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Reproductive Failure in Farm Animals

Proceedings from a symposium at Estonian Agriculture University, Tartu, June 14-15, 2001

Toomas Tiirats *(editor)*

Uppsala 2001

CRU Report 14

CRU CENTRE FOR REPRODUCTIVE BIOLOGY IN UPPSALA

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Introduction

The minisymposium "Reproductive Failure in Farm Animals" was organized in Tartu, 14-15 June, 2001 by the faculty of Veterinary Medicine, the Institute of Animal Husbandry of Estonian Agricultural University (EAU) and the Centre for Reproductive Biology in Uppsala.

The objective of minisymposium was concerned with giving overview of scientific problems and activities in veterinary and animal science. Expected participants were academic staff from Estonia and Sweden, representatives from breeding co-operatives, advisory service etc.

With this minisymposium a co-operative programme for "Improvement of reproductive efficiency in farm animals" between the Estonian Agricultural University, Tartu, Estonia and the Centre for Reproductive Biology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden comes to an end. The final Swedish-Estonian symposium very much reflects successful co-operation during three years period financially supported by Svenska Institutet.

It is most obvious that the general aim of the programme is very well achieved, i.e. to facilitate exchange of academic staff between EAU and CRB at SLU, to promote joint education, research and publication of educational and scientific material. A fertile ground is prepared for the exchange of ideas from which both parties can benefit. We have got an excellent position not to stop, but go on with obtained experiences and keep bringing scientists together improving educational level and research competence of academic staff.

On behalf of Estonian colleagues I would like to extend our gratitude to former director of CRB Dr. Mats Forsberg starting with this idea of co-operation and project itself, and Dr. Ulf Magnusson carrying on this project tirelessly with activeness typical of him.

Toomas Tiirats
Estonian co-ordinator

Breeding for dairy cattle fertility – the Scandinavian experience

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Abstract
Reports are many of a deteriorating fertility of the lactating dairy cow. Poor fertility causes large economic costs to the dairy industries around the world. The Nordic countries have since 25 years ago selected for fertility. This was made possible by the cooperative organisational structure of the Nordic farmers. National recording systems were gradually developed for production, fertility and health traits with a start in the early 60's. A second prerequisite was that a breeding plan with progeny testing of bulls was created with daughter groups large enough (150-300) to calculate Breeding Values with acceptable accuracies for lowly heritable traits such as fertility. The Nordic countries have similar philosophies regarding recording schemes and breeding objectives with minor differences in the traits included and their weightings in the Total Merit Index of bulls. In the Swedish Red and White breed (SRB) the genetic trend in fertility has been unchanged for a long time. Thus, selection for total economic merit has counteracted the expected negative response in fertility from selection for increased production. The SRB trend verifies that it is possible to avoid a deterioration of the fertility of cow if the trait is properly considered in the breeding programme. The Swedish Black and White breed (SLB) has a negative genetic trend in fertility. This can be explained by the Holstein Friesian imports where no information on fertility was available for selection purposes. The trends in fertility are about the same and unchanged for the different Nordic red breeds, whereas the black and white breeds in the Nordic countries all show a deterioration in fertility. Recordings of early reproductive performance such as the interval from calving to first ovulatory oestrus and the occurrence of reproductive disorders are also discussed.

Keywords: dairy cows, reproduction, genetic parameters, genetic trends, reproductive disorders

Introduction
Reports from several countries indicate that the fertility of the lactating dairy cow has deteriorated, especially in the Holstein Friesian (HF) breed. Already in 1994, Hoekstra et al., reported that the introduction of HF genes had decreased the fertility of the Dutch dairy population. In France a decrease in fertility has been observed in the last 20 years (Boichard et al., 1998). In UK, conception rate at first service is now below 40% and thus, the average cow requires more than two inseminations to get in calf (Royal et al., 2000). There are also reports from United States of a declining female fertility of dairy cows (Thompson, 1998; Washburn et al., 2000).

Fertility is an economically important trait in dairy production. It causes lowered production, additional inseminations and veterinary costs, increased culling rate and replacement costs. Impaired fertility is in general one of the two major causes of involuntary culling of dairy cows. In Sweden about 10% of the cows are culled annually because of impaired fertility, corresponding to 26% of all disposals (Swedish Dairy Association, 2000).

It has been questioned whether it is really possible to achieve any substantial genetic improvement in fertility due to its low heritability. This paper presents some experiences from Sweden and the other Nordic countries of breeding for female fertility. A thorough review of the methods practised for categorical traits such as reproduction and health and the results of many years of selection in Scandinavia, was given by Philipsson & Lindhé (2000) at the last EAAP-meeting. It regards definition of traits, the relationships with production, methods for genetic evaluations, selection practised and response obtained. Furthermore, additional measures especially on the early reproductive events are also discussed.

Low heritability but large genetic variation

Female fertility traits usually have a heritability of 5% or less. In spite of this, the additive genetic variation is substantial. Female fertility traits usually show a coefficient of additive genetic variation in the order of 6-9% (Philipsson, 1981) which is only slightly less than for milk production. The large variation has been confirmed in large field data (Roxström et al., 2001a). A low heritability is a serious drawback when the trait is measured only on the individual animal subjected to selection. When measured on many daughters of the bull the accuracy of the Breeding Values (BV) may reach reasonable levels to be used for selection. The Nordic countries have created a breeding structure with 150-300 daughters per bull which give acceptable accuracy of the BV's for the low heritability traits and make selection for these traits possible. Recording of repeated lactations is another possibility to increase the accuracy of BV's. The large influences of management factors on the traits are at least partly corrected for in the statistical models. Thus the genetic change in fertility may be substantial and is, permanent and cumulative, and selection is a long-term strategy to take care of these traits. Likewise, if selection for fertility is ignored substantial deterioration will occur.

Milk yield and fertility is genetically antagonistic

An antagonistic relationship has clearly been shown between fertility and production. Recent studies by Roxström et al. (2001a), show genetic correlations between yield and fertility of 0.2-0.4 (Table 1). Thus selection for yield without consideration of daughter fertility results in a deterioration of daughter fertility. The exact reason for this antagonistic relationship is not known. The needs of energi to maintain a high production after calving have probably priority over reproduction. Genetic correlations between milk yield and fertility become stronger with lactation number as is shown in Table 1, which could be a further strengthening of this hypothesis (Roxström et al., 2001a).

If there is no selection on fertility it is at least important to correct production for a measure of fertility e.g. days open in the genetic evaluations for production. Otherwise cows with no or late pregnancy become favoured due to the negative effect of pregnancy on milk production.

Table 1. Heritabilities and genetic correlations between fertility measures at different ages and correlations with production in the Swedish Red and White breed (SRB) (from Roxström et al., 2001a,b)[a]

Fertility measure	**Parity**	Heritability (%)	r_g fertility parity 1	r_g fertility parity 2	r_g kg ECM_{23}[b]	r_g HI	r_g CFI
No. AI	0	2.5	0.67				
	1	3.0		0.94	0.29	0.14	0.13
	2	3.1			0.31		
	3	2.2		0.93	0.41		
CFI	1	3.4		0.81	0.25	0.36	
	2	2.5			0.30		
	3	1.8		1.00	0.35		

[a] For abbreviations see text
[b] ECM_{23}=mean of energy corrected milk at 2[nd] and 3[rd] test-day

Nordic countries have since 1975 selected for fertility

All Nordic countries have well integrated recording systems on dairy cattle health, fertility and production. In Sweden a national integrated computer based data recording of AI services and milk production was created in the 1960's. Approximately 80% of the 430 000 cows participate nowadays in the AI and milk recording.

The selection of bulls has since long in all the Nordic countries been based on a combination of traits in a Total Merit Index (TMI) of bulls. The most important traits are milk production, reproduction, calving performance, health and conformation. The Nordic countries have similar philosophies regarding recording schemes and breeding objectives with minor differences in the traits included and their weightings in the TMI's. The weighing applied of fertility to milk yield within the Swedish selection index is 0.35 to 1. Usually the weights are chosen to avoid a deterioration in the trait and secondly to maximise the overall economic improvement. Model calculations by Philipsson et al., (1994) show that selection according to TMI will contribute to a 10-25% more economic gain than selection only for milk production.

The evaluation of daughter fertility is to a large extent based on the comprehensive investigations in this area included in the thesis by Janson (e.g. Janson 1980, Janson & Andreasson, 1981).

Fertility is a composite and complex trait. Generally speaking, there are measures of two different kinds of traits, *interval* measures, such as interval from calving to first insemination (CFI) or from first to last insemination (FLI), and measures of *conception rate*. The latter measures are often indirect, such as % non-return (NR), or given as number of inseminations per serviced animal (No. AI). Days open (DO) express the combined effects of both traits but are limited to cows that conceive for at least a second year. Also information about treatments (Tr) and culling (Cull) for reproductive disorders/failures are often used and in some instances also scores for heat symptoms (Heat).

Table 2 shows the measures of fertility used for genetic evaluation in the Nordic countries. Where several measures are evaluated these are combined into a fertility index. The traits differ somewhat between countries. Denmark and Sweden have very similar fertility indexes based on both types of traits, and information is used both from virgin heifers and lactating cows. In Finland only lactating cows are considered, and in Norway the evaluation is based only on non-return rate in heifers.

Table 2. Measures of fertility used for genetic evaluations in the Nordic countries (from Philipsson & Lindhé, 2001) [a]

Country	**Conception rate**		**Interval measure**		**Other**
	Heifers	Cows	Heifers	Cows	
Denmark	NR	NR	FLI	CFI, FLI	-
Finland	-	-	-	DO	Tr, Cull
Norway	NR	-	-	-	-
Sweden	No. AI	No. AI	-	CFI	Tr, Heat

[a] For abbreviations see text

The genetic correlation between different measures of pregnancy and of interval measures is low. For example Roxström et al. (2001a), found a genetic correlation of 0.13 between number of AI's and days from calving to first insemination. The traits are complementary and could therefore be used in a selection index, possibly together with a measure of the ability to show oestrus. The heritability of the ability to show oestrus, measured as a score for heat intensity, is also low (1.5-3.0%) (Roxström et al. 2001b). The trait is important to consider, however, as the ability to show heat is a pre-requisite for the cow to become pregnant. In the same study an unfavourable genetic correlation (r_g=0.36) was found between heat intensity and days to first insemination which means that a shortening of the interval between calving and insemination might lead to impaired heat intensity.

The genetic correlations between the same fertility traits in heifers and first parity cows were only 0.7 in the study by Roxström et al. (2001a), while correlations between cows were higher (Table 1). Therefore measures of fertility should be used both for heifers and for lactating cows. The consequences of genetic correlations being less than 1 between ages were illustrated by Håård et al. (2000), demonstrating considerable re-ranking of bulls between estimated BV's for different parities.

Response to selection

In figure 1 the genetic trends in female fertility for SRB and SLB are shown as average BV's weighted according to the use of bulls. The SRB breed is a Swedish red and white breed with roots in the British Ayrshire and Shorthorn breeds. The SLB breed is a black and white breed with Dutch roots that in the latest four decades has been gradually upgraded with genes from the international Holstein breed (Swedish Holstein). Average milk production varies among countries due to agricultural pricing policies. In Sweden the recorded SRB cows produce on an average 8240 kg energy corrected milk (ECM) and the larger sized SLB cows 8600 kg ECM (Swedish Dairy Association, 2000).

As previously shown the trend is negative for SLB. This can be explained by the Holstein imports. Thus the lack of consideration of daughter fertility in the selection programmes for the North American Holstein breed has caused a deterioration of this trait not only in the US but also in all countries with records on fertility that heavily used this breed. This also illustrates the importance of using the right bulls i.e. those with BV's for daughter fertility as well as for production. The genetic trend in female fertility of SRB is for many years unchanged. The SRB trend verifies that it is possible to avoid a deterioration of the fertility of cow if the trait is properly considered in the breeding programme. The trends in fertility are about the same and unchanged for the different Nordic red breeds, whereas the black and white breeds in the Nordic countries all show a deterioration in fertility.

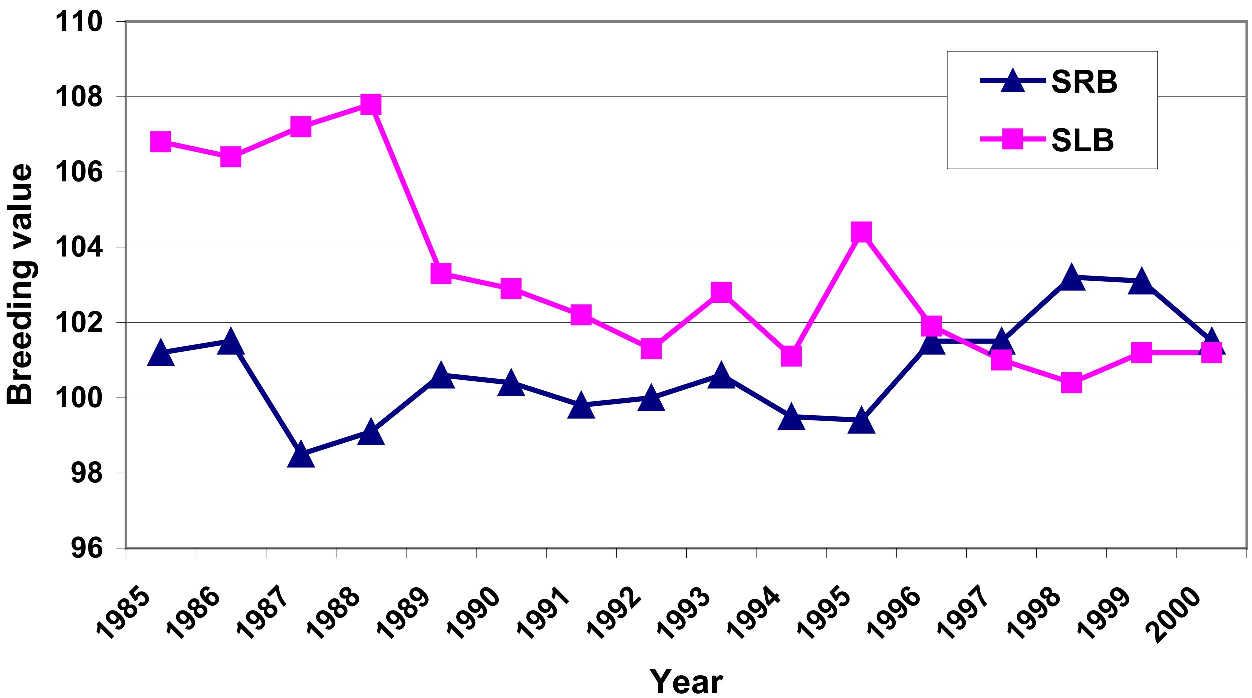

Figure 1. Genetic trends in female fertility of Swedish Red and White breed (SRB) and Swedish Holstein (SLB) (from Lindhé & Philipsson, 2001)

Reproductive disturbances

Reproductive disturbances such as anoestrus, ovarian cysts etc. might increase with short calving intervals and high levels of production (Berglund et al., 1998). Furthermore, Koenen et al. (1994), reported an increase of reproductive disorders in SLB cows with a high proportion of HF genes. A genetic correlation between number of treatments for fertility disturbances and 305 day protein production of 0.16 was found by Roxström et al., (2001a). Although this correlation is weak reproductive disorders could be important to record. The reproductive disturbances are costly (Kossaibati & Esselmont, 1995). The traits probably are of much less value than the other fertility traits for the selection due to their low heritabilities

(Koenen et al., 1994, Roxström et al., 2001a). Nevertheless, the reproductive disorders in first parity cows are included in the Swedish fertility index as an additional information on the reproductive status of the cow.

Possible considerations in future

Early measures of reproductive performance like days to first ovulatory oestrus have higher repeatabilities than the later measures of fertility (e.g. Berglund et al., 1989) and are more independent of management decisions (strategies of insemination, heat detection, culling etc.). An unfavourable association between days to first oestrus and energy balance was shown by Berglund et al., (1989). Recently Veerkamp et al. (2000), demonstrated a genetic correlation of -0.40 to -0.80 between these traits. Veerkamp et al. (1998) also suggested that selection on days to first luteal activity would be possible by analysing progesterone in the monthly milk recording scheme, enabling a ranking of bulls provided a daughter group structure of the bulls. Darwash et al., (1999) suggested that if commencement of luteal activity as an example was desirable by day 21 after calving, three samples within day 17-25 might be enough to rank the bulls. Thus recording of milk progesterone may add information on fertility.

It has been suggested that future breeding programmes should consider a broader range of goal traits especially those that may be possible consequences of metabolic stress. Darwash et al., (1999) suggested that endocrine parameters also may assist in detecting quantitative trait loci for faster genetic gain through marker-assisted selection. The possibility to identify certain genes or QTLs affecting important traits such as reproduction, i.e. functional genomics, is a challenge in the near future.

Along with the more internationalised breeding with dairy cattle there is also a need for an international evaluation for fertility as is performed for milk yield, conformation and now also for mastitis resistance by Interbull. This requires a harmonisation between countries of the routines for recording and genetic evaluation of female fertility traits.

Conclusions

It is important to consider fertility in selection for a high producing cow. Otherwise a further deterioration in fertility can be expected. While fertility has declined in many countries the unchanged genetic trend in female fertility for SRB shows that it is possible to avoid a deterioration in fertility if the trait is properly considered in the breeding programme. Both interval and pregnancy measures are needed in genetic evaluations of fertility. These should be measured at different stages of life i.e. both for heifers and for lactating cows and might be combined into a fertility index in a total merit index selection of important traits. Examples of what future breeding programmes might consider are; consequences of metabolic stress on fertility, more exact measures of the early reproductive performance, possibility to identify genes or QTLs affecting fertility and other important traits, and a harmonisation between countries in the recording and genetic evaluation of the traits.

