The Mink (*Mustela vison*) as an indicator of environmental reproductive toxicity

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CONTENT

SAMMANFATTNING

Det finns en mycket stor mängd kemikalier i vår miljö och vardag, och de flesta hamnar förr eller senare i vatten och ackumuleras och möjligtvis biomagnifieras i fiskar och andra djur genom näringskedjan. På vilket sätt alla dessa kemikalier sammantaget påverkar människor och djurs hälsa är ofullständigt känt. Reproduktionssystemet har i ett flertal fall visat sig vara ett av de känsligaste fysiologiska system vad gäller påverkan från kemikalier. Reproduktionsstörningar är således många gånger det första tecknet på en förorenad miljö. Exempelvis finns det studier som visar att ämnen som PCB och olika östrogena substanser i låga doser kan påverka kroppens fortplantningssystem. Minken lever vattennära och är högt upp i näringskedjan, vilket gör den till en potentiellt högexponerad djurart och därför lämplig för att studera effekter på reproduktionssystemet av kemikalier i miljön. Eftersom minken har varit farmad i många år är den sammanlagda kunskapsbasen kring minkens biologi omfattande och reproduktionssystemet är utförligt studerat. I den här studien utvärderades ett antal parametrar, framför allt morfologiska reproduktionsparametrar, avseende vilka som kan analyseras post mortem på fryst material samt hur reproducerbara mätningarna är. Reproducerbarheten bedömdes genom att utföra upprepade mätningar för en parameter på samma djur. Ett flertal av parametrarna visade sig vara tillräckligt robusta för att användas i framtida miljöövervakning.

SUMMARY

There is a substantial amount of chemicals in our environment and the exposure is continuous over time. It is generally unknown whether these pollutants synergistically or additatively affect human and environmental health. The reproductive system is known to be one of the most pollutant-sensitive physiological systems; therefore signs related to reproductive and developmental toxicity may be one of the earliest indications of environmental chemical impact. For instance, PCB and different oestrogenous substances at low doses are known to affect the reproductive system, in mink as well as in other species. The pollutants will sooner or later end up in streams, lakes and seas where they will bioaccumulate in fish and other aquatic species, making piscivorous animals like the mink highly exposed. Therefore, the mink is suitable as a sentinel species. Also, the general knowledge about mink and mink reproduction has been thoroughly studied since the mink has been kept in fur farms for over a hundred years. In this study, a set of parameters with emphasis on morphological reproductive parameters was analyzed post mortem on frozen/thawed material. The reproducibility of the parameters was assessed by repeated measurements in the same animal. Several of the parameters were found to be reproducible and robust enough to be used in future environmental monitoring.

INTRODUCTION

Rationales

During the last decades, the characteristics of the environmental pollution problem have changed in Western Europe. The release of large amount of chemicals from point sources, such as industries, is increasingly controlled and for instance well known pollutants as PCB and DDT are starting to slowly decline. However, new threats are arising. Particularly, chemicals that reach the environment by diffuse emission, is an emerging concern. Such emission makes the exposure diffuse in both time and space; the source is not defined and may affect the environment for a long time. Also, the number of hazardous identified substances has increased. Examples of toxic substances that are of concern today are brominated flame retardants and plasticizers, as they are either persistent in the environment and therefore have the ability to bioaccumulate and possibly biomagnify, or that there are continuously emitted to the environment. Another concern is that the major part of the heavy metals discharged during the last decades still remains in soil and water (Swedish Chemicals Agency, 2006).

One particular group of chemicals in this context is pharmaceuticals. Apart from oestrogenous substances, very little is known about what effects environmentally spread pharmaceuticals have on biological processes in humans and other organisms. There is only data about environmental load for approximately 10% of the more than 1 200 pharmaceuticals that are approved in Sweden. Pharmaceuticals are compounds that are designed to have high biological activity and selectivity and this means that they can have an effect even at very low concentrations (Medical Product Agency, 2004).

Generally, pollutants in the environment will sooner or later end up in streams, lakes and seas where they may bioaccumulate in fish and other aquatic species, putting piscivorous animals at risk for high exposure.

Risk assessment of the effects of environmental pollutants in human and ecosystem health should primarily be based on experiences from chronic exposure, since the environmental exposure typically is extended over time and at low dose. There are considerable gaps of knowledge how human and environmental health is affected by this kind of exposure compared with acute exposure, especially when the chemicals act in combination. A low dose of a single substance may not have any impact on health, but put together with several other chemicals the effects can be grave. Therefore, measurement of the exposure of specific chemicals may not reflect this possible synergistic or additative effect. For example, when mink were given PCB during a short period of time, the accumulation of cadmium in organs increased, indicating a synergistic effect between PCB and cadmium (Olsson et al., 1979). Another limitation in monitoring for chemical exposure is that no other chemicals than those analysed for will be found. In the present study we therefore have chosen to look for effects on the physiology as a screening method for environmental pollution. Following this screening it will, however, be possible also to analyse for chemical exposure for a set of chemicals.

The mink in environmental assessment

There are several factors that make the mink suitable for risk assessment. The mink is present in most parts of the country and it is not an endangered species but continuously hunted. Also, the mink has a high trophic status and its biology has been thoroughly studied. In addition, there is a possibility to use mink from fur farms for reference values.

