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Sperm test – a useful tool in breeding work of mink

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Abstract. A sperm test was performed over a period of five consecutive years as a fertility check on a mink farm. As a result of the test sterile males were eliminated from breeding, which led to a distinct increase in the number of kits per female. Results of a histological and stereological analysis of testicular tissue from sterile, sub-fertile and fertile males showed clear agreement with the results of the sperm test. In testes taken from sterile males serious disorders were observed in the seminiferous epithelium. The value of the sperm test method as a fertility test is emphasized.

Introduction

A problem of considerable economic importance on mink farms is the presence of sterile breeding males (OLSONI, 1964; ELOFSON, 1981,2; SUNDQVIST, 1982). From 5 to 10 % of the mink males used for breeding in Finland are sterile. As one male usually mates with 4–8 females the negative effect of male sterility rapidly multiplies, leading to groups of unfertilised females. Several attempts to overcome this problem have been made. One method of checking the fertility of mink males is a sperm test conducted on females after interrupted mating (VENGE, 1950; WILLEMS, 1961; ONSTAD, 1967; KANGAS, 1972; ADAMS & RIETVELD, 1981; ELOFSON, 1981,1). The method was used during the 1960's and 1970's, but was abandoned as unprofitable in the late 1970's.

The purpose of this study is to assess the value of this method of testing sperm. The reliability of the method was evaluated by measuring the mean litter size and by a histological and stereological analysis of testicular tissue. The study was made on a conventional farm, not an experimental farm.

Material and methods

Sperm test on the farm

The study was performed on Södersundvik Mink AB (SW Finland) over a period of five consecutive years (1978–1982). The farm consisted of approxi-

Table 1. The total results of the sperm test performed on the test farm, Södersundvik Mink Ab, during five consecutive years.

Mink breed	Year	Sperm quality number of observations			Eliminated from breeding %	$\bar{x}$	Total number of examined males
		0	2	4			
Jet	1978	18	35	141	9.3		194
	1979	4	37	160	2.0		201
	1980	19	38	161	8.7	8.5	218
	1981	29	39	133	14.4		201
	1982	17	33	154	8.3		204
Pastel	1978	10	14	68	10.9		92
	1979	6	16	78	6.0		100
	1980	11	17	80	10.2	10.0	108
	1981	21	32	67	17.5		120
	1982	8	34	101	5.6		143
Sapphire	1978	3	17	36	5.4		56
	1979	5	10	42	8.8		57
	1980	12	3	59	16.2	13.5	74
	1981	10	23	49	12.2		82
	1982	17	10	41	25.0		68
Sapphire	1978	1	5	11	5.9		17
(Carrie)	1979	2	0	14	12.5		16
	1980	1	2	4	14.3	12.3	7
	1981	1	2	4	14.3		7
	1982	1	1	5	14.3		7
Standard	1978	5	9	26	12.5		40
	1979	5	9	23	13.5		37
	1980	2	6	24	6.3	11.3	32
	1981	8	9	25	19.0		42
	1982	3	13	42	5.2		58
Black-Cross	1978	7	4	14	28.0		25
	1979	3	7	26	8.3		36
	1980	5	7	33	11.1	16.9	45
	1981	6	1	14	28.6		21
	1982	1	4	7	8.3		12
Brown-Cross	1978	0	1	9	0.0		10
	1979	3	5	15	13.0		23
	1980	1	4	16	4.8	3.6	21
	1981	0	0	1	0.0		1
	1982	0	1	0	0.0		1
Silverblue	1978	1	0	2	33.3		3
	1979	0	0	1	0.0	16.7	1
Albino	1981	0	1	1	0.0		2
	1982	0	0	1	0.0	0.0	1
$\sum$	1978	45	85	307	10.3		437
	1979	28	84	359	5.9		471
	1980	51	77	377	10.1	10.3	505
	1981	75	107	294	15.8		476
	1982	47	96	351	9.5		494

mately 2000 breeding females and 500 breeding males, representing the most common breeds of mink (see Table 1). The health conditions were satisfactory. During the study the same food kitchen was used and the quality of food was the same throughout the period. The test was performed at the beginning of the breeding season (lst – 2nd week of March). The method described by VENGE (1950) and ONSTAD (1967) was used; however, we would emphasize the following respects in which our practice differed from theirs:

1) The cooperation of three persons is necessary and the work must take place in a warm room.
2) The female mink has to be held firmly during semen collection in order to avoid injury to the genital organs and involuntary discharge of urine.
3) The pipettes, object glasses and the microscope must be kept at 37°C and therefore a heating lamp (AIRAM 250W) was used.

The samples were examined with a Zeiss microscope at a magnification of 125–400×. The sperm quality was classified into three categories (0=sterile; 2=sub-fertile; 4=fertile). During the first year (1978) the males were tested only once, but during subsequent years males classified as sterile were as a rule re-examined in order to certify their status. For details of sampling see SUNDQVIST (1983). Sterile males were eliminated from breeding. The effect of this measure was followed up by counting the number of kits per female.