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The Nordic approach to control of Bovine Viral Diarrhoea Virus infections

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BVDV infections cause huge losses to the cattle production world-wide. In an endemic situation, the prevalence of persistently infected cattle is approximately 1-2% and 60-80 % of the cattle population is antibody positive. No currently used live vaccines against BVDV has proven to be sufficiently safe nor efficient but systematic efforts to eradicate BVDV without vaccination have been implemented, starting in Sweden and Norway in 1993, and in Finland and Denmark in 1994 (Waage et al., 1994, Husu and Kulkas, 1993, Bitsch et al., 1994, Alenius et al., 1997). More schemes are underway in other European countries (Rossmanith and Deinhofer, 1998; Ferrari et al., 1999; Synge et al., 1999).

The reason why none of the Nordic schemes rely on the use of vaccines is that vaccination makes it impossible to use rational screening procedures (e.g. bulk milk sampling) and also because it is regarded as economically unsound – it is a measure that has to be implemented continuously and repeatedly whereas eradication is time-limited and the subsequent monitoring cheap given that the risk of re-infection is kept low. In addition, some vaccines (so called modified live) with adventitious BVDV have in fact been shown to spread the infection (Wensvoort and Terpstra, 1988, Vannier et al., 1988, Kreeft et al., 1990, Løken et al., 1991) and are therefore regarded as a risk for introduction of new, potentially more virulent pestivirus strains.

The Nordic approach relies on a solid knowledge of the epidemiology of the disease – a knowledge which is reflected in the schemes' designs. One example is that emphasis is put on identification of herds without infection, in parallel to finding infected herds. Non-infected herds and their owners are an important target group since the herd incidence of BVDV infection only can be reduced if herds without the infection are protected from achieving it. In addition, free herds form the basis for a more secure livestock trade, which otherwise is the major route for between-herd transmission of the virus. Fortunately, it is very easy to find this group of herds by bulk milk sampling.
Another example is that the strategy for virus clearance of infected herds involves determining the baseline antibody status of all animals, since this will determine the prognosis of the virus clearance programme. Focus is of course on identifying and removing persistently infected (PI) animals, but also on identifying seronegative (female) animals. At the time when herd clearance is initiated, PI animals can be present ”alive” or in the uterus of one or more cows. The only new animals that may get infected (and give birth to a PI) are those who are seronegative at the start of the clearance. Therefore a virus clearance should always be concluded by a follow-up test of such individuals.

Another feature that is prominent in the Nordic approach is that the farmer has to take an active responsibility for the farm's biosecurity. One could say that in order to succeed with a large scale scheme, there must be a persistent change in the attitude to risky behaviour (with respect to introducing BVDV) within the farming community This is because all measures of importance for reducing the risk of introducing BVDV are ”in the hands” of the farmer; e.g. the decision on how to purchase new animals, whether to share pastures, how to look after the fencing, how to provide biosecurity measures for visitors and so on. Therefore, education and information are two crucial elements in large scale BVDV control – so that farmers' become able to make good biosecurity decisions! It should also be noted that information and education are items that have to be iteratively reinforced. New farmers enter the scene, memory is short and therefore the awareness about the infection may decrease otherwise – especially in the final phase of a scheme.

Finally, the Nordic approach to BVDV control is characterised by an active engagement of all (important) stakeholders – not only the farmers and the veterinarians, but also livestock traders, AI personnel, hoof trimmers, milk parlour service staff and any other professional that provides ambulatory services to farmers. It is important that anyone who could potentially transmit the infection to a herd is made aware of that risk and its consequences. However, it should be noted that arguments that motivate a farmer to comply with the scheme may not motivate other groups. For example, a farmer may be motivated by learning about the effects of BVDV infection and the potential economic losses, whereas the livestock trader may be more motivated if he finds that there is a market for BVDV-free animals, if they pay better and if providing BVDV-free animals gives him/her goodwill. It is important to identify what is motivating each important target group and to use this when BVDV information is communicated.

Today, the Nordic countries are in their final phases of BVDV eradication. In Sweden, the prevalence of infected herds have decreased from approximately 50% to 5% in less that 8 years. The reason why the approach has worked is manyfold. We have been able to use cheap and efficient screening tools (eg. for bulk milk antibody) which made it possible to identify free herds and to create a market for controlling traded livestock at a very early stage in the scheme. Also, we were well acquainted with the diagnostics for programme testing and the procedures for virus clearance well before the scheme was launched in 1993. In addition, we have had suitable field organisation which is competent with respect to disease knowledge and large volume sampling. However, one of the most important factors for the progress have been that both farmers and other stakeholders have good knowledge about the disease and its control – a result of an iterative spread of information in order to keep the awareness at a high level.

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Pig health programmes in practise

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Introduction with a survey from the nineteen forties to the beginning of the twenty-first century

In Sweden, pig health programmes were initiated in the beginning of the nineteen forties. Promoters of the first pig health programmes were the then district veterinarian in Mjölby, Harald Sandstedt, and the then director Torsten Sallnäs, ÖAF, Linköping, both working in the province of Östergötland (7).

In the nineteen fifties, under the leadership of the Swedish Agricultural Societies ("Hushållningssällskapen"), Swedish veterinarians had successfully eradicated the brucellosis and the tuberculosis in dairy cattle, and were now prepared for new challenges in the field of preventive veterinary medicine. At that time the production of fattening pigs were established in specialised farms and requirements were raised of a more uniform health status of the weaned pigs which were purchased. Two classical swine diseases, atrophic rhinitis and swine enzootic pneumonia, were considered to be important contributors to disease-associated losses in swine production.

In 1970 and in accordance with a parliament resolution, the Swedish Meat Marketing Society ("Slakteriförbundet") was again appointed to be the responsible organisation (7). At that time the health service programmes were mostly focused on preventing enteric, respiratory and parasitic diseases as well as nutritional disorders among growing young pigs, sows and livestock animals. During the last two decades the health service- and control programmes have also been provided the sheep husbandry and the beef cattle production. The control programmes were now extended aiming to completely eradicate diseases such as the Aujeszky´s disease in pigs (5), Maedi Visna in sheep, John´s disease in cattle and tuberculosis in farmed deer. The eradication of the Aujeszky´s disease was completed in the midst of the nineties and encouraged by this success other control programmes were designed to decrease or completely eradicate other frequently occurring pig diseases such as atrophic rhinitis, swine dysentery, mange etc.

The first of January 2001 the Swedish Animal Health Service organisation was converted into an independent subsidiary company. The co-operative slaughterhouses (Swedish Meats and KLS) as well as the private breeding organisation ("Avelspolen") are now part owners in the new company. The Swedish Board of Agriculture states that the new company will be a more autonomous company providing Swedish pig-, sheep- and beef cattle producers with advisory service aiming to maintain a high health status within the Swedish animal husbandry.

The Swedish District Veterinarian organisation belonging to the Swedish Board of Agriculture has during all these years provided the pig producers with emergency veterinary care. Twenty to thirty years ago all medical treatments of diseased pigs as well as vaccinations of the sows, mostly against erysipelas, were carried out of the responsible district veterinarian or in south of Sweden of private veterinarians. In the last twenty years the district veterinarian has also been able to work with preventive pig health care, often in close collaboration with the Swedish Animal Health Service organisation. The new assignments for the district veterinarians were rendered possible thorough a new announcement (C 15) from the Swedish Agricultural Board in 1979, where the veterinarians were allowed to delegate well defined medical treatments of frequently occurring disease conditions in sows and pigs. The district veterinarian was now obliged to visit the farm monthly. The new assignments enabled the district veterinarian to suggest measures aiming to prevent unsatisfactory management of the sows and pigs. Other preventive measures such as various vaccination programmes facilitate the immunisation of sows against erysipelas, diarrhoea among new-born piglets and reproductive failure among sows caused by parvovirus infection. The "pig herd veterinarian" has got a new task when continuously supervising all these new preventive measures and guarantee that they are carried out in a professional way.

Health programmes in Swedish pig herds – ongoing or recently finished

Ongoing health programmes, such as BIS (Best In Sweden; strict translation), have been designed to improve the general health condition and the welfare among sows and pigs and thereby stimulating the production to be more effective and profitable. Other health control programmes have been designed to eradicate specific infectious diseases. When efforts were made to eradicate the infectious viral disease of Aujeszky´s, these efforts were rewarded with success. Sweden is declared free of the Aujeszky´s disease since 1996 (5). All health control programmes where the goals are to eradicate diseases based on serological tests require laboratory service of high standard. These prerequisites are fulfilled in Sweden.

Control programmes aiming to eradicate a specific disease

Aujeszky´s disease or pseudorabies (PR) has been a threat to pig production in many countries. Specific inactivated vaccines have been developed to control the situation in countries where the disease is endemic. In Sweden, already in the sixties and seventies, boars and sows in all breeding nucleus herds were serologically tested for antibodies. Despite the efforts to control the PR, there was a tendency of spread between herds. In Denmark as well as in the United Kingdom efforts to eradicate the PR were successful. These successes encouraged the Swedish pig industry, the Swedish Agricultural Board, the National Veterinary Institute and the Swedish Animal Health Service to implement a similar eradication program. The eradication program was initially voluntary but the eradication programme was declared compulsory in July 1994. The eradication programme was successfully carried out and in 1996 Sweden were declared free of the Aujeszky´s disease (5). Since 1997 there has been an annual testing of 5000 sows and 1600 boars (5).

Health control programmes aiming to minimise some diseases hazardous to the pig industry

The **salmonella** control was initiated in Sweden in the beginning of the nineteen-sixties. Severe outbreaks in humans in the middle of the nineteen-fifties had drawn attention to the hazardous of the disease. The salmonella control is carried out from slaughtered pigs (lymph-nodes) in the abattoirs as well as from faeces samples collected from sow herds with growing weaned pigs and/or from herds with production of fattening pigs. This work at herd level is often carried out of the responsible "pig-herd-veterinarian". The results from these monitoring programmes show that salmonella is extremely rare in Swedish pork. The annual number of salmonella infected pig herds has been less than 5 during the last ten years (8).

Progressive **atrophic rhinitis** (PAR) caused by toxigenic *Pasteurella mutocida* has to some extent been spread in pig herds in Sweden. Vigorous efforts have been carried out to eliminate

PMT carriers among gilts and boars delivered from nucleus herds. Clinical outbreaks of PAR seem to be minimised, especially in pig-herds in the middle of Sweden.

The **sarcoptic mange** (*Sarcoptes scabie* var. *suis*) is unquestionable a welfare problem among pigs worldwide. The most significant economic effect of mange is reduced growth rate and feed utilization (6). Ivermectin and doramectin are modern chemicals acting as powerful tools when we are trying to control mange or even trying to eradicate the parasite. Efforts are now carried out in Sweden aiming to eradicate the sarcoptic mange, at least in a longer perspective.

Implementation of general health programmes

From the beginning the pig health programmes focused on health problems associated with the production of weaned pigs and the demands for healthier weaned pigs for the specialised fattening-pig-units (4). Complaints about the specialised production system for fattening pigs were discussed during the eighties in different medial forums. In 1987 health programmes were also implemented in the production of fattening pigs and improvements have been achieved. The later health programme was based on records from the inspection of the carcasses and the viscera of the slaughtered pigs at the abattoirs. The records indicate which diseases or other bad conditions that are most prevalent in a herd. Different preventive measures have been taken to improve the condition for this category of production.

In the "BIS-programme" (Best In Sweden) for sow herds with production of weaning pigs, production as well as economical efficiency have been highlighted. In the fattening pig production these parameters have been merged together with the prevalences of different disease lesions recorded at gross examination at slaughter.

Many investigations have been performed in Swedish pig herds with the objectives to increase the general health of a specific age category of pigs or category of sows. As Swedish pig producers banned the use of feed additive in 1986, criticised by the consumers, efforts have been carried out to analyse risk factors for the most common health problems associated with a limitation of feed additive, particular the higher risk for post weaning diarrhoea (PWD) (2). Investigations in pig production have also been focused on disorders such as mammary gland diseases among sows (3), claw and leg injuries in group-housed dry sows (1) etc. Put together, all the results obtained from these and other investigations carried out during the years, are the platform for the spreading of knowledge to practising veterinarians, counsellors but above all to our pig producers.

Summary

Health programmes aiming to improve the general health conditions among pigs, or in particular specific programmes aiming to eradicate specific infectious diseases among pigs must be based on a co-operation between animal health organisations and the board of agriculture. Other important prerequisites are solid financial support, laboratory service and last but not least acceptance and knowledge about the specific programmes among our farmers and swine-producers.

Acknowledgement

Professor Martin Wierup, Head of the Swedish Animal Health Service, for kindly having supported me with necessary information about different aspects on health care service for all breeders of pigs. The aim of the organisation is “Sound and competitive production of healthy animals”, prestige words reflecting the Swedish awareness of animal welfare and ethics as well as awareness of the need to support the Swedish pig breeders with relevant and updated knowledge.

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The PRRS-threat to pig production

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Introduction

The Porcine Reproductive and Respiratory Syndrome (PRRS), also called Blue Ear Disease or Mystery Swine Disease was first described in the USA 1987 and in Europe 1990. The disease is spread world-wide: the US, Canada and several European as well as Asian countries. However, no cases have been reported from Estonia, Sweden and Australia.

So, why is this disease such a threat to pig production world wide? Firstly, it is very infectious, is maintained in a herd or an individual for a long time and it affects all age groups of pigs. Secondly, it predispose for severe secondary infections at it is somewhat difficult to vaccinate efficiently against.

The virus, symptoms and pathology

The PRRS virus is a RNA-virus that mainly infects macrophages and monocytes. The virus is very infectious, 20 – 40 virions is an infectious dose, and has a quick replication cycle. With current methodology it is hard to detect the virus in specimen. Further, infected animals shed virus for a few weeks up to several months, it has also been suggested that some pigs are chronic carriers. Finally, the virus is genetically divers, in contrast to parvovirus for instance, and prone to change.

The clinical symptoms occur 2-4 days after infection and there is a large variation in signs of disease between age-groups. Most common are reproductive and respiratory affection and inappetence: Stillborns, abortions, weakborns and preweaning mortality has been reported in 100% of affected herds, inappetence and fever in approximately 50% of affected herds. The “blue ear”, cynosis, seems to be quite common on a herd-basis, about 70% has been reported, however, very few individuals seem to be affected (1-5%). The duration of symptoms varies considerable between herds and secondary infections are common.

Gross pathology lesions are typically subtle and non-specific, or not present at all, in adults. In addition, there are few consistent findings associated with reproductive failure. In growing pigs interstitial pneumonia is the most common finding, the severity depends on secondary infections. Histopathology may reveal an interstitial pneumonia, with mononuclear cell infiltrate and intact epithelium.

Immunology and epidemiology

The immune response to the virus is characterized by a rapid antibody response, even though it takes 4-6 weeks before neutralizing antibodies are detected. Unfortunately there is limited cross-reaction between sera for different strains of the virus. Hence, immunity to one strain doesn’t necessarily protection against infection with another strain. There are also reports that the virus is “immune suppressive”, likely due to the fact that it infects macrophages and monocytes.

An epidemic of PRRS in a herd could be a short or long term history and the severity of clinical symptoms seem to vary by viral strain. It seems that only seropositive animals shed virus and that the virus is spread by pig to pig contact (oro-nasal secretions). Also the use of contaminated has been reported to spread the disease. Finally, wildlife has been suspected to transmit the disease from one herd to another.

Diagnosis and prevention

The diagnosis should be based on the typical clinical signs and disease history described above, followed by postmortem examination and serological analysis (by Elisa). Virus isolation may be difficult to perform.

The key element in the prevention is to hinder contact between herds and to apply an “all in, all out” management. Vaccination programmes are unfortunately not that efficient. In conclusion, the disease is difficult to eradicate or prevent if established in a region. Thus, due to it the great losses it causes the PRRS is a considerable threat to pig production

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The Panorama of Diseases and Differences in Fur Thickness of Tied Dairy Cows According to the Zonal Distribution of Microclimate

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The productivity, health and welfare of cows is considerably influenced by the microclimate of the cowshed in which they live. Rapid temperature changes lessen milk output more than a low mean temperature of the environment (Anderson, 1997). Such a situation takes place in tied housing insulated cowsheds during feeding and manure removal in winter, when these procedures will be carried out using a tractor, and the doors should be opened and closed periodically (Aland *et al*., 2001).

The microclimate in the cowshed influences the thermoregulation of cows, which use behavioral, physical and chemical mechanisms for keeping heat flux between the environment and their bodies in order to maintain a normal body core temperature. If the environmental temperature is low for a longer period the cow's organism will adapt to the lower temperatures by forming a thicker fur layer. If the temperature falls below the critical temperature, cows may become sick.

To date, the zonal differences of microclimate within the cowsheds and the welfare of cows have been studied separately without quantitative analysis of their interactions (Vutt, Nõmm, 1983). The relationship between fur thickness and the location of cow within the cowshed should characterize the adaptation of the animal to the zonality of microclimate during the cold period of the year. If the zonality of the microclimate becomes a stressor it should be reflected in the panorama of diseases. Thus fur thickness and the panorama of diseases could be used as criteria for evaluation of the adaptation and welfare of cows.

The aim of the current study was to explore the influence of cows' location within the cowshed to fur thickness and the panorama of diseases.