In addition, free-ranging wildlife can integrate ecological factors, such as stress associated with disease and temperature, in a way that is more difficult in controlled laboratory studies. It has been found that cold stress and PCB both enhances the toxic effects of methyl mercury in mink (Wren et al., 1987). Temperature is a stress factor that can influence reproductive success, and the cost/gain ratio of hunting can become critical even at quite mild conditions (Bronson, 1985). For example, weight loss in both female and male farmed mink during the winter caused negative effects on their reproductive performance (Tauson, 1985; Tauson & Alden, 1985). It is important to take stress factors into consideration when studying effects at an ecological level, for example by performing a full autopsy to rule out diseases and by estimating nutritional condition. Measuring faecal content of glucocorticoids or their metabolites can be used as an indicator of chronic stress (Möstl & Palme, 2002). Disease in any form can cause stress. Aleutian disease is a well known viral disease in mink fur farms. The infection is persistent and the disease is progressive and fatal (Hunter & Lemieux, 1996). Antibodies have been found in free ranging mink, for example in Spain (Manas et al., 2001), in France (Fournier-Chambrillon et al., 2004) and in southern England (Yamaguchi & MacDonald, 2001). Stress factors are not unusual but a part of nature and therefore, studying free-ranging wildlife offers a unique opportunity to look at true effects of pollutants.

The mink is known to be sensitive to a variety of toxicants, for example PCB. In a study with environmentally relevant doses the whelping frequency and kit survival was reduced (Brunström et al., 2001). Reduced reproductive success can partly be explained by an increase in both late fetal death (Bäcklin & Bergman, 1992) and early fetal death (Jenssen et al., 1977). Kits from females fed PCB-contaminated carp from Saginaw Bay in USA showed reduced survival and body weights. Females given a 40% carp diet showed signs of nervousness, listlessness, anorexia and melena. They also appeared to have lack of interest in their kits. In addition to PCB, the fish also contained a variety of pesticides in lower doses (Heaton et al., 1994). Effect on the reproductive system of the mink has also been seen after exposure to lindane (Beard & Rawlings, 1998), phytoestrogens (Yang et al., 1995; Ryökkynen et al., 2005), and hexachlorobenzene (Bleavins et al., 1984). The response of the mink to toxic substances is generally similar to other commonly used species as well as humans. This has found to be true for several dozens of toxic substances (Calabrese et al., 1992).

For the survival of a species, the reproductive system has a central biologic function, but it is also very sensitive to disruption. The reproductive system in mink has been studied thoroughly (Hansson, 1947; Enders. 1952), as it has been raised in captivity by the fur industry ever since 1866 (Basu et al.. 2006). This

makes the mink, combined with the fact that it exhibits several features suitable for a sentinel species, a potential indicator of environmental reproductive toxicity.

General biology of the mink

The mink population in Sweden is comprised of feral American mink (Dunstone, 1993). The first mink farm in Sweden started in 1928, and in the same year the first free ranging mink was observed. About 35 years later the mink was found all over the country, except for in the northern mountain region (Gerell, 1972). Today, the number of trapped mink is estimated to almost 20 000 every year. Hunting is permitted throughout the year with the aim to prevent damage by wild animals. However, a referral has been put forward from the Swedish Association for Hunting and Wildlife Management to the Swedish Government, to limit the hunting season to the time of year that no offspring is nursed, except for areas in the archipelago and near poultry farms etc (Swedish Association for Hunting and Wildlife Management, 2006).

The mink is a small carnivore mammal belonging to the weasel family (*Mustelidae*). It has elongated body and flattened head. The fur is dark brown and usually a white ventral spot (Dunstone, 1993). The body weight varies with gender; an adult male mink weights 850-1800 g while the female is about half that mass, weighing about 450-810 g. Juveniles weights approximately 690-1330 g and 440-740 g for males and females respectively (Chanin, 1983). In males, the body-weight seems to change throughout the year. It increases during the autumn, peaks before mating period and then decreases during the spring (Dunstone, 1993). The habitat is usually situated near fresh or saltwater shores. The mink is an opportunistic predator; the diet varies with type of locality and what is commonly available, and consists mainly of fish, crayfish, rodents, frogs and waterfowl (Gerell, 1972).

The average life span of a wild mink is often said to be three years. A capture-mark-recapture study in Sweden 1968-1970 indicated a generation interval of three years (Gerell, 1972). In a demographic study of culling data from UK and Estonia, juvenile (3-6 months old) and adult (> 1 year) mortality differed substantially between populations, and in continuously culled populations the proportion of younger minks was higher. The mean age in the studied populations before breeding varied between 0.78 to 1.54 years. In the same study, the maximum age was 6 years old. The difficulty to estimate age structure in a population using culling data is that juveniles are more easily trapped than adults (Bonesi et al., 2006).

The size of the home range is most commonly expressed as a distance along water where a female or male roam. Much of the activity of the mink occurs within 100-200 m from the water, so the estimation is probably fair. Males have a longer mean home range than females; approximately 1.5-2.5 km compared to 1-2 km for females. The size of the home range depends on how dense the population is. For example, eutrophic waters can support a larger population than oligotrophic waters. The mink generally tries to avoid open areas and prefers vegetative cover, which may affect the distribution of the population. Due to the fact that the mink is an opportunistic predator, it may tolerate human disturbance to some extent,

especially at locals where human activity has a positive effect on hunting success (Dunstone, 1993).