Histological and stereological analysis

Immediately after the breeding season some of the mink males on the farm were slaughtered. Samples of testicular tissue were collected from 53 minks, 36 of which had been classified as sterile (0), 6 as sub-fertile (2) and 11 as fertile (4). The testes were excised immediately after the males were killed, washed in physiological salt solution and fixed in Bouin's fixative or in 10 % formaldehyde in 70 % alcohol. The material was dehydrated and embedded in histowax. Sections were cut at 6 μm and stained with Delafield's Haematoxylin Eosin according to HUMASON (1962). The sections were examined with a Leitz Ortholux microscope (no.II) and a stereological analysis of the tissue was performed according to WEIBEL (1979) and WIKGREN (1981). A Leitz counting ocular was used.

To avoid non-random sampling in the stereological analysis the following steps were taken:

Level of selection	Comments
1. Selection of male minks	Random
2. Organ	Both testes were cut into many pieces and fixed.
3. Organ pieces	3 pieces/male were selected at random
4. Object glasses	Out of 9 glasses/male 2 were selected at random. The label of the glasses was covered. Faultless sections were selected at random using 100× magnification.
5. Tubules	Tubules were examined with 900× and the number of spermatogonia and spermatocytes were counted per tubule. Depending on tubule size 5–15 fields were studied. A total of 80 fields/male were counted.

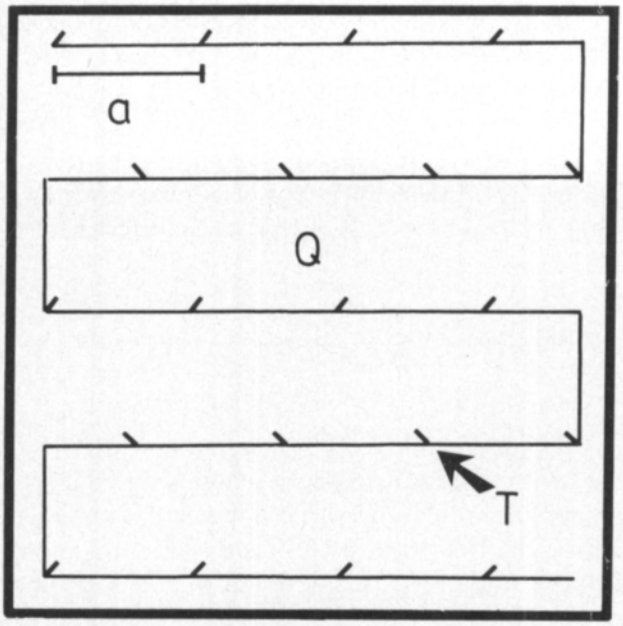

Fig. 1. The Leitz'counting ocular consisted of a hexagonal net of points and a square field. Spermatocytes and spermatogonia in testicular tissue of mink were counted.
a = distance between two points on the net
n = number of fields counted per male
T = number of hits congruent with the cells counted
Q = number of cells within the square field

The values obtained were processed according to the formulae below. For meanings of Q, T, a and n see figure 1.

Number of particles/area $N_A = \frac{Q}{17.32\ a^2 n}$

Mean diameter of the particles

$$\bar{D} = 1.337\ a\ \sqrt{\frac{T}{Q}}$$

The following statistical methods were used:
Mean and standard error of the mean
Student–Newman–Keul's test
Student's t-test
Analysis of variance

Results

Sperm quality

Table 1 shows the results of the sperm test. All nine different breeds of mink on the farm were tested. Some of the breeds are represented in very low numbers and the results are therefore subject to great variation from year to year. Only the results from the larger groups will be analyzed.

Fertility is known to vary in minks of different breeds. The lowest sperm quality was observed in Black-Cross and Sapphire minks. On average, 17 and 13 % respectively of these males were classified as sterile (0) and eliminated from breeding. The corresponding values for minks of Jet, Pastel and Standard breeds were 8, 10 and 11 %.