Method

The data were collected from an insulated cowshed for 300 animals (Fig. 1a) in the following manner:

1. The microclimate parameters and their distribution within the cowshed were registered in October, January and March. For measurements of temperature, humidity, air speed and ammonia concentration the Testo 615 thermo-hygrometer, thermo-anemometer Testo 425 and gas analyser DrägerPac III were used.
2. During the coldest period (in January) a monitoring of temperature and humidity was carried out to estimate the influence of opening doors during manure removal. For these purposes the parameters of the microclimate were registered from 6 points (door, A1, A8, A12, A15 and A38) at a height of 0.7 m.
3. During the indoor period all of cows' disease incidences were registered. For the evaluation of udder health status, the somatic cell count of milk (SCC) was also used.

4. Fur thickness was measured in 36 cows by removing hair from a roughly 4-cm^2 area behind the scapula. The mass of the given hair probes was estimated by an analytical scales and the partial mass of hair for one cm^2 was calculated. This parameter was used as the hair thickness index during analysis.
5. The location of every cow within the cowshed was registered. During analysis, the places of cows in different rows but on the same distance from the doors (leading to the open manure storage) was equalised and entered on a conditional axis (Fig. 1b).

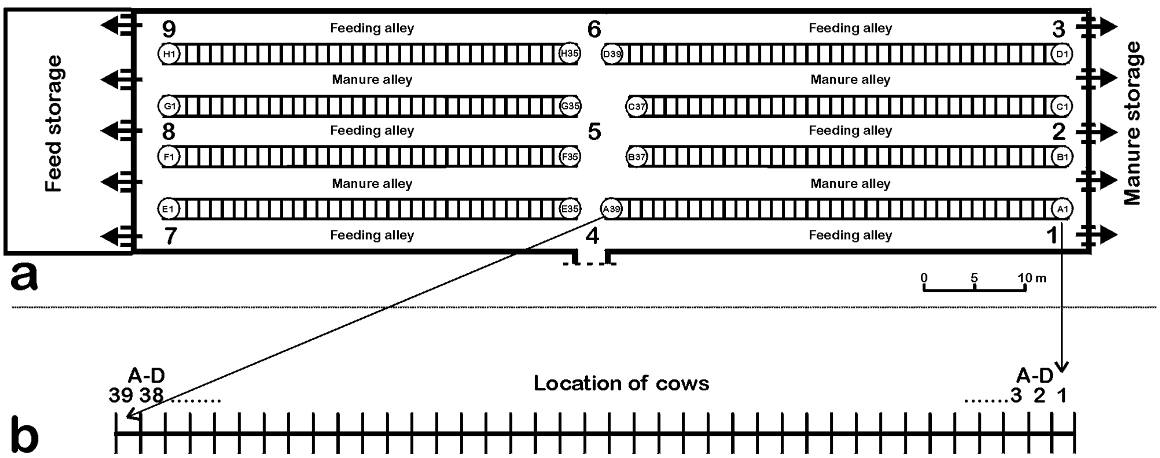


Figure 1. a – layout of the cowshed, the location of cows (A1-H35) and the measurement points of the microclimate parameters (1-9); b – the conditional axis for the cows' location at the right side of the cowshed (next to the manure storage)

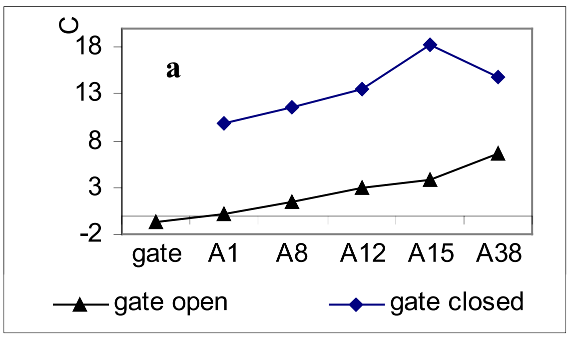
For the analysis of fur thickness, the following groups of cows were formed on the conditional axis: 1 – A1D1; 2 – A5D5; 3 – A10D10; 4 – A15D15; 5 – A20D20; 6 – A25D25; 7 – A30D30; 8 – A35D35; 9 – A39, B37, C37, D39. For the SCC analysis the cows were grouped to the conditional axis in the following manner: group 1 – A1D6; group 2 – A7D12 etc.; group 6 – A31D39.

Results

The values and distribution of the microclimate parameters concerning the whole cowshed is given in table 1. The temperature within the cowshed was quite high during the whole indoor period. The air temperature on the right side of the cowshed was lower than on the opposite side. This was determined by the existence of a closed feed storage at the end of left side, which lessened the influence of outdoor temperatures. There were greater zonal differences at the right side of the cowshed (Tab. 1, Fig. 1a). The humidity of the air reached an undesired level at the centre of the cowshed more frequently than in other parts. There were no considerable zonal differences in air movement in most parts of the cowshed. The ammonia concentration remained at a normal level (up to 6 ppm) in all parts of the cowshed. The opening of doors during manure removal had a considerable influence on zonal differences in temperature and air movement (Fig. 2).

Table 1. The microclimate parameters of the cowshed during the indoor period

Parameter	Month	Points of measurement									Average
		1	2	3	4	5	6	7	8	9	
Temperature ºC	November	16,8	15,0	12,4	18,3	19,0	19,1	19,1	17,3		17,1
	January	15,7	15,7	15,8	17,2	17,5	17,6	16,6	16,5		16,6
	April	16,8	17,8	17,0	20,4	20,5	20,9	20,4	20,9	20,9	19,5
Relative humidity %	November	73,5	74,3	81,3	90,0	83,2	83,5	87,2	83,4		82,1
	January	83,6	82,7	83,0	90,6	85,5	82,7	80,0	75,1		82,9
	April	77,5	76,5	76,7	72,9	76,5	72,2	77,1	79,3	76,3	76,1
Air speed m/s	November	0,1	0,1	0,3	0,1	0,1	0,2	0,2	0,4		0,2
	January	0,2	0,1	0,1	0,0	0,0	0,0	0,1	0,1		0,1
	April	0,3	0,1	0,2	0,4	0,3	0,5	0,2	0,1	0,1	0,2



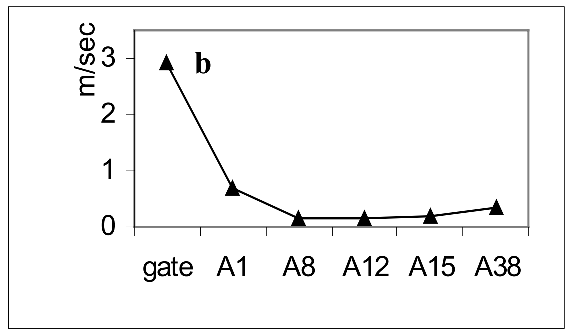
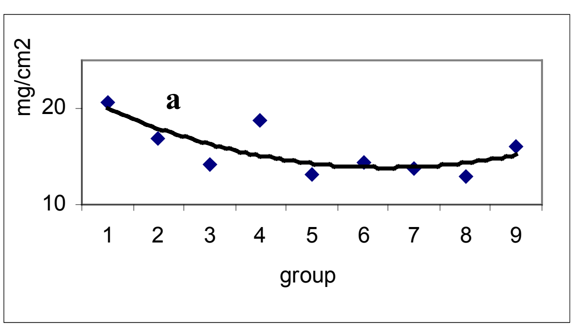


Figure 2. The influence of opening the doors to temperature (a) and air movement (b) at the right side of the cowshed

The fur layer was thicker on cows situated closer to the doors (Fig. 3a). The relationship between thickness of fur layer and location of cows within the cowshed refers to adaptation to lower temperatures. The SCC of milk (which reflects udder health) appeared to be higher for those cows located father from the gates leading to the manure storage (Fig. 3b). The worse udder health of cows in the central part of the cowshed might be explained by better conditions for the development of mastitis pathogens there.



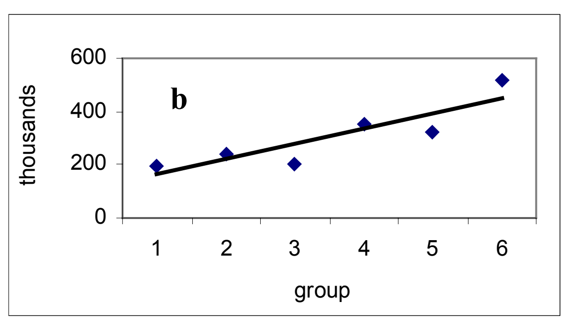


Figure 3. The influence of zonality on fur thickness (a) and SCC (b)

The frequency of diseases is presented in table 2. Of these diseases, during the indoor period endometritis and retained placenta were diagnosed more frequently on the right side of the cowshed. Cases of mastitis and teat disorders were more frequent in the centre of the cowshed. Other diseases mentioned in table 2, were distributed equally throughout the cowshed. The large number of calcium metabolic disorders also includes the prophylactic injections of Ca after calving.

Table 2. Incidences of diseases during the indoor period

Diseases	Number of incidences	Percentage
Endometritis	16	5.5
Dystocia	7	2.4
Retained placenta	22	7.6
Parturient paresis	22	7.6
Ca metabolic disorders	159	54.8
Mastitis	42	14.5
Teat disorders	2	0.7
Other udder diseases	12	4.1
Limb disorders	2	0.7
Other diseases	8	2.8
Total	292	

Conclusions

There are considerable zonal differences in tied housing insulated cowshed with mobile technology for feeding and manure removal, which influence the fur thickness and health of cows. The adaptation of cows to colder zones is reflected by their thicker fur layer. The extensive fluctuations of microclimate near doors seem not to influence the udder health of cows in winter. The relationship between the location of cows and the panorama of diseases needs to be investigated further.

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Changes in the Structure of the Bovine Pelvis, Udder and its Suspensory Apparatus

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Abstract
In order to study developmental changes, the pelvic structures of the cows from the Middle Ages, the beginning of the 20th century, and the end of the 20th century were compared. Increased body mass, including the mass of the organs of the abdominal cavity and the udder, has brought about changes in the pelvic structure. From the point of view of delivery, a number of undesirable changes have occurred.

Estonian Holstein cows (EHF) have well-developed udders, with symmetric quarters. About 60% of the udder base is attached to the ventral abdominal wall, and 40% is attached to the pelvic floor by means of the symphysial tendon. By comparison with the previous data, the distance between the fore and hind teats has increased, and the distance between the hind teats has decreased. When looking from aside, in the majority of cows the hind teats are covered by the shin. On the basis of their location it is expedient to divide the principal components of the suspensory apparatus of the udder, the lateral and medial laminae, into the abdominal and pelvic parts. In functional terms, the abdominal part of the medial laminae and the pelvic part of the lateral laminae are more important. The role of the pelvis in the suspension of the udder has increased considerably. In the contemporary EHF cow the udder is attached to the pelvis by means of a strong, plate-like symphysial tendon. The abdominal part of the medial laminae has been supplemented by the connective tissue funiculus, located on the median line, connective tissue plates, and the root of bands above the udder's centre of gravity.

Selection of cows for the backward-sloping pelvis in the future would result in changes in the pelvis position and structure that are favourable for delivery as well as in the attachment of the suspensory apparatus.

Introduction
Improved feeding and keeping conditions and successful breeding activity have increased the milk production per cow. This has been accompanied also by the increase of body size and mass of the cow.

Over the past fifty years milk production per cow has increased especially rapidly. In the year 2000 the average milkyield per cow amounted to 4,960 kg in Estonia. In better EHF farms the production amounts to 7,000–8,000 kg, the average body mass of cows is 600–700 kg, and the withers height reaches 140–145 cm. The proportion of the EHF breed has witnessed a constant increase in Estonia – from 31,8% in 1964 to 70,3 % in 2000 (Pentjärv, 2001).

Although the breeding activity has been successful, it has brought along some negative side effects as well. The breeding of high-production bovine breeds with a large body mass has made their pelvic structure less suitable for delivery, thus aggravating the course of the delivery and contributing to the genesis of a number of complications (Nickel *et al.*, 1984).

One might assume that as the milk production (udder mass) has increased, so has the structure of the suspensory apparatus changed. The increasing use of udder supporters points to the fact that the suspensory apparatus has not strengthened along with the increase in the udder mass.

The aims of the present work were:
1) to reveal the developmental changes in the structure of the bovine pelvis; 2) to determine the positioning of the udder and the teats in the EHF cows; 3) to study the structure of the suspensory apparatus of the udder in high-production EHF cows; 4) to suggest on the basis of the research results such selection criteria for the breeding that will make the bovine pelvis more suitable for delivery and strengthen the suspensory apparatus of the udder along with an increase in milk production.

Materials and methods

Our studies were carried out in the herd of the joint-stock company "Estonia" (2,000 EHF cows, average milkyield 7,500 kg, average body mass 640 kg and withers height 143 cm).

For determination of the developmental changes in the bovine pelvis, the bones from the Middle Ages found in Estonia during archeological excavations, two aboriginal bovine skeletons and three pelvises from the beginning of the 20^{th} century were used. Pelvimetric studies were conducted also on 9 primiparous and 11 multiparous cows. To obtain general data about the exterior of the udder, a group of 50 milking cows in the second to fourth lactation and second to third lactation months was selected. From 4 to 5 h after milking the following measurements were taken: udder height from the base of the teats at the hind legs standing side by side on a horizontal surface, the greatest diagonal length and circumference of the udder, the location of the front and rear teats in relation to the horizontal surface and vertebrae, and the length of the teats and the distance between them. The suspensory apparatus of the bovine udder was examined in 3 heifers 1,5-2 years old and 23 adult cows culled for various reasons.

Results

In medieval cows the pelvis-forming hipbones are slender, have thin walls and smooth surfaces. The attachment points of the muscles, marked by rough surfaces, were almost absent, which points to the fact that the musculature was underdeveloped. The ischial spines were short, thin, and low, and they were pointed dorsally in the sagittal plane. The pelvic inlet and outlet were oval and the symphysis pelvis remained unossified until an advanced age. The pelvic floor (and thus the birth canal) had an almost direct course. Since the symphysial eminence and the symphysial crest, allowing the udder to be attached to the pelvis, had not developed, then it proves that the udder was attached only to the abdominal wall in the region of the posterior abdomen (abdominal udder). The modest development of the udder is supported by the data about the low milk-production of cows.
The pelvises of the **cows that lived at the beginning of the 20^{th} century** were quite similar to the pelvises of medieval cows. However, one should point out one difference – the hipbones have more signs of attachment points of the muscles, the symphysial eminence is better-developed, and there is a shallow depression in the pelvic floor in the caudal region of obturator foramina. Their pelvic inlet and outlet had an oval shape, and the ischial spines were low. From the point of view of delivery such a pelvis could be regarded as satisfactory.

The pelvic structure of **present-day high-producing cows** has witnessed important changes. The hipbones have developed thick walls and have become massive. Their outer surface reveals numerous tubercles,tuberosities, and crests, marking the detachment points of the muscles. All this points to a well-developed musculature. The ischial spines have thick walls and are high, and their medial part is arched towards the pelvic cavity. The pelvic floor proceeds horizontally as far as the caudal part of the obturator foramina and then rises in the caudodorsal direction, thus changing abruptly the previously direct course of the birth canal.

In EHF cows the **udder** is well developed and has symmetrical quarters. In the majority of the cows (66%) the tips of teats lie on the same horizontal line, or the tips of the hind teats are up to 2 cm lower (34%). The horizontal line passes through the level of the tip of the tuber of the calcaneus or is a few centimetres higher. On average the udder height is 31±7 cm from the base of fore teats and 35±7 cm from the base of the rear teats. The greatest diagonal length of the udder is 49±6 cm and the circumference is 146±19 cm. The average length of the fore teats was 6.2 cm and the distance between the tips of the teats was 14.7 cm. The length of the rear teats was 5 cm, and the distance between the tips of the teats was 4.5 cm. The distance between the fore and rear teats was 11 cm. Thus, the distance between the tips of the rear teats was three times shorter than in the case of the fore teats. It is caused by the fact that the hind quarters of the udder are pressed between the shins, and the axis of the rear teats is directed ventromedianly downward, especially in the cows having the first or second lactational periods. In some cows the tips of the rear teats are even in contact, which makes the positioning of milk cups more inconvenient. Comparison of our results with the earlier data (Kurm, 1981) shows that the distance between the fore and rear teats has increased, and the distance between the rear teats has decreased. According to Kurm (1981), the distance between the rear teats was twice as short as that of the fore teats, and the distance between the fore and rear teats was 7–8 cm.

Taking the prepubic tendon as the line in EHF cows 60% of the udder is attached to the ventral abdominal wall and 40% is attached to the pelvic floor by means of the symphysial tendon.

The main components of the **suspensory apparatus** of the udder are the lateral and medial laminae, equipped with suspensory lamellae, that surround the udder halves. The lateral laminae cover the udder from the outside, and the medial laminae are located on the medial sides of both halves of the udder. These laminae were divided on the basis of their position into the abdominal parts and the pelvic parts. The lateral and medial laminae of the suspensory apparatus are associated with each other on the cranial and caudal surfaces of the udder. The udder base is connected to the ventral abdominal wall by means of the connective tissue plate that lies above the fore quarters. Thus, both udder halves are surrounded by a protective connective-tissue capsule *(capsula uberis)*, which is closely related to the glandular tissue by means of the suspensory lamellae.

In functional terms, the more important parts are the abdominal parts of the medial laminae and the pelvic parts of the lateral laminae (Jalakas *et al.*, 2000).

