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REPRODUCTION IN WILD VERTEBRATES

Proceedings from a symposium in Uppsala, Sweden
February 10, 2011

Björn Brunström, Jonas Malmsten, Bodil Ström Holst & Galia Zamaratskaia
(editors)

Uppsala, 2011

CRU Report 25



CRU

CENTRE FOR REPRODUCTIVE BIOLOGY IN UPPSALA

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Contents	
Foreword	7
List of participants	9
Programme	13
Abstracts:	
Eva Axnér	
<i>Collection of reproductive data in the lynx</i>	15
Cecilia Berg	
<i>Environmental pollution – a threat against reproduction in frogs?</i>	17
OA Fadeyi, DVM, OO Ajala OO, CE Obudu CE' WD Nafarnda	
<i>Studies on some reproductive parameters of the grasscutter (Thryonomys swinderianus)</i>	19
Judit Győri, László Szendrei, Lajos Juhász	
<i>Effect of indoor keeping on wild boar reproduction in Hungary</i>	21
László Kövér, Lajos Juhász,, Péter Gyüre	
<i>The nesting of hooded crow (Corvus cornix) in Debrecen (Hungary)</i>	23
Katrin Lundstedt-Enkel	
<i>Multivariate methods used during the multivariate research chain – from design of experiments to data evaluation</i>	25
Hanna Løvlie	
<i>Cryptic female choice against inbreeding in teh red jungelfowl, Gallus gallus spp.</i>	27
Sara Persson	
<i>Wild mink (Neovison vison) reproductive data for environmental monitoring</i>	29
Anna Roos	
<i>Improved reproductive success in grey seals, otters and sea eagles in relation to decreased PCB concentrations</i>	31
CRU Publication Series	33

Foreword

On 10 February 2011 the symposium "Reproduction in wild vertebrates" is arranged by CRU, Centre of Reproductive Biology in Uppsala. CRU is a multidisciplinary network of almost 100 scientists with different academic backgrounds at SLU, Swedish University of Agricultural Sciences, and UU, Uppsala University.

Reproduction in wild vertebrates is an area that interests researchers within different fields. Knowledge on basic reproductive physiology is limited for several species, and research of interest not only for these species, but also of a comparative value. Some wild species are used for studies on the effect of environment on reproduction, an issue that is of increasing importance not only for wild vertebrates but also for the domestic animals and humans. We wish you welcome to take part of the symposium that covers varying aspects of reproduction in wild vertebrates, and look forward to fruitful discussions.

Uppsala, February 2011

Björn Brunström, Jonas Malmsten, Bodil Ström Holst and Galia Zamaratskaia (editors)

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CRU SYMPOSIUM ON

REPRODUCTION IN WILD VERTEBRATES:

A meeting on ongoing research on all aspects of reproduction in wild vertebrates

Thursday 10 February 2011,
Ultuna main building, Uppsala, Lecture Hall L

Programme:

08.30-09.00	Registration
09.00-09.10	Welcome
09.10-09.35	Collection of reproductive data in the lynx <i>Eva Axné, SLU</i>
09.35-10.00	Environmental pollution – a threat against reproduction in frogs <i>Cecilia Berg, UU</i>
10.00-10.25	Improved reproductive success in grey seals, otters and sea eagles in relation to decreased PCB concentrations <i>Anna Roos, Swedish Museum of Natural History</i>
10.25-10.50	Coffee
10.50-11.15	Multivariate methods used during the multivariate research chain – from design of experiments to data evaluation <i>Katrin Lundstedt-Enkel, UU</i>
11.15-11.40	Cryptic female choice against inbreeding in the red junglefowl, <i>Gallus gallus</i> ssp. <i>Hanna Løvlie, UU</i>
11.40-12.05	Wild mink (<i>Neovison vison</i>) reproductive data for environmental monitoring <i>Sara Persson, SLU</i>
12.05-12.15	Concluding remarks
12.15	LUNCH

