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REPRODUCTIVE PERFORMANCE IN MARES IN FINLAND

A study of seasonality, ovulation and postovulatory breeding

Selostus: TAMMOJEN LISÄÄNTYMISTOIMINNOT SUOMESSA

Tutkimus vuodenaikojen vaikutuksesta, ovulaatiosta ja siemennyksestä munarakkulan puhkeamisen jälkeen

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Academic dissertation
To be defended in public, with the permission of
the College of Veterinary Medicine in Auditorium
Maximum, Hämeentie 57, 00550 Helsinki,
on 10th April, 1991, at 12 noon.

PREFACE

This investigation was carried out in The Equine Research Station, Ypäjä, during the years 1981—1991. I would like to express my warmest thanks to M.Sci.Agr. Leea Rauvala, the former Head of the Horse Breeding Institute, and M.Sci.Agr. Helena Jansson, Head of the Equine Research Station, for placing the facilities and resources of the Station at my disposal.

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Finally I express my heartfelt gratitude to my wife Leena for her supportive optimism and my six children who had to share their time for computer games with my time for preparing the manuscript.

LIST OF ORIGINAL PAPERS

I Koskinen E & T. Katila. Onset of luteal activity in non-foaling mares during the early breeding season in Finland. Acta Vet. Scand. (in press)

II Koskinen E. Post partum ovarian activity in Finnhorse mares with special reference to seasonal effects. Acta Vet. Scand. (in press)

III Koskinen E., E. Kurki & T. Katila. Onset of luteal activity in foaling and seasonally anoestrous mares treated with artificial light. Acta Vet. Scand. (in press)

IV Koskinen E., H. Kuntsi, H. Lindeberg & T. Katila. Predicting ovulation in the mare on the basis of follicular growth and serum oestrone sulphate and progesterone levels. J. Vet. Med. A 36, 299—304 (1989).

V Koskinen E., H. Lindeberg, H. Kuntsi & T. Katila. Milk and serum progesterone levels in mares after ovulation. Acta Vet. Scand. (in press)

VI Koskinen E., H. Lindeberg, H. Kuntsi, L. Ruotsalainen & T. Katila. Fertility of mares after postovulatory insemination. J. Vet. Med. A 37, 77—80 (1990).

ABBREVIATIONS AND DEFINITIONS

E1	oestrone
E2	oestradiol
EED	early embryonic death
ERS	Equine Research Station
1 foot-candle	10.8 lux
N	number of animals
nmol/l	nanomoles per litre
N.S.	not significant
P4	progesterone
PG	prostaglandin
RIA	radioimmunoassay
SD	standard deviation
warm-bloods	mainly American standard bred trotters and half-bred riding horses such as German Hannoverians

CONTENTS

ABSTRACT

The purpose of the work described in this thesis was to investigate the reproductive patterns of mares under Finnish climatic conditions, especially in Finnhorses, mainly on the bases of serum and milk progesterone determinations, and ultrasonography. The work covered the seasonality of Finnish mares; the effect of artificial illumination on seasonality; ovarian changes post partum; possibilities for predicting ovulation; use of progesterone analysis to determine times of ovulation retrospectively; and the maximum time after ovulation for insemination still be succesful.

The investigations related to 186 mares in seven stables in Southern Finland from 1985 to 1989.

Seasonality

Nearly one non-foaling mare in four was found to have high progesterone concentrations even in winter. After February 15th, increasing numbers of mares ovulated. Half of the mares were cycling during the first fortnight of April. All mares were cycling in June. Our results indicate that the fact that foalings take place in Finland about a month later than in other countries for which monthly foaling percentages have been published is caused by the later ovulatory season in Finland.

The factors influencing onset of cycling early in the season were the use of the mare, foaling in the previous year and the time of onset of cycling in preceding years.

In foaling mares, the time from parturition to first ovulation was shorter in Summer than in Spring. Very long post partum intervals occurred mainly before May.

Seasonal effects were found also in relation to follicular growth rate. The preovulatory follicle reached ovulatory size faster in relation to foalings during Summer than during Spring. This information is relevant to prediction of ovulation on the basis of size of preovulatory follicles.

Artificial illumination

Long-day treatment (14.5 hours light, 9.5 hours dark) began on December 1. Anoestrous non-foaling mares ovulated for the first time during the second fortnight of February. Sensitivity to long-day treatment was least in Finnhorses and decreased with age.

All long-day-treated post partum mares ovulated within 18 days of parturition, except the mare which foaled first. She ovulated in February, within 32 days of parturition in January.

Insemination of 14 long-day-treated non-foaling mares in the second oestrus of the year in March resulted in a foaling rate of 86 % during the subsequent year.

Long-day treatment of non-foaling and pregnant mares is an effective way of starting the breeding season in Finland at the beginning of February.

Sizes of ovaries and follicles during post partum period

The left ovary was more active after parturition in terms of follicular activity. Two days post partum follicles larger than 10 mm were more frequently found in left than in right ovaries. The first post partum ovulation also occurred more often in the left than in the right ovary.

In general, the ovulatory ovary and its largest follicle were statistically significantly larger than the ovary and follicle on the non-ovulatory side five days and more post partum. During the last week before foal-heat ovulation the growth rate of the preovulatory follicle was on average 3 mm a day.

Prediction of ovulation

During second or subsequent post partum oestrous periods, serum progesterone concentrations decreased to less than the detection limit 1—10 days before ovulation.

Serum oestrone sulphate usually reached its highest concentration 0—2 days before ovulation. The peak was not, however, statistically significantly different from values obtained on other days of oestrus.

The preovulatory follicle grew by 3 mm a day from day 5 to day 2 before ovulation. The average size of the preovulatory follicle 1—3 days before ovulation was 43 ± 4 mm. Times of ovulations were equally distributed throughout the day. Ovulation occurred most often in the left ovary. In terms of prediction of time of ovulation, changes in shape and flaccidity of the preovulatory follicle were not informative. The best method of prediction appeared to be determination of follicle size.

Progesterone levels after ovulation

There was a large variation in the time of the first measurable increase in progesterone concentration after ovulation. Increases in progesterone concentrations occurred within 6 to 54 hours in milk and within 6 to 60 hours in serum. The correlation coefficient ($p < 0.05$) between progesterone concentrations in serum and milk was 0.7. The results indicate that progesterone concentrations can be used to distinguish between mares in luteal and non-luteal phases but that the method is unable to determine times of ovulation to an accuracy greater than within two days.

Postovulatory breeding and early embryonic death

Postovulatory insemination resulted in good conception rates for up to 18 hours after ovulation but not 24 hours after ovulation.

In mares examined once a day and mated within 0 to 27 hours of ovulation, the 16-day conception rate was 46 %. Some mares, however, exhibited embryonic death between days 16 to 25, resulting in a final conception rate of 29 %. In cases of limited semen availability or a short lifespan of spermatozoa, insemination after ovulation can therefore be a good alternative to insemination before ovulation. However, there could be an increased risk of early embryonic death.

AIMS OF STUDY

to investigate the reproductive patterns of mares under Finnish climatic conditions, especially in Finnhorses

to describe the natural seasonality of Finnish mares and the effect of artificial illumination on seasonality

to examine ovarian changes post partum

to examine the possibilities of predicting ovulation

to determine the value of progesterone measurements for determining the time of ovulation

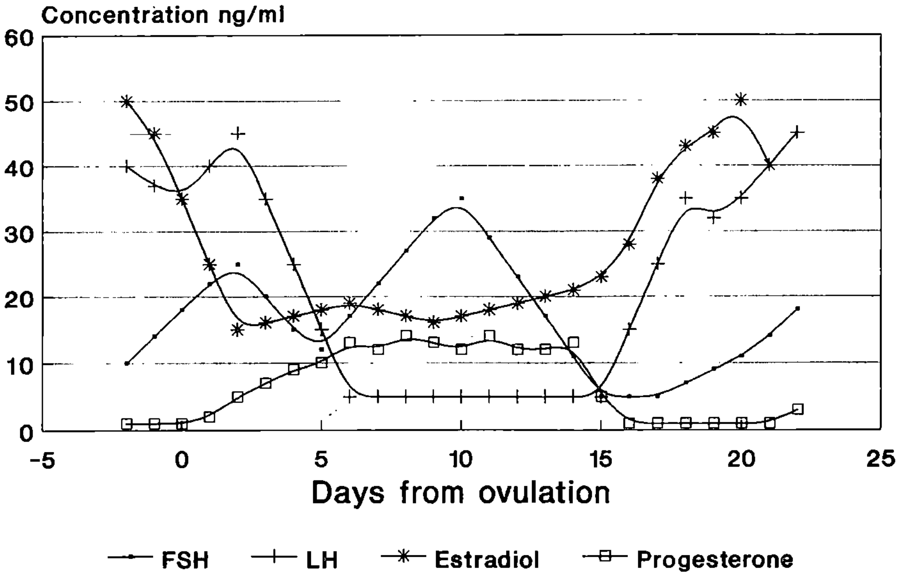
to discover for how long time after ovulation an egg can remain capable of being fertilized.

There is ample evidence to describe mares as seasonal breeders. In Winter mares usually exhibit sexual inactivity. The gonadal steroid levels are low resulting in endometrial atrophy and small ovaries without follicles. Oestrus signs are rare. In Spring mares are in transitional stage. Ovaries increase considerably in size. Hormonal activity increases and follicles appear in ovaries. Finally one follicle starts to grow faster than other follicles and after reaching a size of 40—50 mm ovulates. Oestrous cycles begin. Regular changes in hormone concentrations can be measured in serum as shown in the hormonal scheme.

The effect of season on the oestrous cycle is manifested in mares in three ways (LOY 1968): 1) A minority of mares are continuously polyestrous, cycling fairly regularly throughout the year. 2) Most mares are seasonally polyoestrous, with definite cyclic and non-cyclic periods during the year. 3) Some mares are seasonally polyestrous, exhibiting erratic reproductive patterns such as ovulation without oestrus or oestrus without ovulation. In the northern hemisphere the physiological breeding season starts in late April or early May and peaks in late June or early July (KENNEY et al. 1975). However, because of the official birth date of 1 January and claims regarding better performances by early foals in sales and first races, breeding early in the year has become common in all countries of current major equine importance.

Breeding early in the year has led to problems. A considerable amount of unproductive time and effort is devoted to teasing and examinations in January, February, March and even April (KENNEY et al. 1975). Mating early in the year leads to foalings in late Winter and Spring, usually a time of sexual repose in mares. The time from parturition to first ovulation increases if foaling occurs early (LOY 1980). Work relating to diagnosis of ovulation is increased.

Hormonal changes in serum during the oestrous cycle in the mare



Adapted from Neely (1983)

It is important for clinicians to know about the timing of foal-heat ovulation, and especially about any influence of season on such ovulation. There are, however, only a few published reports on the interval between parturition and first ovulation, fewer than reports on numbers of days to first signs of oestrus, as the review by BELLING (1983) shows.

The high demand for foals born early in the year has led to pressures to shorten Winter anoestrus. Various kinds of hormone treatments have been tried, for instance progesterone withdrawal therapy (ALLEN et al. 1980) or administration of GnRH (TURNER and IRVINE 1990). However, artificially long days have yielded the most promising results (PALMER et al. 1982). Long-day treatment from around the time of the winter solstice is common nowadays, e.g. in the UK (ALLEN 1985) and US (LOY 1990). Mares begin cycling between 6 and 12 weeks after the start of light therapy (ALLEN 1985). In Finland, long-day treatment has not yet been widely used. It is not known whether light treatment in Finland could have different effects in different breeds (e.g. Finnhorses and Finnish warm bloods) or whether or not it's outcome could be altered by the depth of anoestrus resulting from the dark, long winter in Finland.

The percentages of ovulatory oestrous periods in studies based on rectal palpation are probably lower than those in studies based on progesterone determinations. In one study, 20 % of first increases in progesterone concentrations were not associated with a diagnosis of ovulation on rectal palpation (PALMER and JOUSSET 1975). Serum progesterone determinations are useful in oestrus and ovulation control. Progesterone levels increase within 24—36 hours of ovulation. (STABENFELDT et al. 1972, PLOTKA et al. 1972, ALLEN and HADLEY 1974, GINTHER 1979). Progesterone measurements once a week can be used to detect the luteal phase after the Winter anovulatory season, or after parturition. In addition, because progesterone concentrations increase after ovulation in the mare, frequent analyses can also be used to allow it to be decided when matings should cease, and, for instance, to determine the time of flushing in embryo transfer programmes. This requires accurate information on progesterone concentrations after ovulation. To be accurate to within 6 hours, mares need to be rectally palpated and samples need to be taken 4 times a day but this has not been done in most of published studies.

One problem relating to equine reproduction is that the availability of semen from good stallions is often limited. The number of services per pregnancy should therefore be as low as possible. Mares are usually mated every other day during oestrus, until ovulation. If ovulation could be accurately predicted, the number of services would approach unity. Prediction of ovulation is usually based on the size and consistency of the preovulatory follicle as evaluated by rectal palpation and ultrasonic scanning (GREENHOFF and KENNEY 1975, PIERSON and GINTHER 1985). Oestrogen and progesterone analyses can also help in detecting ovulation. Oestrogen seems to peak two days before ovulation (PALMER and TERQUI 1977, MAKAWITI et al. 1983). Although the size of the preovulatory follicle is commonly regarded as the best criterion, there are no certain ways of predicting times of ovulation (GINTHER 1986).

Another possibility of reducing numbers of services is mating of mares after ovulation. Conception can be brought about by natural or artificial insemination after ovulation (SALTZMAN 1939, ZIVOTKOV 1940, HUGHES and LOY 1970, ALLEN 1981, BELLING 1984). However, postovulatory breeding requires frequent examination of mares, to allow the time of ovulation to be detected. Because continuous palpation is impracticable, accurate information on how late mares can be mated after ovulation for an acceptable conception rate and minimization of side effects such as early embryonic death is needed.

REVIEW OF THE LITERATURE

1. Seasonality

1.1. Non-foaling mares

As in other seasonal breeders, the seasonal regulation of reproduction in the mare ensures that the young are born in environmental circumstances optimal for their survival (LOY 1967).

LOY (1968) reported that constant ovulatory oestrous cycles occurred from April to September. From January to February only 20—25 % of mares cycled. Van NIEKERK (1967) kept 75 light farm-type mares on grass in South Africa. Although the mares exhibited signs of oestrus fairly constantly throughout the year, ovulation occurred mainly in the Summer. Results fairly similar to those of van NIEKERK were recorded in the study of SHARP (1980). OSBORNE (1966) showed, in a large slaughterhouse study in Australia, that about 20 % of mares ovulate during winter. During Spring months, the frequency of ovulation increases. A maximum is reached at the beginning of Summer. Similar figures relating to slaughterhouse material have been reported from the United Kingdom by ARTHUR (1958) and from the Nordic area by NYBORG (1953).

There are few reports concerning the seasonality of mares at the equator. However, SALTIEL et al. (1982) reported that mares also exhibit a seasonal reproductive pattern near the equator, at latitudes between 15° and 22° N. Although convincing evidence for differences in the onset of cycling attributable to latitude is apparently not available, GINTHER (1979) concluded that the peak incidence of foaling in Standard-bred mares occurred one month earlier at latitudes below 40° than at latitudes above 40°.