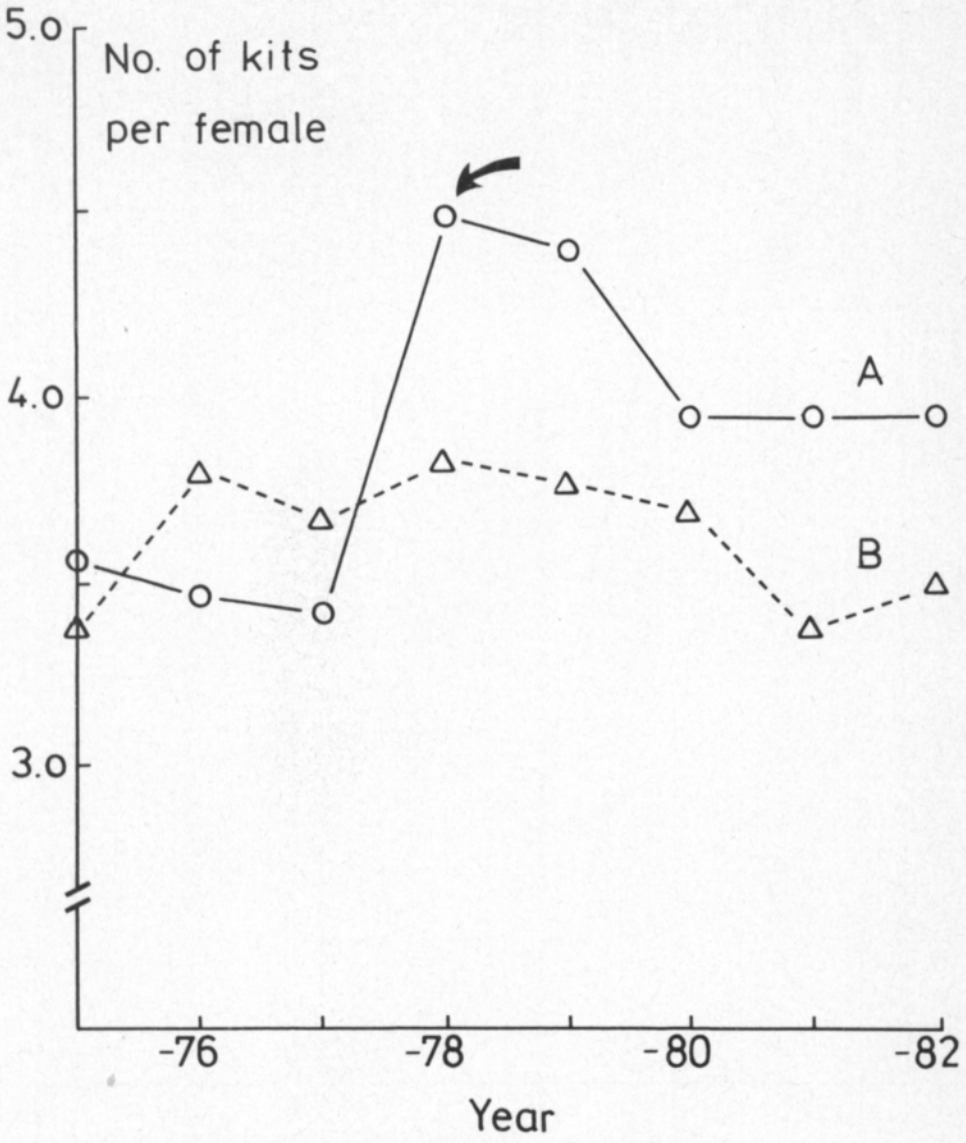

Fig. 2. Graph showing litter size on the test farm (o ——— o, A) and the corresponding value for Finland (△ - - - - - - △, B) over an eight-year period. Note the clear increase in number of kits per female on the test farm after the introduction of the sperm test (arrow; 1978).

Litter size

'Litter size' here means the mean annual number of kits per female on the farm. Figure 2 shows the litter size before and after beginning the sperm test. The elimination of sterile males resulted in an increase in litter size. The difference between litter size on the test farm and the mean value for Finland is statistically significant ($p<0.05$) for the years 1978, 1979 and 1981. In 1980 and 1982 the difference did not reach the level of significance.