COLLECTION OF REPRODUCTIVE DATA IN THE LYNX

Eva Axnér

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The Eurasian lynx is the only wild felid in Scandinavia. It is listed as vulnerable in the red list of Swedish species. Although reproduction is of central importance in the development of a population the knowledge about reproductive physiology in the lynx is limited. The pregnancy rate and litter size varies between age groups and regions although the exact causes of these variations are largely unknown. Failure of breeding also occurs at Zoos and can therefore not only be attributed to nutrition. Environmental disturbances and climate changes are likely to affect reproduction of wild animals. Because the lynx is a predator on top of the food chain it would be a suitable animal to monitor for such effects. All lynx that are found dead or culled during hunting are to be sent to the Swedish Veterinary Institute. Last year (2010) 169 lynx were killed at legal hunting. The availability of organs that can be examined contributes to the suitability of the lynx as a species to be continuously monitored to detect effects on environment on reproduction. To be able to detect changes in reproductive parameters it is, however, necessary to first collect basal data.

ENVIRONMENTAL POLLUTION – A THREAT AGAINST REPRODUCTION IN FROGS?

Cecilia Berg

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Amphibian populations are declining world-wide and one of the suggested reasons is environmental pollution. The aim of the study was to investigate if exposure to ecologically relevant concentrations of estrogenic environmental pollutants impairs sex organ development and fertility in frogs. We characterized effects of the estrogenic environmental pollutant ethynylestradiol (EE₂) on the reproductive system in two amphibian species; a temperate species, *Rana temporaria* (Common frog) and a tropical model species, *Xenopus tropicalis* (African clawed frog). Frog tadpoles were exposed from shortly after hatching until metamorphosis to EE₂ concentrations found in the environment. Effects on gonadal differentiation were assessed by evaluating gonadal histology at metamorphosis and one month later (in *Rana temporaria*). A subset of *Xenopus tropicalis* individuals were held unexposed for eight months after metamorphosis until they were sexual mature. Their fertility were assessed by mating them with unexposed frogs and evaluating fertilisation rates and gonadal histology. The results show that environmental concentrations of EE₂ caused female-biased sex ratios, indicating male-to-female gonadal sex-reversal in both species. The susceptibility of the two species to EE₂ was comparable, which supports the use of *Xenopus tropicalis* as a model organism for research on developmental reproductive toxicity of estrogenic pollutants. Female-biased sex ratios were found at concentrations as low as 6 pM (1.8 ng/L) and were shown to persist in adult frogs. EE₂-exposed adult males that were not sex-reversed had a significantly reduced fertilization rate compared with the control males. In addition, histological evaluation revealed that the EE₂-exposed males had a reduced amount of spermatozoa in the testes. Among the EE₂-exposed frogs with ovaries a significantly higher proportion of individuals lacked oviducts (and therefore were sterile) compared with control females. In conclusion, the results show that environmental concentrations of EE₂ disrupted testicular differentiation and impaired fertility in male frogs. Considering that several estrogenic chemicals are present in contaminated surface waters this type of environmental pollutants may pose a risk to reproduction in wild frog populations.

STUDIES ON SOME REPRODUCTIVE PARAMETERS OF THE GRASSCUTTER (*THRYONOMYS SWINDERIANUS*)

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A survey of seven grasscutter farms located in Ibadan, Lagos and Badagry was carried out in order to analyse farm records concerning some reproductive parameters such as gestation length, litter size, sex ratio, birth weight and weaning age and weight. The overall aim of the study was to provide base-line information to prospective farmers with a view to encouraging domestication and reducing the rate of environmental degradation associated with bush burning which is the usual mode of capture of the animal from the wild. Results indicated that the gestation length of grasscutters was 156.61 ± 1.02 days, litter size of 4.6 ± 0.28 , a sex ratio of 1.45 ± 0.13 males : 1 female, weaning age of 28.56 ± 0.38 and birth weight of 133.55 ± 42.31 g. This study shows that with effective and efficient management practices, grasscutters can be weaned at 4 weeks of age in this environment without any adverse effects on the survivability of the young ones, instead of the 6 weeks advocated by some schools of thought. This practice would make it possible for farmers to breed their stock twice a year for enhanced profitability. Domestication would also reduce the labour and time expended capturing the animals from the wild, make them available on demand and eliminate smoke and greenhouse gases associated with the capture of grasscutters from the wild.