1.2. Foaling mares

Mares usually exhibit their first signs of oestrus one week after parturition (BELLING 1983). In 1984, BELLING reported that foal-heat ovulation occurred between 9 and 30 days post partum. The time from parturition to first ovulation has been reported to be 13.3 days in six thoroughbred mares (LOVELL et al. 1975), and 12.3 days in six mares of mixed breed (NODEN et al. 1978). LOY et al. (1975) examined 7 mares, 4 of which ovulated around day 11 post partum. Three mares failed to exhibit oestrus or to ovulate within 3 weeks of foaling. In 1980, LOY reported that 43 % of 470 mares had ovulated by day 9 post partum, 95 % by day 15, and 97 % by day 20.

MATTHEWS et al. (1967), in Australia, observed no differences in time of onset of foal-heat during (Australian) Spring, from September to November. On the other hand, in large pony mares GINTHER (1974) found the mean length of oestrus to be statistically significantly shorter in the Summer months of July and August than in Spring or early Summer months, from April to June.

In a clinical study, LOY (1980) showed that the time from parturition to first ovulation is influenced by the foaling month. Ninety-seven per cent of mares had ovulated by day 20 (mean 10.2 ± 2.4 days) but there was an apparent shift from longer intervals from January to February to shorter intervals in May. Intervals of more than 14 days occurred mainly from January to March (in 37 out of 40 cases). The results of PALMER and DRIANCOURT (1983) were broadly similar to those of LOY.

PALMER and DRIANCOURT also found that the incidence of ovarian inactivity after the first post partum ovulation increased early in the breeding season. However, parturition was regarded as a powerful stimulus to ovulation in early foaling mares which would not otherwise have completed the cycle (LOY 1980, PALMER and DRIANCOURT 1983).

1.3. Artificial illumination

BURKHARDT demonstrated in 1947 the effects of light on ovarian activity in anoestrous mares. Since then, the ovulatory season has been changed from early Summer to early Spring by exposure of mares to artificial light for a few months before mating (LOY 1968, COOPER and WERT 1975, KOOISTRA and GINTHER 1975).

It has been suggested that the increase in the time of exposure to light inhibits the pineal secretion of melatonin, thus stimulating GnRH secretion, one of the first events leading to the ovulatory season (SHARP 1988).

Exposure of anovulatory mares to light for 15 to 16 hours a day has been found effective (FREEDMAN et al. 1979, SCRABA and GINTHER 1985). On the other hand, PALMER et al. (1982) found a one-hour photosensitive phase in the morning, 9.5—10.5 hours after dusk, during which light is necessary for effective stimulation.

According to SCRABA and GINTHER (1985), the most effective time of the year for light treatment to be started is the beginning of December. Mares usually begin cycling between 6 and 12 weeks after the start of light therapy (LOY 1968, ALLEN 1985).

Light intensities have varied between trials. According to OXENDER et al. (1977) one 250-watt bulb in each stall is effective. Oxender measured a light intensity of 160 lux at a height of 150 cm in the centre of the box. LOY (1968) recommended a 200 W bulb in each stall, or at least two footcandles of light.

Early breeding of horse mares leads to foaling during Winter, a time of deep anovulation in many mares. There is a risk of further conception being delayed because mares sometimes become anovulatory immediately after foaling or after the first postpartum ovulation (PALMER and DRIANCOURT 1983) or, more often, because the average time from foaling to first ovulation is longer during early Spring than during Summer (LOY 1980). It has been shown that such risks can be overcome by exposing pregnant mares to light from the beginning of the 10th month of pregnancy (PALMER and DRIANCOURT 1983).

2. Prediction of ovulation

Prediction of the time of ovulation in the mare has long been a problem (ANDREWS and McKENZIE 1941). According to HUGHES et al. (1972), it is easiest to judge when to mate a mare on the basis of behaviour of the mare when she is teased by the stallion, the condition of the cervix when palpated and viewed using a speculum, and follicle development in the ovary as determined by palpation. Although follicle development is now often followed using ultrasonography, there is no visible predictor of impending ovulation (PIERSON and GINTHER, 1985).

2.1. Follicle growth

The preovulatory follicle becomes the largest follicle one week before ovulation. It grows by 3—5 mm a day until ovulation takes place (HUGHES et al. 1975, GINTHER 1979, NEELY 1983, PIERSON and GINTHER 1985, PALMER 1987).

The diameter of the ovulating follicle varies from 2 to 10 cm (GREENHOFF and KENNEY 1975) but is, on average, about 4 cm (ANDREWS and McKENZIE 1941, GINTHER & PIERSON 1984). PALMER (1987) reported that ovulation often occurs 5 days after a diameter of 30 mm is attained. Palmer also noted a linear growth rate of 3 mm/day in the preovulatory follicle, followed by a plateau, and a slight decrease in size near ovulation.

The effect of season on follicles the day before ovulation was studied in 102 non-foaling riding-type horse mares by GINTHER and PIERSON (1989). They found statistically significant differences in mean diameters of preovulatory follicles in April (46 mm) and May (48 mm) as compared to July (40 mm). More recently, GINTHER (1990) has reported that the ovulatory follicle at the end of the transitional period grows more slowly, by 2.6 mm a day, than that before the second ovulation of the year (3.6 mm a day).

2.2. Follicle consistency

Softness of a preovulatory follicle on palpation has frequently been proposed as a criterion of impending ovulation. In ARTHUR's opinion (1969) softness is the best clinical sign of imminence of ovulation. KLUG and ANDRES (1987) found a soft follicle on palpation in 79 % of mares within 12 hours before ovulation. Although Parker, in 1971, as cited by GINTHER (1979), found a very soft follicle on palpation in 40 % of mares 12 hours before ovulation, a soft follicle in 51 % and a turgid follicle in 9 % of cases, he concluded that the degree of softness was not a reliable basis for predicting times of ovulation in individual mares.

2.3. Follicle shape

Follicular softening can be associated with a change in follicular shape (GINTHER and PIERSON 1984, KÄHN and LEIDL 1987, MCKINNON et al. 1987). Large follicles impinge on the ovulation fossa because bands of connective tissue radiate towards the fossa and can provide boundaries forcing piriform follicular gowth (PRICKETT 1966). PIERSON and GINTHER (1985) reported that 85 % of follicles exhibited a pronounced change in shape, from approximately spherical to nonspherical (pear-shaped or conical) at some time during the preovulatory period.

2.4. Side of ovulation

Horse mares ovulate slightly more commonly from the left ovary, usually in 50—60 % of cases (ANDREWS and MCKENZIE, 1941; OSBORNE, 1966; BELLING, 1984). In pony mares, the ovarian activity tends to be similar in both sides (WESSON and GINTHER 1981).

2.5. Oestrogen measurements

In the mare, the presence of oestrogens has been reported in urine by ZONDEK (1930); in follicular fluid by KNUDSEN and VELLE (1961) and by SHORT (1961); and in plasma by NETT et al. (1973) and PATTISON et al. (1974). It has been suggested that luteal tissue also produces conjugated oestrogens (DAELS et al. 1990). The major oestrogen in equine follicular fluid is 17 β-oestradiol (E2), which is not metabolized further once it has entered the follicular fluid (SHORT 1961) but is metabolized in liver to oestrone (E1), the main oestrogen excreted by the mare in the urine. Because oestrogens are not stored in the body, their urinary excretion rates are thought to be good indices of follicular secretion rates (HILLMAN and LOY 1975). RAESIDE et al. (1990) has reported that modern methodology allows faecal samples to be used for E1 analysis and diagnosis of pregnancy in the mare. Although methods of determination of conjugated oestrogens usually involve several time-consuming steps, fast and, consequently, more practical methods have been reported (FRANEK et al. 1988).

Positive feed-back effects of the oestrogens are thought to be important in regulating LH secretion in the mare. Although free E2 seems to be most effective, certain conjugated oestrogens may also be important for regulation of LH secretion (BALDWIN et al. 1990).

Both urinary and plasma oestrogen measurements have been used in attempting to predict ovulation. Urinary oestrogen peaks within three days before ovulation (HILLMAN and LOY 1975, PALMER and JOUSSET 1975). Plasma E2 concentrations are highest 2 days before the LH peak (PATTISON et al. 1974) or 24—36 hours before ovulation (PIPKIN et al. 1987), E2 and androstenedione concentrations 2 days before ovulation (NODEN et al. 1975). Total oestrogens are at levels 100 times higher than those of E2 and seem to peak 2 days before ovulation (PALMER and TERQUI 1977, MAKAWITI et al. 1983).

3. Milk and serum progesterone levels

As early as 1959, SHORT measured elevated progesterone (P4) concentrations in the plasma of midluteal non-pregnant mares. In 1964 he found significantly increased P4 secretion into the ovarian vein within the first 24—36 hours after ovulation.

In 1968 van RENSBURG & van NIEKERK discovered P4 18 hours after ovulation, in a histological study of the ruptured follicle wall.

SMITH et al. (1970) demostrated a cyclic pattern of peripheral plasma P4 concentrations in the mare. In that study, the first increases in P4 levels occurred 2—3 days after ovulation.

In general, P4 plasma concentrations seem to increase on the second day following ovulation (STABENFELDT et al. 1972, PLOTKA et al. 1972, ALLEN and HADLEY 1974). More recently PIPKIN et al. (1987) have shown that the mean serum P4 concentration increased ($p<0.05$) within 12 hours of ovulation.

P4 patterns have been reported to be similar in milk and plasma (HUNT et al. 1978, GUNTHER et al. 1980).

4. Postovulatory mating

Mating 24 to 36 h before ovulation has been regarded as optimal in mares (DAY 1957). However, it has also been reported that an ovum remains capable of being fertilized over various times, ranging from 4 to over 48 hours, after ovulation (CHENG 1961, ROSSDALE and RICKETTS 1980). Daily examination, allowing insemination 0—24 hours after ovulation, has resulted in pregnancies in 70—75 % of cases (SALTZMAN 1939, ALLEN 1981, BELLING 1984). Insemination with frozen semen up to 12 or 24 hours after ovulation has resulted in pregnancy rates of 63 and 67 %, respectively (ALIEV and OCHKIN 1979).

MATERIAL AND METHODS

Data was collected at the Finnish Agricultural Research Centre, Equine Research Station and at six trotting stables in southern Finland, at a latitude of 61° N, between 1985 and 1989.

1. Animals in the Equine Research Station (ERS)

The ERS houses 200 to 250 horses, mostly Finnhorses. Throughout this thesis, other breeds are called warm-bloods. These equate to standardbred and half-bred riding-horses such as German Hannoverians. There are many types of horse in the ERS: brood mares, stallions, foals, colts, fillies, and horses in training. The horses live in three heated stables, one for brood mares, in separate stalls. However, 2 to 3 year-olds not yet in training are kept as herds in unheated barns.

The daily care of the horses begins between 6.00 and 6.30 a.m. and ends between 6.00 and 7.00 p.m. Usually, all horses are exercised daily. They are either trained or put into paddocks. Horses are fed according to the recommendations of the Finnish Board of Agriculture as reported by PELTONEN (1986).

2. Animals in trotting stables

In the six trotting stables, the horses were warm-blood trotters, mainly American standard-bred. They were only trained and raced. Daily care began between 6.00 and 6.30 a.m. and ended between 6.00 and 7.00 p.m. The horses were fed in accordance with the nutritional needs of horses in training.

3. Summary of materials and methods

Table 1.

Paper	Number of mares	Subject of investigation	Samples and examinations
		Seasonality	
I	186	Frequency of anovulatory season, onset of cycling	Weekly serum P4 determination for 5 months
II	55	Time from parturition to first ovulation,	Milk P4 determination, 3 times a week
	21	Growth of preovulatory follicle in foal-heat	Rectal palpation, ultrasound scanning
		Long day treatment	
III	37	Onset of cycling,	Weekly serum P4 determination
	27	Time from parturition to first ovulation	Daily rectal palpation, ultrasound scanning
	14	Foaling rate	Insemination
		Prediction of ovulation	
IV	30	Prediction of ovulation in accuracy of 6 hours	Rectal palpation, ultrasound scanning every 6 hours, daily P4 (serum and milk) and serum E1 determination
		Post-ovulatory progesterone levels	
V	24	Increase in P4 levels in serum and milk in accuracy of 6 hours	Serum and milk samples for P4 determination every 6 hours
		Post-ovulatory breeding and early embryonic death	
VI	44	Conception in post-ovulatory insemination,	Post-ovulatory insemination
	44	Early embryonic death after post-ovulatory insemination	Ultrasonographic pregnancy diagnosis

4. Progesterone radioimmunoassay

Progesterone concentrations in mare's serum and whole milk were determined using direct radioimmunoassay (RIA).[1] Standards were prepared in buffer when the serum progesterone kit was used, and in nonluteal phase mare's milk when the milk progesterone kit was employed. Antiserum was produced by immunizing rabbits with BSA-11α-progesterone. Its cross-reactivity with other endogenous pregnene derivates and related steroids was negligible. Bound and free antigen were separated by precipitating antibody-antigen complex with a second antibody (sheep anti-rabbit IgG) and polyethylene glycol.

5. Seasonality

5.1. Non-foaling mares

In the ERS, records relating to mares were divided into three categories, depending on their use and stable conditions:

1. Three-year-olds (N = 38) not yet in training, stabled in an unheated barn
2. Mares in training (N = 47, mean age 4.9 years, range 4 to 8 years)
3. Brood mares (N = 21, mean age 9.5 years, range 5 to 23 years) kept in separate, heated stables, in individual boxes.

The study was conducted over several years but only the first record for each mare was included in each category. The data covered Finnhorses and warm-bloods.

In the trotting stables, the mares (N = 73, mean age 5.1 years) were American standardbreds. They were used only for training and racing and housed in separate boxes in stables.

The onset of cycling was based on blood samples collected once a week for progesterone determination, beginning in January. Two subsequent samples with elevated (> 10 nmol/l) progesterone concentrations were considered as the onset of cycling.

5.2. Foaling mares

First records relating to 55 foaling Finnhorse mares were analysed. The mean age of the mares was 10.0 years (range 4 to 22 years). They had foaled 1 to 10 times, but 55 % of the mares were foaling for the first time. Milk samples were collected for progesterone determination three times a week to allow the time from parturition to first ovulation to be assessed. Milk progesterone concentration of over 2 nmol/l, and higher concentrations in subsequent samples were considered to indicate the beginning of luteal activity.

5.3. Post partum ovarian development

During Spring 1985 the ovaries and follicles of 21 Finnhorse mares (mean age 13.1 years, range 4 to 23 years) were examined by rectal palpation and ultrasonic scanning[2] on the second and fifth days post partum and then every other day until first ovulation. The greatest diameter of the ovaries was estimated digitally, during rectal palpation. The diameter of the largest follicle in each ovary was measured ultrasonically.

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[1] Direct SPECTRIA, Farmos Diagnostica, Turku, Finland.

[2] Aloka SSD-210 DX-scanner, 5 MHz.

6. Artificial illumination

Light therapy for non-foaling and foaling mares began in the first week of December and lasted until Summer. In the case of non-foaling mares records relating to 37 anoestrous mares, 28 Finnhorses and 9 warm-blood mares, were used. The 13 youngest were 3-year-olds not yet in training (12 Finnhorses, one warm-blood). The mean age of the mares over three years old was 10.3 years (range 4 to 18 years). Five mares were lactating at the beginning of the trial (one Finnhorse mare, four warm-blood mares).

The intensity of light was 70 to 150 lux. The lights went on at 6.00 a.m. and were turned off at 20.30, i.e. there were 14.5 hours of light and 9.5 hours of darkness. Onset of cycling was determined by analysis of weekly serum progesterone samples.

During long-day treatment in March 1988, 14 mares were inseminated with a stallion of known fertility in their second oestrus of the year.