In comparison with the studies conducted sixty years ago (Swett et al., 1942), the role of the pelvis in the suspension of the udder has increased considerably. In the EHF cow the symphysial tendon is strong, plate-shaped, with a thickness of 3.5 mm at the attachment point, and is attached to the 1–2.5 cm-high symphysial crest that has developed on the symphysis pelvis. The abdominal part of the medial laminae has been supplemented as well. The additions include the connective tissue funiculus on the median line of the abdominal tunica flava, the connective tissue plates, and the root of bands above the udder's centre of gravity. In the EHF cows the udder's centre of gravity is located ventrally on the prepubic tendon.

<u>Discussion and the future development of the bovine pelvis, udder, and its suspensory apparatus</u>

For a long time the breeders have preferred animals with straight backline and a horizontal pelvis and sacrum. The pelvis has become more massive because the weight of the udder, the organs of the abdominal cavity, and the muscles have increased. In the adult cow the entire symphysis pelvis is ossified, and the pubic spine that supports the prepubic tendon and the symphysial crest have appeared. Since the angle between the ischial plates *(angulus intertabulares)* has decreased and the ischial spines have elongated, the bony birth canal has become tunnel-shaped. The height of the caudal pelvic aperture has decreased. By preferring the cows with horizontal pelvises, the breeder unintentionally prefers cows with a smaller dorsally open angle *(angulus soli pelvis)* on the median line of the pelvic floor above the symphysial eminence. As a result, the bovine pelvic axis has broken above the caudal edge of the obturator foramina, and it proceeds diagonally upwards from there as far as the ischial arch. The caudodorsally ascending birth canal makes the bovine delivery much more difficult than in other domestic animals.

In the case of the possible future changes we will proceed from two premises – the cow will be bred with the higher milk production in mind and the breeder will prefer cows with a backward-slating pelvis. Then the bovine pelvis will become more massive and the pelvic floor will be either horizontal or slightly backward-inclined as far as the caudal edge of the obturator foramina. The pelvic floor will take a straighter course and the height of the caudal pelvic aperture will further increase, which will facilitate the delivery. The symphysial crest is strengthened, and it will also develop on the symphysis pelvis formed by the ischial plates.

The symphysial tendon become plate-like in the region of pelvic symphysis that is formed by the ischial plates and the udder base will elongate backwards. The rear teats will shift to the caudal line of the shin or backwards from it. The distance between the rear teats as well as between the fore and rear teats will increase, the tips of the teats will rise higher in relation to the horizontal plane.

The changes in the udder's structure and position call for some changes in the structure of the milking machines. In the case of this kind of udder it is easier to attach the milk cups from behind, therefore parallel milking parlours will be preferred.

We can conclude, that using approriate selection criteria in dairy cattle breeding could induce the changes in pelvic structure more suitable for delivery and the udder's suspensory apparatus will strengthen along with the increased milk production.

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Kokkuvõte

Muutused lehma vaagna, udara ja selle kandeaparaadi ehituses.

Vaagna arengulooliste muutuste uurimiseks võrreldi keskaja, XX sajandi alguse ja kaasaja lehma vaagnate ehitust. Suurenenud kehamass, sealhulgas just kõhuõõne organite ja udara mass, on põhjustanud muutusi vaagna ehituses. Sünnituse seisukohalt on kaasnenud mitmed ebasoovitavad muutused. Udar on eesti holsteini tõugu (EHF) lehmadel hästi arenenud, sümmeetriliste veeranditega. Udarabaasist 60% kinnitub ventraalsele kõhuseinale ja 40% sümfüsiaalkõõluse abil vaagnapõhjale. Udara raskuskese paikneb ventraalselt süleluude-eessele kõõlusele. Võrreldes varasemate andmetega, on suurenenud eesmiste ja tagumiste nisade vahekaugus ning vähenenud tagumiste nisade vahekaugus. Küljelt vaadates jäävad enamus lehmadel tagumised nisad sääre varju. Udara kandeaparaadi põhilised koostisosad, lateraalsed ja mediaalsed lestmed on otstarbekas paiknevuse alusel jagada kõhu- ja vaagnaosadeks. Funktsionaalselt on olulisemad mediaalsete lestmete kõhuosa ja lateraalsete lestmete vaagnaosa. Vaagna osatähtsus udara kandes on märgatavalt suurenenud. Kaasaja EHF lehmal kinnitub udar vaagnale tugeva, plaadikujulise sümfüsiaalkõõluse vahendusel. Mediaallestmete kõhuosa on täienenud mediaanjoonel kulgeva sidekoeväädi, sidekoeplaatide ning sidemetejuurega udara raskuskeskme kohal.

Kui tulevikus veise valikul eelistada tahapoole luipa laudjat, siis vaagna asendis ja ehituses tekkivad muutused on soodsad nii sünnituse kui ka udara kandeaparaadi kinnitumise seisukohalt.

The Changes in BVDV Situation in Estonia Through the Years 1993-2000

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Summary
The BVD situation has been monitored in Estonia since 1993 through serological testing of cattle herds. The changes in the spread of the BVDV infection observed during these years are natural as there is no control program for BVD in Estonia.

The LPB ELISA from Danish Veterinary Institute for Virus Research was used as a diagnostic test.

During the first study (1993-1995), a total of 315 randomly selected cattle herds from all over the country were tested serologically for BVDV infection. The blood samples of a 'random spot sample' of 10 cows and 10 young animals (6-24 months of age) from each herd (189 herds) or bulk milk samples (178 herds) were taken for serological testing.

In the second study period (1997-1998) pooled milk samples from 336 herds were tested for BVDV antibodies. In 1999 228 herds and in 2000 244 herds were tested from bulk milk samples for BVDV infection.

The results of these surveys demonstrate the remarkable reduction in the spread of BVDV and in the prevalence of acute BVDV infection (the PI-herds) in Estonia during the years 1995-1999. However, the results of the year 2000 study, show increase in the proportion of herds having acute spread of the BVDV (see Figure 1). This might be a sign of a re-emerging epidemic of BVD among Estonian cattle.

The mane factors limiting the spread of BVDV in Estonia, which have made possible the self clearance of the herds from the BVDV infection are the following:

1. The high prevalence of the infection in the beginning of 1990's resulted with the development of high herd immunity to BVDV.
2. The cattle herds are in most cases managed as closed herds. Large cowsheds are mostly situated several kilometers apart from each other.
3. Animals introduced from other herds have always been brought directly from the herd of origin as there are no animal markets in Estonia, and animal collecting points are not used for the within-country live animal trade.
4. The large farms have bought animals only for breeding reasons, which means that the number of animals moving from one herd to another has never been very high.
5. For economic reasons, the trade in animals has been extremely low in recent years.
6. Due to the low price of beef on Estonian market, the male calves, which do not have breeding value, are slaughtered soon after birth, thus eliminating about half of PI-calves born in the herd.

However, there are also management practices present in Estonia favoring the long term persistence of the BVDV infection in the farm. Most farms in Estonia, which have more than 100 cows raise young stock in separate buildings from the cows. In PI-herds, where cattle are kept in many different units the herd immunity level in these units may be remarkably different. This means, that there are always susceptible animals in some part of the farm and favourable conditions exist for foetal infection and production of new PI-animals. In Estonia, herds having more than 400 cows normally house animals in several units. Our data show that the "self clearance rate" is lowest among these herds.

In the somewhat specific conditions in Estonia, the infection has appeared to be self-limiting (at least to some extent) on the level of national cattle population. This gives us the opportunity to begin the eradication of the disease from a very favourable situation. On the other hand, if control measures are not implemented a new epidemic of BVDV is highly likely to occur.

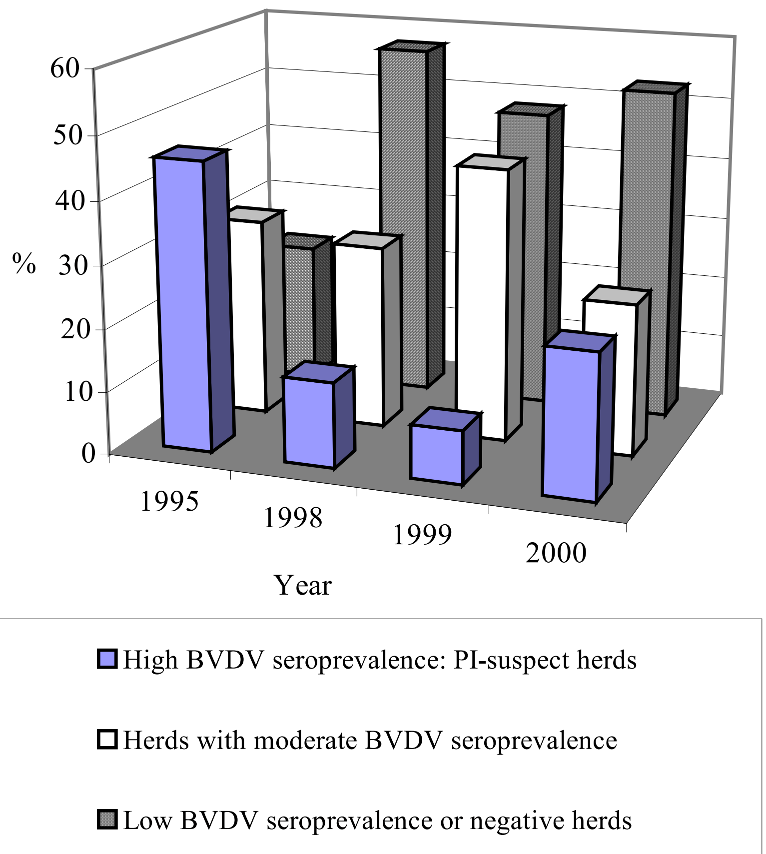

Figure 1. Changes in BVDV situation in Estonia during 1995-2000 according to the results of serological investigations

Investigations on Body Condition Scores in Estonian Primiparous Dairy Cows

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Introduction

Over the whole production and reproduction cycle of the dairy cow, energy demand and supply are in balance. Dairy cows go into negative energy balance (NEB) after parturition because a marked increase of their energy requirements, primarily due to high milk production, is not always balanced by feed intake. Therefore, cows mobilize their body tissue, via lipolysis and proteolysis and lose weight. About 10 weeks after calving high-producing cows possess a negative energy balance and their highest deficiency occurs during the first weeks. The amount of body reserves a cow has at calving has strong influence on potential complications at or immediately after calving, milk production and reproductive efficiency for the upcoming lactation (Wattiaux,1996). Cows with excess body weight or overcondition at calving are likely to have lower feed intake postpartum than cows that have normal body condition. Overconditioned high producing dairy cows inevitably go into a more severe NEB postpartum than do cows that have normal appetite (Rukkwamsuk,1999). Severe negative energy balance induces changes in metabolic pathways that are responsible for production, maintenance of health, and reproduction of the postpartum dairy cows. Thus the goal is to have cows in "good" condition at calving – not too thin and not too fat. Among other indicators of the metabolic status of dairy cows, body reserves of cows expressed by body condition score (BCS) and BCS changes have been used to monitor the amount and mobilisation of body adipose tissue. Body condition scoring is a subjective method of indicating the amount of body fat cover or the amount of stored energy the cow carries.

The system of body condition scoring commonly used for dairy cows today was devised by E.E. Wildman. The method of Wildman is based on palpating relevant parts of the back and rump of the cow, to determine the amount of fat tissue covering these areas. The systems of Edmonson and Ferguson visually appraise the same relevant parts. Cows are ranked on a scale from 1 to 5. A cow with a condition score 1 is considered emaciated, 2 is thin, 3 is average, 4 is fat, and 5 obese. Often this scale will be divided into quarter point increments (Ferguson,1996). For every whole unit loss in BCS, about 56 kg of body tissue are lost in mature Holstein cow (Otto et al.,1991).

The risk of post parturient problems may be avoided when dry cows score a 3.25 to 3.50. Body condition loss in early lactation should be less than 1 unit, thus early lactation cows should be above a 2.50 in condition. Body condition loss should be maximal by 4 to 6 weeks (Ferguson,1996).

Because of the subjective nature of body condition scoring, our first interest was to investigate the repeatability of body condition scores given by different observers.

Material and methods

(Study 1). Repeatability of the BC scoring of different Estonian breeds was examined. Body condition was scored on the same farm on 40 Estonian Holstein, 24 Estonian Red, 20 Red Holstein and 4 Estonian Native breed pregnant heifers and primiparous cows every two weeks during the 10 week period. Each time all the animals got two scores: one by an evaluator, who used Edmonson´s evaluating chart (score A) and the other by a pair of evaluators (score B) using Ferguson´s decision chart for BCS. The evaluators had some preliminary experience in body condition scoring, they had evaluated BCS on different farms, but had not been trained together.

(Study 2).The BCS dynamics of different breeds before and after parturition was investigated. BCS was evaluated by one observer every two weeks using Edmonson´s BCS chart. 24 Estonian Red, 20 Red Holstein and 40 Holstein primiparous cows were evaluated before and after parturition. 40 Holstein Cows were divided into two groups according to their genetic merit index.

(Study 3).The relationship of BCS with body weight change of 42 Estonian Holstein and 24 Estonian Red pregnant heifers and primiparous cows was analyzed. The animals were BC scored using Edmonson´s BCS chart every fortnight and weighed once a month starting from calving to 22 weeks post-partum.

Results and discussion

Examining the repeatability of the body condition scoring between the observers we found that observers agreed (r=0,88) with the absolute score 37,7% of all the cases, deviating by 0,25 units in 45,3% of the cases. Thus 83% of the scores deviated by either zero or a quarter point unit. The results confirmed the reliability of the method. The best coincidence of the scores was between the values from 2,0 to 3,5 BC units (Figure 1).

The average BCS before parturition was 4,0 for RHF group, 3,9 for ER and 3,7 for both Holstein groups (Figure 2). After parturition the BCS started to decline. The recession continued up to the 12-14th week during the postpartum period. The BCS loss was greatest in the EH- top group and EH-medium group, where the BCS declined up to 2,7 and 2,75 units respectively. In the RHF group the BCS declined up to 3,2 units and in the ER group up to 3,1 units. In conclusion the BCS loss was greatest in both Holstein groups where the body condition loss was up to one unit. In the ER and RHF groups the BCS loss was 0,8 units.

Regression analyses revealed significant correlation ($p<0,05$) between body weight change and BCS change in the breeds during the period of postparturient BCS decline. According to our preliminary data 1 BCS point change corresponds to 72 kg change in Estonian Holstein and 52 kg in Estonian Red postparturient cows. In both breeds 1 BCS point change corresponds to 12% of BW change.

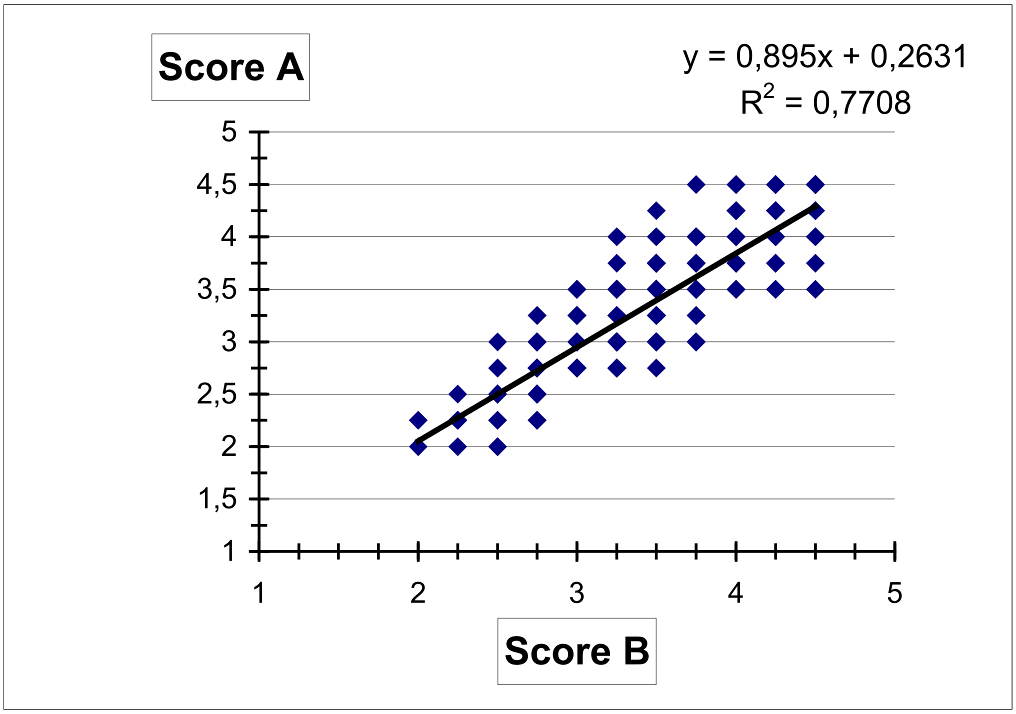

Figure 1. Comparison of the BCS results scored by different observers. Score A: J.D. Fergusons BCS system (2 observers), Score B: A.D. Edmonson´s BCS system (1 observer).