EFFECT OF INDOOR KEEPING ON WILD BOAR REPRODUCTION IN HUNGARY

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The Carpathian basin is great habitat for big games. Hungary is well known of its high level game-management. Nowadays the demand of more profitable and sustainable game-management is increasing. Because of that fact game-preserves are being established all over the country. According to The National Data Base of Game Management (OVA) in 2009-2010 a vast number of big games in Hungary are kept indoors: 7,2% of red deer, 22,5% of fallow deer, 0,38% of roe deer, 29,5% of mouflon, and 19,8% of wild boar. There are 118 game-preserves in Hungary, more than half of them are established specifically for wild boar keeping.

In case of indoor game keeping the continuous feeding and the higher game density cause differences in some parameters of the game population compared to the game stock found in the nature. This difference can be clearly seen in the variation of the reproductive characteristics. The main period of wild boar mating in nature is between October and January. Contrarily in game-preserves the reproductive period can hold during the whole year. One of the main reason of this phenomena is the forage that is provided whole year. In practice that means hogget can be fertilized any time after reaching the sexual maturity. On one hand it can be positive, because sows can be fertilized more times in a year. On the other hand sows can be too early sexually mature and their gruntlings are weaker than the older sow's offspring. The rate of gestation can be influenced by supplementary feeding. The results of researches in this topic are very diverse. Náhlik and Sándor (2004) found positive correlation between the age and weight of sow and the natality rate. In contrast according Jánoska's (2008) results in wild boar game-preserve the condition of the sow has no significant effect on gestation.

An other important fact, that in game-preserves we find a much higher density of games than in nature. This can cause a great stress in the population, and can influence reproductive parameters in a negative way. According Kőhalmy's (1979) examination the number of gruntling in one litter is 6,7 and 5,8 in nature and 3,5 in game-preserves. This effect could be offset by the fact that sow can be fertilized more time in a year.

Indoor wild boar keeping can satiate the demand of great bag, which would be difficult to ensure in nature. Because of this demand in game-preserves the owners used to bring domesticated pigs in rearing to improve reproduction parameters. In Hungary this issue is regulated by law.

Indoor big game keeping is an artificially maintained community, which would not be viable without continuous feeding. Therefore it's really important to realize a proper, well planned feeding.

THE NESTING OF HOODED CROW (*CORVUS CORNIX*) IN DEBRECEN (HUNGARY)

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In the past decade Hooded Crows showed a significant growth in Debrecen. The aim of this study was to become acquainted with Hooded Crows nest-site selection in urban circumstances. The research revealed that Hooded Crows are not choosy when it comes to selecting the species of the trees, but we noticed differences regarding nesting height - Hooded Crows living in the city build their nests in higher regions than those living in places outside Debrecen. We also discovered a slight, insignificant difference between nesting heights and the tree species, which is probably due to the different characteristics shown by the given tree species. As for nesting heights, we found that in typical urban habitats there were no relevant differences shown. However, when we compared these habitats in pairs, it came to light that nesting heights -when comparing lonely trees - wood segments and tree rows – wood segments- did show significant differences, which can be explained by the various conditions provided by the habitats mentioned.

To sum up, during our research the following results emerged:

1. Hooded Crows prefer approximately the same nesting heights in all kinds of habitats, urban environment and tree species.
2. Nesting height does not significantly depend on the habitat itself and the tree species.

MULTIVARIATE METHODS USED DURING THE MULTIVARIATE RESEARCH CHAIN - FROM DESIGN OF EXPERIMENTS TO DATA EVALUATION

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Examples of several multivariate techniques will be given from my research regarding effects of contaminants on fish reproduction and other end-points as well as when modelling bioaccumulation and biomagnification of contaminants in Baltic Sea animals.

Multivariate design (MVD)

Herring (Clupea harengus) example¹. Fish was collected in the Baltic Proper and in order to get samples that spanned numerous biological variables (using the natural variation regarding fish ages, body weights, body lengths) the biology was determined for 381 individuals. From this large number, 60 individuals were selected using MVD for further chemical analyses of the contaminant concentrations (figure 1).