Twenty-seven pregnant mares (22 Finnhorses, 5 warm-blood mares) were subjected to long-day treatment. Because the first mare foaled at the end of January and the last at the beginning of June 1989, the length of light treatment pre partum varied from two to six months. The mean age of the mares was 11.7 years (range 4 to 20 years). Seven mares were nulliparous. The remaining 20 mares had foaled on average 3.9 times (range 1 to 10 times). The first ovulation after foaling was detected by daily rectal palpation and ultrasonic scanning. The day of disappearance of the ovulatory follicle was regarded as the day of ovulation.

7. Prediction of ovulation

Thirty Finnhorse mares were studied, in relation to 38 oestrous cycles. The average age of the mares was 9.1 years (range 3 to 23 years) and the average number of foalings was 3.0 (range 1 to 10). The study was conducted from June to August. Foal heats were excluded. The start of the follicular phase was detected by progesterone analysis of thrice weekly milk or serum samples. On detection of a mature follicle (diameter ≥ 3.5 cm), rectal palpation and ultrasound scanning were performed every 6 hours, to determine the time of ovulation to within 6 hours. There were three cases of double ovulations. The degree of follicle maturity was evaluated by fluctuation, softness, and how far the follicles protruded above the ovarian surface and the sharpness of their shoulders (GREENHOFF and KENNEY, 1975). The diameter of the preovulatory follicle was measured from the ultrasound scan.

Blood samples for oestrone sulphate (E1) determination were collected daily, beginning in late dioestrus or early oestrus. E1 was determined using Sephadex-RIA, as described by FRANEK et al. (1988).

8. Milk and serum progesterone measurements

Twenty-four Finnhorse mares were studied. Their average age was 9.8 years (range 4 to 23 years). The mean number of foalings was 3.5 (range 1 to 10). The mares had foaled between mid-April and late July. First post partum oestruses were excluded. The start of the follicular phase was detected by progesterone analysis of thrice-weekly whole milk and serum samples. Daily rectal palpation began once the progesterone level had fallen to 1 nmol/l. On detection of a mature follicle (diameter ≥3.5 cm) rectal palpation and ultrasound scanning were performed every 6 hours, to determine the time of ovulation to within 6 hours. In two cases of double ovulations, which occurred 6 and 18 hours apart, the earlier ovulation was excluded. Milk and blood samples were collected simultaneously every 6 hours, for up to 3 days after ovulation, in some mares for longer, until progesterone concentrations increased markedly.

9. Postovulatory breeding

In one experiment, 11 recently foaled Finnhorse mares (mean age 8.5 years, range 4 to 23 years, mean number of foalings 3.1, range 1 to 10) were inseminated after ovulation over 1, 2 or 3 cycles but not during foal heat. On detection of a mature follicle (diameter ≥3.5 cm), rectal palpation and ultrasound scanning were undertaken every six hours, to determine the time of ovulation. Insemination was carried out 6—12 hours, 12—18 hours, 18—24 hours and 24—30 hours after ovulation. There was one case of double ovulation in these mares. The time between the ovulations was 6 hours and the mare was inseminated 24—30 hours after the latter ovulation. The mares were examined for pregnancy 16 and 21 days after insemination. All pregnancies were terminated by prostaglandin (PG) injection at 21 days. Some mares were inseminated again during subsequent cycles.

In a second experiment, 14 mares (12 maiden three-year-olds and two five-year-olds) were examined every day at about the same time in the forenoon and inseminated in the afternoon, if ovulation had occurred, i.e. within some 0—27 hours of ovulation. Pregnancies were terminated by PG injection on day 25 and the animals inseminated again during PG induced oestrus. Another 19 mares were inseminated daily until ovulation. These were used as a comparison group.

10. Statistical methods

Statistical analysis of postovulatory increases in serum progesterone concentrations was conducted using the paired t-test. Progesterone concentrations in samples collected during 0—6 hours after ovulation were compared with subsequent concentrations in the same individual.

The frequency of anoestrus in mares with foal at foot versus maiden mares was analyzed. Variance was analyzed,[3] with dependent and independent variables as follows:

Model	Dependent variable	Independent variables
1	Weeks from Jan 1st	Age
2	Weeks from Jan 1st	Use of mares
3	Weeks from Jan 1st	Mare
4	Days post partum	Season
5	Weeks light treatment	Breed
6	Weeks light treatment	Age
7	Weeks light treatment	Lactation
8	Days post partum	Season
9	Ovarial diameter	Side of ovulation
10	Follicular diameter	Type of ovary (ovulatory or not)
11	Oestrogen concentration	Day of cycle

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[3] CATMOD and GLM procedure in SAS, SAS Institute Inc., SAS Circle, Box 8000, Cary, NC 27512-8000, U.S.A.

RESULTS

1. Seasonality

1.1. Non-foaling mares (I)

In non-foaling mares, 23.7 % of mares cycled throughout the year. Increasing numbers of anoestrous mares began to cycle from the end of February. The last mares to enter the ovulatory season did so at the beginning of June (Fig. 1). Mares which had been in winter anoestrus ovulated for the first time, on average, in the middle of April, 16.8 ± 4.6 weeks from the beginning of the year.

In the all groups studied in the ERS, Finnhorses cycled later than warm-blood mares but the difference was not statistically significant (Fig. 2).

In Finnhorse mares which had foaled the previous year, old brood mares tended to cycle later ($0.05 < p < 0.10$) than young brood mares (model 1, Fig. 3).

The effect of use was evident in two ways. Forty per cent of Finnhorse mares in training cycled throughout the year as compared to no mare not in training. Anoestrous mares in training exhibited significantly earlier onset of cycling after winter anoestrus than three-year-olds or brood mares not in training (Fig. 4). On average, three-year old Finnhorse mares started to cycle three weeks later ($p < 0.01$) and Finnhorse brood mares five weeks later ($p < 0.01$) than mares in training (model 2).

All 12 5-year-old Finnhorse mares which had foaled the previous year were anoestrous in

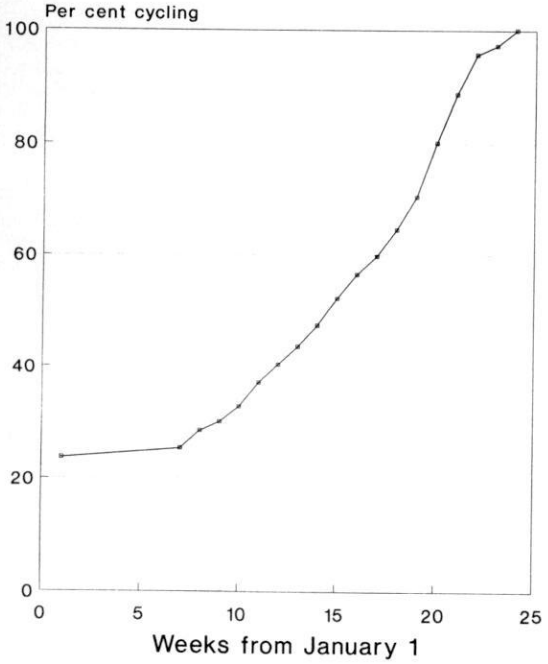

Fig. 1. Cycling of mares in seven stables in southern Finland on the basis of high progesterone concentrations in weekly blood samples. Mares (N = 186) were of different breeds, ages and were being used in different ways. Week 9 = March 1st, week 22 = June 1st.

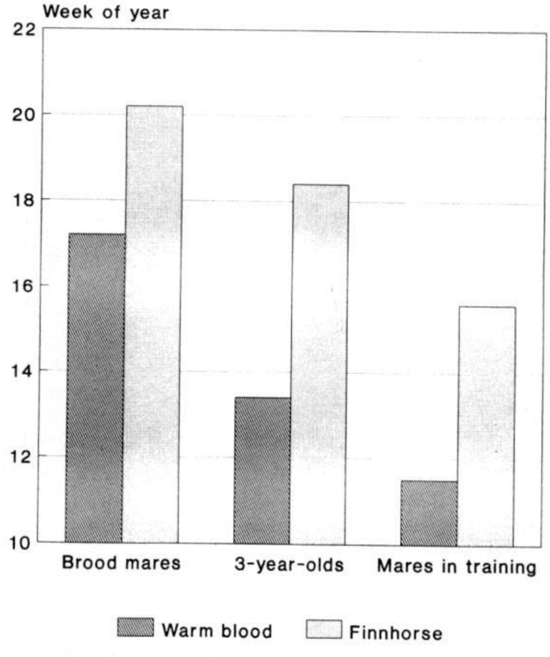

Fig. 2. Breed effect on onset of cycling in different groups of mares in the Equine Research Station. The categories of brood mares, three-year-olds and mares in training covered 5, 3 and 4 warm-bloods and 21, 35 and 28 Finnhorse mares, respectively ($p > 0.05$, N.S.).

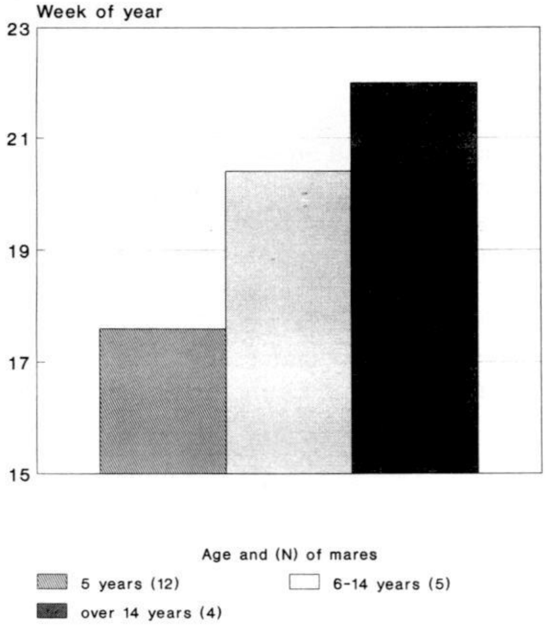

Fig. 3. Age effect on onset of cycling in different groups of Finnhorse mares which had foaled the previous year in the Equine Research Station ($0.05<p$ 0.10, N.S.).

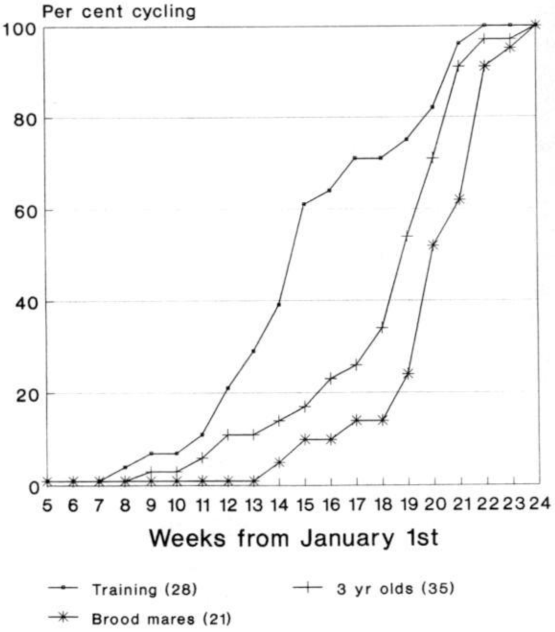

Fig. 4. Effect of different kind of use of Finnhorse mares in the Equine Research Station on onset of cycling ($p<0.01$).

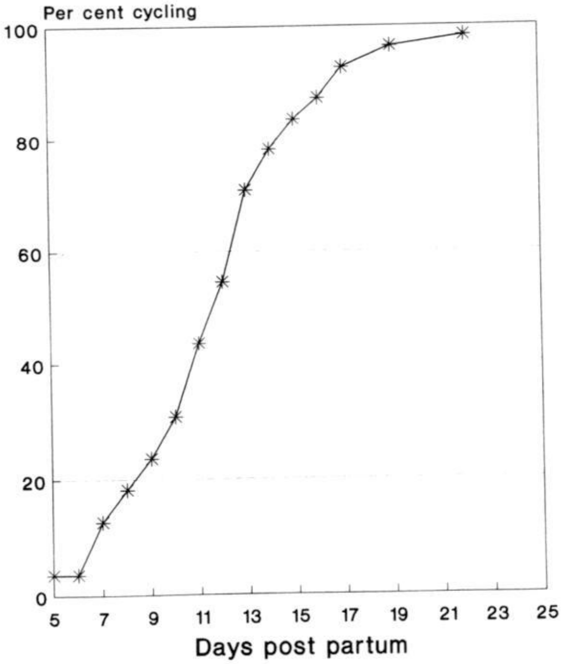

Fig. 5. Time from parturition to first ovulation in Finnhorse mares in the Equine Research Station, N = 55.

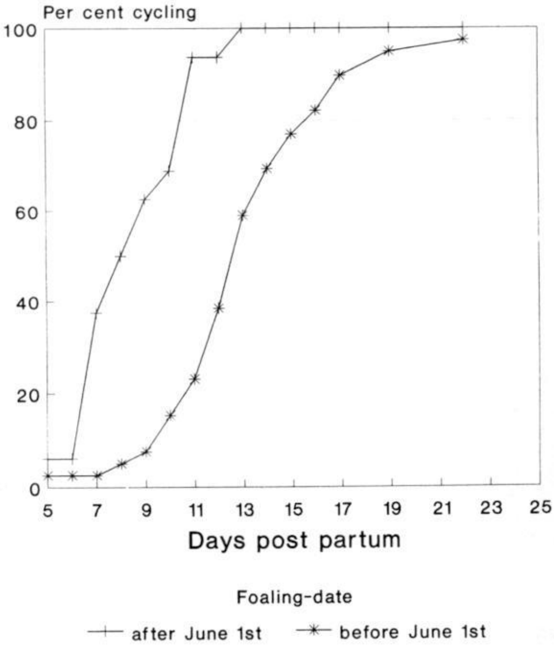

Fig. 6. Time from parturition to first ovulation in the Equine Research Station in Finnhorse mares which had foaled after June 1 (N = 16) or before June 1 (N = 39) ($p<0.001$).

Winter. Only 37 % (7/19) maiden mares of the same age cycled throughout the year ($p<0.01$).

The preceding seasonal pattern influenced the onset of cycling. Mares started cycling about the same time in Spring from year to year (model 3, $p<0.01$).

1.2. Foaling mares (II)

Over 95 % of Finnhorse mares ovulated by day 20 post partum (Fig. 5). However, 90 % of the mares which had foaled after 1 June first ovulated by day 11 post partum but 90 % of early foaling mares ovulated as late as by day 17 post partum (Fig. 6). On average, the time from parturition to first ovulation was shorter in Summer (8.8 ± 2.2 days) than in Spring (13.0 ± 3.1 days) (model 4, $p<0.001$). Intervals of 10 days or less were seen in 15.4 % of cases (N = 39) of foaling before 1 June but in 68.7 % of cases (N = 16) of foaling after 1 June. Intervals of over 16 days occurred mainly before May, in 6 out of 7 cases.

In post partum mares, the growth rate of the preovulatory follicle during foal-heat was faster in the case of foaling during the Summer (3.7 mm a day) than in Spring (1.8 mm a day) (Fig. 7).