The home range of females and males may sometimes overlap. A certain area of the home range is defended as a territory towards other individuals. Generally, a male does not allow other males in their range. Females can allow their daughters to stay in the home range during a part of the daughters first year in life. During the breeding season the movement of males increase, as females in heat attract them. This makes the risk for male intraspecies aggression leading to fights larger. Wounds around the cheeks and neck are most common. Females can sometimes get neck wounds as a result of mating fights, as the male holds the female by biting her neck. The pattern of diurnal activity varies throughout the year, but generally the activity peaks during dusk and dawn. The activity at nighttime is sometimes high, but at daytime the activity is usually at lower levels (Dunstone, 1993).

Reproduction of the mink

The mink is a seasonal breeder. The morphological and physiological alterations throughout the year can be separated into four periods: July to October – early anoestrus; October to December – late anoestrus; January to February – prooestrus; Late February and March – oestrus (Pilbeam et al., 1979).

During early anoestrus, both female and male gonadal activity is minimal. The mink is a short-day breeder; the decreasing photoperiod in the autumn stimulates gonadal activity (Jallageas et al., 1994). Plasma androgens, oestradiol and progesterone slowly increase during the second half of anoestrus and peak during prooestrus. Ovarian and testicular weight increases during late anoestrus and prooestrus and reaches maximum size prior to mating (Pilbeam et al., 1972). These seasonal changes also apply on mink less than one year old. Both males and females become fertile in their first year, by February or March at approximately 10 months of age (Hansson, 1947; Enders, 1952).

The breeding season starts in late February and ends in the beginning of April. The female is not in oestrus throughout this period, but once she enters oestrus she remains in that state until the breeding season is over. Copulation, ovulation or pregnancy does not shorten the oestrous period. If ovulation takes place, oestrus is followed by pregnancy if fertilized, or pseudo-pregnancy if not fertilized. If no ovulation occurs, the female goes straight to anoestrus (Enders, 1952).

The length of gestation period varies between 39-74 days (Hansson, 1947) and the mean length of pregnancy is approximately 51 days. Kits are born early in May and grow rapidly during their first 8 weeks in life (Hansson, 1947; Enders, 1952).

During the spring, a gradual regression in gonadal activity takes place. By summer, the activity is at an absolute minimum and remains so until late autumn, when the decreasing day length stimulates the preparations for a new breeding season (Pilbeam et al., 1979).

Reproductive biology of the male

The scrotum is situated close to the anus, as in cats, and it is not sharply defined as in bulls, rams and bucks. The testis has a round-oval form and cauda epididymis can be felt as a small, soft prominence in the caudal pole of the testes (Onstad, 1967). The penis is relatively large, 5-6 cm long, and contains a baculum, which distally has a dorsal bend. During copulation the baculum is thought to be pressed against the female cervix to induce ovulation (Klingener, 1972).

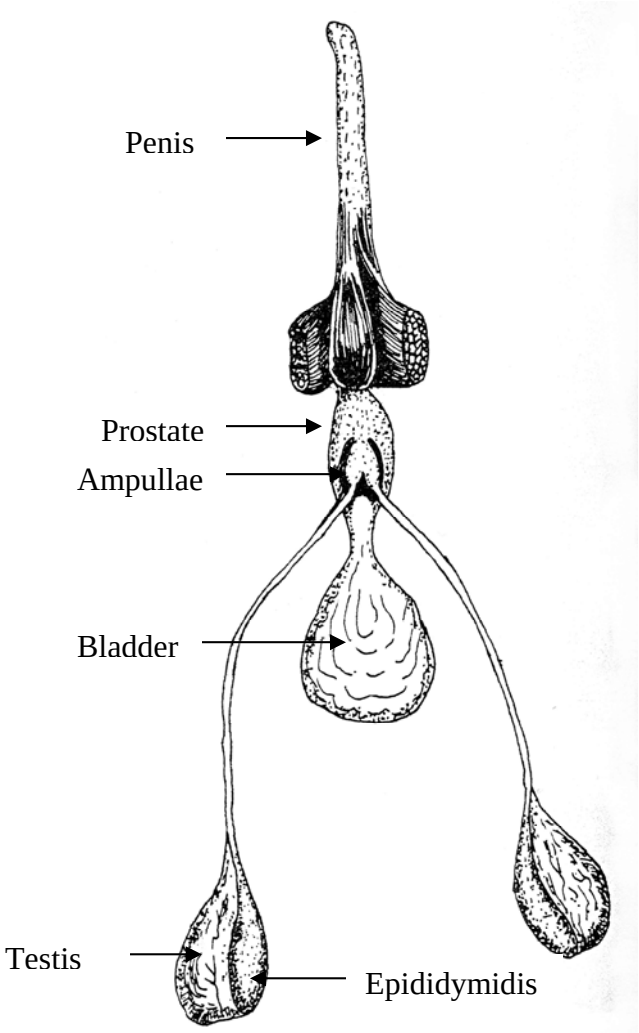

Figure 1. Male reproductive tract (adopted from Basrur & Ramos, 1972).

The prostate is situated at the base of the bladder and a pair of ampullae is associated with the urethra distal to the prostate (Basrur & Ramos, 1972). In the subadult mink the prostate is not very distinct and the accessory glands are hard to identify macroscopically (Klinginer, 1972). The size of the prostate reaches its maximum size during the breeding season (Basrur & Ramos, 1972).

The testicles descend during the first month of life (Lundh, 1961). Only a small change in testicle weight has been seen in kits from June to November. In a study by Onstad the testis weight increased with 0.2 g per month from 1 to 6 months of

age. After that, in November, the weight of the testis started to increase with more than 1 g per month (Onstad, 1967). Pilbeam found that the volume of one testis increased from 12 mm^3 in September, to 1425 mm^3 in March. Later in the spring, when the breeding season is over, the volume decreased continuously (Pilbeam et al., 1979). The testicular regression begins at the end of March (Lundh, 1961).