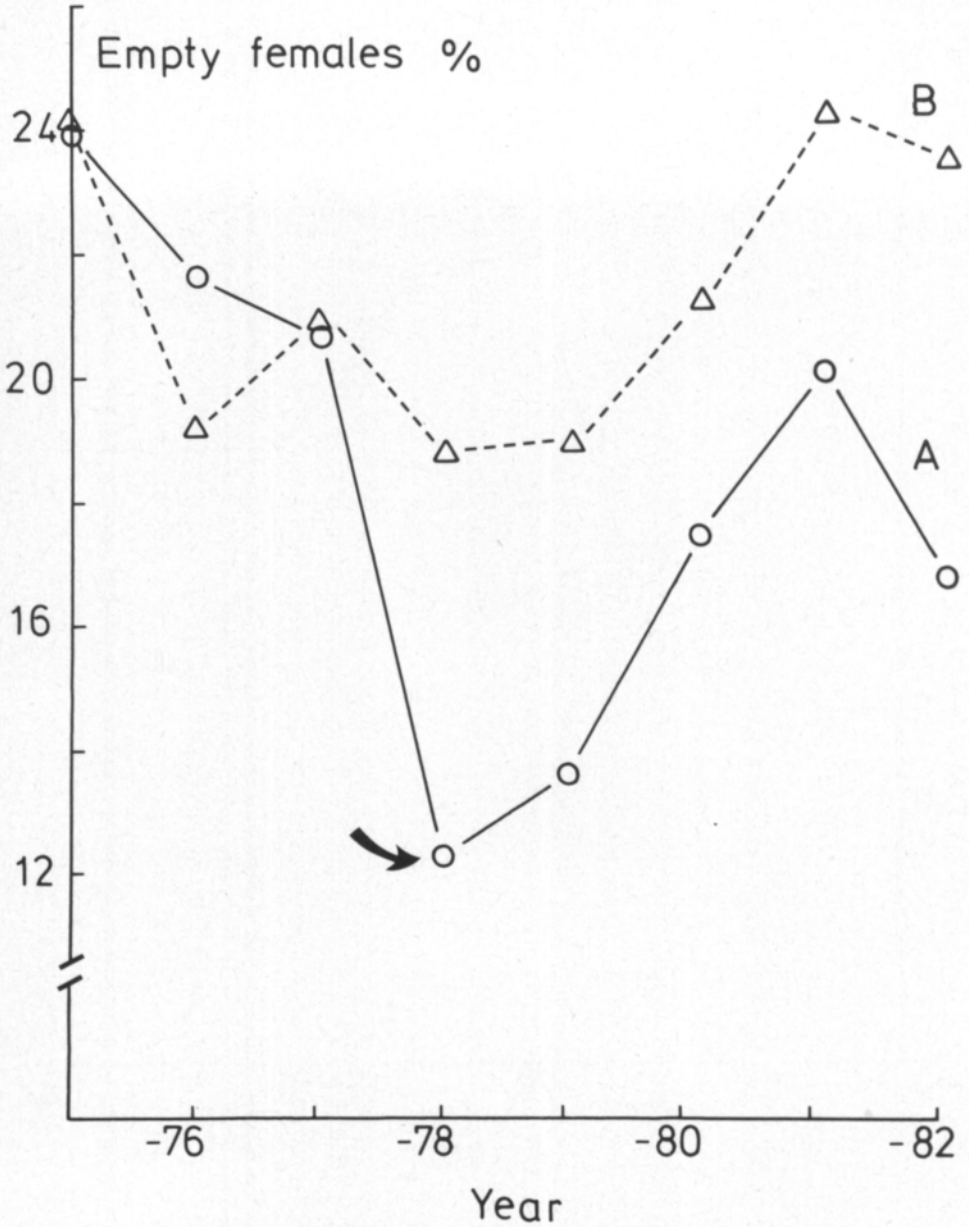

Fig. 3. Graph showing the proportion of empty females on the test farm (o——— o, A) and the corresponding value for Finland (△ - - - - - △, B). Note the drastic fall in the number of empty females in 1978 (arrow), i.e. at the introduction of the sperm test.

Empty females

Figure 3 shows the effect of the sperm test on the proportion of empty females. The number of mink females lacking kits was greatly reduced after the introduction of the sperm test and remained lower than the corresponding value for Finland during the test period.

Figures 4, 5 and 6 show representative sections of testes from minks classified as sterile (0), sub-fertile (2) and fertile (4). Clear histological differences are apparent between the three categories of males. The histological picture of the testicular tissue from minks classified as sterile also varies greatly and three categories could be distinguished. 22 % of sterile males had grave disorders in the seminiferous epithelium, with strong vacuolization of the cells (Fig. 7). 44 % exhibited clear indications of delayed spermatogenesis (Fig. 4). Spermatids and free-swimming spermatozoa were found in only small numbers. However, spermatogonia and spermatocytes occured. Semi-