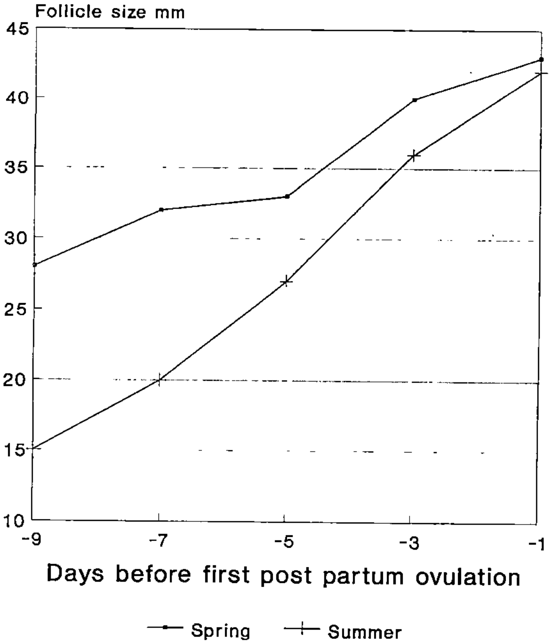

Fig. 7. Growth rate of foal-heat pre-ovulatory follicles in Finnhorse mares in the Equine Research Station during Spring (N = 7) and Summer (N = 13).

2. Artificial illumination (III)

2.1. Non-foaling mares

In the ERS, long-day-treated non-foaling mares ovulated for the first time, on average, during the second fortnight of February, 11 weeks after the beginning of light treatment. Mares first started to cycle at the end of January. All mares were cycling in the middle of March (Fig. 8).

Sensitivity to long-day treatment was influenced by breed and age.

Long-day treated 4—8 year old Finnhorses started to cycle, on average, 11.8 weeks after the beginning of the trial. Warm-bloods started to cycle 9.8 weeks after the start of the trial (model 5, $p<0.02$, Fig. 9).

Three-year-old Finnhorse mares cycled on average 10.5 weeks after the beginning of long-day treatment but mares aged 10 ± 4.6 years cycled 11.8 weeks after long day treatment began (model 6, $p<0.03$, Fig. 10).

Previous lactation slightly delayed the onset of cycling. Warm-blood mares which had had a foal the previous year began to cycle 10.8 weeks after the beginning of the trial. Mares which had not foaled started to cycle 9.8 weeks ($0.10<p<0.20$) after the beginning of the trial (model 7, Fig. 11).

Insemination of 14 long-day-treated mares during the second oestrus of the year resulted

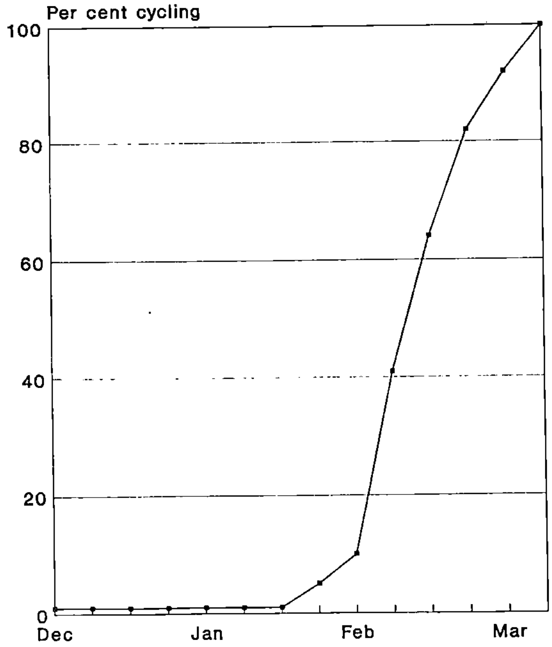

Fig. 8. Onset of cycling in anoestrous non-foaling mares after artificial long-day treatment from the beginning of December (N = 37).

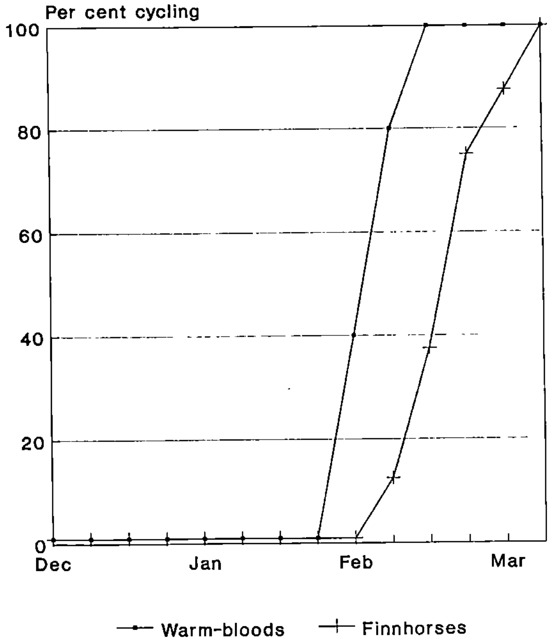

Fig. 9. Effect of breed on onset of cycling in anoestrous long-day-treated warm-blood mares (N = 5) and Finnhorse mares (N = 7) ($p<0.02$).

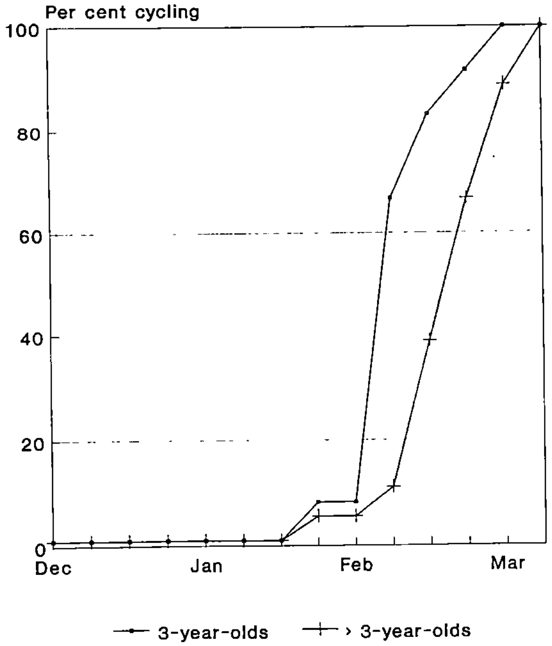

Fig. 10. Effect of age on onset of cycling in anoestrous long-day-treated Finnhorse mares (N = 12 in the group of 3-year-olds and 16 in the group over 3 year olds ($p<0.03$).

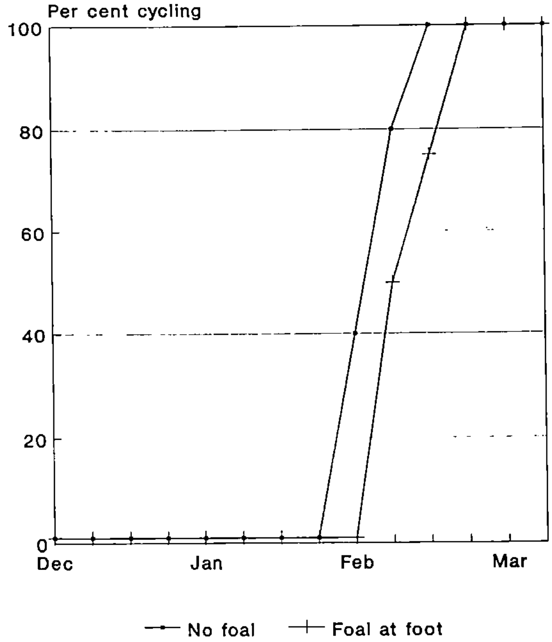

Fig. 11. Effect of previous foaling on onset of cycling in anoestrous, long-day-treated warm-blood mares (N = 5 and 4 in non-lactating and lactating mares, respectively) ($0.10<p<0.20$, N.S.).

in 12 foalings (86 %) during the subsequent year.

2.2. Foaling mares

The time from foaling to the first post partum ovulation was, on average, 17.0 days in long-day-treated mares foaling before 15 February, as compared to 12.1 days in long-day-treated mares foaling after 15 February (model 8, $p < 0.01$).

3. Sizes of ovaries and follicles post partum (II)

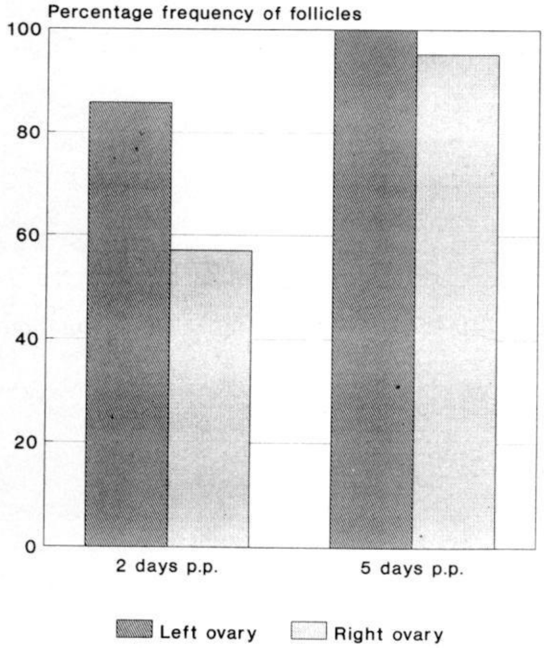

Fig. 12. Frequency of follicles over 10 mm of size in ovaries of 21 Finnhorse mares during the post partum period.

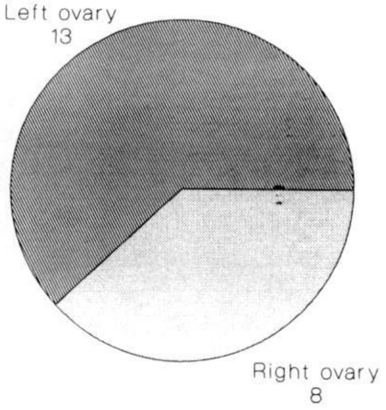

Fig. 13. Ovarian side of first ovulation after parturition in 21 Finnhorse mares.

Follicles over 10 mm in size were found more frequently in left ovaries than in right ovaries two and five days after parturition (Fig. 12).

The first post partum ovulation occurred in the left ovary in 13/21 (62 %) of cases (Fig. 13).

In post partum mares the ovulatory ovary was larger ($p < 0.01$) than the non-ovulatory ovary five days post partum (model 9, Fig. 14).

Five days post partum, the preovulatory follicle was larger ($p < 0.01$) than the largest follicle of the other ovary (model 10, Fig. 15).

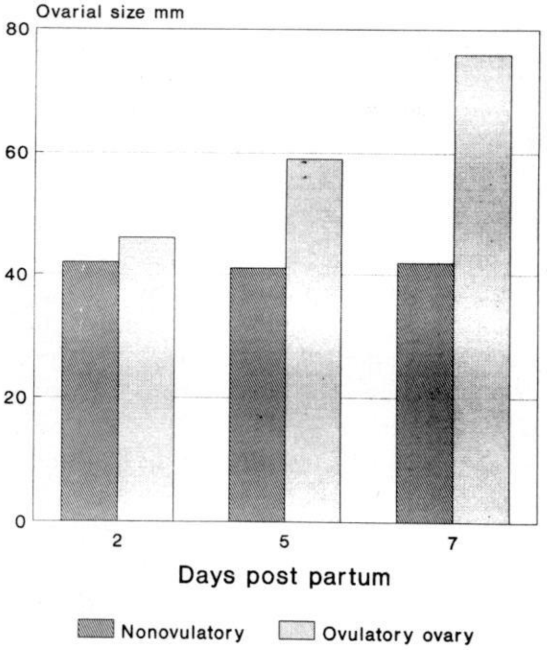

Fig. 14. Observations of ovarial size in Finnhorse mares (N = 18, 20 and 5 in examinations 2, 5 and 7 days after parturition, respectively).

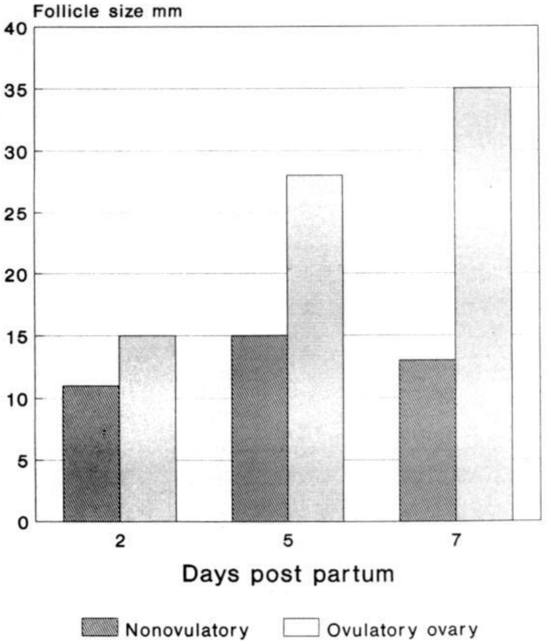

Fig. 15. Size of pre-ovulatory follicle and the largest follicle in the nonovulatory ovary in 21 Finnhorse mares after parturition.

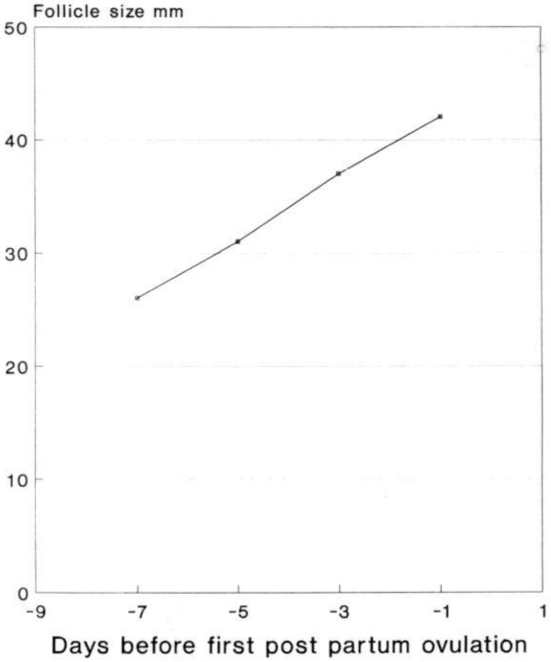

Fig. 16. Growth rate of the pre-ovulatory follicle during foal heat in 20 Finnhorse mares.

During the week before the first post partum ovulation the largest diameter of the preovulatory follicle increased from 26 mm to 42 mm. The growth rate was therefore on average 2.7 mm a day during foal heat, N = 20 (Fig. 16).

4. Preovulatory follicle in other oestrous periods (IV)

The preovulatory follicle grew by 3 mm a day from day 5 to day 2 before ovulation. After a plateau it diminished in size by 2—3 mm before rupturing (Fig. 17). The preovulatory follicle was greatest (43 ± 4 mm) 1—3 days before ovulation. Ninety per cent of follicles achieved a size of 40 mm.

Frequencies of ovulations in different six-hour periods throughout the day did not differ statistically significantly from each other. The frequency was highest in the evening (Fig. 18).

Ovulation other than foal heat ovulation occurred in the left ovary in 62 % of cases (Fig. 19).

Follicles were usually round in shape but some time before ovulation irregular shapes were seen in 89 % of cycles. However, only 26 % of the follicles appeared irregular 0—6 hours before ovulation. Kidney-shaped follicles with a depression in the centre were seen in 24 % of ovulations. A mature follicle was palpated in over 95 % of mares during the last 24 hours before ovulation.