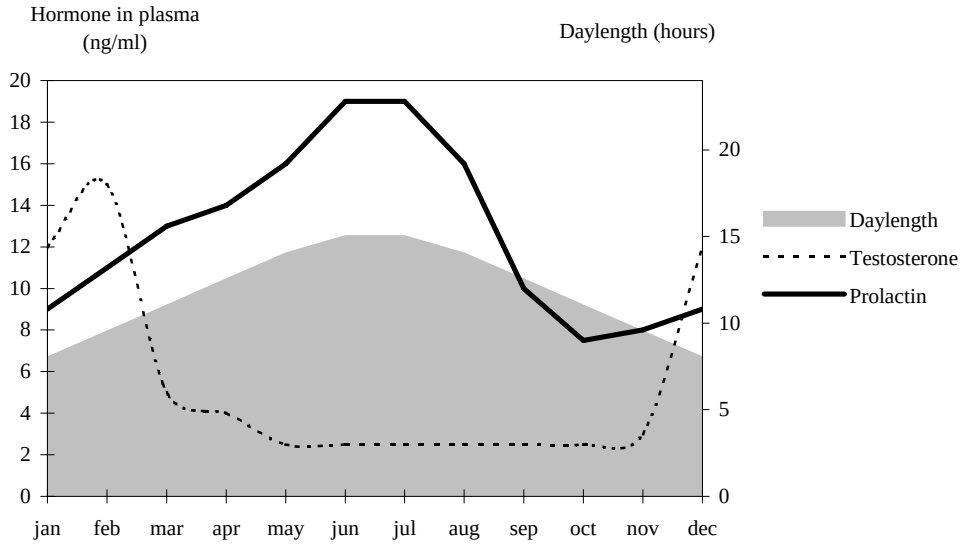

Figure 2. Diagram showing seasonal changes in reproductive hormones in male mink (adopted from Sundqvist et al., 1988).

During December the blood concentration of testosterone starts to increase (Pilbeam et al., 1979). There is a correlation between serum testosterone levels and testicular development. The testosterone levels decline sharply in February. Sundqvist et al found that males that still had high plasma testosterone concentrations in early February also had defective sperm quality by the time of the breeding season in March. The reason for the prolonged testosterone production in sterile male is unknown (Sundqvist et al., 1984). However, testicular testosterone concentrations did not decline in March in a study by Blottner et al. The testicular concentrations were as high in March as in February (Blottner et al., 2006).

There is a progressive increase in spermatogenic activity, starting in October. Lundh found that spermatozoa are present in 75% of the mink in December, and in all minks in January (Lundh, 1961). When the breeding season is over the activity decreases and by May, no spermatozoa are seen in the testes (Onstad, 1967). During December a functional blood-testis barrier is formed, and after the breeding season is over it undergoes partially breakdown and becomes permeable to some extent (Pelletier, 1986). The barrier is reformed during the following season (Sundqvist et al., 1988). Mink spermatozoa have a head length of 5.8-7.0 µm and an average width of 6.1 µm. The total length is 43 µm (Cummins & Woodall, 1985).

Defective testes in farmed mink are quite often found. In a study by Onstad, cryptorchism occurred in 1.3% of the males, delayed puberty (small testes) was

found in 6% and hypoplasia in 1.9%. Poorly developed spermatogenic epithelium in older, previously fertile males occurred in 4.4% (Onstad, 1967).

The regulation of the seasonal changes in testicular activity is not fully known. Development and regression of the activity can be stimulated by light experiments (Boissin-Agasse et al., 1986). The roles of prolactin and thyroxine have been discussed (Sundqvist et al., 1989), but no definite conclusions have been made.

Reproductive biology of the female

The uterus of the mink is a true uterus bicornis. The uterine horns are approximately 3-4 cm and the corpus about one third the length of the horns; 1.5 cm long and 0.7 cm in diameter. The cornua are round in cross-section while the corpus is dorso-ventrally compressed. The cervix is closed except during oestrus and parturition. As the breeding season approaches, the uterus elongates and the wall thickens, and approximately at the time of implantation, the uterine horns are no longer straight but twisted into many loops (Enders, 1952).

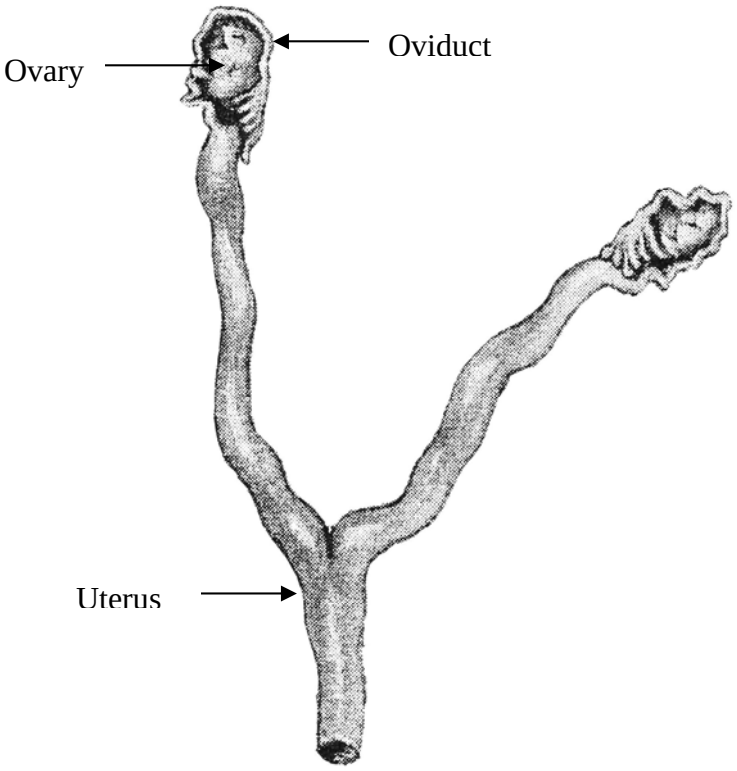

Figure 3. Female reproductive tract in prooestrus (Enders, 1952).