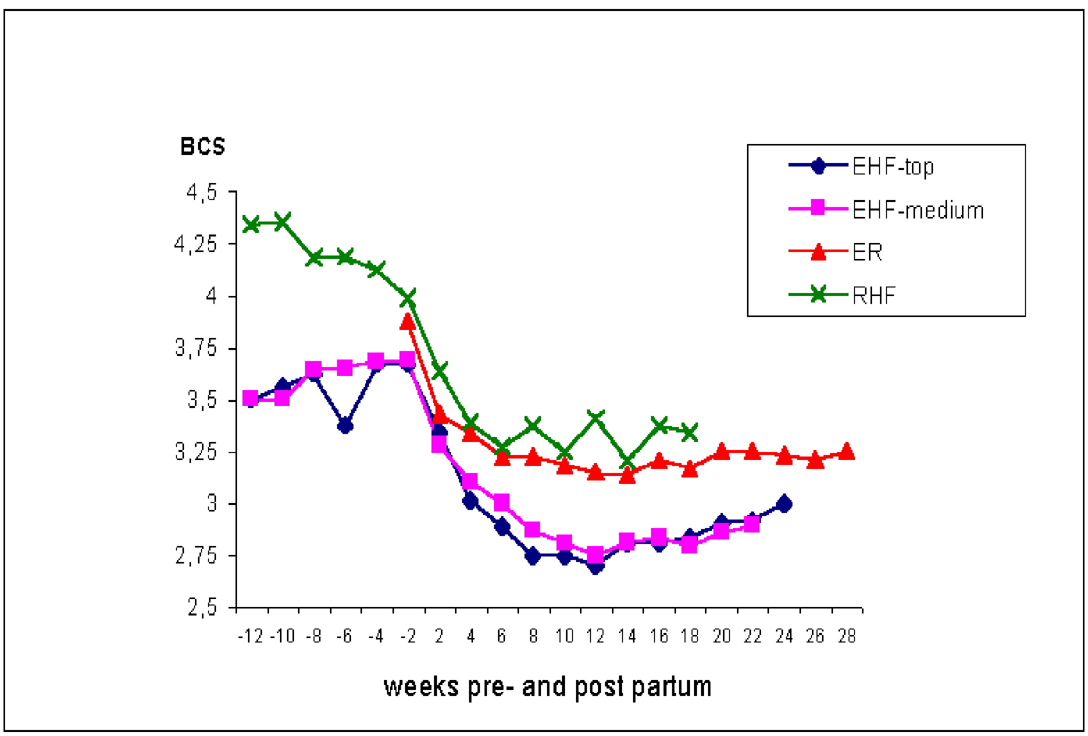

Figure 2. Dynamics of the BCS of EHF-top, EHF-medium, ER and RHF cattle.

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On Blood Biochemistry Reference Values in Veterinary and Animal Science

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Introduction

Laboratory testing of blood analytes is used for a variety of reasons. Clinical testing and nutritional assessment are two possible occasions. Whatever is the purpose of laboratory investigations and whatever blood analyte concentration is measured, comparison basis for the analyte is necessary. The comparison basis – this is the essence/core of "reference values". Terms "normal values", "normal range", "reference values" or "reference range" all figure in special literature of veterinary and animal science. "Reference values" is recommended by International Federation of Clinical Chemists due to statistical-terminological reasons (Lumsden, 1998). Which of the previously mentioned terms is used, it implies the same – to provide a comparison basis for a blood parameter.

There are two different aspects concerning reference values. These aspects are the establishment and the using of reference values. The establishment of reference values can be a solitary and a final purpose as well. Clinical or nutritional assessment assume previous establishment of reference values or using already established values from special literature.

Reference Values as Aids in Diagnosing

To carry out laboratory testing of animals for diagnostic purposes, following prerequisites must be fulfilled – appropriate analytes or clinicopathologic markers for certain diseases must be selected and reference values established. Comparison of the test results with reference values could be a valuable aid in diagnosing.

How to choose a suitable analyte to diagnose certain disease? For clinicopathologic purposes the ideal analyte must be strongly influenced by the present disease and not by others. Sometimes only one marker may not be enough. Generally, analytes that are not under rigid homeostatic control and can vary widely have a good diagnostic merit.

Establishment of reference values can seem quite simple, however, some important aspects must be considered. The basis of reference values concept is the statement that for establishment of reference values reference animals must be "normal" or clinically healthy. Sometimes it might be difficult to find the right measure or standard of normality. Another point to be considered selecting reference animals for testing is the randomness of selection. Animals selected randomly/at random are likely to represent more possible values of the analyte in population. To get reliable results a sufficient number of animals must be tested. Their number can be calculated using appropriate statistical techniques (Morris, 1999). The rule of the thumb is that 20…30 animals are necessary as a minimum.

When a sufficient amount of clinically healthy animals selected randomly is tested the distribution of the analyte concentrations can be visualised graphically. For the most of the blood analytes the graphic image of the distribution is a bell-shaped curve known as

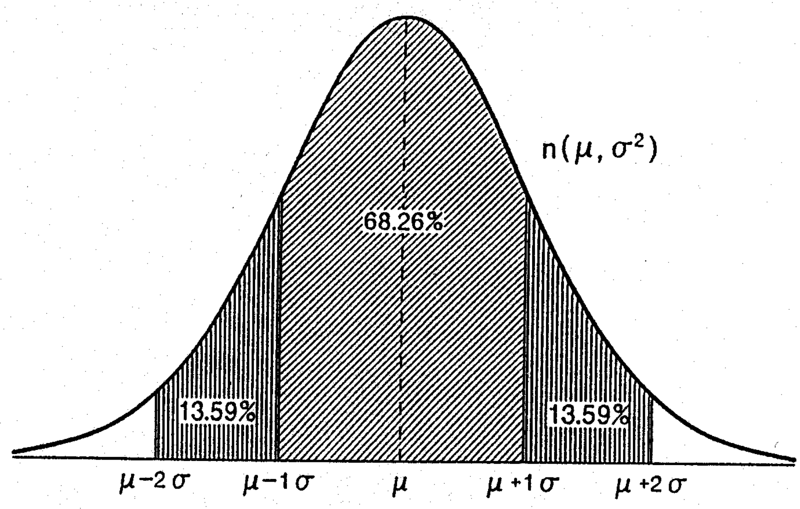

Figure 1. Gaussian distribution. μ: average; σ: standard deviation (from Kaneko et al., 1997).

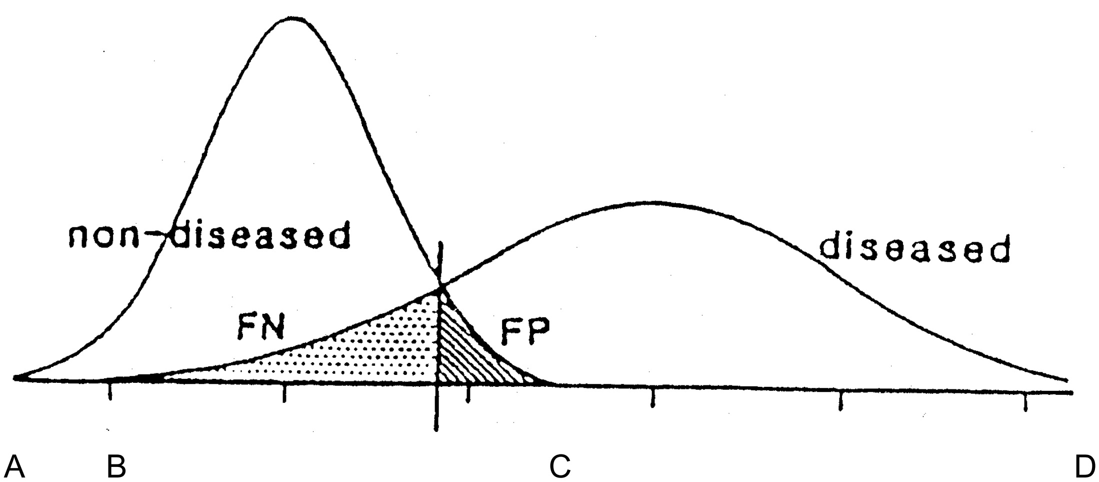

Figure 2. Overlapping of reference values (non-diseased animals) and pathological values (diseased animals). Dotted and striated areas indicate the overlapping. Range between A and C covers two standard deviations around average. Animals having analyte concentrations from A to B or from C to D can be diagnosed properly, healthy or diseased respectively. From B to C two types of mistakes can be made – normal animals can be diagnosed as diseased (false positive) and diseased animals can be diagnosed as normals (false negative). From Magid, 1992, revised.

Gaussian or normal distribution (Figure 1). The most important characteristics of Gaussian distribution are average and standard deviation. These characteristics must be calculated to obtain reference values.

In the case of Gaussian distribution average shows the concentration of analyte that is most frequent in tested animals. The average of established reference values will be more close to true average of animal population if the sample size is high. The average of reference values and true average would have the same value if all the animals in population were tested.

Standard deviation characterizes the variance of individual values in tested animals. The amount of tested animals falling within one or two standard deviations to the right and left of the average is a fixed value – approximately 69% and 95% respectively, despite the number of animals tested (Figure 1.). It is an important conclusion for clinical pathologists – when reference values are established testing multiple animals, only 5% of normal animals can be diagnosed as diseased if values between two standard deviations around average are used as reference values. Only 2% of animals may be diagnosed improperly, if the range of three standard deviations around average is used. Unfortunately this causes a wide overlapping in reference values and diseased animals analyte values (Figure 2). This problem will be discussed more closely a little bit later.

Although the majority of analytes in blood have Gaussian distribution not all do. Especially, distributions of some enzyme activities can be asymmetric and must be previously transformed to more Gaussian and after that statistical characteristics may be calculated like for Gaussian distribution. Proper techniques are available. Another possibility is to use methods, like percentiles, not dependent on the distribution of the parameter (Kaneko, 1997; Herrera, 1958).

When reference values are obtained clinicopathologic testing can be carried out. However, one important aspect must be considered. If the reference animals differ by age, sex, genetic merit etc., the variance of reference values is quite large. Fortunately the variance caused by disease is usually large compared with other factors, hence, the disease can be diagnosed. Suspicion can arise when analyte concentration measured in patient's blood lies near the borderline of reference values. Figure 2. indicates how in this case some overlapping of reference and pathological values can occur. It can lead to two types of right and wrong decisions (Kaneko, 1997; Magid, 1992; Boyd, J. W., 1984). Fortunately, the probability of correct diagnosis can be calculated using proper statistical techniques. The probability of diagnosing truly normal animal as normal is called the sensitivity and the probability to diagnose truly diseased animal as diseased the specificity of the test.

Chemical method used to determine the analyte is an important factor in clinical testing affecting the results and their interpretation. Every assay includes some sources of possible mistakes which can be calculated and considered in evaluating or using reference values.

Reference Values in Nutritional Assessment

Nutritional assessment refers to the evaluation of the status of the body in relation to nutrition. In nutritional assessment the approach to reference values is quite different comparing with clinical testing. Reference values for clinical testing are obtained from a population of healthy animals. In the population a number of defined and

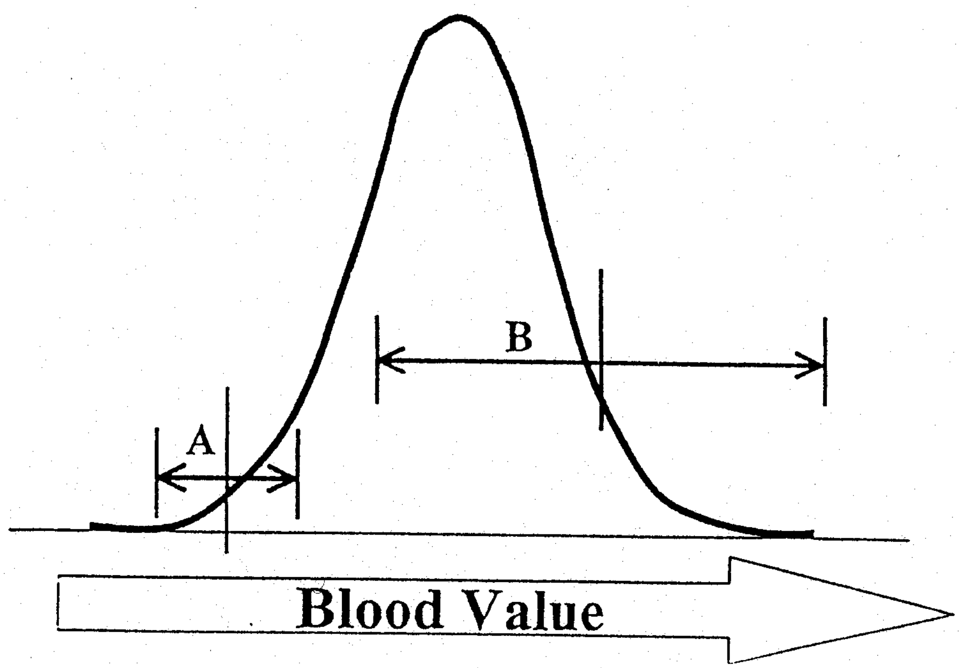

Figure 3. Gaussian distribution of herd means in a population of herds with averages and confidence intervals of the same analyte in two different herds. Herd A has a small confidence interval and it is likely that true average lies on the left tail of the reference values. Herd B has a large confidence interval and the true average can fall within a large portion of reference values. It is evident, that nutritional status of herd B can not be detected with confidence because of difference between the herd average and the average of reference values can not be detected. Testing more animals from herd B will result in a smaller confidence interval and solve the problem (from Herdt, 2000, revised).

undefined sources of variation influences analyte concentration in blood. Defined sources include sex, age, physiological state, nutrition, environment and others. Undefined sources of variation are referred to as biologic randomness. Reference values for clinicopathologic assessment include all these sources of variation. As mentioned before, the variability associated with disease is usually very large compared with other sources of variation and thus the disease can be detected. Whereas variation associated with nutrition is a part of the total analyte variation in population, analyte concentrations in animals tested for nutritional assessment fall within clinicopathologic reference values, hence effect of nutrition is undistinguishable from effects of other factors. To establish reference values for nutritional assessment the proportion of variation associated with nutritional effects must be maximized and the proportion of variation associated with non-nutritional effects minimized. Defined non-nutritional sources of variability (age, sex, stage of gestation or lactation, milk production and others) can be minimize grouping animals based on these factors. Feeding time in relation to the time of sampling is a non-nutritional source of variability as well and can be minimized by unifying sampling time. Undefined sources of variability (biological randomness) can be minimized by testing multiple animals from multiple populations (herds). The reference population should be a population of herds selected at random where average and standard deviation should be calculated from the herd means, not individual animal values. Thus variability is reduced and standard deviation will be less as well (Sokal et al., 1981; ref. Herdt, 2000). By Herdt (2000) the best practical solution to establish reference values for nutritional assessment is to choose at random from herds with good production performance and a low incidence of nutritionally related problems or diseases.

How to choose appropriate analyte for nutritional assessment? Like in clinicopathologic practice, an analyte not under rigid homeostatic control may have a good detective value in nutritional assessment as well. Blood calcium, for example, is not a good analyte for nutritional assessment, as in normal animals it varies only 20% around the mean. On the contrary, non-esterified fatty acids have a good potential, especially for assessment of energy status. They show large variation – 100% around the mean indicating high influence of environmental factors, including nutrition, to this analyte concentration. Large variation of non-esterified fatty acids is also pointed out in the authors' earlier publication (Jaakson and Ling, 2000). Glucose may also be a good characteristic of nutritional status when variation associated with feeding time is eliminated by unifying sampling time in relation to feeding time.

In statistical characterization of reference values confidence interval, besides average and standard deviation, is of great importance. It is usually calculated on 95% confidence and shows the range of values within which true average is likely to fall. Confidence interval is influenced by the number of sampled animals and will diminish when the number of tested animals increases. The importance of this parameter in nutritional assessment will be discussed hereinafter.

There are two different approaches to nutritional assessment. If only one animal with nutritional disorder is tested the core of the action will be the same as in clinical testing. But the approach must be quite different if the purpose is to evaluate nutritional level of animal groups or herds. In this case animals under investigation must also be grouped by age, sex etc. as described earlier (to establishing reference values).

Sample size is a critical factor affecting the results in nutritional assessment. At least seven animals must be tested for a blood analyte to detect with 95% confidence a difference of one standard deviation between the test herd mean and the mean of reference population or reference values (Herdt, 2000). Consequently, difference less than one standard deviation between the test herd mean and the mean of reference population can not be detected if the sample size is only seven. The fact indicates the necessity of statistical evaluation of test results. Figure 3 illustrates how sample size and confidence interval can influence the interpretation of the results in nutritional assessment.

Concluding this discussion it must be stressed once more that any biochemical investigation, clinical as well as nutritional, carried out on whatever purpose must rely only on correctly established and statistically evaluated reference data.

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IGF-1 and Thyroid Hormones in Cattle

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Introduction

In cattle breeding on milk performance, the great relevance have several factors, like the animal pedigree and environmentally dependent factors. However, some physiological parameters show even higher correlation with milk production, than the pedigree breeding value (Panicke et al., 2000). Some general growth controlling factors, like GH and IGF-1 are playing an important role in the development of the mammary gland. Pituitary hormone prolactin is crucial for development of the mammary gland during puberty and pregnancy, and it acts as a lactogenic factor in the lactating mammary gland (Dovč, 2000). Also, there is necessity for emphasising the importance of a thyroid gland. Generally, the TSH assay is preferred in diagnostics of thyroid disease (Kaneko, 2000), whereas thyroxine and triiodothyronine are particularly important as regulators in development. Consequently, it is worth to study potential regulatory factors in respect of T3 and T4. Since 90-s the number of reports in physiology and biochemistry of farm animals has been rapidly raising. This trend bases on purpose to work out the methods for manipulation the quality and quantity of animal production using different treatments, what can affect the animal metabolic and hormonal status (Bauman, 1998). A high milk performance associates a stabile and healthy metabolism with sufficient fertility of dairy cows. It depends on a balanced distribution of energy in body and well functioning regulatory system.

The aim of the present study was:

- to characterise cattle serum hormones in development;
- to obtain respective information for clinical purposes;
- to investigate the relationships between physiological and productivity data.