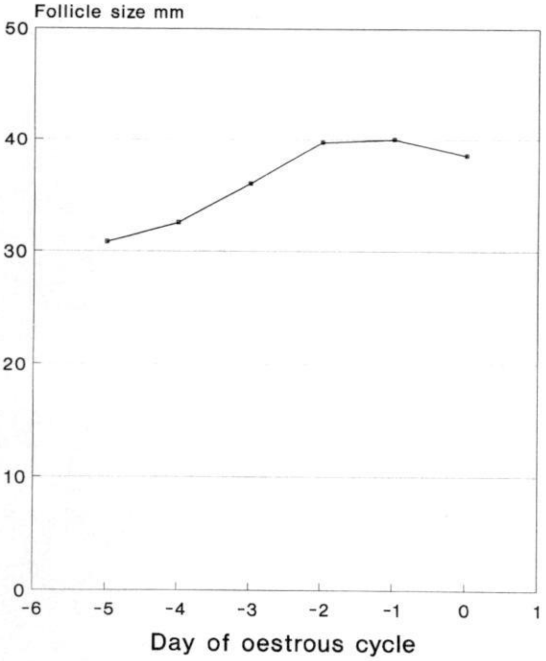

Fig. 17. Growth rate of the pre-ovulatory follicle in 12 oestrous cycles of Finnhorse mares. In post partum mares foal heat was not included.

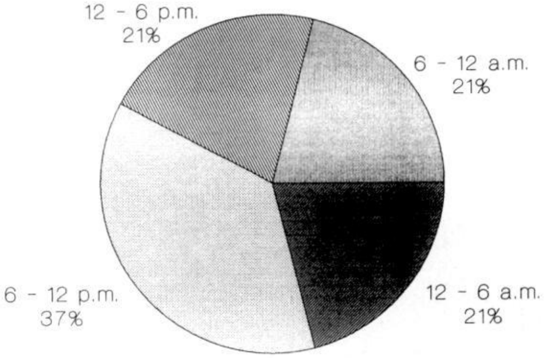

Fig. 18. Distribution of ovulations in 38 oestrous cycles of Finnhorse mares. In post partum mares foal heat was not included.

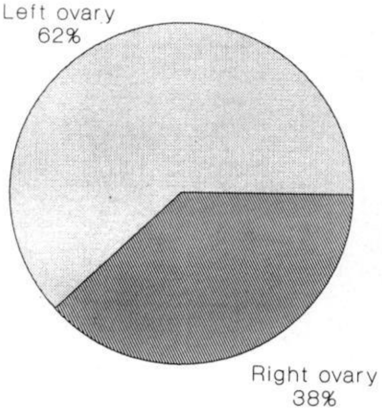

Fig. 19. Ovarian side of ovulations in 38 oestrus cycles of Finnhorse mares. In post partum mares foal heat was not included.

5. Serum oestrogen and progesterone concentrations before ovulation (IV)

Serum E1 concentrations peaked 1 to 2 days before ovulation. However, peak concentrations did not differ significantly from values on other days (model 11, Fig. 20).

The first decrease in E1 level occurred most commonly around ovulation (Fig. 21).

The concentration of serum progesterone decreased below the detection limit of the method 4.6 days (range 1 to 10 days) before ovulation.

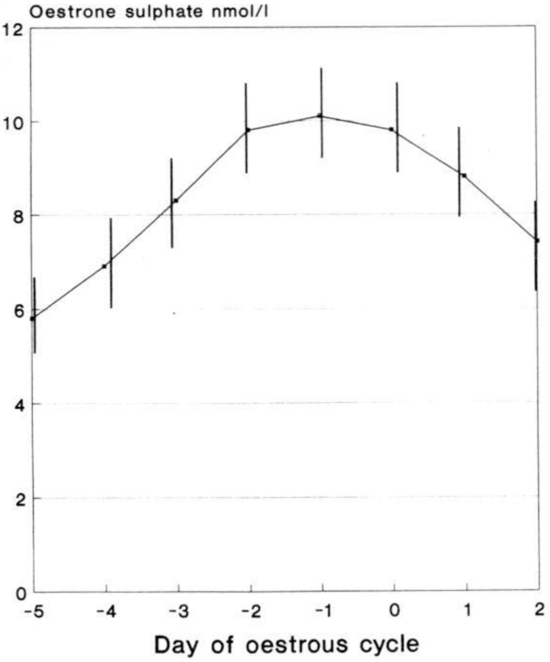

Fig. 20. Concentration of oestrone sulphate during 38 cases of oestrus in Finnhorse mares. In post partum mares foal heat was not included.

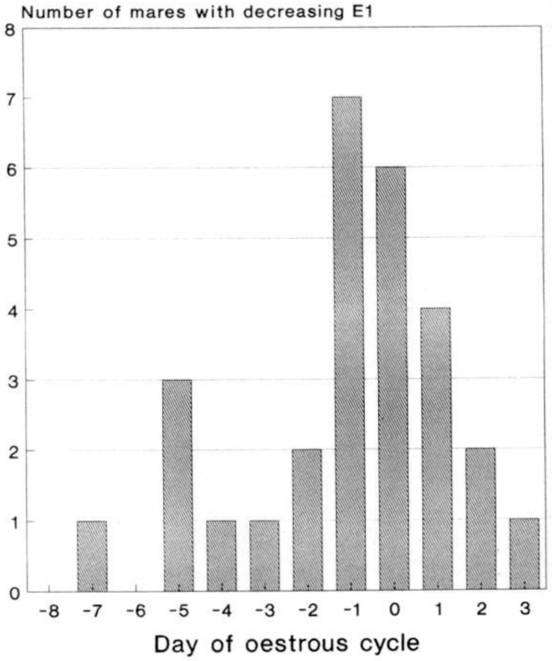

Fig. 21. Day of decrease in serum oestrogen (E1) level during 38 cases of oestrus in Finnhorse mares. In post partum mares foal heat was not included.

6. Prediction of ovulation (IV)

Follicle size was the best criterion for predicting the time of ovulation (Table 2). If insemination had been started when the diameter of the largest follicle was 3.6 cm, no ovulation would have been missed. If the decision had been based on the first low value for progesterone no ovulation would have been missed but the mares would have had one insemination more per oestrus.

Table 2. Comparison of different criteria for prediction of ovulation in mares (n = 35, double ovulations excluded)

Inseminations started on the first day when	Number of inseminations per ovulation when the last insemination was carried out		Percentage of ovulations missed	
	Before ovulation	Within 24 h of ovulation	When mated before ovulation	When mated within 24 h of ovulation
Diameter of largest follicle was >36 mm	1.9		0 %	
Diameter of largest follicle was >40 mm	1.5		9 %	
Serum progesterone <1 nmol/l	2.9		0 %	
Serum oestrone sulphate had started to decline during low progesterone phase	1.6	2.0	24 %	10 %

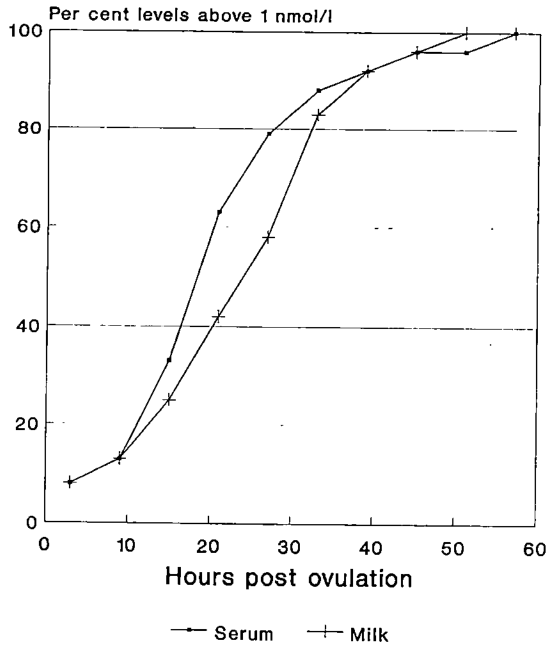

Fig. 22. Cumulative percentage frequency of progesterone levels above 1 nmol/l in 24 post partum Finnhorse mares.

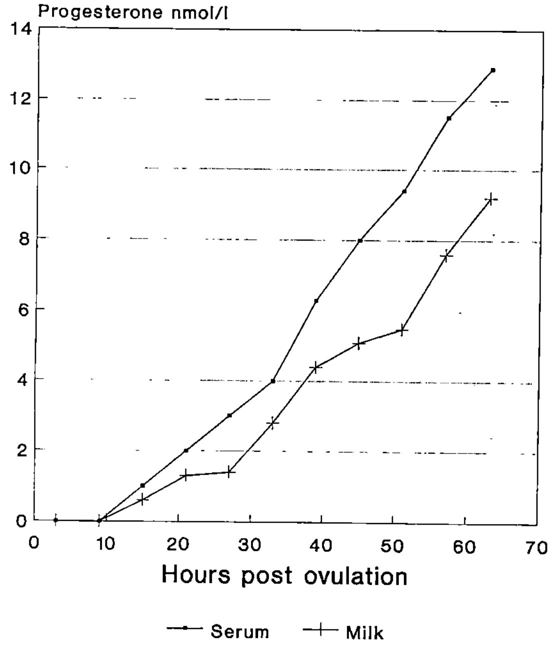

Fig. 23. Increase in serum and milk progesterone levels in relation to levels 0..6 hours of ovulation in 24 post partum Finnhorse mares.

7. Progesterone after ovulation (V)

Statistically significant increases in progesterone concentrations were measured for the first time, on average, within 18 to 24 hours (range 6 to 54 hours) of ovulation in milk and within 12 to 18 hours (range 6 to 60 hours) in serum (Fig. 22).

With the methods used in this study concentrations were consistently higher in serum than in milk (Fig. 23). The correlation coefficient between serum and milk progesterone concentrations was 0.7 ($p<0.05$).

8. Postovulatory breeding and early embryonic death (VI)

The conception rate was good up to 18 hours after ovulation. Two mares out of five conceived when inseminated 18 to 24 hours after ovulation. Mares did not conceive in inseminations more than 24 hours after ovulation.

Nearly half of the mares (13/28) which had been examined once a day and bred within 0 to 27 hours of ovulation conceived. However, early embryonic death (EED) between days 16 to 25 resulted in a final conception rate of 29 %. In the preovulatory control group, 15 out of 19 mares (79 %) conceived. No instances of EED were observed (Table 3).

Table 3. Pregnancy rates for mares.

Time of insemination (hours after ovulation)	Number of cycles resulting in pregnancy/numbers inseminated	Pregnancy rate at 16 days (%)	Early embryonic loss between days	
			16—21	21—25
Experiment 1				
6—12	5/5	100	0	
12—18	5/5	100	0	
18—24	2/5	40	0	
24—30	0/5	0	0	
Experiment 2				
0—27	13/28	46	4	1
Controls (preovulatory insemination)	15/19	79	0	

DISCUSSION

1. Materials and methods

1.1. Animals

In Finland there are currently some 15 000 Finnhorses, 15 000 warm-blood trotters (American standard-breds) and 6 000 warm-blood saddle horses. There are 5 000 ponies.[4]

The material which formed the subject of this thesis consisted mainly of Finnhorses. The horses in the ERS were previously almost exclusively Finnhorses, however, the percentage of warm-bloods is being increased to reflect more closely the distribution of breeds in the country. The low number of warm-blood mares in the ERS made comparisons between breeds difficult. In equine research it is always difficult to include more than a few tens of horses in any one study. On the other hand, horses in the ERS with regard factors such as age and use correspond well with the horse population in Finland in general. The ERS breeds, trains and races horses.

1.2. Examination methods

Reliable diagnosis of ovulation is of fundamental significance to studies of cyclicity and of determination of times of ovulation in mares. Possibilities to distinguish between anovulatory and ovulatory mares on the basis of rectal palpation varies from mare to mare. If a mare is easy to examine, the increased tonus of the uterus and cervix and the increased size of the ovaries and possible follicles indicate cycling or transitional phase from anovulatory season to ovulatory season. Rectal palpation is relatively laborious way of searching ovulatory mares in Spring as compared to taking serum samples for progesterone determination.

In a living animal, ultrasonography is the most accurate method for following follicular development and ovulation in ovaries (PALMER & DRIANCOURT 1980). Ovulation was confirmed by ultrasound scanning in all studies covered by this thesis except those dealing with seasonality in non-foaling and foaling mares.

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[4] Monthly Review of Agricultural Statistics. No 1. National Board of Agriculture. Helsinki 1990.

Appearance of the first signs of oestrus does not always coincide with the onset of the ovulatory season (BERLINER 1959). Nor it is possible to determine accurately the day of first post partum ovulation on the basis of external signs of oestrus, because of the large variation in lengths of foal heat (BELLING 1983). Progesterone levels, which relate well to the follicular and luteal phases of the ovarian cycle (PALMER and JOUSSET 1975) were determined in the studies covered by this thesis to investigate the onset of cycling after winter anoestrus in non-foaling mares and the time of first post partum ovulation in foaling mares.

1.3. Frequency of examinations

Blood samples were collected once a week for progesterone determination in the study of onset of cycling after winter anoestrus. GINTHER (1979) averaged results in several published reports and concluded that the average length of the luteal phase in the mare is 14.9 days (SD 2.8 days). It is highly probable that weekly progesterone sampling is frequent enough to differentiate cycling from non-cycling mares.

QUINLAN et al. (1951) has proposed that a period of over 45 days without ovulation might be termed an anovulatory period. In the studies reported here the onset of the anovulatory season was not investigated but, since investigations began at the start of January each year and cycling first began at the end of February, a distinct anovulatory period was evident in these mares.

Milk samples for P4 determination were collected three times a week in post partum mares to ascertain the day of ovulation. Published results usually relate to ovulation, not to elevations in progesterone concentrations. Because there is a delay of some 24 hours resulting from thrice weekly sampling and another delay of about 24 hours in the post ovulatory increase in milk progesterone concentration, ovulation was estimated to have taken place two days before any increase detected in milk progesterone concentration. Daily sampling would probably have improved the accuracy of the result.

Follicle development in post partum ovaries was examined on the second and fifth days and then every other day until ovulation. More accurate results relating to follicle development would have resulted from daily examination. In the long-day-treated mares, daily rectal examinations were carried out during the study relating to time of first post partum ovulation.

The animals were examined every six hours in the studies relating to prediction of ovulation, post ovulatory progesterone concentrations and post ovulatory mating. Such frequent examination is impractical for the clinician but was needed in the study of post ovulatory mating, in particular, to determine whether once daily or more frequent diagnosis of ovulation is needed to allow an acceptable conception rate to be achieved and in the other studies, to allow accurate description of follicle development and increases in progesterone concentrations after ovulation.

1.4. Artificial illumination

Long-day treatment of 14.5 hours of light and 9.5 hours of darkness was the method chosen for this study because it has been found highly effective by other investigators (PALMER et al. 1982, SCRABA and GINTHER 1985).

1.5. Exclusion of foal heat data

Foal heats were not taken into account in studies relating to the prediction of ovulation, progesterone levels after ovulation and post ovulatory breeding. KATILA et al. (1988) have reported that the conception rate after foal-heat mating may be slightly lower than that in relation to mating after subsequent heats in Finnhorse mares in the ERS.

2. Results

2.1. Onset of cycling

2.1.1. Non-foaling mares

Seasonality is important in relation to the reproductive performance of mares. However, current terminology is somewhat confusing. Although the terms breeding season and anoestrous season are commonly used, ovulatory and anovulatory seasons would be more precise (GINTHER 1979).

On the basis of several published articles, GINTHER (1979) concluded that most but not all horse mares are anoestrous during Winter months. KENNEY et al. (1975) concluded that reproductive activity is minimal after the Winter solstice and maximal just before the Summer solstice. These conclusions are supported by our results, which showed that nearly one mare in four is cycling in Finland throughout year and that anovulatory mares start cycling at the beginning of June at the latest. In addition to that the results reported here showed that the cause for the later foaling in Finland, as compared to other countries at smaller latitudes, is the later start of ovulatory season in Finland.