The ovaries of the mink are roughly bean-shaped and sometimes a formation of lobules can be seen. In the prepubertal and the anoestrous ovary, follicles with the maximum size of 0.4-0.6 mm in diameter can be found, before they become atretic. During oestrus the size of the ovaries increase, leading to less distinct lobular character, and follicles may project from the surface of the ovary. The anoestrous ovary appears to differ from the prepubertal ovary mainly in its larger size and greater number of developing follicles. The formation of new follicles is not completely suppressed by either anoestrus or the presence of a corpura luteum (Enders, 1952). Ligamentum ovarii proprium is short, resulting in fixation of the

ovary near the uterotubal junction, and the tuba uterina runs round the ventral surface of the ovary forming 10-11 regular coils (Hansson, 1947).

Prepubertal and pubertal females have generally lower ovary and oviduct weights compared to adults. The weight of the ovaries, oviducts and uterus change with season in both adult and juvenile females. The weight of each part increases during late anoestrus and prooestrus and reaches maximum size prior to mating (Pilbeam et al., 1979). The length of the vagina also increases as oestrus approaches. Ovary weight during breeding season is 0.3-1.7 g, the average weight approximately 0.6 g. An ovary weighing less than 0.3-0.4 g at this time of year is inactive. The average ovary weight during anoestrus is 0.3 g (Enders, 1952).

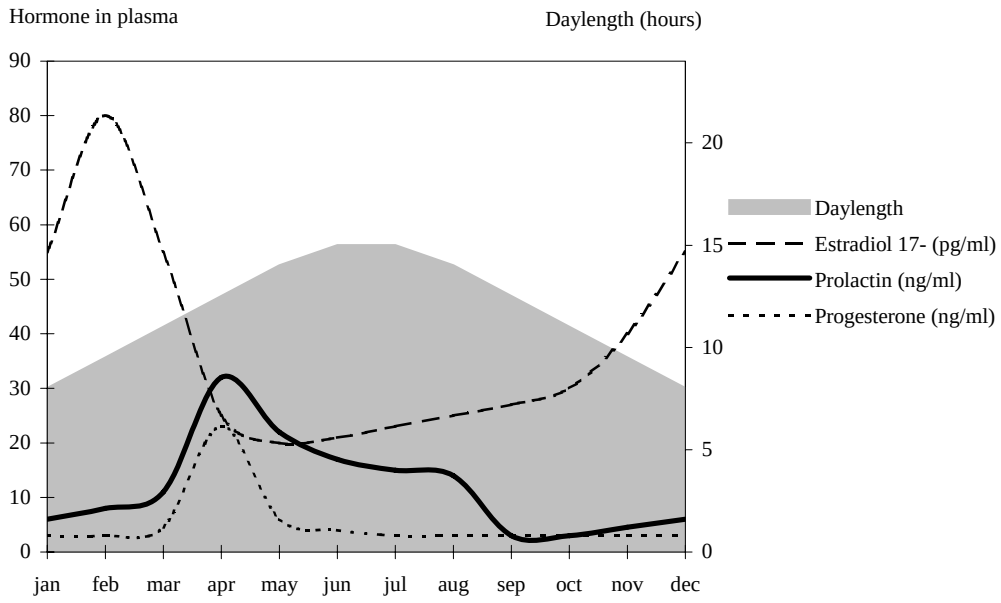

Figure 4. Diagram showing seasonal changes in reproductive hormones in female mink (adopted from Sundqvist et al., 1988).

Plasma oestradiol is low in May and June but slowly increases during the autumn and reaches a peak in late January. The levels then decrease and remain low during gestation and lactation (Pilbeam et al., 1979). A pregnancy-associated serum protein has been isolated in mink (Larsen et al., 1971).
During mating season waves of follicles mature at approximately 8-day intervals (Enders, 1952) and LH stimulates ovulation. Ovulation is induced by mating and takes place 28-72 hours after mating (Hansson, 1947). Follicles in the ovary with the size of 0.7 to 1 mm can ovulate. The corpus luteum does not produce progesterone initially, allowing a second oestrus and a new mating-induced ovulation to occur after 8-10 days (Enders, 1952). Ovulation can also be induced by fighting with a male or from frequent collection of vaginal smears. Stimulating the cervix mechanically is used in artificial insemination techniques (Enders, 1952).

Fertilization takes place in the oviduct (Enders, 1952). The fertilized egg undergoes partial development (to blastocyst stage) and it remains inactive until it is implanted in the uterus (Hansson, 1947; Enders, 1952). Embryos in diapause from the first mating will be in the uterus when the second mating occurs several days later. The newly fertilized eggs will develop to blastocyst stage and join the

first group in the uterus, a phenomenon called superfetation. The result is that a female may give birth to kits from different ovulations and males (Enders, 1952).

