Significant differences were noted in the relative frequency of each step among the six different sites from both testes ($p<0.05$). In the mink (TIBA et al.)[14] and coyote (KENNELLY), it was found that the relative frequency of the stages of the seminiferous epithelium cycle varied in testes from the same animal. These findings may indicate something common in the animals and in their seasonal variations of sexual activity.

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Explanation of plates

Plate I

Figures 1~8 were photomicrographs of a cross-section of the seminiferous tubules in a 16 month old mink.
PAS-haematoxylin stained. × 450

Fig. 1 Step 1

Fig. 2 Step 2

Fig. 3 Step 3

Fig. 4 Step 4

Fig. 5 Step 5

Fig. 6 Setp 6

The degenerating type-I spermatogonia (arrow)

Fig. 7 Step 6

The pyknosis of primary spermatocytes at the pachytene phase (arrow)

Fig. 8 Step 7

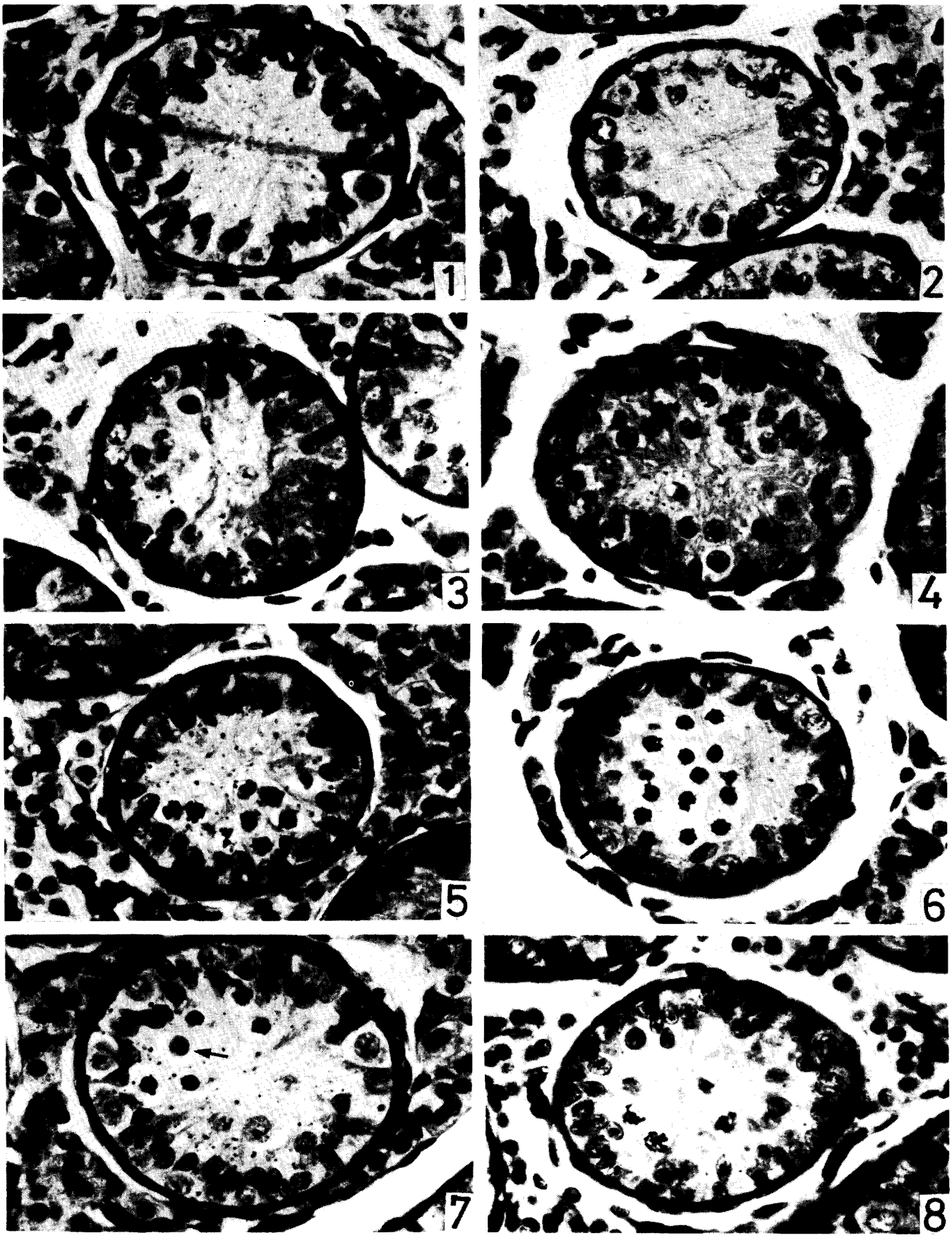
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PLATE II

Figures 9~12 were photomicrographs of a cross-section of the seminiferous tubules in a 16 month old mink.
PAS-haematoxylin stained.

Fig. 9 Step 7

The pyknosis of primary spermatocytes at the pachytene phase (arrow) × 450

Fig. 10 Step 8

The pyknosis of primary spermatocytes at the pachytene phase (arrow) × 450

Fig. 11 Cellular association GAP

The pyknosis of primary spermatocytes at the pachytene phase (arrow) × 450

Fig. 12 Gonocyte-like cell (G) × 550


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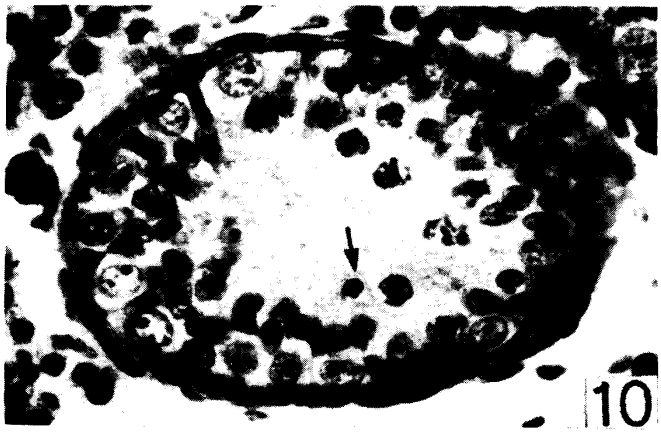
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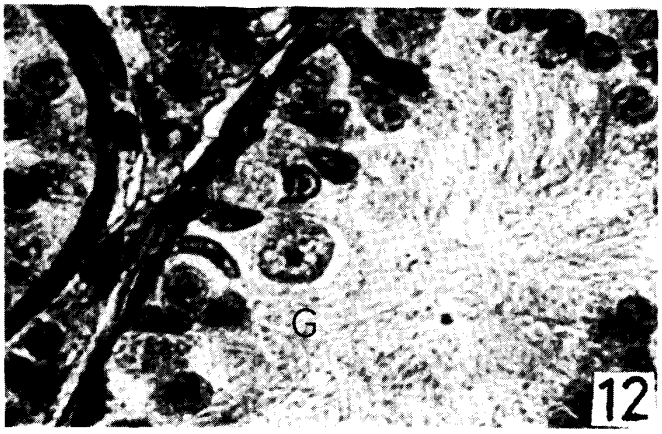
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