There is a marked difference between the more primitive pony breeds and horse breeds. PALMER reported, in 1978, that not one of 57 pony mares cycled continuously throughout the Winter but 34 % of 24 French saddle mares cycled throughout the Winter. In our study, 40 % of Finnhorse mares in training cycled during Winter. They therefore behaved more like horse than pony mares. However, anoestrous Finnhorse mares seemed to start cycling later during Spring, and were significantly less affected by long-day treatment during the anovulatory season than our warm blood mares.

It has been suggested that the age of a mare influences its reproductive pattern, with reduced activity in older mares (WESSON and GINTHER 1981). Although old Finnhorse mares started cycling in the Spring later than young mares, the difference was not statistically significant. The number of old mares was not great, however. Old mares were significantly less affected by long-day treatment than three-year-olds.

The effects of different levels of activity in mares (exercise, rest) on onset of cycling after the Winter anovulatory season have not been widely reported in the literature. Other factors, such as light and nutrition, may mask any specific effects, e.g. of training. According to BENGTSSON and KNUDSEN (1963) the frequency with which mares in training exhibit inactive ovaries is significantly higher if an unbalanced oat diet is fed than if feeds are well balanced.

In the study reported here mares in training were cycling significantly more often at the beginning of the year and anovulatory mares in training exhibited a significantly earlier onset of cycling than mares not in training. These differences could be explained by differences in levels of nutrition and, more particularly, by different levels of illumination between the groups.

SCRABA and GINTHER (1985) found that lighting from midsummer to Winter resulted in some pony mares cycling throughout the year. Lighting in our training stable late in the evenings might have stimulated some mares to cycle throughout the year.

It has been found that light treatment for 14.5 to 16 hours a day hastens the onset of cycling after the Winter anovulatory season (PALMER et al. 1982) and that it is effective even if not undertaken every day (SCRABA and GINTHER 1985). Low intensity lighting (2 footcandles) has also been claimed to be effective (LOY 1968). It is possible that the illumination in the training stables was sufficient to stimulate ovarian activity in anovulatory mares with early onset of cycling.

An adverse effect of previous foaling on the yearly number of ovulations has been reported by PALMER and DRIANCOURT (1983). In our study the effect of previous lactation was studied in 5-year-old Finnhorse mares. A significantly greater proportion of mares with foals at foot (12/12) exhibited anovulatory periods than maiden mares (12/19). Mares with foals were also more resistant to long-day treatment, cycling on average 10.8 weeks after the beginning of the long-day treatment, as compared to 9.8 weeks in non-lactating mares. However the difference is not statistically significant.

Similarity in yearly seasonal patterns was reported by PALMER (1978), who found that same mares had anovulatory periods from year to year. Our results showed another similarity in yearly seasonal patterns. Mares tended to begin cycling about the same time every year.

2.1.2. Foaling mares

In the study reported here, results concerning the time of first ovulation after parturition are broadly similar to those reported by LOY (1980) and BELLING (1984). A shift to shorter intervals between parturition and first ovulation was evident as the season advanced. However, in the study by Loy, the proportion of mares which ovulated within fewer than 11 days post partum was 64.8 % in April. About the same percentage, 68.7, was reached by our mares only in June. In Finland, only foalings in June or later are therefore suitable for the traditional foal heat breeding, 8 or 9 days after parturition without further monitoring of ovulation. KENNEY et al. (1975) stated that short oestrous period was a major sign of high fertility in mares. Our finding of decreasing length of time from parturition to first ovulation as Spring proceeded was analogous.

2.2. Artificial illumination

The onset of cycling occurred markedly earlier in long-day-treated animals than in untreated animals. All non-foaling, long-day-treated mares cycled in mid-March rather than at the beginning of June, as mares normally do under our conditions. Long-day treatment therefore hastened the onset of cycling after the Winter anovulatory season by over two months. This finding is consistent with the advancements of nearly three months in pony mares reported by KOOISTRA and GINTHER (1975), two and half months in pony mares reported by SCRABA and GINTHER (1985) and a few days over two months in horse mares reported by PALMER et al. (1982). The interval of 11 weeks from starting light treatment to the beginning of cycling corresponds to findings by others (OXENDER et al. 1977, ALLEN 1985).

The small number of long-day-treated post partum mares and the wide distribution of foaling months makes statistical analysis of results relating to any shortening effects of long-day treatment and the natural increase in length of day on time from parturition to first ovulation pointless. However, all mares ovulated within 18 days of parturition but the first mare foaling in January ovulated within 32 days of parturition.

The good conception rate of 86 % after long-day treatment is in agreement with the findings of van NIEKERK (1967), who concluded that the fertility of an ovum is unaffected by the time of year when ovulation occurs. The cause of low conception rates in Winter without long-day treatment is likely to be difficulty in diagnosing ovulation during long Winter oestrus.

It would seem that long-day treatment from the beginning of December would be an effective way of starting the breeding season in Finland as early as the beginning of February.

2.3. Sizes of ovaries and follicles post partum

The difference between growth rates in relation to early foalings (1.8 mm a day) and late foalings (3.7 mm a day) indicates that follicles develop slowly early in the season. This finding is in agreement with those of GINTHER (1990), and is worth remembering when predicting times of ovulation on the basis of size of the preovulatory follicle. The interval from parturition to first ovulation is presumably influenced by the growth rate of the preovulatory follicle. In Spring the slower follicular growth rate increases the interval from parturition to first ovulation.

Unlike GINTHER and PIERSON (1989), we found no difference in the size of the preovulatory follicle just before ovulation as between early and late season foalings. GINTHER and PIERSON noted a small preovulatory follicle in July. Only one of the mares in our studies foaled after June.

The ovulatory ovary was significantly larger than the other ovary 5 days post partum. This was to be expected, because the preovulatory follicle starts rapid growing usually 6 to 7 days before ovulation (PIERSEN and GINTHER, 1987) and on the other hand the first post partum ovulation occurs 10 days after parturition (LOY 1980. According to ARTHUR (1966) the main cause of variation in ovarian size is follicular content.

The finding of greater frequency of ovulation in the left ovary is in agreement with reports by OSBORNE (1966) and BELLING (1984). We also found a greater frequency of follicles over 10 mm in size two days post partum in the left ovary.

The sizes of the preovulatory follicle shortly before the first post partum ovulation (43 mm) and three days before ovulation (37 mm) were similar to sizes in other oestrous cycles in non-foaling Finnhorse mares (43 mm and 36 mm), respectively. This indicates that the same criteria apply to both foaling and non-foaling mares as regards, e.g., deciding the time of mating on the basis of diameter of the ovulatory follicle.

2.4. Preovulatory follicle in other oestrous periods

The observed growth of the preovulatory follicle by 2.7 mm a day during the week before ovulation is in agreement with the findings of PIERSON and GINTHER (1985), and PALMER (1987). The change in the shape and the decrease in the size of the preovulatory follicle close to ovulaton might have been caused by pressure of the ultrasound probe on the soft follicle. To avoid errors resulting from flattening PIERSON and GINTHER (1985) took the average of the longest and widest dimensions of the follicle.

2.5. Serum oestrogen before ovulation

It has repeatedly been suggested that the time of ovulation could be predicted from the significant decline of oestrogens which usually occurs on the day preceding ovulation (HILLMAN and LOY 1975, PALMER and JOUSSET 1975, PALMER and TERQUI 1977, PALMER 1978, MAKAWITI et al. 1983). In the method of FRANEK et al. (1988) E1 can be determined in a short time. Since this is advantageous in routine practice, it was the method chosen for this study. Although E1 levels were highest two days before ovulation and on the day of ovulation, no sharp E1 peak could be demonstrated using this method.

2.6. Prediction of ovulation

There is widespred interest in possibilities for predicting ovulation in mares. At the moment the best criterion for predicting ovulation is follicle size. The method is not, however, such as to allow the number of inseminations to be reduced to one per oestrous cycle, which would be highly advantageous in many cases. A quick and accurate method, based, for instance, on serum oestrogen measurements, which could reliably allow the day before ovulation to be determined would be of great benefit.

In this study a follicular diameter of 3.6 cm was the critical limit for not missing ovulation. Had there been a wait until follicles attained a diameter of 4.0 cm, the average number of inseminations would have decreased from 1.9 to 1.5 but 9 % of the mares would have ovulated without having been mated. Because of the great variation among mares, no other method accurately predicted impending ovulation. Further studies are needed to allow a quick and reliable method for predicting ovulation to be developed.

2.7. Progesterone after ovulation

The results reported in this study support the general view that progesterone levels increase within 24—48 hours of ovulation (STABENFELDT et al. 1972). The correlation coefficient of 0.7 ($p<0.05$) found by us between serum and milk progesterone concentrations is consistent with the correlation coefficient 0.7 ($p<0.01$) found by HUNT et al. (1978). Either serum or milk can therefore be used for progesterone determinations in lactating mares. The advantage of measuring milk progesterone is that milk is relatively easy for owners to collect. The fat content of mare's milk sample is obviously not a cause of any large variation in P4 concentrations because of the low fat content in mare's milk in general and because of the continuous suckling of the foal, which causes no regular changes in fat content as compared to great variation in fat content in cow's milk during first and last phases of milking.

The accuracy of progesterone determination was decreased, instead, by the relatively large interassay variation. Concentrations just after ovulation are some nano moles per litre but the standard curves in P4 kits were best linear between 10 and 40 nmol/l.

Because the time from ovulation to the first detectable rise in serum progesterone level varied considerably among mares, the time of ovulation could be estimated only to within two days. Progesterone determinations seem to be a fairly inaccurate way of timing ovulation. They are primarily a useful adjunct to rectal examination in oestrus control in brood mares. Weekly progesterone determinations can also be used on their own to detect mares cycling after the Winter anovulatory season, and to differentiate follicular-phase mares from dioestrous mares.

2.8. Postovulatory breeding and early embryonic death

Because the mares were examined at six-hourly intervals, more accurate information on the viability of ova after ovulation was obtainable than previously published. The results of the study of PALMER (1984) indicated that the conception rate may start to decrease 12 hours after ovulation. Our results showed that mating up to 18 hours after ovulation was successful. Thereafter, the conception rate decreased rapidly.

BELLING (1984) achieved a first cycle pregnancy rate of 74 % with daily examination, and insemination after ovulation had been detected. ALLEN (1981) achieved a similar pregnancy rate (76 %) using postovulatory natural covering. Early embryonic death (EED) decreased the 16-day conception rate of 46 % in the mares, which were examined daily in our study, to only 29 % on pregnancy diagnosis 25 days after insemination.

There were no cases of EED in the mares which were examined at six hourly intervals, nor in the control mares which had been mated before ovulation. MOBERG (1975) reported an EED percentage of 16 in relation to 213 pregnancies after natural matings between 1965 and

1974 at the ERS. Further investigations are needed to show whether or not post ovulatory breeding increases the frequency of EED.

Our results indicate that postovulatory insemination could be considered as a breeding practice in mares. In published studies on post ovulatory mating, the animals have been examined at intervals of 12 hours or more. In our study examination of mares at six-hourly intervals enabled us to determine how long an ovum remained capable of fertilization. It was shown that the viability of the ovum starts to decrease 18 hours after ovulation. ALLEN suggested, in 1981, that if mares are examined once a day matings furthest from ovulation will be found to be the least fertile. This suggestion is supported by the results reported in this thesis, which indicate that twice-daily examination of mares is desirable.

In conclusion, this thesis contains information about reproduction in mares, mainly Finnhorse mares but also mares of other horse breeds, in Finland. Some differences between Finnhorses and warm-bloods were evident but in many cases the differences were not statistically significant. As so often in equine research, numbers of animals were low. It would seem that the reproductive patterns are basically similar everywhere: minimum ovarian activity just after the Winter solstice with maximum ovarian activity starting just before the Summer solstice.

A new finding related to follicular development early in the season was reported. In post partum mares the preovulatory follicle grows more slowly in the Spring than in the Summer. This is of practical importance to clinicians trying predict impending ovulation on the basis of follicle size.

A system allowing relatively rapid progesterone measurements was developed during the study. Its main object was to allow clinicians to distinguish between cycling and noncycling mares. Our results show that reproductive patterns in mares are not regular in Finland before June. Sorting them by luteal activity saves the clinician work. However, it was also shown that long-day treatment from the beginning of December caused the mares to start their ovulatory season over two months earlier than usual. The value of progesterone measurements and the effect of long-day treatment need to be effectively communicated to breeders in Finland. Further studies should be carried out to determine the critical intensity of the light used. As timers are now available at low cost it would be easy to arrange artificial illumination in ordinary stables for brood mares.

CONCLUSIONS

In the study reported here nearly one nonfoaling mare in four was cycling at the beginning of year. All mares were cycling by the Summer solstice. The results also indicate that the late foaling in Finland is a result of late onset of cycling.

Factors which delayed the onset of cycling early in the season were type of use of the mare and late onset of cycling in preceding years.

In post partum mares there was a shift to shorter intervals from parturition to first ovulation as the season progressed. Only foalings in June or later are therefore appropriate to traditional foal heat breeding 8 or 9 days after parturition unless ovulation is monitored.

Artificial arrangements for there to be 14.5 hours of light and 9.5 hours of darkness a day from the beginning of December would be an effective way of starting the breeding season in pregnant and nonpregnant mares in Finland as early as the beginning of February.

In post partum mares the growth rate of the preovulatory follicle during foal heat is faster in foalings in Summer than in Spring.

In studies of the size, flaccidity and shape of the preovulatory follicle and the use of progesterone and oestrogen determinations, the best criterion for the prediction of ovulation was found to be follicle size.

Progesterone determinations are a fairly inaccurate way of timing ovulation but can be used to select cycling mares after the winter anovulatory season and to differentiate follicular phase mares from dioestrous mares.

Postovulatory insemination can be successful with twice daily examination of mares but whether or not once daily examination is sufficiently frequent remains to be investigated.

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SELOSTUS

Tammojen lisääntymistoiminnot Suomessa. Tutkimus vuodenaikojen vaikutuksesta, ovulaatiosta ja siemennyksestä munarakkulan puhkeamisen jälkeen.

ERKKI KOSKINEN

Maatalouden tutkimuskeskus

Tutkimukseen käytettiin 186 tammaa Maatalouden tutkimuskeskuksen hevostalouden tutkimusasemalla ja kuudella ravitallilla Etelä-Suomessa vuosina 1985—1989. Tarkoitus oli selvittää tammojen lisääntymisfysiologiaa suomalaisissa olosuhteissa.

Kiimakiertojen alkamista keväällä tutkittiin progesteronimääritysten avulla sekä luonnollisessa valossa olleilla että lisätyssä valossa olleilla tammoilla. Munasarjojen muutoksia varsomisen jälkeen ja ennen ovulaatiota seurattiin peräsuolen kautta tunnustelemalla ja ultra-äänilaitteella. Ovulaation jälkeen siementämisen onnistumista tutkittiin siementämällä tammoja kuuden tunnin välein munarakkulan puhkeamisen jälkeen.

Yhdellä tammalla neljästä oli kiimakiertoja tammikuussa, sen sijaan kaikki tammat sykloivat yleensä kesäkuun alussa. Tammojen varsominen myöhään johtuu Suomessa siis kiimakiertojen myöhäisestä alkamisesta suomalaisissa olosuhteissa.

Varsallisilla tammoilla aikaväli varsomisesta ensimmäiseen ovulaatioon lyheni kesää kohti. Vain toukokuun jälkeen tapahtuvissa varsomisissa varsakiima oli niin säännöllisesti 8—9 päivää varsomisen jälkeen, että tiineyttäminen olisi voitu suorittaa viikon kuluttua varsomisesta ilman tarkempaa ovulaation kontrollia.