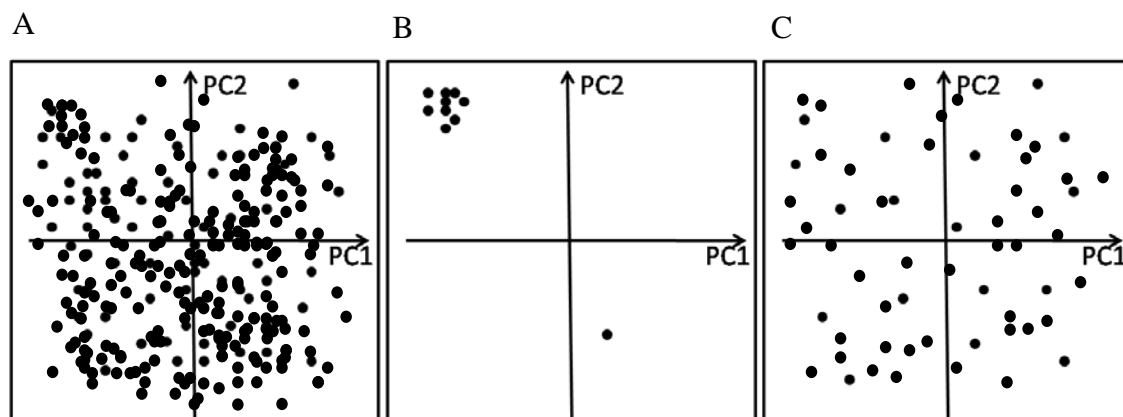


Figure 1. Multivariate design (A), Score plot from a principal component analysis (PCA) including all observations (here all individually measured fish). PC1 and PC2= principal component 1 and 2. (B), Skewed sample selection. (C), Optimal sample selection spanning the whole properties domain for herring biology.

Design of Experiments (DoE)

Stickleback (Gasterosteus aculeatus) and rat examples². A chemometric approach was used to study interactions between ethynylestradiol (EE₂) and an AhR-agonist (beta-naphthoflavone (βNF)) in stickleback and between two flame-retardants (polybrominated diphenyl ethers (Bromkal 70-5 DE) and chlorinated paraffins (Cereclor 70L)) in the rat. The substances were given to the animals after determining the exposure concentrations using a Doehlert design³ that resulted in 8 different exposure groups. Interestingly, the results showed that exposure to intermediate concentrations led to significant increase in the fish sperm motility variables and similarly, that intermediate concentrations caused a marked effect in the rat *e.g.* lowering the thyroid hormones' levels.

Multivariate Data Analysis (MVDA)

The guillemot (*Uria aalge*) and goldfish (*Carassius auratus*) examples^{4, 5}. An animal can be described multivariate, *i.e.* numerous variables determined/animal. Principal component analysis (PCA) extracts and displays the systematic variation in the data and allows groupings, trends and outliers to be found. Partial Least Squares regression (PLS) is similar to PCA, as both are projection methods that work by reducing the number of dimensions in the data. The difference between PCA and PLS is that PLS is used to model relationships between two matrices, Y and X, that can both be multidimensional⁶⁻⁸.

For the special case of exploring one Y variable and its relationship to the X matrix, the method orthogonal PLS (OPLS) is preferred^{1, 9}. OPLS is able to separately model variation correlated and uncorrelated (orthogonal) to Y. In OPLS, an extra filtering step is added to the well-known Nonlinear Iterative Partial Least Squares (PLS-NIPALS) algorithm developed by Herman Wold¹⁰. Chemometrics is an integral part of the research strategy within animal reproduction biology by providing organized multivariate statistical approaches for MVD, DoE, and MVDA.

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CRYPTIC FEMALE CHOICE AGAINST INBREEDING IN THE RED JUNGLEFOWL, *GALLUS GALLUS SSP.*

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When inbreeding reduces offspring fitness, individuals are selected to prefer unrelated mating partners to avoid these costs. In polyandrous species, this can occur through a range of behavioural and physiological mechanisms occurring before, during and after copulation. However, particularly post-copulatory mechanisms of cryptic choice are typically hard to investigate and therefore remain largely unresolved. I have investigated the mechanisms through which female red junglefowl (*Gallus gallus ssp.*) bias fertilisation in favour of sperm from unrelated males. Female fowl are commonly forced into copulation by males, thus have limited pre-copulatory mate choice. However, due to polyandry and prolonged female storage of sperm in numerous sperm storage tubules, there is a great potential for female driven post-copulatory mechanisms in favour of certain males. By experimentally removing female pre-copulatory choice, I show that in natural copulations, female red junglefowl bias their sperm utilisation against sperm of brothers. This bias was not due to male differential sperm allocation, or female ejaculate ejection. In addition, the bias against brothers is lost when ejaculates are inseminated artificially, suggesting a pre-copulatory cue driving female post-copulatory bias against inbreeding. Taken together, paternity is mediated by multiple mechanisms in the red junglefowl, with several mechanisms occurring at a post-copulatory stage, and that these processes may be influenced by pre-copulatory cues in a complex manner.