The termination of embryonic diapause is initiated by prolactin (Murphy et al., 1981). Short photoperiods, or injection of melatonin, inhibit prolactin secretion (Martinet et al., 1983). The increasing day length in the spring increases prolactin that in turn initiates luteal progesterone secretion and the onset of implantation (Martinet et al., 1981). Additionally, LH probably plays an important luteotrophic part in the activation of the corpus luteum (Douglas et al., 1998). A high ratio of progesterone to oestradiol in plasma is necessary for production of uterine prolactin receptors (Rose et al., 1996), but results that suggests that prolactin upregulates its own receptor have also been found (Douglas et al., 1998).

Plasma progesterone starts to increase about 40 days before parturition. Maximum concentrations are observed early in April (about 10-25 days after start of increase), when the implantation ends. After that, the progesterone levels slowly decrease and by the time of parturition the levels are low and remain so throughout the year (Pilbeam et al., 1979).

Implantation always occurs after the beginning of April. Once the embryo is attached to the uterus wall the embryo complete its development in 28-30 days. Therefore, most kits are born in early May regardless of when mating occurred (Enders, 1952). This explains the variable duration of pregnancy seen in mink. The placenta is zonodiscoidal (Hansson, 1947).

After ovulation, the diameter of the corpus luteum lies between 0.5 and 0.9 mm. Generally, the fewer the corpora the larger they are. When the breeding season is over, the corpus luteum rapidly increases in size. After implantation the diameter is 1.4 to 1.8 mm. The corpora maintain their size or continue to grow throughout pregnancy and early lactation. Post partum the corpora change in character but persists undiminished in size for at least five weeks (Enders, 1952).

Parturition usually occurs from the last week of April to the middle of May (Chanin, 1983). In mink farms the average litter size is about 5. One of the largest litters reported contained 17 kits, but litters of 10 or more are not common (Enders, 1952). There are little reliable data on litter size in the wild. Gerell reports a litter size of 3-6 (Gerell, 1972). Dunstone found the mean number of active nipples to be three, and estimates that the typical production in wild mink is 2-3 kits per female (Dunstone, 1993).

OBJECTIVES

- Evaluate the free-living mink as a sentinel species and its reproductive system in order to reflect environmental health.
- Examine which general and reproductive parameters that are feasibly measured at dissection of frozen/thawed material.
- Assess how reproducible the measurements of some of these parameters are and identify possible compromising factors in making the measurements.

MATERIAL AND METHODS

General parameters

Seventeen mink from different parts of Sweden were dissected. They were trapped along the lake Funbosjön in Uppsala county, the lake Rovakka järvi near Övertorneå in Norrbotten county and also along small lakes near Linghem in Dalarna county. All were trapped between August and December in 2005 and 2006, and stored at -20ºC until dissection was performed.

Before necropsy, the condition of the carcasses was assessed as mild, moderate, substantial or rotten. The substantially affected carcasses or rotten were excluded. The carcasses were sexed and weighed and any gross lesions were recorded. The body was measured with a steel tape measure, from nose to tip of tail and from nose to base of tail. The length of the tail was also measured. Liver, spleen, kidney, adrenal gland, thymus, omentum were weighed using a precision balance with a readability of 0.001g. The abdominal subcutaneous fat was collected and weighed. This fat is situated on the ventral part of the abdomen, between the hind legs. It is easily removable in a standardized fashion with the animal lying on its back when dissected.

An ocular assessment of nutritional condition was performed according to the score table below.

0	emaciated, serous atrophy of fat around the coronary arteries and/or bone marrow
1	below average
2	Average
3	above average
4	obesitas – intramuscular fat

The adrenal glands were fixed in 10% phosphate buffered formaline for histological examination. Stomach and intestines were refrozen for analysis of content later on. Spleen, liver, kidney, brain, lung, subcutaneous fat, adrenal gland, musculature and mesenteric lymph node were stored in freezer for analysis.

Urine, when present in bladder, was collected with plastic pipette and stored at -70ºC. Faeces was collected from rectum and colon descendens and stored at -70ºC. Blood was collected with a plastic pipette directly from an incision in the aorta and frozen. For cementum analysis, the lower jaw was collected and refrozen at -20ºC.

Reproductive parameters

Any gross lesion (abnormal external genitalia, aplasia, cysts etc) was recorded. Reproductive organ (ovary, oviduct, uterus, testis, epididymidis and penis including the baculum) weights were recorded. The baculum length and penis width and length were measured with vernier callipers. The penis was separated from the body where it enters the pelvis caudally and measured from the tip of the

baculum to the cut off base. Penis width was measured approximately at the middle of and at the widest part of the baculum. The preputium was removed before width measurement and weighing. The ovaries and oviducts were separated from the surrounding tissues before weighing.