Materials and Methods

Estonian Red (**ER**) and Estonian Black-and-White (**EBW**) breed cattle were studied. For an investigation 98 healthy neonate calves were selected. Serum was separated within three hours after blood collection and it was kept frozen by –24°C. Triiodothyronine (T3), thyroxine (T4), growth hormone (GH) and prolactin (prl) was measured using an immunochemoluminescence assay. An insulin-like growth factor (IGF-1) was measured by radioimmunoanalyse, using the human DSL-5600 IGF-1 IRMA Kit. SPSS SYSTAT 10 was used for statistical analysis and graphical design of results (SYSTAT 10, 2000). In text medians and standard errors are given.

Results and Discussion

The characterization of our selection, the results of measurements of IGF-1, hormones and cattle age, is given in two figures. First of all it is important to mention, that we did not succeed to observe any statistically significant difference between studied breeds, although the figure 2 shows a variance in animal development, and especially in IGF-1. In our study of individual animals, hormones showed relatively high values on the second postnatal month. However, the concentrations of studied thyroid hormones remain relatively stable in the first year. At the end of second month, the T3 level was 152 ± 8 ng/dl and T4 level 5.24 ± 0.18 μg/dl.

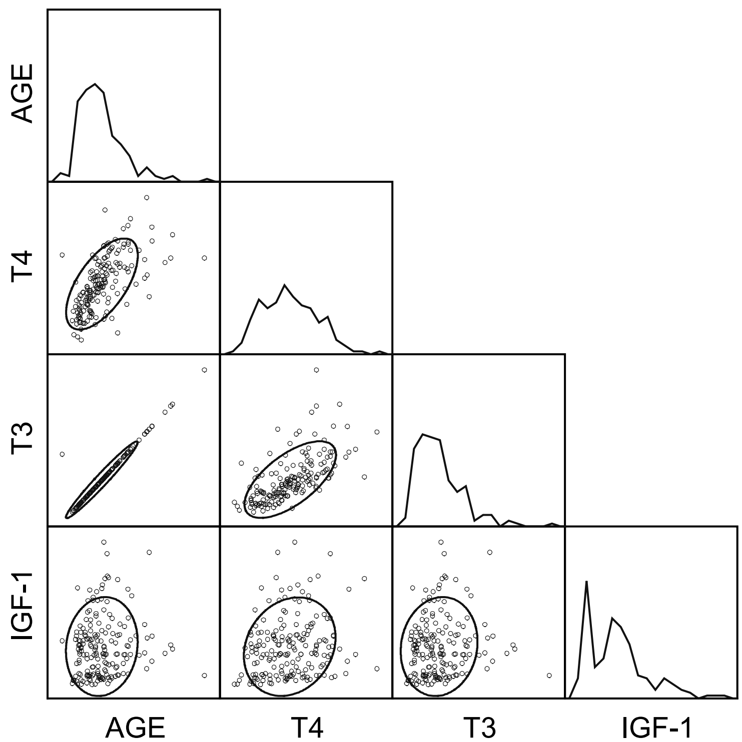

Figure 1. Confidence ellipses for parameters in studied selection
Joonis 1. Uuritud parameetrite jaotus valimis

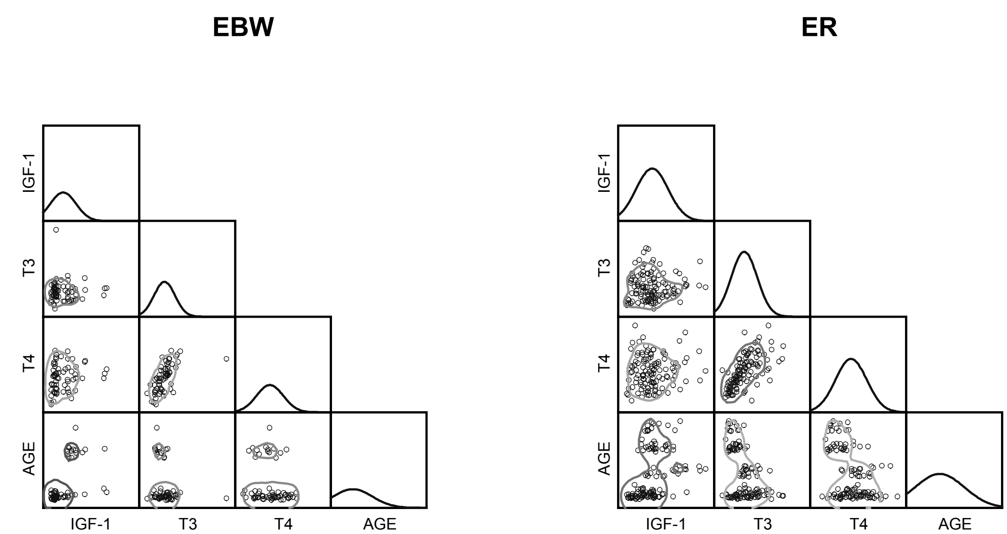

Figure 2. Confidence Kernel contours for parameters, separately for Estonian Black-and-White and Estonian Red breed
Joonis 2. Uuritud parameetrite jaotus Eesti Mustakirjul ja Eesti Punasel tõul

Our measurements show a weak tendency to decrease in the second half of the first year. The T3 in serum attains to the end of the first year an average value of 22% under the maximum value of T3 concentration for this individual animal, although, the T4 lose only 3% of its maximum value.

In the first postnatal year, the content of IGF-1 in serum shows the tendency to increase, and the maximum value of IGF-1 in bovine serum achieved by 13 - 14 month ages. The content of IGF-1 in this period was 180 ± 7 µg/l. That can be associated with the cell differentiation in this period. Moreover, the IGF-1 level in bovine serum can be predicted from a linear equation of the animal age (in days) and T4 (Equation 1).

Equation 1. IGF-1 calculation from animal age and serum T4
Võrrand 1. IGF-1 väärtuse arvutamine looma vanuse ja seerumi T4 alusel

$$C_{IGF\text{-}1} = 0.275 * age + 18.072 * C_{T4} - 23.343;$$
$$p<0.001.$$

It may indicate the direct causal relations between IGF-1 and T4. On the other hand, T4 can be calculated from T3 (Equation 2).

Equation 2. Serum T4 and T3 correspondence
Võrrand 2. Seerumi T4 ja T3 arvväärtuse seos

$$C_{T4} = 0.0154 * C_{T3} + 2.744;$$
$$p<0.001.$$

However, we could not find any significant statistical links between IGF-1and T3. The growth hormone in lactating cows correlates with total milk production (0.41; $p<0.05$), milk fat production (0.58; $p<0.01$), milk protein production (0.58; $p<0.01$) and highly with milk protein content (0.73; $p<0.01$). Serum IGF-1 level shows significant correlation with total milk fat production (0.48; $p<0.05$) and milk protein production (0.42; $p<0.05$). However, no significant correlation between IGF-1 and milk fat or milk protein content was found (Karus, 2000). The possible prognostic value of all studied characteristics for animal breeding remains to be calculated.

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Keywords: cattle, T3, T4, IGF-1

Kokkuvõte
Veiste IGF-1 ja türeoidhormoonid.
Töös uuriti Eesti Punast ja Eesti Mustakirjut tõugu veiste vereseerumi kilpnäärmehormoonide trijodotüroniini, türoksiini ja insuliini-sarnase kasvufaktori-1 sisaldust. Statistiliselt usaldusväärset erinevust tõugude vahel ei leitud. Loomade individuaalsel uurimisel täheldati kilpnäärmehormoonide maksimumi teise elukuu lõpul. IGF-1 tase suurenes seevastu 13 – 14 elukuuni. IGF-1 ja kilpnäärmehormoonide kontsentratsiooni dünaamika seosed majanduslikku kaalu omavate näitajatega vajavad täiendavaid uuringuid.

Relationships Between the Grade of Holstein Genes and Sperm Morphology in Estonian Holstein Bulls

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Abstract
The aim of the current study was to reveal the relationships between the grade of Holstein genes and the morphological characteristics of bull semen, and evaluate if the semen of poor morphological quality could be used for A.I. after the adjustment of sperm number in the insemination dose.

The results of the study showed that the increasing grade of Holstein genes is accompanied by the decline in the morphological quality of bull semen (P<0,0001) and the sperm morphology of the bulls with 100% of Holstein genes is more sensitive to the fluctuation of the seasonal temperature (P<0,0001).

The results demonstrated also that in some cases bulls with high breeding value but with high incidence of abnormal spermatozoa in semen can be used in breeding after adjustment of the number of normal spermatozoa per insemination dose.

Introduction
Sperm morphology has been shown to be one of the most important quality characteristics of bull semen which correlates well with the NRR of the cows (Söderquist *et al*, 1991; Barth, 1992; Zhang *et al*, 1998; Padrik, 2000). Semen quality evaluation using morphological characteristics is quick and perspicuous and fits well to AI station for it’s simplicity and low cost.

The aim of the current study was to determine if the grade of Holstein genes is related to the morphological characteristics of bull semen, and does it influence the results of AI. We studied also the effect of season on the morphological quality of bull semen and clarified the conditions enabling to use the semen with poor morphological quality in breeding.

Materials and methods
Altogether 2645 ejaculates from 78 bulls collected since March, 1999 to March, 2001, were analyzed. Five to eight ejaculates from each tested bull and 3-4 ejaculates from young bulls were collected per month during that period.

To study the influence of the grade of Holstein genes on sperm morphology the bulls were divided into 3 groups according to the grades: 75,0-87,5%; 87,6-96,9% and 100%.

Frozen-thawed semen from 66 ejaculates of 19 AI bulls with established sperm morphology prior to freezing were used to study the relationship between the sperm morphology and bull's fertility. Totally 4610 cows were inseminated (242 inseminations per bull and 70,0 inseminations per ejaculate on an average). The NRR in cows was determined 60 days post-insemination.

Semen of different morphological quality from three bulls was used in a experiment to evaluate if the semen of poor morphology could be used for AI after the increasing of sperm number per insemination dose.

Bull A had 88,0% of morphologically normal sperms per insemination dose on an average (range 80,0-91,0%). The relevant figures for bull B were 57,4% (44,0-69,0%) and bull C 18,25% (15,0-33,0%). The ejaculates were collected during spring-summer and the inseminations were done in autumn-winter period. Each of 15 ejaculates from three bulls was used by 4 AI technicians in 4 herds. Totally 980 cows were inseminated (326 inseminations per bull on an average) and NRR recorded 60 days post-insemination.

For evaluation of sperm morphology air dried smears were fixed in ethanol and stained with SPERMAC™ (Stain Enterprises, South Africa) according to the recommendations of the manufacturer. One hundred spermatozoa in each preparation were examined under the phase contrast microscope (x 1000). Different morphological abnormalities (detached heads, abnormal heads, abnormal necks, proximal and distal cytoplasmic droplets, abnormal midpieces and normal tails) were registered in each preparation as a percentage of the total number of counted spermatozoa.

In order to determine the effect of the grade of Holstein genes differences between mean values of sperm abnormalities were evaluated using analysis of variance. The relationship between the incidence of normal spermatozoa in the semen samples and 60-days NRR was examined using Pearson correlation analysis.

Results and discussion

1. Relationship between the grade of Holstein genes and morphological quality of bull semen.

The results presented in Table 1 showed that there was a significant difference in the incidence of abnormal sperms between the bull groups with the different grade of Holstein genes ($P<0,0001$). Generally, the increase in the grade of Holstein genes was accompanied by the increase in the incidence of abnormal sperms. The bulls with 87,6-96,9 % of Holstein genes had the highest incidence of sperm abnormalities in our study. The significant differences in the incidence of abnormal heads, detached heads and abnormal midpieces were recorded between the groups.

The remarkable changes in the shape of sperm heads occur most often in the group with 100% Holstein genes. Figure 1 shows main types of abnormal sperm heads found in semen of this group. These types of abnormalities were seldom found in semen of bulls with lower grade of Holstein genes.

As 9 (100% Holstein genes) of 39 tested bulls have similar pedigree from the sire side we cannot exclude that such differences in sperm morphology may be partly influenced by an inbreeding effect.

Table 1. The incidence of sperm abnormalities depending on the grade of Holstein genes.

Sperm abnormalities	Grade of Holstein genes 75,0-87,5%	87,6-96,9 %	100,0%
Bulls	16	23	39
Ejaculates	425	546	1674
1. Abnormal heads %	2,19***	2,60***	2,97***
2. Detached heads %	2,45***	3,19***	2,21***
3. Abnormal acrosomes %	0,47	0,40	0,51
4. Neck defects %	0,63	0,63	0,66
5.Proximal&distal cytoplasmic droplets %	0,96	1,18	1,08
6.Abnormal midpieces %	2,42***	3,43***	3,34***
7.Abnormal tails %	0,90	0,98	0,95
8.Total abnormalities %	10,02***	12,41***	11,72***

Values in a row with a superscript are significantly different (* P<0,05; **P<0,001; ***P<0,0001)

Figure 1. Shape of abnormal heads occurred in semen of bulls with 100% grade of Holstein genes.

2. Effect of season of semen collection on the incidence of sperm abnormalities.

The results of the study shown on Figure 2 demonstrate that the changes in sperm morphology depend on a season of semen collection and are most expressed in the group of bulls with 100% of Holstein genes (P<0,0001).

Several authors have found that semen collected in summer contains more abnormal sperms than semen collected in cold period of a year (L.Söderquist *et al*, 1996; Slaweta, 1986). L.Söderquist *et al.* (1996) have explained a higher incidence of sperm abnormalities in summer by heat stress. It is well known that increase of temperature of the testicles influences directly spermatogenesis and thereby the quality of semen (Vogler *et al*,1991; Barth and Bowman, 1994; Malmgren and Söderquist, 1998). In on study, the incidence of abnormal sperms in the semen of 100% Holstein bulls increased by 4% in summer if compared to winter. The respective increase for the bulls of 75,0-87,5% and 87,6-96,9% of Holstein genes was 2,56 and 3,10 %, showing that increase in the grade of Holstein genes might be accompanied by the increase in sensitivity of spermatogenesis to heat stress.

Figure 2. Relationship between sperm morphology, grade of Holstein genes and season of semen collection.

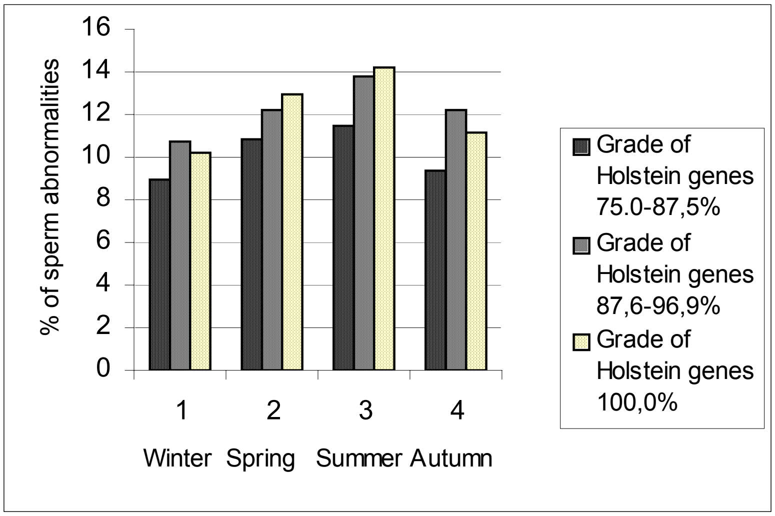

Table 2. The relation between the sperm morphology and 60-days-NRR.

Bull	A		B	C
No of ejaculates	4	1	6	4
1. Sperm abnormalities				
Abnormal heads %	2,25***	3,00***	16,16***	70,75***
Detached heads %	1,25**	1,00**	10,50**	4,75**
Abnormal acrosomes %	-	-	2,00	-
Neck defects %	1,25	2,00	2,00	-
Proximal&distal cytoplasmic droplets %	1,25	-	2,33	0,75
Abnormal midpieces %	3,50	10,00	9,33	5,5
Abnormal tails %	1,00	4,00	0,16	-
Total abnormalities %	11,36***	20,00***	42,48***	81,75***
2. Sperm concentration per straw (10^6)	40	50	65	65
3. NRR %	64,6**	69,0**	43,4**	10,9**

Values in a row with a superscript are significantly different (* P<0,05; **P<0,001; ***P<0,0001)

3. Correlation of sperm morphology and 60 days NRR in cows

In our study the incidence of normal sperms in fresh semen was generally in a range of 80,0-95,0%. Different insemination doses (30-65 x 10^6 spermatozoa per straw) produced from those ejaculates had no significant effect on NRR(r=0,004 P>0,1). However, increasing of the total amount of spermatozoa per insemination dose could influence NRR if the morphological quality of semen was poor (Table 2).

Figure 3. Normal sperms in fresh semen of bull A

Figure 4. Abnormal sperm in fresh semen of bull B

Figure 5. Abnormal sperm in fresh semen of bull C

Fresh semen of bull B contained 57,5 ±9,1% abnormal sperms in fresh semen and was characterized by the high incidence of abnormal heads, detached heads and abnormal midpieces. Increasing the total sperm number up to $65x10^6$ sperms per A.I.dose enabled to achieve NRR of 43,4%. On a contrary, such effect was not gained with bull C, yielding only 18,3±8,5% of normal sperms on an average (Figure 5). Increase in AI dose up to $65x10^6$ sperms resulted only in 10,9% NRR and further increase of sperm number per dose is not feasible in A.I. practice.