Valojen pitäminen päällä tallissa 14.5 tuntia päivässä joulukuun alusta lähtien aloitti tehokkaasti tammojen kiimakierrot maaliskuun alkuun mennessä. Valohoidon avulla voitaisiin siitoskausi aloittaa Suomessa sekä varsallisilla että varsattomilla tammoilla helmikuun puolenvälin jälkeen.

Varsallisilla tammoilla ensimmäinen varsomisen jälkeinen munarakkula kasvoi nopeammin kesällä kuin keväällä. Tällä olisi merkitystä ovulaation kontrollissa, koska munarakkulan koko osoittautui parhaaksi mittariksi ennustettaessa ovulaation ajankohtaa.

Progesteronimäärityksellä voitiin tehokkaasti erottaa sykloivat tammat talvisessa seksuaalilevossa olleista tammoista sekä keltarauhasvaiheessa olevat tammat follikulaarivaiheessa olevista tammoista.

Siementämällä vasta ovulaation jälkeen väheni siemennyskertojen määrä kiimaa kohti yhdeksi. Tiinehtyminen säilyi hyvänä 18 tuntiin saakka ovulaation jälkeen. Voitiin päätellä, että ovulaatiota tulisi seurata vähintään kaksi kertaa päivässä hyvän tiinehtymistuloksen varmistamiseksi. Tulevaisuudessa tulisi tutkia, onko kerran päivässä tapahtuva ovulaatiokontrolli riittävän usein. Tällä olisi merkittävä kustannuksia alentava vaikutus.

ANNALES AGRICULTURAE FENNIAE, VOL. 30: 41—55 (1991)

Seria ANIMALIA DOMESTICA N. 99 — Sarja KOTIELÄIMET n:o 99

PROLACTIN IN FINNISH DAIRY CATTLE. 1

Long-term and daily variations of blood prolactin in heifers and lactating cows and effect of TRH-injection on prolactin and thyroid hormone levels

VAPPU KOSSILA, RITVA MÄKELÄ-KURTTO and ANU OSVA

KOSSILA, V., MÄKELÄ-KURTTO, R. & OSVA, A. 1991. **Prolactin in Finnish dairy cattle. 1. Long-term and daily variations of blood prolactin in heifers and lactating cows and effect of TRH injection on prolactin and thyroid hormone levels.** Ann. Agric. Fenn. 30: 41—55. (Agric. Res. Centre of Finland, Inst. Anim. Prod., 31600 Jokioinen, Finland.)

Long-term and daily variations in blood prolactin level as well as prolactin, T3 and T4 responses to TRH injection were investigated in heifer triplets and identical heifer twins (long-term variation & TRH response) and in two unrelated cows (daily variation & TRH response). Radioimmunoassay (RIA) method was used for the hormone analyses.

The circulating basal PRL level varied between 4—20 ng/ml, and the differences between animals were statistically significant. During the long-term follow up of the heifer triplets and the twins, prolactin level varied with the physiological state of the animals, eg. age, puberty, stage of gestation and stage of lactation. The identical twins responded in a similar pattern and so did two of the triplets. One of the triplets showed greater fluctuation.

Diurnal rhythmic variation was observed in prolactin levels, the lowest values were found in the morning. The most stable PRL values were measured between 8—10 a.m. and 2—3 p.m. Milking increased prolactin levels 2—9 fold the basal level.

TRH injection (1 μg/kg live weight) increased plasma prolactin to the level of 38—345 ng/ml, which was 6—38 fold the animals' basal level. The two non pregnant cows in late lactation had the most marked increases. The twins responded similarly to each other and so did triplets ($P < 0.005$). The TRH injection also increased the level of circulating thyroid hormones.

The PRL content of milk was about 27 % of that in blood. TRH response was observable in the milk of the subsequent milking, but not in urine.

Index words: heifers, lactating cows, twins, triplets, prolactin, thyroid hormones, blood, variation, TRH response.

INTRODUCTION

Background

In 1970, prolactin (PRL) studies in bovine were introduced as the Finnish part of an inter-Scandinavian hormone project (NKJ-22). During 1970—1975, a radioimmunoassay (RIA) method was developed for bovine blood prolactin and during the subsequent inter-Scandinavian hormone project (1975—1979, NKJ-34), the method was expanded to apply to bovine milk, urine and semen as well.

The main emphasis was on research in the following areas: 1) basic variation in blood, milk and urine prolactin levels in heifers and cows, 2) associations between blood PRL levels and blood enzyme and mineral levels in lactating cows, 3) blood PRL levels in A.I. bulls and association with male fertility, 4) blood PRL levels in A.I. bulls and association with blood minerals, trace elements and enzymes, 5) heritability of blood PRL level and correlation with milk production characteristics or other important traits.

With the exception of the RIA method developed, the results have not been systematically published. In this article the most important results on the first group of experiments are reported.

Prolactin and lactation

Prolactin is secreted by acidophilic cells (mammotrophs) of the anterior pituitary. In ruminants, prolactin is the predominant hormone in the adult anterior pituitary, contrary to most mammalian species in which the growth hormone is a predominant hormone both in the fetal and adult pituitary (NILSON et al. 1983). Prolactin has been detected inside the mammary cells accumulated in the rough endoplasmic reticulum, Golgi apparatus and secretory vesicle (MALVEN and KEENAN 1983).

It has been well demonstrated that PRL plays an important role in lactogenesis (TUCKER 1981). During lactation, prolactin is essential for casein gene expression in the bovine mammary gland (CHOI et al. 1988, TUCKER 1981). Research results also indicate that prolactin is important in gut absorption and bone mobilization of some minerals including calcium (BAUMAN and McCUTCHEON 1985), and possibly in lipid metabolism (BAUMAN and CURRIE 1980). It has been argued that PRL together with the growth hormone, participates in nutrient partitioning between mammary gland, growth and body reserves. However, for PRL, the evidence for such a role is insufficient (BAUMAN and McCUTCHEON 1985).

Prolactin concentrations in blood are positively correlated with milk yield although the coefficients are low (KOPROWSKI and TUKCER 1973). The same authors found a higher correlation between milk yield and milking-stimulated release of PRL. Selection for high milk yield also has failed to change the basal PRL level in blood (KAZMER et al. 1986, BONCZEK et al. 1988).

Variation in blood PRL levels

Individual animals seem to have their own basal blood PRL level, varying between 5 and 30 ng/ml in heifers and cows. Diurnal rhythm of the animals, milking, feeding and ambient temperature are among the factors causing variation in PRL levels (SCHAMS 1974, MOLLET and MALVEN 1982). Increases are quick and of short duration. In addition, blood PRL level varies by season, stage of lactation and parturition (SCHAMS 1974, AKASHA et al. 1987, CHEW et al. 1979).

The repeatability of basal male PRL level in a single blood sample has been reported to be very low (b = 0.13) (TUCKER et al. 1973). Higher estimates have been calculated for TRH induced release of PRL in prepubertal heifers (b = 0.61) (VINES et al. 1976). In this experiment, the repeatability of basal PRL level was 0.27. Estimates for heritability have been close to zero (TUCKER et al. 1973), which rather reflects the unknown nature of the variation in blood PRL levels than the genetic control of circulating PRL.

Objectives of the research

The extreme climatic and daylength conditions in Finland probably affect the endocrine status of cattle. The objectives of the present research were to obtain basic information on daily and long-term variation in blood PRL levels. Prolactin levels in milk and urine were studied as well as PRL, T3 and T4 response to a thyrotropin-releasing hormone (TRH) injection. TRH is known to release PRL and other hormones. Triplets and identical twins were used to increase the reliability of results and to give an idea of the variations in blood PRL levels when genotypes and/or a considerable part of the environment are the same.

MATERIAL AND METHODS

Analytical methods and samples

A radioimmunoassay method for bovine prolactin determinations developed by MÄKELÄ and KOSSILA (1976) was used for the PRL analyses. The dilution curves of the blood and milk samples were parallel to the standard curve. The dilution curve of the urine samples was not completely parallel to the standard curve. The recoveries of the bovine prolactin added into blood, milk and urine were 90, 110 and 50 %, respectively. Synthetic TRH (Thyrefact, Hoechst AG) did not cross-react with the antibodies against bovine prolactin. Sensitivity of the method was 0.1 ng/ml. Within the series of samples analyzed at one time the coefficient of variation was 8 % and between the series 14 %.

Thyroxine (T4) and triiodothyronine (T3) analyses were carried out at the Laboratory of Minerva Foundation by the RIA method.

The experimental animals were fitted with an indwelling catheter in the vena jugularis. A catheter was used for hourly or more frequent blood sampling. Less frequent samples were taken with a needle. Serum or heparinized plasma was employed for the prolactin determinations.

The cows were machine-milked. Milking was between 7 and 8 a.m., as well as 2 and 3 p.m. Whole milk from each milking was used for the analyses.

Urine was collected totally and weighed twice a day: between 7 and 8 a.m., as well as 3 and 4 p.m. It was centrifuged before the analysis.

Identity of genotypes was tested at the Blood Laboratory of the Central Association for the A.I. Centres in Finland.

Experiments

Long-term variation. Prolactin levels in the blood serum of triplet heifer calves and identical twin heifers were followed up 18 months for the twins and 8 months for the triplets. One blood sample per animal was taken once a month between 9 a.m and 10 a.m. At the beginning of the PRL control period, the twins were 12 months and the triplets 4-months-old. The animals were housed indoors year round and had individual feeding. The animal breeds, feeds, and information on lactational performance are given in Table 1.

Daily variation. Daily variation in blood prolactin concentration was studied in two genetically unrelated, non-pregnant cows in the late stage of lactation. The cows used were named Appeli and Sipuli. Breed, age, lactational performance and information on feeding are shown in Table 1. The daily variations in blood, milk and urine prolactin levels were monitored for seven succesive days. Blood samples were collected daily between 8 a.m. and 3 p.m.. The interval of blood sampling was one hour.

The response of blood PRL to milking was studied on the cows Appeli and Sipuli. The

Table 1. Experimental animals.

IDENTICAL TWINS:	ELSA	HELKA
Breed	Ayrshire	Ayrshire
Born	May 1975	May 1975
First parturition	July 6, 1977	June 25, 1977
Sex of the calf	male (35.5 kg)	female (37 kg)
Weight loss after calving	70 kg	84 kg
Live weight before calving	460 kg	474 kg
Total milk yield		
FCM 4 %, 28 weeks	1903 kg	1813 kg
fat	5.17 %	5.22 %
protein	4.27 %	4.29 %
lactose	4.77 %	4.91 %
Length of lactation	329 days	294 days
Feeding	According to the production level: hay, barley, concentrate (protected prot.)	hay, barley, concentrate (non-protected prot.)
Second parturition	July 12, 1978	July 4, 1978
Sex of the calf	male (37 kg)	male (39 kg)
Live weight before calving	484 kg	498 kg
Weight loss after calving	78 kg	73 kg
Peak yield after parturition (FCM 4 %)	14.3 kg	14.5 kg
Days from the parturition to the peak yield	28	15
Total milk yield		
FCM 4 %, 28 weeks	1723 kg	2028 kg
fat	5.12 %	5.08 %
protein	4.17 %	4.24 %
lactose	4.74 %	4.86 %
Feeding	According to the production level: hay, barley, concentrate (protein non-protected)	hay, barley, concentrate (protein protected)

TRIPLETS:	TIUKU	TÄHTI	TIMANTTI
Breed	Finncattle-Friesian crosses		
Born	February 1977		
First parturition	Feb. 21, 1979	Jan. 27, 1979	Jan. 18, 1979
Length of gestation	270 days	277 days	269 days
Sex of the calf	female (34 kg)	male (41 kg)	female (20 kg) male (24 kg)
Live weight before calving	533 kg	519 kg	507 kg
Weight loss after calving	95 kg	82 kg	77 kg
Total milk yield			
FCM 4 %, 28 weeks	3 025 kg	3 164 kg	2 809 kg
fat	3.90 %	3.46 %	3.50 %
protein	3.29 %	3.36 %	3.20 %
lactose	4.98 %	4.90 %	4.91 %
Feeding	According to the production level: straw, barley, molasses, urea	straw, barley, concentrate (protected prot.)	straw, barley, concentrate (non-protected prot.)

TWO COWS:	APPELI	SIPULI
Breed	Ayrshire	Ayrshire
Born	Feb. 16, 1974	April 20, 1969
Age at the time of PRL experiments in Aug. 1977	2 years 6 months	8 years 4 months
Number of calvings	2	6
Last calving	March 21, 1977	March 27, 1977
Live weight	503 kg	456 kg
Month of lactation	9	9
Daily milk yield	11.6 kg	15.3 kg
Feeding	According to the production level: hay, concentrates	

blood samples were taken 0, 1, 4, 6, 8, 10, 12, 17, 22, 32 and 62 minutes from the onset of milking. This experiment was carried out three days after the seven-day test period described above.

The experiments were performed in August. At that time the daylength was about 15 hours. The average amount of sunshine per day was 7 hours, and the temperature in the cowshed varied between 9 °C (morning) and 20 °C (afternoon).

Response to TRH stimulation. The TRH stimulation experiment with the cows Appeli and Sipuli was carried out on the day after the daily variation experiment had ended. A separate experiment for the twins and triplets was conducted in August of the following year.

Prolactin response to TRH stimulation was studied by injecting about 1 μg of the TRH/kg live weight into the vena jugularis. From 2 to 4 blood samples were taken before the TRH injection. After TRH injection the samples were taken first at intervals of 10 minutes, then every 20 minutes, every 30 minutes, and finally once an hour. The total number of samples per animal was 17 or 18. T3 and T4 levels were determined in 8 or 9 samples. For the order and number of samples see Figs. 5, 6 and 7.

During the TRH stimulation experiment the triplets were pregnant (1st pregnancy, 3rd or 4th month of pregnancy), the twins were in the 6th and 7th lactation week (first lactation, not pregnant) and the cows Appeli and Sipuli were in the 9th month of their 3rd and 7th lactation (not pregnant), correspondingly. For further information on the animals see Table 1.

RESULTS

Long-term variation

The long-term variations of blood PRL level in twins and triplets are presented in Figs. 1 and 2.

Prolactin levels of the triplet calves decreased from the average level of 20 ng/ml to that of 5—7 ng/ml at the age of 4 to 10 months. In pu-

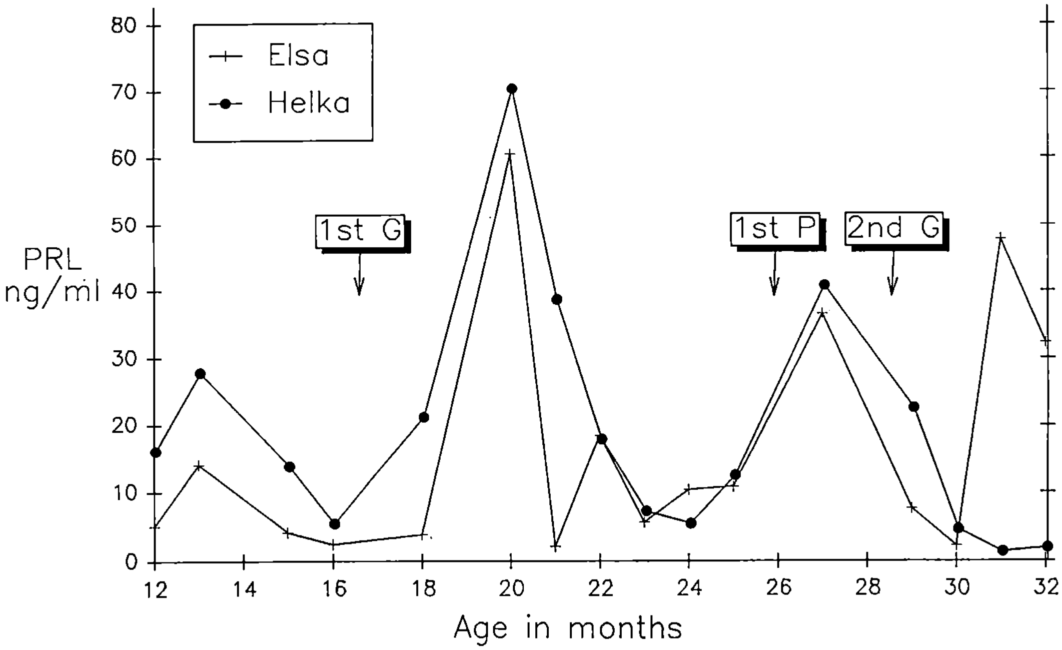

Fig. 1. Long-term variations of prolactin (PRL) in the blood serum of identical heifer twins (Elsa and Helka). G = gestation, P = parturition.