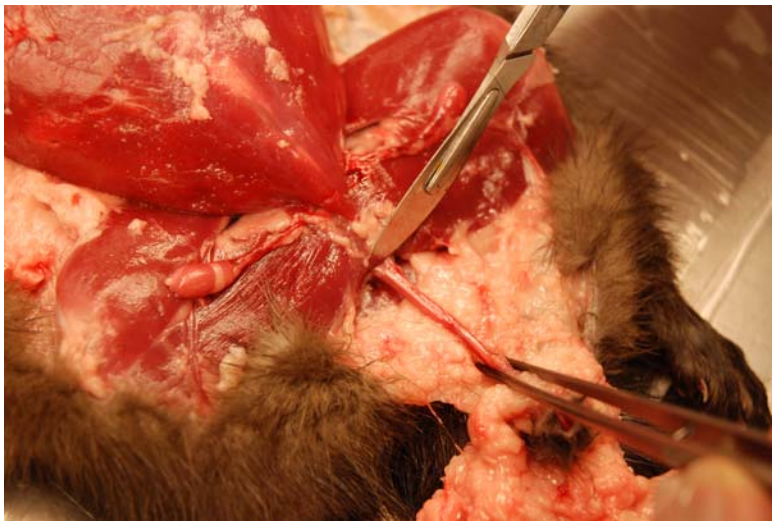

Figure 5. The penis was removed from the body as close to the pelvis as possible.

Some developmental parameters as preputial separation (set as the ability of penis to protrude at least 5 mm from the preputium) and testis descent were examined. In females, the vagina was cut open for general examination as well as control of vaginal opening. The anogenital distance, AGD, was measured with vernier callipers (accuracy ± 0.02 mm) in both sexes. The distance between anus and opening of the preputium was measured. In females, measurement was made between anus and vaginal opening.

A 25 gauge needle aspiration biopsy from cauda epididymidis was taken and put in formol-saline (Hancock's solution). For future histological examination, pieces of testis and epididymidis were fixed in Bouin's solution for 24 h and followed by storage in 70% alcohol. The prostate gland and the ampullae were fixed in 10% phosphate buffered formaline, as well as the uterus and the right ovary and oviduct. Testis, epididymidis, baculum, ovarium and ductus ovarica were stored at -70ºC for future examination.

A mean coefficient of variation (C.V.) was generated for several of the parameters. From each animal, the C.V. for the respective parameter was calculated from the triplicate measurement where a mean and standard deviation were recorded.

Correlations between anogenital distance, AGD, and body weight and body length (measured from nose to base of tail) respectively were calculated for male mink.

RESULTS AND DISCUSSION

Evaluation of parameters

Ten of the seventeen minks were male and seven were female. The females had a generally lower body weight as shown in table 1. At least four of the males and four of the females were presumed to be juvenile, based on total body weight (generally low), appearance of teeth (not worn down) and presence of thymus. For some of the carcasses it was difficult to make a fair assumption of age. The lower jaw was collected for future cementum analysis, as this is the only accurate method of age determination of mink (Matson, 1981; Dunstone, 1993).

Table 1. Body weight (kg) of males and females

	N	Min	Max	Mean	SD
Female	7	0.38 -	0.55	0.48 ±	0.06
Male	10	0.53 -	1.15	0.94 ±	0.18

By comparing the subjective score of body condition to the weight of abdominal subcutaneous fat and the weight of the omentum respectively, the score system appears to offer a generally fair estimation of the amount of fat found in the body, although it is a rough measurement. When studied on a larger population, the "average" and "below average" score will probably, as seen in this small study, tend to become a larger group with a wide range, while the above average score will be rare. The weight of the abdominal subcutaneous fat as well as the omentum weight generally reflected the body condition score. Obviously, the weight of the fat is a more objective parameter. Therefore, weighing the subcutaneous abdominal fat or the omentum appears to be the most accurate way to make a more precise estimation of the condition of nutrition.

Table 2. Body condition score compared to weight of omentum and abdominal subcutaneous fat

Body condition score		Min	Max	Mean	SD
1 (below average)	s.c. fat (g)	0.00	3.86	1.74 ±	1.92
n= 6	omentum (g)	0.27	1.32	0.82 ±	0.36
2 (average)	s.c. fat (g)	2.51	16.16	7.67 ±	4.19
n=10	omentum (g)	1.13	4.89	2.15 ±	1.24
3 (over average)	s.c. fat (g)		35.99		
n=1	omentum (g)		10.30		

Two of the females were almost rotten, which made dissection and for example weighing of ovaries and oviducts difficult. No tissues for histological examination were taken from these carcasses.

It was only possible to collect urine from seven of the seventeen carcasses (41%) and sometimes the amount of urine was scarce. Faeces were always present and collectable, but sometimes there were only enough faeces for one sample (less than approximately 1 ml). At least one sample containing approximately 1,5 ml of

clotted blood from the heart and large vessels was collected from each mink, except for one single mink. These collected samples will be further used for hormone and chemical analysis. For example, faecal content of steroids as oestrogen and testosterone has been determined in several species (Schwarzenberger et al., 1996). In pregnant female mink, progesterone in faeces has been monitored (Möstl et al., 1993), as well as urinary excretion of cortisol and oestrone sulfate (Madej et al., 1992).

The average coefficient of variation, C.V., indicates low variation in the measurements recorded with vernier callipers. These parameters seem to be reproducible and may be used in the future.

Table 3. Mean coefficient of variation for the triplicate measurements

Parameters	Mean C.V.
Male	
Anogenital distance	2.63%
Penis length	1.00%
Penis width	3.12%
Baculum length	1.04%
Nose to tip of tail	0.40%
Nose-base of tail	0.50%
Tail	1.19%
Female	
Anogenital distance	4.58%
Nose to tip of tail	0.25%
Nose to base of tail	0.48%
Tail	0.74%

The anogenital distance of male mink was significantly correlated with weight (R=0.75, *p*=0.01) and less correlated with body length (R=0.63, p=0.05). Therefore, a weight-normalized index for anogenital distance was calculated [AGI =AGD/weight (mm/kg)] as previously done by Schwan (Schwan et al., 2005).