Conclusions

- Increase in grade of Holstein genes is accompanied by the increase in incidence of abnormal sperms in fresh semen.

- Increase in grade of Holstein genes is accomponied by the increase in bull sensitivity to heat stress

- In some cases, bull semen with poor morphological quality can be used in breeding if the number of sperm in insemination dose is corrected according to the morphology test results.

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Porcine Cerebral Neuropeptides and Resistance

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Abstract

It has been demonstrated that CNS and immune system are functionally reciprocally connected (J.E.Blalock, 1989), and peptides may effect especially the innate immunity (D.Barra et al., 1998). We succeeded to demonstrate that porcine brain cortex peptides (NP) CCb diminishes markedly the mortality of Swiss white mice on the background of experimental salmonellosis (*Salmonella typhimurium* $LD_{\sim 100}$)(J.Kumar et al., 1998). Optimal dose of NP is between 1.6—3.6 ng/g (30—70 ng/mouse). Current study reveals that the subcutaneous way of challenge is preferred to compare the intraperitonal one. The subfractions *slow* and *fast* of frontal lobe (Fl) peptides challenged five days before the artificial infection had the same effect on the mortality of mice during five postinfection days (i.e. 72.5%, contr. 95%), while during ten days the mortality was correspondingly 87.5 and 95% (contr. 100%). Concerning the lateralization effect of Fl dextra and Fl sinistra it was observed, that during the first five postinfection days the mortalities were correspondingly 67.5 and 77.5% (control 95%). During ten days the mortalities were correspondingly 90, 92.5 and 100%. It shows, that NP manifested the protective effect mainly during the first five postchallenge days. The practical aspect of the use of NP consists in our mind in the protection of animals in the period when the vaccination effect is not yet established.

Introduction

Neuroimmunology involves the function of immune system (IS), central nervous system (CNS) and endocrinology (Blalock, 1997). During the last two decades the number of publications in boundary areas of neurobiology and immunology is rapidly raised. So it has been found a lot of data about presence of immunomodulatory nature neuro- and immunopeptides in central nervous system and lymphoid tissues (O'Dorisio, Panerai, 1990; McCann et al., 1998). It has been demonstrated that CNS and immune system are functionally reciprocally connected (Blalock, 1989). Mostly the studies have revealed that for certain peptides the immunomodulatory activity is characteristic, which, depending on different circumstances may be inhibitory or stimulatory (Scharrer et al., 1994). Both extreme activities are in importance especially in point of view of medicine and veterinary medicine, because in case of some pathologies is a need to depress the immune system. In many cases concerned with the malfunctioning of IS, the exploitation of immunostimulants is unavoidable (see Sharrer et al., 1994). So also some peptides, like myelopeptides are nowadays involved in the clinical practice (Petrov et al., 2000). A lot of neuroimmunological studies are devoted to study the functioning and the structure of the immune system, but this is a theme for an another, separate report. In position of animal husbandry and veterinary medicine the great importance have neuroimmunological studies, that aimed to replace the microbial antibiotics used in treatment of animal. The current report is also mainly focused on this problem. It is important to mention, that our knowledge about the pig brain cortex neuropeptides is modest. So the data about pig brain cortex GTP-binding proteins and their relations to the lymphoid system (Kovaru et al., 1998), newborn piglets blood plasma prolactin relations to animal IS functions (Brownborg et al., 1997), IS and CNS relations by piglets stress (Hicks et al., 1998) have been published. Works of C.L.Nilsson (1998; Nilsson, Brodin, 1998) are dedicated to the pig cerebral cortex P substance, related peptides and their molecular nature.

The aim of this study is based on two circumstances. First – to find alternatives to the use of antibiotics in veterinary practice, and second – to characterise the architectonics of pig brain cortex, consider the distribution of immunoprotective neuropeptides in cortex.

Methods

The isolation of porcine brain cortex neuropeptides (NP) is performed as previously described (Karus et al., 1998). The NPs were extracted from different locations of the pig brain cortex with buffers: 25 mM phosphate-buffer (+0.1M KCl) pH 7.4, and 0.1 M acetate-buffer pH 5.4. For this purpose the tissues were chopped with 1.5-fold volume of cold phosphate-buffer during 5 min. From first extract after the centrifugation (8000g 15 min) the precipitate was used for extraction with the acetate-buffer, and the supernatant was used for NP precipitation with 2-volumes of cold acetone. NPs separated using centrifugation getting as result the NP crude fractions (CCa, CCb, HHa and HHb). From crude fractions NPs (for following studies) were separated using gel-chromatography.

The dose of NP was usually near 60-70 ng (i.e. 3.6 ng/g) per mouse. Mice were challenged subcutaneously (s.c.) 5 days before the experimental infection. As the pathogen in all experiments was chosen *Salmonella typimurium* strain 34-96 mainly in high dose, LD near 100%, i.e. 1.4×10^4 cells intraperitoneally (in experiments No 3 and 4 the dose was 1.0×10^3 cells). Mortality was registered during ten postinfection days. As the host was chosen Swiss white mice population, which is genetically very heterogeneous and makes able to assess the amount of refractory mice. According to the use of genetic heterogeneous hosts (mice) and the high infective dose (LD~100%) of *Salmonella typimurium* 34-96 the results of experiments appeared usually statistically not significant. Statistical analyse was done using SigmaStat 2.0.

Results

Results of the study indicate the marked immunoprotective effect of isolated NP. High doses of NP (>600 ng in experiments 3 and 5) demonstrated a harmful effect to the host immune system – the number of mortality was the same as in the control group or even greater. The high dose of HHb and CCb appeared to be toxic. Concerning the approximately hundred times lower dose of NP (experiment 4), the HHb in the dose of 7.5 ng had no immunoprotective effect at all compearing the mortality in control group. The best result gave NP CCb (NP from the crown of the head) the mortality only 58.3% against 91.6% of the control animals (see table 1).

Now let us see the experiments in which the mortality of challenged mice were observed during two stages – during 1-5 and 10 days after the experimental infection. The number of experimental animals in groups were increased. While the study of NP in gel-electrophoresis demonstrated that NP crude fractions contained two main fractions – fast and slow, and to us was interesting to assess the possible lateralization effect of NP, we performed special experiments (see Table 2 and 3).

Table 1. Immunoprotective effect of NP
Tabel 1. Neuropeptiidide immunoprotektiivne efekt

Experiment	NP, dose	No of mice	Mortality in 10 days (%)	Protective index [2]
1+2	CCa, ca 60 ng	12	83.3	0.09
	CCb, ca 60 ng	12	58.3	0.36
	HHa, ca 60 ng	12	75.0	0.18
	HHb, ca 60 ng	12	75.0	0.18
	control	12	91.6	-
3	CCb, 675 ng	10	90.0	-0.13
	HHb 747 ng	10	70.0	0.13
	control	10	80.0	-
4	HHb, 7.5 ng	10	70.0	0.00
	HHb[1], 7.5 ng	10	0	-
	control	10	70.0	-
5	CCb, 675 ng	10	90.0	0.00
	CCb[1], 675 ng	10	0	-
	control	10	90.0	-

Remarks In Experiments 3 and 4 mice were infected 5 days after challenge with $1.0x10^3$ cells of *S.typhimurium* while in other experiments the dose was $1.4x10^4$ cells
[1] not infected controls
[2] Protective index = (Mortality of control – Mortality of exp.mice) / Mortality of control

Table 2. Comparison of fast and slow fractions of NP frontal lobe (Fl)
Tabel 2. Esisagara NP fast ja slow fraktsiooni võrdlus

NP, dose ng	No of mice	Mortality (%)		Protective index	
		in 1-5 days	Total in 10 days	in 1-5 days	Total in 10 days
Fld+Fls fast, 63 ng	40	72.5	87.5	0.24	0.13
Fld+Fls slow, 63 ng	40	72.5	95.0	0.24	0.05
Control	20	95.0	100.0	-	-

From Table 2 it is seen that during the first postinfection days, i.e. in the main period of NP effect, the mortalities were the same. Or, there was no difference in the effect of fast and slow fraction in this period. Concerning the effect of location, i.e. lateralization of NP (Fl sinistra and Fl dextra) the data are shown in Table 3. Here we may constate a marked difference in the Fl immunoprotective activity during the first five days after infection advantage to the Fl dextra. But also this difference proved not to be statistically significant.

At last we tried to assess the effect of the route of challenge of NP (CCb) either intraperitoneally or subcutaneosly. In this last experiment we used as the infection dose of *S.typhimurium* $1.0x10^3$ living cells (see Table 4).

Table 3. The lateralization effect of NP Fl (Fld and Fls)
Tabel 3. Esisagara NP lateralisatsiooni efekt (Fld ja Fls)

NP, dose ng	No of mice	Mortality (%)		Protective index	
		in 1-5 days	Total in 10 days	in 1-5 days	Total in 10 days
Fl dextra, 63 ng	40	67.5	90.0	0.29	0.10
Fl sinistra, 63 ng	40	77.5	92.5	0.18	0.08
Control	20	95.0	100.0	-	-

Remark. Dextra – right lobe; sinistra – left lobe.

Table 4. The effect of the route of challenge of CCb
Tabel 4. CCb manustamisviisi mõju

NP, dose, route	No of mice	Mortality (%)		Protective index	
		in 1-5 days	Total in 10 days	in 1-5 days	Total in 10 days
CCb, 70 ng, i.p.	20	70.0	70.0	0.07	0.07
CCb, 70 ng, s.c.	20	55.0	65.0	0.27	0.13
Control	20	75.0	75.0	-	-

Remark. i.p. - intraperitoneally; s.c. – subcutaneously.

Conclusions

- Isolated porcine cerebral cortex neuropeptides (NP) CCb, CCa, HHb, HHa, Fld and Fls demonstrate marked immunoprotective qualities.

- The near optimal dose of NP for Swiss white mice is near 70 ng (3.6 ng/g).

- The effect of NP is expressed if it is challenged five days before the artificial infection with *Salmonella typhimurium* in host dose near LD 100%.

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Kokkuvõte

Sea peaaju neuropeptiidid ja resistentsus.
Töös uuriti sea peaajust isoleeritud neuropeptiidide immunoprotektiivset toimet Shveitsi valgete hiirte populatsioonis *Salmonella typhimuriumi* LD~100-ga nakatamise taustal. Võrreldi erinevatest ajupiirkondadest isoleeritud ja erinevate füsiko-keemiliste omadustega NP toimet ning saadava immuunoprotektiivse efekti sõltuvust manustamise viisist. Määrati neuropeptiidide soovituslik doos (~ 70 ng).

The Normal Morphology of Domestic Cats Reproductive Organs

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There are many animal clinics founded in Estonia lately. Many families in Estonia have cats or dogs. In this study we examined the normal morphology of domestic cats internal reproductive organs. The reproductive organs are very important and we are interested in biological and clinical aspects of reproduction. The present study of normal morphology is the first step of our investigations.

Materials and methods

The reproductive organs of the female 11 domestic cats were used in this study. The cats age was 8 months to 6 years. The cats were operated in the Animal Clinic. Domitor (medetomidin hydrochlorid), Torbugesic (butorphanol), 10% Ketamin were used for narcosis. The histological material (ovaries, oviducts and uterus) for light microscopy was embedded in paraffin and cuts stained with H&E, Van Gieson, PAS. The material for transmission electron microscopy was fixed in cold (4°C) glutaraldehyde solution (2,5%) buffered with sodium cacodylate buffer at pH=7,4 for 2 hours, postfixed for 1 hour in 1% osmium tetroxide solution at the same temperature and pH, dehydrated in alcohol and embedded in Epon 812. Semithin sections stained according to Richardson were examined using Olympus BX-50 microscope. Philips Tecnai 10 electron microscope was used for viewing and photographing.

Results

The cat ovaries are paired oval bodies, approximately 0,93 cm long, 0,38 cm wide and 0,27 cm thick. The surface of the ovaries (fig.1, 2) is covered with cuboidal epithelium - *epithelium superficiale*. Under the epithelium is the *tunica albuginea*. The ovary is composed of a *cortex* and a *medulla*. Ovarian follicles are distributed of the cortex. The follicle in the ovaries is primary, secondary and mature. In the cortex are great number of the primary follicles (fig.1, 2). Primary follicles had primary oocyte with large nucleus and a single layer of follicular cells. The oocyte in secondary follicle (fig. 3) had several layers of follicular cells. In the *folliculus ovaricus crescens* (fig.1) theca surrounding the follicle, the thickened area (*cumulus oophorus*) embedded the ovum and follicle antrum filled with follicular fluid.

The oviducts are paired muscular tubes, divided into infundibulum, ampulla, isthmus and junctura. The oviducts composed of three layers (*mucosa, muscularis, serosa*). A cross section through the ampulla of the tube is in fig. 4. The epithelium of the uterine tubes consists of secretory, ciliated and non-ciliated cells. Electron microscopic study of the secretory cells demonstrate on the luminal surface secretory granules. The secretory cells are prismatic and have large, elongated nucleus. The ciliated cells are prismatic and have on the luminal surface microvilli. Non-ciliated cells are columnar cells with large nucleus.
The epithelium of the cats uterus (fig. 5) is simple columnar and consists of ciliated and secretory cells. In the lamina propria are present the uterine glands (glandulae uterinae).

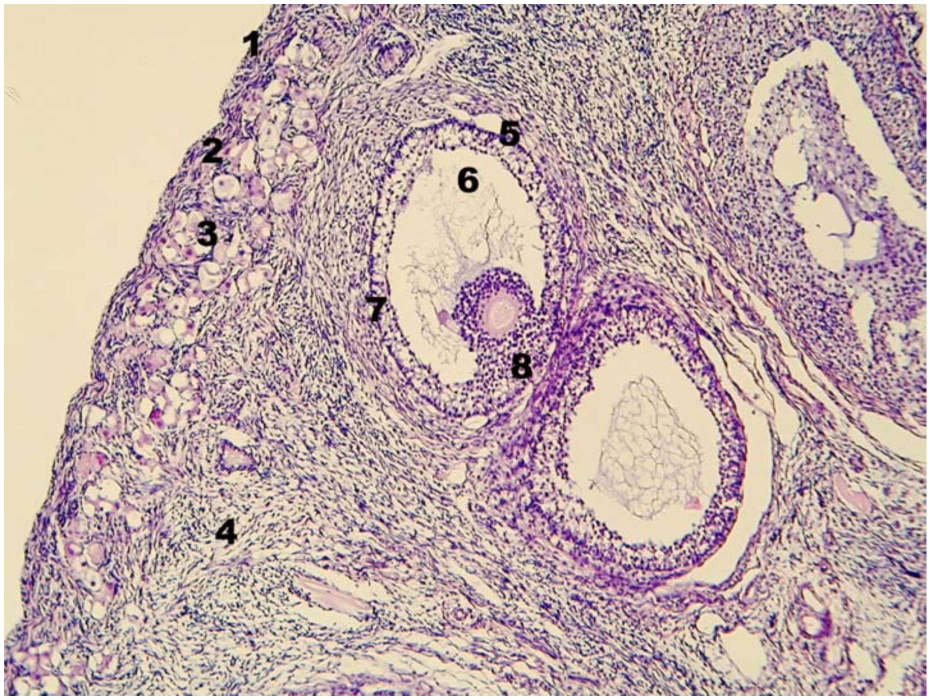

Figure 1. Part of the ovary. 1- epithelium superficiale, 2 - tunica albuginea, 3 - folliculus ovaricus primarius, 4 - cortex ovarii, 5 - folliculus ovaricus vesiculosus, 6 - cavum folliculi, 7 - theca folliculi, 8 - cumulus oophorus. H&E 168x

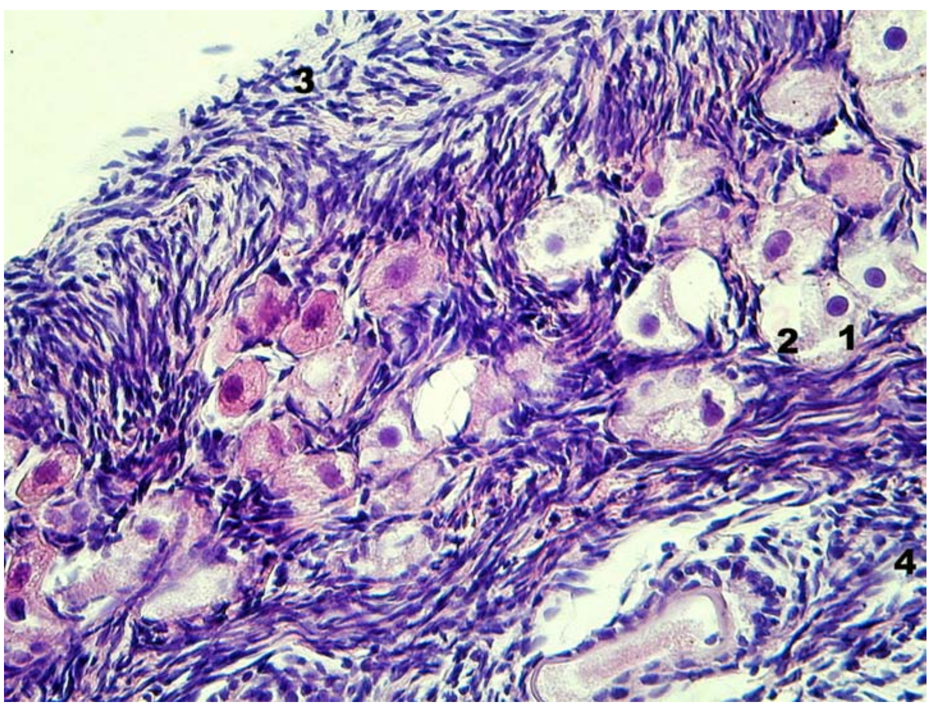

Figure 2. Part of the ovary. 1 - folliculus ovaricus primarius, 2 - epithelium folliculi, 3 - tunica albuginea, 4 - cortex ovarii. H&E 368x