WILD MINK (*NEOVISON VISON*) REPRODUCTIVE DATA FOR ENVIRONMENTAL MONITORING

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The wild mink is presumably highly exposed to pollutants as it is high up in the food chain and has a semi-aquatic lifestyle. This, together with other qualities such as it has a restricted home range and a widespread distribution in Sweden, makes the mink a suitable sentinel species in environmental monitoring. As many pollutants affect reproduction, 117 wild male mink from August to the end of April (year 2005 to 2008) were collected from all over Sweden and the reproductive system was examined on autopsy. The influence of age, nutritional status and season on the reproductive system was calculated by least squares using the general linear model (GLM) procedure of SAS. Age was determined by cementum analysis and mink were grouped as juvenile, one year old or two years and older. Nutritional status was set as subcutaneous fat (g)/body weight (kg). Hours of day-light at the specific capture date and site for each mink was used to construct three seasonal groups; autumn (17-9 hours of day light before winter solstice), winter (<9 h) and spring (9-17 h after winter solstice). There was a clear effect of age and season on all reproductive organs. The weights of testes, epididymides and penis were lower in juveniles compared with the other age categories ($p < 0.0001$ – $p = 0.0023$) There was no difference for these traits between one-year-old mink and those that were two years or older. In addition, these traits increased from autumn to winter and also from winter to spring. Penile and baculum length increased ($p = 0.0095$ and $p = 0.0003$, respectively) from juveniles to the one-year-old mink, and increased further in animals that were two years old or more ($p = 0.012$ and $p = 0.0182$, respectively). These two organs increased significantly from autumn to winter ($p = 0.015$ and $p = 0.005$ respectively) but not between winter and spring. Surprisingly, nutritional status had no significant effect on the reproductive organs except for baculum length and anogenital distance. In conclusion, when assessing male reproductive data in wild mink populations, season must be taken into account for all reproductive traits. In contrast, the nutritional status seems not to be of any major significance. Our data indicate that the development of most parts of the reproductive system in the wild male mink is completed after one year.

IMPROVED REPRODUCTIVE SUCCESS IN GREY SEALS, OTTERS AND SEA EAGLES IN RELATION TO DECREASED PCB CONCENTRATIONS

Anna Roos^{1,2}, Britt-Marie Bäcklin¹, Björn Helander¹, Frank Rigét³, Lillemor Asplund⁴ and Ulla Eriksson⁴

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Grey seals (*Halichoerus grypus*), otters (*Lutra lutra*) and white-tailed sea eagles (*Haliaeetus albicilla*) were common in Sweden but decreased dramatically in numbers after the 1950s. The underlying cause is believed to be organochlorines in their diet. Since the 1980s, the populations have increased. The population trends and health condition in Baltic grey seals has been studied since the beginning of the 1970s, and a disease complex, including uterine obstructions and leiomyomas have earlier been described. The health status of otters has also been investigated, although not in as much detail. Sea eagles have been monitored in Sweden since 1965, including reproduction, population trends and investigations of unhatched eggs.

Three aspects of adult grey seal uteri were studied: 1) pregnancy rate, 2) uterine obstructions and 3) uterine leiomyomas. The prevalence of uterine obstructions and leiomyomas have decreased and the pregnancy rate among the investigated adult grey seal females (n=92), found dead between August and March 1976-2009 has increased over time and is nowadays close to 100%. In another study on PCB concentrations (sum of 7 CBs: CB 52, 101, 118, 138, 153, 156 and 180) in blubber from juvenile grey seals (0-3 years old, collected 1968-2005, n=242) a decrease of PCB-concentrations with approximately 5 % yearly was seen (Figure 1A).