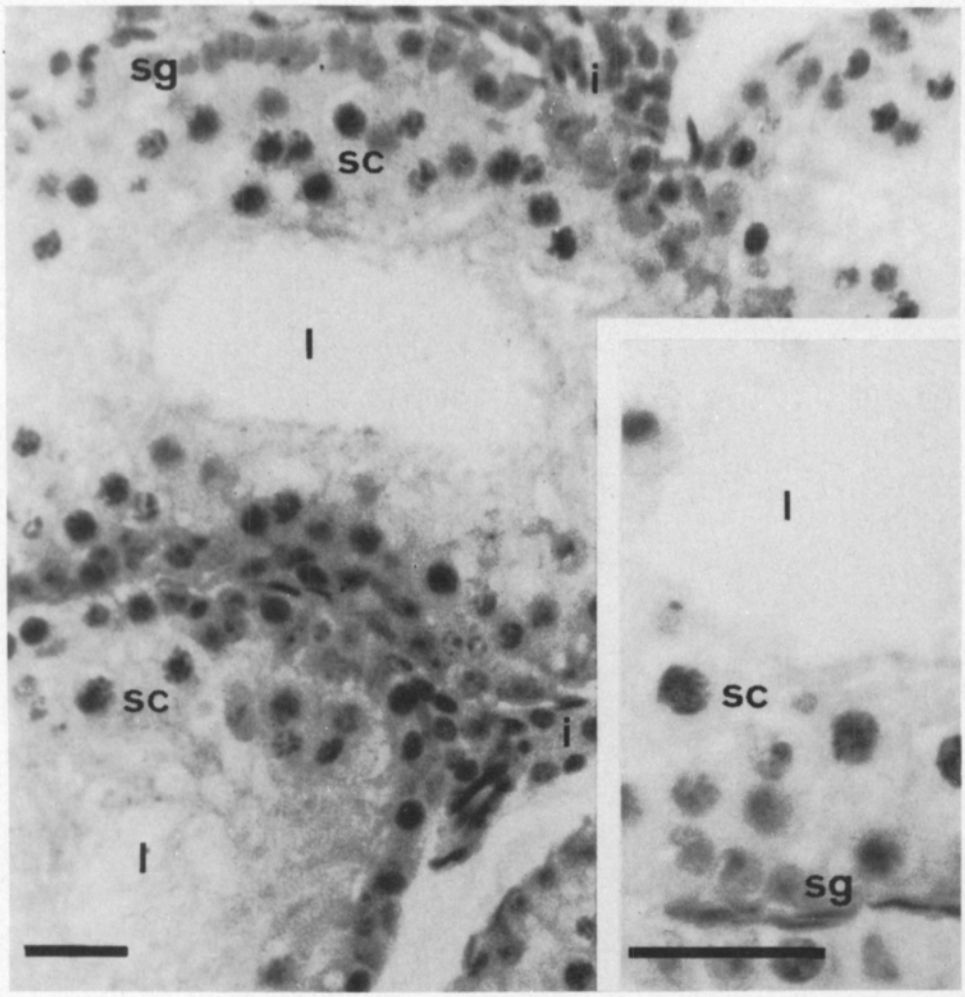

Fig. 4. Micrograph of seminiferous tubules from a mink male classified as sterile (0). The seminiferous epithelium is low. Spermatozoa and spermatids are absent. Spermatogonia (sg) and spermatocytes (sc) are present in small amounts. Note the threadlike material in lumen (1). Interstitial cells of Leydig (i).
Inset. Detail of seminiferous epithelium.
Bar = 25 μm in both figures

niferous tubules with lumina filled with threadlike material were observed in this group. The third group appeared to be normal. All the testes taken from fertile and sub-fertile males were normal in morphology and free-swimming spermatozoa occured in large quantities.

Figure 8 shows the number of spermatogonia and spermatocytes per area in the three categories of mink males. It is obvious that the number of cells rises with the degree of fertility. Figure 9 shows the mean diameter of the nuclei in the seminiferous tubules of sterile, sub-fertile and fertile males.

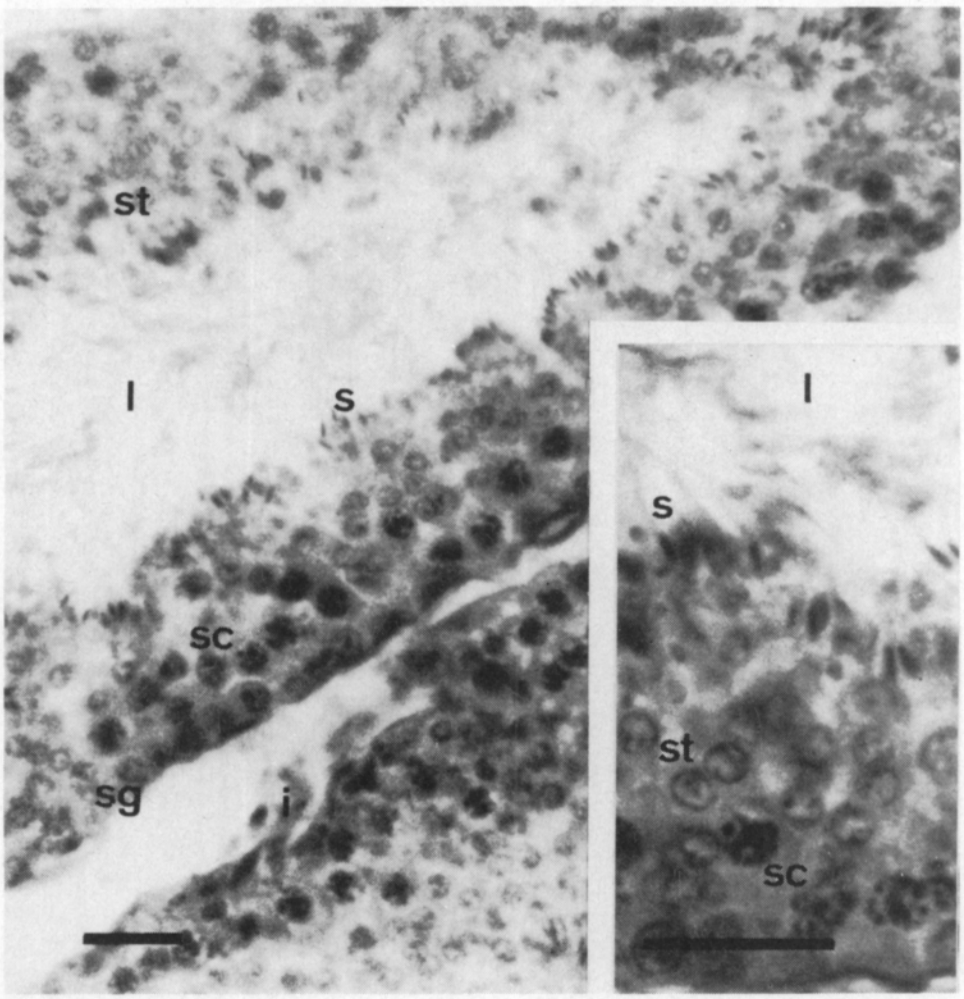

Fig. 5. Micrograph of seminiferous tubules from a mink male classified as sub-fertile (2). The seminiferous epithelium is of medium height. The population of cells show normal appearance with spermatogonia (sg), spermatocytes (sc), spermatids (st) and spermatozoa (s). However, spermatogonia and spermatocytes occur in only medium amounts.
Inset. Detail of seminiferous epithelium.
Bar = 25 μm in both figures.