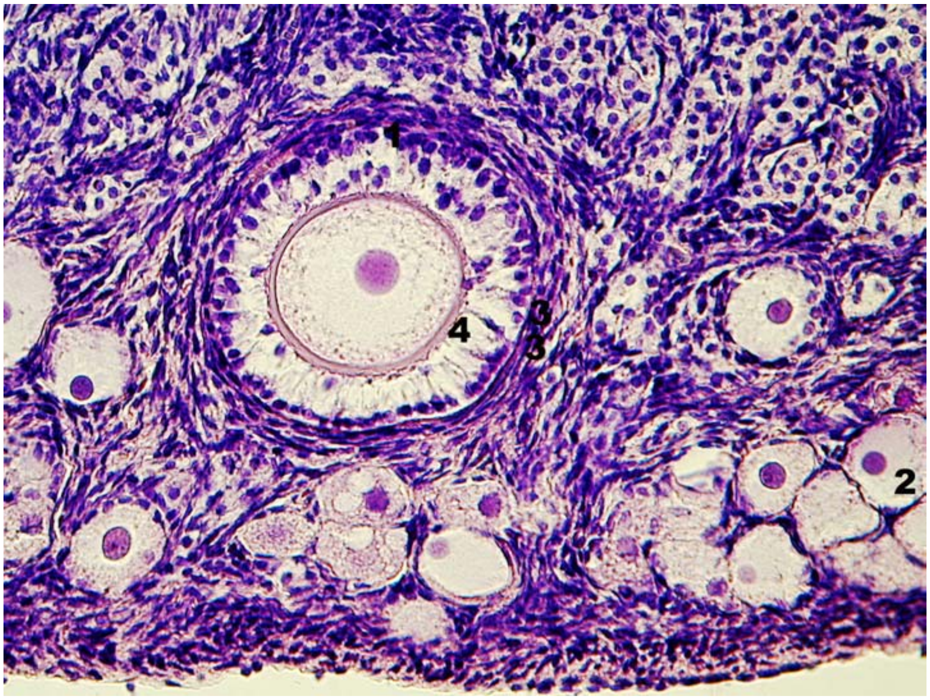

Figure 3. ***Folliculus ovaricus crescens*** **in the cortex of the ovary. 1 - folliculus ovaricus crescens, 2 - folliculus ovaricus primarius, 3 - theca folliculi, 4 - zona pellucida. H&E 368x**

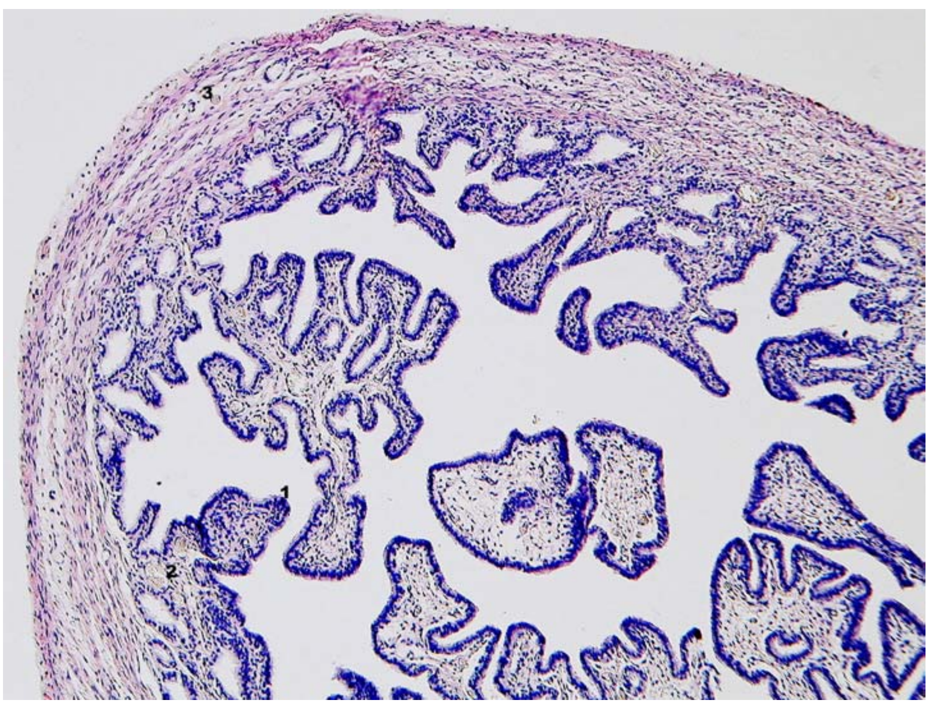

Figure 4. Cross-section of the ampulla. 1 - epithelium, 2 - propria, 3 - tunica muscularis. H&E 168x

Figure 5. Cross-section of the uterus. 1 - epithelium, 2 - stroma endometrii, 3 - glandulae uterinae. Van Gieson 168x

Discussion

The light and electron microscopic examinations in the present study demonstrated the reproductive organs normal morphology. The surface of the ovaries is covered with cuboidal epithelium (Brück *et al*., 1999). In the *folliculus ovaricus vesiculosus cumulus oophorus* embedded the ovum, the cumuli oophori were attached to the follicular wall (Brück *et al*., 1999). The oviducts epithelium is columnar and consists of secretory, ciliated and non-ciliated cells (Bareither *et al.,*1981; Kühnel *et al*., 1981, 1974; Odor 1974). The secretory cells have on the luminal surface secretory granules (Kühnel *et al*., 1974). The epithelium of the uterus is simple columnar (Beier *et al*., 1976) and consists of ciliated and secretory cells.

The results of our studies show structurally normal ovaries, oviducts and uterus.

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Kokkuvõte

Kodukassi reproduktsiooniorganite morfoloogia normis.
Viimastel aastatel on kasvanud väikeloomade osatähtsus veterinaararstide praktikas. On tekkinud hulgaliselt väikeloomade kliinikuid nii Tartus, kui ka mujal Eestis. Tutvudes viimastel dekaadidel Eestis ilmunud veterinaariaalase kirjandusega, konverentside teesidega selgus, et väikeloomade reproduktsiooniorganite morfoloogia valdkonnas uurimistöid tehtud ei ole. Meie poolt uuritud materjal on võetud üheteistkümnelt EPMÜ Loomakliinikusse toodud emaselt kassilt vanuses 8 kuud kuni 6 aastat. Materjal on fikseeritud histoloogiliseks ja elektronmikroskoopiliseks uuringuks klassikaliste metoodikate järgi. Histoloogiliselt ja elektronmikroskoopiliselt uuriti munasarju, munajuhasid ja emakat. Saadud tulemused vastasid meie ootustele ja võimaldavad uurida edaspidi kodukassi reproduktsiooni bioloogilist, kliinilist ja immunoloogilist aspekti.

Tuberculosis of Birds in Estonia: Transmission and Diagnostics

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Abstract
The tuberculosis of the chicken in Estonia was eliminated in 1968. The improvement of epizootic situation was helped by spread of the technology of keeping chicken in cages on large state farms which did not allow a transmission of infection by alimentary way. Since 1995 there were diagnosed 5 sporadic cases of tuberculosis of the chicken in small farms with free containment of birds, and also number of cases of a tuberculosis of birds in Tallinn Zoo (peacock, Japanese crane, pheasant) and of synanthropos birds. The results of PCR-identification of *Mycobacterium avium* correlated with results of conventional methods and biotest. Epidemiological importance of a MAC-infection is extending on many species of mammals. Isolation of mycobacteria from eggs of the chicken indicates the possibility of transmission of MAC-infection to man with immunosuppressive disorders.

Keywords: tuberculosis of birds, Mycobacterium avium complex, PCR-identification, transmission of mycobacteria.

Introduction
Tuberculosis of fowls is now often called as "old fashioned" disease, but quite often there has been diagnosed tuberculosis in farms with of free contaniment of birds in the developed countries quite often (Veterinary investigation division, 1997). In Estonia tuberculosis was eliminated in 1968 when the technology of cages for keeping of birds in large state farms was distributed and the transmission of the infection by alimentary way was prevented. The long-lasting period of well being ended in 1995, when we diagnosed two cases of tuberculosis in hens (Sudakov et al., 1998). It is important to note, that in 1995 drastic increases of the cases of tuberculosis of hens in Russia were found (Avilov et al., 1997).

M. avium was originally identified as a pathogen of birds, but both *M. avium* and *M. intracellulare* (*M. avium-intracellulare* complex - MAC) are environmental saprophytes and play a main role in transmission of NTM-mycobacteria (non-tuberculous mycobacteria) infections to man and animals (Inderlied et al., 1993; Augustijn et al., 1997; Duerrling et al.,1998).

The greatest susceptibility to NTM have children and the immunocompromised individuals, especially AIDS patients. The number of cases of atypical mycobacterioses infections have increased rapidly after spreading of HIV-infection. It causes most frequently the death of the AIDS patients (Elvira et al., 1998; Czachor and Gopalakrishnan, 1997; Wark et al, 1998). The possibilities of control of MAC-mycobacterioses depend on clearing up of sources and paths of a transmission of the given infection.

Materials and methods

Materials for bacteriological analysis were collected from clinical samples of birds, eggs and milk, which have been sent by Veterinary and Food Laboratory. The samples were treated by conventional methods with subsequent crop on the Lewenstein-Jensen medium (Becton Dickinson).

Bacterial DNA isolation.

CTAB (cetyltrimethylammoniumbromide) method. A loop of mycobacteria grown on Lowenstein -Jensen solid medium was suspended in 400 µl of TE (10 mM Tris-HCl, 1mM EDTA; pH 8) and was heat inactivated for 20 min at 80°C. After inactivation 50 µl of lysozyme (10 mg/ml) was added and was incubated at 37 °C for 1 h. Then 75 µl of 10% SDS/proteinase K mix was added and incubated for 10 min at 65 °C. 100 µl of 5 M NaCl and 100 µl of CTAB/NaCl (10 % CTAB in 0,7 mol of NaCl/l) solution were added, and the sample was incubated at 65 °C for 10 min. Proteins were removed by phenol-chloroform (1:1) extraction, and DNA was precipitated with 0,6 volumes of isopropanol. The DNA was pelleted by centrifugation (12,000×g for 15 min.), washed once with 70 % (vol/wol) ethanol, and air dried for 15 to 20 min. The final pellet was resuspended in 20 µl of TE (10 mM Tris-HCl, 1mM EDTA; pH 8).

Heat extraction of DNA. Mycobacteria colonies from Lowenstein -Jensen agar were suspended in 100 µl of TE (10 mM Tris-HCl, 1mM EDTA; pH 8) containing 1 % Triton X-100 and incubated at 100 °C for 30 min. Two microliters of these lysates were used as DNA sources without futher purification.

PCR.: The composition of PCR mixture (50 µl) was 50 mM KCl, 10 mM Tris-HCl (pH-8,3), 1.5 mM $MgCl_2$, 200 µM (each) deoxynucleoside triphosphate, 0.5 µM (each) primer, and 1U of *Taq* polymerase.

On the basis of the sequence IS1245 , two primers were used: P1 (5'-GCC GCC GAA ACG ATC TAC) and P2 (5'-AGG TGG CGT CGA GGA AGA C) for amplification of the 427 bp. segment(Guerrero et al. 1995). PCR was performed as previously described (Telenti et al. 1993) under the following conditions : 30 cycles of 1 min at 94°C , 1 min at 65°C and 1 min at 72°C and one final extension cycle of 10 min at 72°C. The reference strain of *M.avium* D4ER was as a positive control and a water blank was used as a negative control for each PCR mixture. PCR products were analyzed by electrophoresis on 1.8 % agarose gel and stained with ethidium bromide. Gels were visualized by using a 302-nm UV transilluminator.

There were identified mycobacteria in the laboratory of mycobacterioses at Tartu University (dr. Annika Kryner) by using the commercial DNA tests (GenProbe Inc.). The serotypes of the three MAC-complex isolates of chicken were determined in Denmark (dr. S. Giese, Danish Veterinary Laboratory, Copenhagen).

Results

During 1995-2001 bacteriologic research of clinical samples and slow growth cultures from hens with suspicion on tuberculosis and five outbreaks of *M. avium* infection were determined: twice in 1995 and once in 1997, 2000 and 2001 (Table 1).

The patients of tuberculosis were from a small farms (4) of Estonia. The results of the identification of the chicken isolates by PCR conformed with GenProbe (AccuProbe) analyses, Schaefer's serotyping and biotest.

As a result of bacteriological testing of clinical samples from the chronic patients of birds in Tallinn Zoo, 10 cultures of the slowly growing mycobacteria were isolated. Nine of them were isolated from the exotic birds, containing in cells living birds and one crow living on the territory of Zoo. By results of PCR-identification *M. avium* was detected in many cases including peacock, Japanese crane, three pheasants and crow (Table 2). Three strains isolated from pheasants were identified as *M. intracellulare*. It is necessary to note, that by results of autopsy, *M. intracellulare* strains isolated from pheasants were less virulent compared to *M. avium* strains.

Table 1. Cases of tuberculosis of chickens in Estonia between 1995-2001.

Date of isolation	Country	Strain number	Serotyp MAC*	Accu Probe MAC	PCR-identification	Results of biotest
1995	Koeru, Jarvamaa	M177	3	+	*M. avium*	+
1995	Kadrina, Lääne-Virumaa	M178	3	+	*M. avium*	+
1997	Reola, Tartumaa	M202	3	+	*M. avium*	+
2000	Halliste, Viljandimaa	M266	x	x	*M. avium*	x
2001	Ardja, Lääne-Virumaa	M276	x	x	*M. avium*	+

MAC* - *Mycobacterium avium complex*
x - investigations not performed

Table 2. Results of bacteriological analysis of clinical samples from birds in Tallinn Zoo and PCR- identification of isolated strains.

Source of samples	Strain number	Site of isolation	Morphological lesions	PCR-identification
Japanese crane	M248	liver, spleen	liver, spleen	*M. avium*
Peacock	M232	intestine	intestine	M. avium
Pheasant	M247	liver, lung	liver, lung	*M. avium*
	M260	liver, lung	liver, lung	*M. avium*
	M263	liver, spleen	liver, spleen	*M. avium*
	M259	liver, spleen	-	*M. intracellulare*
	M261	liver, spleen	-	*M. intracellulare*
	M262	liver, spleen	-	*M. intracellulare*
	M258	liver, spleen	-	not belong to MAC
Crow	M249	liver	liver	*M. avium*

x - PCR-investigations not performed

Table 3. Results of bacteriological analysis of samples from milk and eggs and identification of isolated strains.

Source of samples	Number of samples	Number of isolated	Strain number	PCR-identification	AccuProbe
Milk	32	2	M205	*M.avium*	*M. avium*
			M206	*M. avium*	*M. avium*
Eggs	111	3	M204	M. avium	MAC
			M209	MAC	x
			M219	*M. avium*	*M. avium*

For determining the possibilities of transmission of MAC-infection with animal products we tested bacteriologically samples from eggs (111 cultures) and from milk (32 cultures). The cultures of mycobacteria were isolated from 3samples of eggs and from 2 samples of milk, and by PCR-identification in four of five cultures *M. avium* was detected (Table 3).

Conclusions

At present epidemiological importance of *M. avium*-infection is not limited only to birds, but also plays a main role in an etiology of NTM-mycobacterioses of humans and wide variety of animals (Andre, 1996; Thorel et al., 1997; Morita et al., 1999). In Estonia there has not been detected the tuberculosis of cattle since 1986, however MAC-mycobacterioses of swine and cattle are widely spread. There were detected five cases of the avian-tuberculosis in the hens during 6 years, but they didn't have epizootical importance. Until 1995 the tuberculosis of hens has not been diagnosed for 27 years, but the changes of epidemiological situation may be caused by appearance of many small farms with free containment of birds during 1990 - 1995.

During 1999-2000 several cases of *M. avium*-tuberculosis (5) were diagnosed in birds in Tallinn Zoo. The spread of the tuberculosis in cages of exotic birds is possibly caused by the weakening of the immune system when living in stressful conditions (Singbeil et al., 1993; Gyimesi et al., 1999).

During 1999- 2000 we diagnosed *M. avium* infection in crow living on the territory of Zoo. Heilicek et al. (1993) have shown that various species of the free living birds play an important role in the epidemiology of a MAC. The same article shows concern about infected pheasants having ability to contaminate the environment and to transfer mycobacterial infection even after relatively short contact with pigs and poultry.

Compared with *M. tuberculosis* (the only reservoirs of *M. tuberculosis* are other humans) both *M. avium* and *M. intracellulare* are environmental saprophytes and can survive well in soil, in water, and in food. Therefore possibility exists that birds and animals play potential role in the transmission of *M. avium* infection to person with immunosuppressive disorders (Montali et al., 1998). The selection of mycobacteria from eggs and milk supports the possibility of such transmission (Cornejo et al., 1998).

The application of molecular methods for identification of mycobacteria gives new possibilities both for fast diagnostics of MAC-infection, and for clearing up of a transmission.

The diagnostics of a tuberculosis of birds is necessery for distinguishing *M. avium* and *M. intracellulare.*

PCR is fast and accurate method for identifing mycobacteria, but it is impotant to find out DNA sequences responsible for virulence of *M. avium* making possible to detect virulence by PCR.

Acknowledgements
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