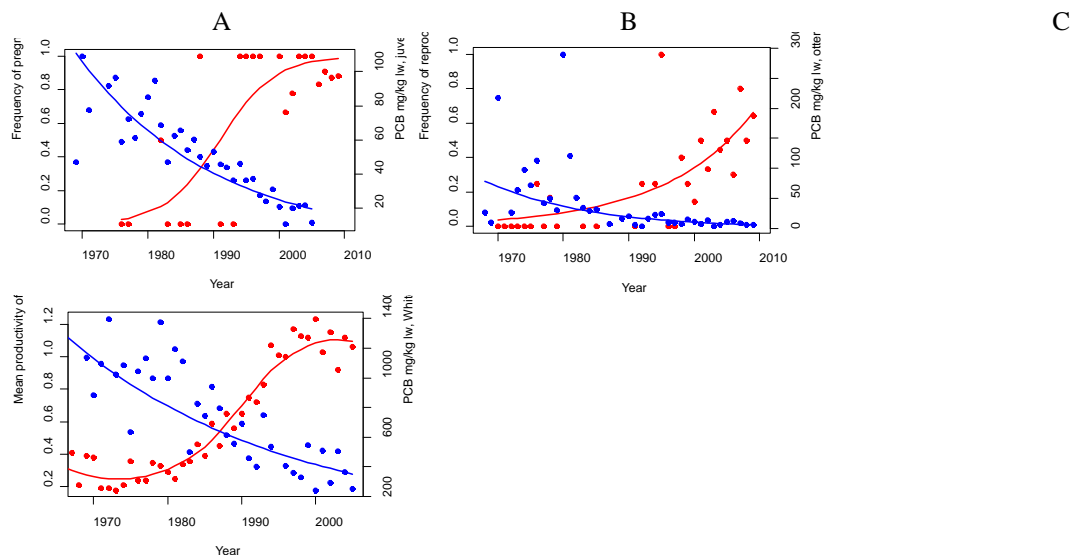


Figure 1A. Frequency of adult pregnant grey seals and concentrations of sum of 7 CB (mg/kg l.w.) in juvenile grey seals.

B) Frequency of adult female otters with signs of reproduction and concentrations of total PCB in otter muscle tissue (both sexes). C) Mean productivity of white tailed sea eagles (number of fledglings/checked pair) and concentrations of PCB in eggs. Red dots represent adult females (A and B) and mean productivity (C), red lines represent their logistic curves estimated by non-linear regression. Blue dots represent concentrations of PCB, blue lines a log-linear regression of annual geometric mean concentrations.

Otters found dead were investigated and signs of reproduction was noted in most adult otter females *i.e.* pregnancy, lactating or with uterine signs indicating a recent birth (n=145). Signs of reproduction were very rarely seen in otters from the 1970s-1980s. During 1968-2010,

PCB in otter muscle tissue (both sexes) has decreased with approximately 6 % yearly in otter muscle tissue (Figure 1B).

Finally, the temporal trend of mean productivity of white tailed sea eagle (i.e. number of fledglings/checked nest) and PCB concentrations in dead eggs is shown in Figure 1C.

The diagrams for the three species are quite similar and support the hypothesis that when concentrations of PCB decrease, reproductive success improves at approximately the same time for all species. It also implies that PCB has had an effect at a population level.

CRU Publication series

Report 1

Proceedings of the Inauguration of the Centre for Reproductive Biology, 1997, by *Andersson H, Kindahl H, and Neil M (editors)*

Report 2 (In Swedish)

Svinforskning vid SLU - Presenteras för Sveriges grisproducenter vid föreningens årsmöte på Ultuna 25-27 juni, 1998 by *Andersson K (editor)*

Report 3

Advances in Canine Reproduction - Minisymposium at SLU, September 3, 1998, by *Linde-Forsberg C (editor)*

Report 4

Sperm behaviour prior to fertilization in animals - Special symposium at SLU, November 16, 1998, by *Larsson B and Rodriguez-Martinez H (editors)*

Report 5

Aspects of Equine Reproduction- symposium at SLU, April 15, 1999, by *Rodriguez-Martinez H (editor)*

Report 6

Impaired Reproductive Performance in Postpartum Cows- symposium at SLU, May 26, 1999, by *Kindahl H, (editor)*

Report 7

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