Table 4. Results of measurements on male mink

Parameters	*n*	Mean		SD
AGD (anogenital distance) (mm)	10	62.43	±	7.84
AGI (anogenital index)	10	67.93	±	12.81
Penis length (mm)	10	52.57	±	6.15
Penis width (mm)	10	3.83	±	0.51
Baculum length (mm)	10	41.00	±	4.41
Penis weight (g)	10	0.93	±	0.50
Testis weight (g)	19	0.63	±	0.75
Epididymidis weight (g)	19	0.18	±	0.19
Body weight (kg)	10	0.94	±	0.18
Nose to tip of tail (cm)	9	57.71	±	4.19
Nose-base of tail (cm)	10	40.38	±	2.62
Tail (cm)	9	18.65	±	2.02
Weight of subcutaneous fat (g)	10	9.94	±	10.02

Table 5. Results of measurements on female mink

Parameters)	*n*	Mean	SD
AGD (anogenital distance) (mm)	7	12.44 ±	1.22
Ovary weight (g)	14	0.04 ±	0.02
Oviduct weight (g)	14	0.04 ±	0.02
Uterus weight (g)	7	0.23 ±	0.17
Vagina weight (g)	7	0.56 ±	0.31
Body weight (kg)	7	0.48 ±	0.06
Nose to tip of tail (cm)	7	49.62 ±	2.56
Nose-base of tail (cm)	7	34.21 ±	2.51
Tail (cm)	7	16.05 ±	1.33
Weight of subcutaneous fat (g)	7	3.39 ±	4.22

Difficulties when dissecting the oviduct from the surrounding tissues due to small size and decay is probably the reason for the high weight relative to the ovaries.

The prostate weight was not recorded as it was too difficult to dissect because of rather diffuse margins, especially on juvenile males. However, this measurement might be useful for adults during certain periods of the annual reproductive cycle, when the prostate is enlarged and more prominent. Also, measurement of the prostate might be performed by recording length as previously done by Basrur & Ramos (Basrur & Ramos, 1972).

Some specific findings

One mink had unilateral hypoplasia of both testis and epididymidis. Especially the cauda epididymidis seemed hypoplastic at macroscopical examination. The hypoplastic testes could not be felt in the scrotum at palpation, but when dissection was performed it was found in the inguinal canal. Both testes and epididymidis were collected for histological examination. The mink was a small male, probably juvenile, with scarce fat storages.

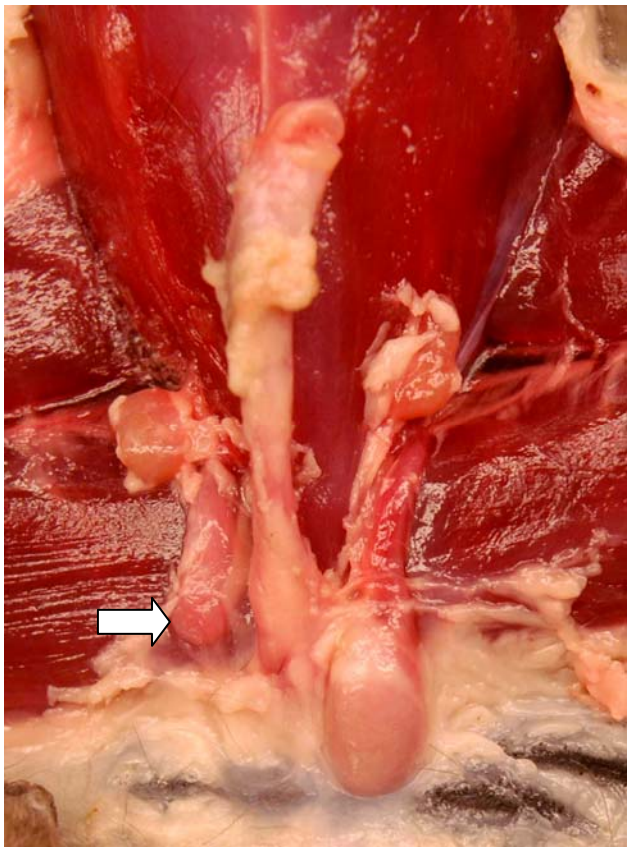

Figure 6. The small right testis was found near the base of the penis in the inguinal canal.

A male mink had a lesion on one of its testicles. It appeared to be a wound, presumably an old bite wound. Post-mortem findings on otters in Great Britain show that intraspecies fights often results in bite wounds in the neck and genital area (Simpson, 2006). Another male mink had a previously fractured baculum that had not healed properly.

CONCLUSIONS

The dissection protocol was easy to follow for most of the parameters and can be used in future reproductive toxicology studies. However, although the carcasses had been frozen, the decay was sometimes substantial and made handling and weighing of the organs difficult. Organ measurements were successfully recorded, but in juveniles where the prostate was to discrete to dissect and weigh in a standardized manner. Urine was only present in 41% of the carcasses and this might limit the use of the parameters recorded in urine. On the contrary, clotted blood and faeces were collectable in almost all of the carcasses.

In some cases it was possible to make an assumption whether the mink was juvenile or adult, but cementum analysis is necessary for accurate age determination. Furthermore, weighing the abdominal subcutaneous fat was found to be the most accurate way to estimate condition of nutrition. The average intraindividual coefficient of variation was low for all the parameters examined with vernier callipers, suggesting a high reproducibility of the measurements. The AGD was strongly correlated to the body weight, suggesting a calculation of a weight adjusted AGI for future assessment of male genital development.

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