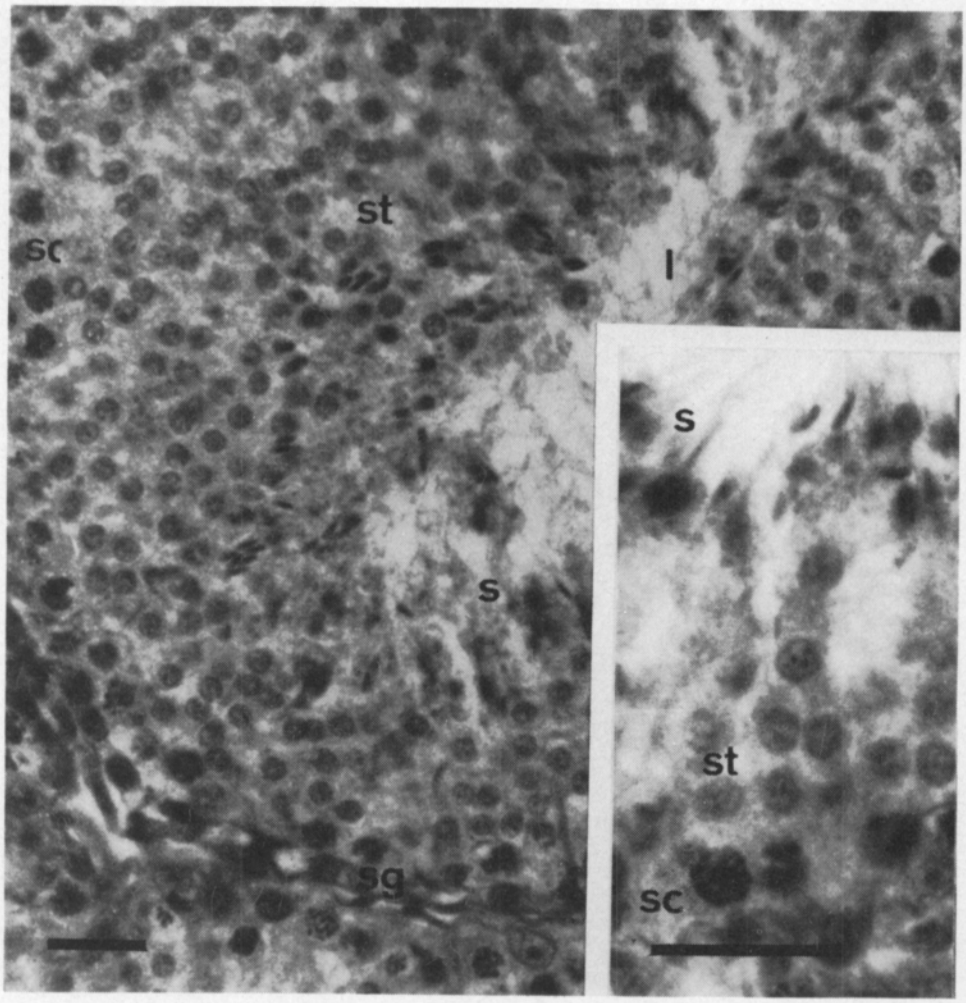

Fig. 6. Micrograph of seminiferous tubules from a mink male classified as fertile (4). The seminiferous epithelium is high. Spermatozoa (s) and spermatids (st) occur in large amounts.
Inset. Detail of seminiferous epithelium.
Bar = 25 μm in both figures.

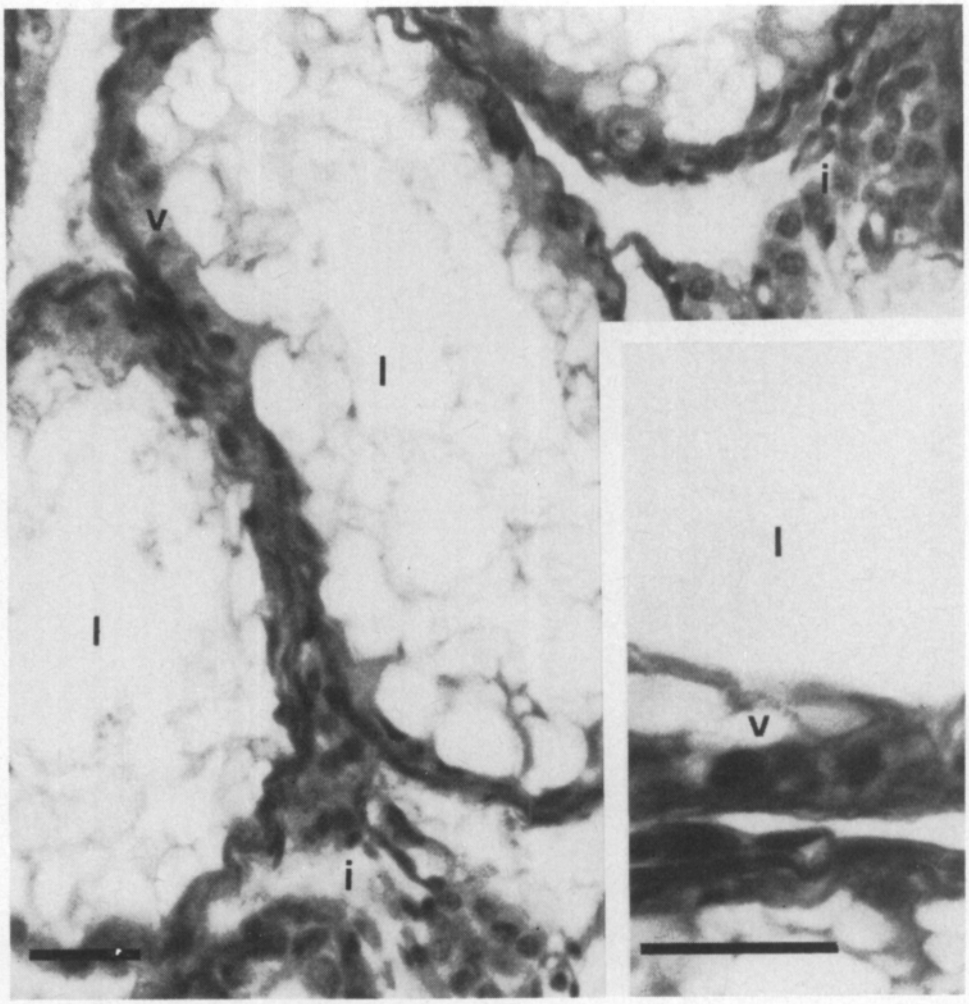

Fig. 7. Micrograph of seminiferous tubules from a mink male classified as sterile (0). The seminiferous epithelium shows grave disorders with vacuolized cells. Note the complete absence of normal epithelium. The interstitial cells of Leydig (i) are present.
Inset. Detail of seminiferous epithelium.
Bar = 25 μm in both figures.

No. of cells/mm^2

A.

	0	2	4
$\bar{x}$	2905	3836	5073
n	36	6	11
SD±	908	608	797

B.

	0	2	4
0			
2	*		
4	***	**	

Fig. 8A. Graph showing the mean number of cells per mm^2 ($\bar{x}$) in seminiferous tubules of sterile (0) sub-fertile (2) and fertile (4) mink males. n = number of males tested. SD = standard deviation.
8B. Diagram showing the level of significance for the values given in fig. 8A. * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

Mean diameter of nuclei (μm)

A.

	0	2	4
$\bar{D}$	8.0	8.9	9.4
n	36	6	11
SD±	1.1	0.8	0.5

B.

	0	2	4
0			
2	ns		
4	**	ns	

Fig. 9A. Graph showing the mean diameter of nuclei (μm, $\bar{D}$) in seminiferous tubules from sterile (0), sub-fertile (2) and fertile (4) mink males.
9B. Diagram showing the level of significance for the values given in fig. 9A. ns. = non-significant; ** = $p<0.01$.

Discussion

The value of the sperm test has been questioned several times. ROTTENSTEN (1959) found no significant difference in litter size after matings with males of good and bad sperm quality and declined to recommend the method for general use. VENGE (1973) considers the method of limited value, at least as usually performed. ONSTAD (1967), KANGAS (1972) and ELOFSON (1981,1) regard it as valuable but point out that it has to be performed with care. The sperm test was used in Finland during the late 1960's and 1970's but was then abandoned as impractical and unprofitable. Currently the method is gaining in respect and interest is increasing.

The results of the present study clearly show that the sperm test can be a valuable tool in breeding work. The reliability of the method was tested in two ways.

The first and most direct way was to count the number of kits. The litter size increased markedly after the introduction of the sperm test. The number of females without kits also decreased considerably, as might be expected. Females without kits still occured, but singly – not relative to one specific male. These females fail to produce kits for reasons not dealt with here. The absence of groups of unfertilised females after one single male shows that the sterile males were effectively removed by the sperm test.

Of interest in this connection is the known fact that more highly bred minks – i.e. Black-Cross and Sapphire – have a higher percentage of sterile males. This means that checks on the fertility of these males are very important.

The results of the histological and stereological analysis of testicular tissue from sterile, sub-fertile and fertile minks provide scientific support for the reliability of the sperm test. The testes of minks classified as fertile and sub-fertile have the same morphological appearance. In both, free-swimming spermatozoa occur in large quantities. The stereological analysis, however, shows the number of cells per area in the seminiferous tubules of sub-fertile males be significantly less ($p<0.05$) than for fertile males. The mean diameter of the nuclei is the same for both groups, indicating normal progress to meiosis (ONSTAD, 1967). The lower number of cells in the tubules is reflected in less concentrated semen, a condition which can be of importance at the end of the breeding season.

Mink males classified as sterile represent a heterogeneous group that varies greatly from the histological point of view. Highly vacuolized epithelium and no spermatozoa were observed in 22 % of the sterile males. The second group showed indications of delayed spermatogenesis (ONSTAD, 1967). Spermatozoa occured in part of the tubules, but in small amounts. Only a small number of spermatids were observed. The third group of sterile males had normal testicular morphology. These males were from the group tested for fertility only once (1978), which indicates the importance of double testing of males with unsatisfactory sperm quality. After double testing 30 % of the males initially classified as sterile "rose" to sub-fertile or fertile levels. The change was aspecially marked for Sapphire males, which mate later than

other types of males, and for males used for breeding for the first time. We would add that all males classified as sterile mated normally.

According to LOHI (1976) a rise of 0.5 in the number of kits per female results in a reduction of 5 % in food costs per produced skin. In our study the number of kits per female exceeded the mean value for Finland by 0.2–0.5, which would translate into considerable savings in food costs (2–5 %).

Our investigation confirms the reliability of the sperm test method. A prerequisite for success is that it is performed with skilled personnel and under strictly controlled conditions. The microscope has to be of good quality and there must be close cooperation on the part of workers on the farm.

References

ADAMS, C. E. & RIETVELD, A. A. 1981. Duration of copulation and fertility in the mink. Theriogenology 15(5): 449–452.

ELOFSON, L. 1981,1 Dags att kontrollera mink- och rävhanarnas avelsduglighet. Våra Pälsdjur 52(2): 33–36.

— 1981,2. Avel för bättre fruktsamhet. Våra Pälsdjur 52(10): 246–256.

HUMASON, G. L. 1962. Animal tissue techniques. W. H. Freeman and CO, San Fransisco, USA.

KANGAS, J. 1972. Siitostulokseen vaikuttavista tekijöistä. Turkistalous 44(2): 105–116.

LOHI, O. 1976. Aktuellt. Finsk Pälstidskrift 48(3): 97–98.

OLSONI, B. 1964. Siitosurosten hedelmällisyydestä. Turkistalous 36(6–7): 324–327.

ONSTAD, O. 1967. Studies on postnatal testicular changes, semen quality and anomalies of reproductive organs in the mink. Acta endocrinol. suppl. 117: 1–117.

ROTTENSTEN, K. 1959. Saedkvalitet og ufrugtbarhed hos minken. Dansk Pelsdyravl 22: 215–217.

SUNDQVIST, C. 1982. Tre olika sätt att undersöka minkhanars avelskvalitet. Skärgård spec. nr. 1: 34–38.

SUNDQVIST, C. 1983. Om spermier, befruktning och spermaundersökning av mink, del II. Finsk Pälstidskrift 55(2): 108–112.

WEIBEL, E. R. 1979. Stereological Methods. Vol. 1. Academic Press, London.

VENGE, O. 1950. Spermakontroll hos mink. Våra Pälsdjur 21: 79–80.

VENGE; O. 1973. Reproduction in the mink. Den Kgl. Vet. Landb. sk. Årsskr.: 95–146.

WIKGREN, B.-J. 1981. Cellbiologiska arbetsmetoder. Kurskompendium, Åbo Akademi.

WILLEMS, C. M. T. 1961. Investigations into semen of minks and its relationship with fertility. 4th Int. Congr. Anim. Reprod., The Hague 4: 902.

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Siemennestetutkimus – käytännöllinen apukeino minkin paritustyössä

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Maamme minkkitarhoja rasittava ongelma on steriilien siitosurosten yleinen esiintyminen. Monin paikoin 5–10 % siitosuroksista on steriilejä. Kun otetaan huomioon että jokainen uros parittelee 4–8 eri naaraan kanssa, johtaa steriliteetin esiintyminen tyhjien naaraiden runsaaseen esiintymiseen ja näinollen heikkoon pentutulokseen. Eräs keino urosten siitoskelpoisuuden tarkistamiseksi on siemennestetutkimus. Tätä menetelmää käytettiinkin Suomessa melko yleisesti 1960- ja 1970-luvulla, mutta tästä menetelmästä luovuttiin heikon kannattavuuden takia. Esillä olevan työn tarkoituksena oli arvioida siemennestetutkimuksen arvoa pentutulosten kohentajana. Kivesten histologinen ja stereologinen analyysi suoritettiin jotta voisimme todeta saadaanko steriilit urokset tehokkaasti poistettua siitoksesta käyttämällä siementutkimusmenetelmää. Työmme suorituspaikkana oli vuosina 1978–1982 keskisuuri minkkitarha (Södersundvik Mink Oy) Lounais-Suomessa.

Siemennestetutkimuksen tulokset näyttävät että urosten siemennesteen laatu vaihteli jonkin verran eri minkkityyppien välillä ja vuosivaihtelua oli myös todettavissa. Laadullisesti heikointa siemennestettä esiintyi Black-Cross- ja Safiiriminkeillä joista edellisistä 16 % ja jälkimmäisistä 13 % poistettiin siitoksesta. Jet-, Pastel- ja standardminkkien vastaavat arvot olivat 8, 10 ja 11 %. Pentutulos on siemennestetutkimusten aloittamisen jälkeen (1978) ollut säännöllisesti korkeampi kuin maan keskiarvotulos ($p<0.05$ vuosina 1978, 1979 ja 1981). Vuosina 1975–1977 Södersundvik Mink Oy:n pentutulos oli suurin piirtein yhtä korkea kuin maan keskiarvotulos. Tyhjien naaraiden määrä on merkittävästi laskenut vuoden 1978 jälkeen.

Kivesten histologinen ja stereologinen analyysi näyttää siemennestetutkimuksen merkityksen. Urokset, jotka tutkimusten jälkeen pidettiin steriileinä, osoittivat selviä häiriöitä ($p<0.05$) kivesten kehityksessä verrattaessa uroksiin jotka todettiin fertiileiksi.

Työmme osoittaa selvästi, että siemennestetutkimuksesta parituskauden aikana on hyötyä, jos tutkimuksen suorittaa huolella ja käyttää etevää henkilökuntaa.