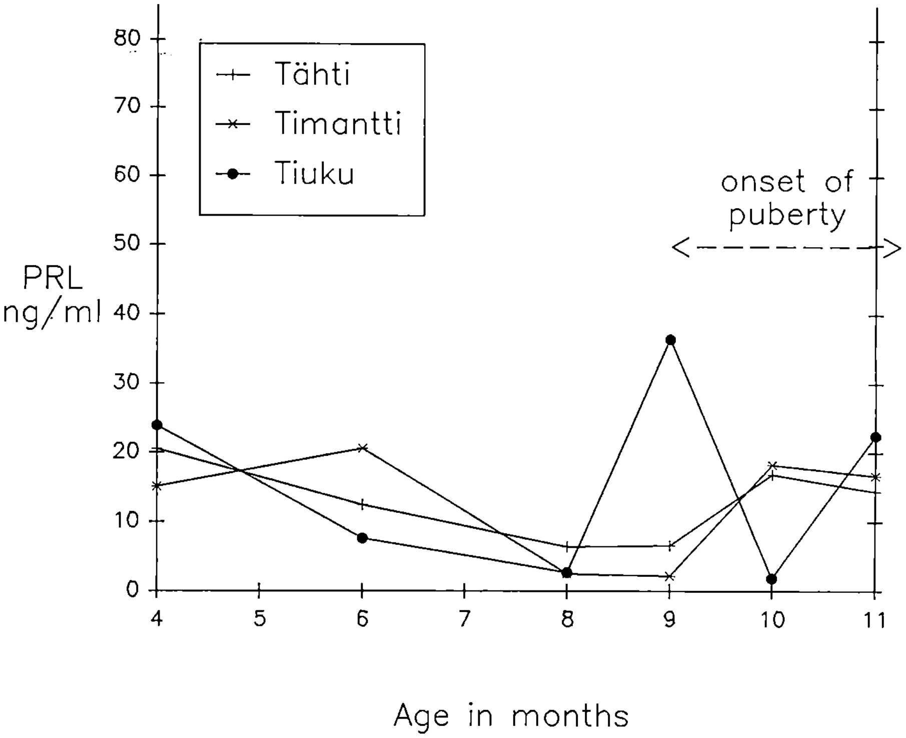

Fig. 2. Long-term variations of prolactin (PRL) in the blood serum of heifer triplets (Tähti, Timantti and Tiuku).

berty the PRL levels increased, and especially one animal (Tiuku) showed large fluctuation.

Highly correlated values in blood PRL levels were found between the twins. Simple correlation between the observed PRL levels was 0.82 (last two observations excluded) with a 68 % confidence interval of 0.48—0.94 (bootstrap approach, ref. DIACONIS and EFRON 1983). At about mid-pregnancy, a peak was observed in the prolactin levels of both animals. Blood samples were not collected in the parturition context. Observed prolactin values in blood serum were still high six (Helka) and five (Elsa) weeks postpartum.

Seasonal variation in blood PRL levels of twins and triplets could not be detected.

Daily variation

The daily variation in the blood PRL levels of the cows Appeli and Sipuli is presented in Fig. 3. The means, standard deviations and coefficients of variation in prolactin levels in blood, milk and urine of both cows are presented in Table 2.

Diurnal rhythmic variation was observed in the prolactin levels of lactating cows. Prolactin levels in the blood were generally low in the morning and gradually rose towards the afternoon ($P<0.025$).

Prolactin response to milking seemed to be more significant in the evening than in the morning, although the cow Sipuli was more irregular compared to the cow Appeli (Fig. 4).

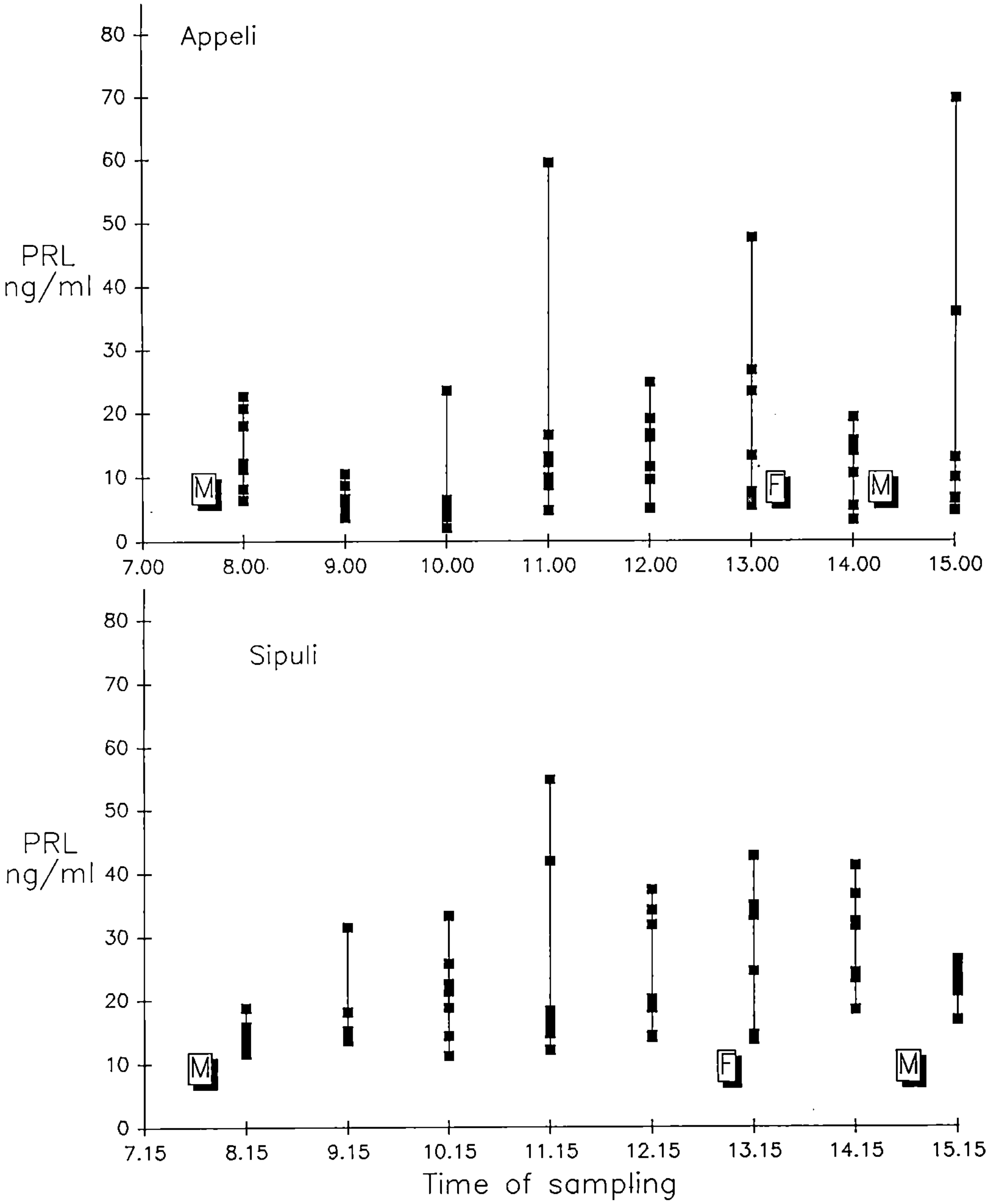

Fig. 3. Daily variations of prolactin (PRL) in the blood plasma of two non-pregnant cows (Appeli and Sipuli) in late lactation. F = feeding, M = milking.

The mean prolactin levels in morning and evening milk of cows Appeli and Sipuli are presented in Table 2. Evening milk contained more prolactin than morning milk ($P < 0.005$). The diurnal rhythm observed in milk agreed with the diurnal rhythm noted in blood. Total

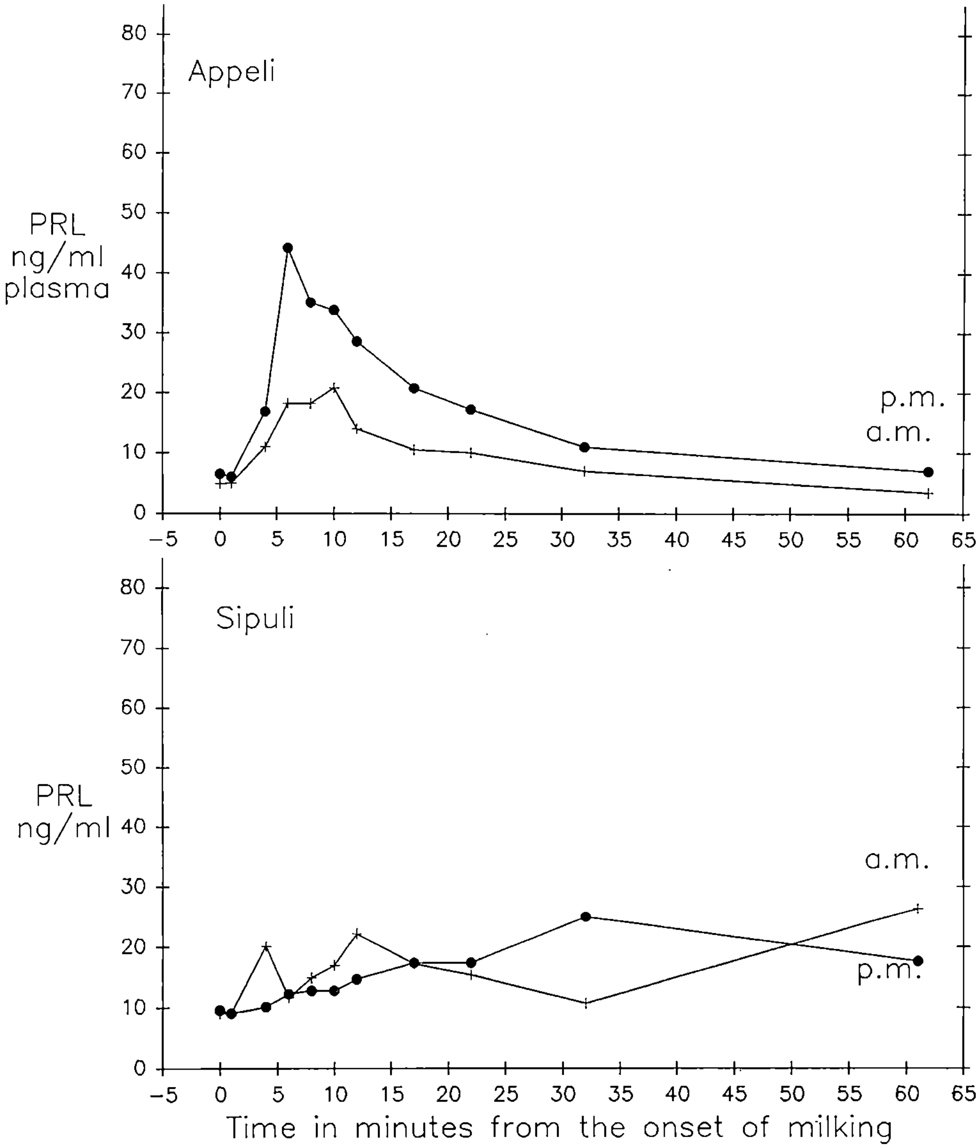

Fig. 4. Prolactin (PRL) responses to milking (in the morning = a.m., in the afternoon = p.m.) in blood plasma of two non-pregnant cows (Appeli: whose milk yield 8.5 kg a.m. and 3.2 kg p.m. and Sipuli: whose milk yield 11 kg a.m. and 4.5 kg p.m.) in the late stage (9th month) of lactation.

daily excretion of prolactin in milk was 45.0 (Appeli) and 76.4 µg (Sipuli). Within each animal, the correlation between blood PRL level and level in milk was not significant and was close to zero.

Prolactin or immunologically prolactin-like-activity was found in urine (Table 2). In both cows, the daily excretion of prolactin in urine was about 15 µg.

Table 2. Daily variations of prolactin (PRL) level (ng/ml) in blood plasma, milk and urine of two cows (Appeli and Sipuli). n = number of succesive days, X = mean, s = standard deviation, V% = coefficient of variation.

	Appeli				Sipuli			
	n	$\bar{X}$ ng/ml	s ng/ml	V%	n	$\bar{X}$ ng/ml	s ng/ml	V%
MILK								
morning	6	3.3	0.8	24.2	6	4.2	1.4	33.3
evening	7	4.7	1.3	27.7	7	7.2	1.1	15.3
URINE								
morning	6	1.2	0.2	16.4	6	1.2	0.1	8.3
evening	7	1.3	0.2	15.4	7	1.2	0.3	25.0
BLOOD								
8 a.m.	7	22.7	6.3	27.8	7	14.7	2.4	16.3
9 a.m.	7	6.4	2.4	37.5	7	17.5	6.3	36.0
10 a.m.	7	7.1	7.4	104.2	7	21.0	7.3	34.8
11 a.m.	7	22.4	20.3	90.6	7	24.8	16.6	68.3
12	7	14.7	6.5	44.2	7	24.3	9.8	40.3
1 p.m.	7	18.7	15.1	80.7	7	28.0	10.9	38.9
2 p.m.	7	11.9	5.6	47.1	7	29.5	8.0	27.1
3 p.m.	7	20.9	24.0	114.8	7	22.6	3.2	14.2
Daily* averages	7	14.5	4.9	33.8	7	22.8	3.1	13.6
OVERALL MEAN IN BLOOD	56	14.5	13.9	93.8	56	22.7	9.7	42.7

* 8 samples per day

TRH response

After administration of TRH, blood prolactin levels increased in each animal studied (Figs. 5, 6 and 7). Differences between animals in PRL levels before and after the TRH injection were statistically significant ($P < 0.005$). The levels were maximal 20 minutes after the TRH injection.

TRH increased prolactin levels most markedly (to 345 and 176 ng/ml) in the two non pregnant cows in late lactation. Differences in basal PRL level between the animals were statistically significant ($P < 0.05$), but not under the influence of TRH ($P > 0.05$). The cow Appeli, with the higher PRL level in the milking context, had a higher PRL level also after TRH stimulation compared to the cow Sipuli.

Both the twins compared to each other and the triplets compared to each other responded equally to the TRH injection ($P < 0.005$). In comparison with the twins, the triplets released more PRL in total due to a longer period of increased release (Figures 6 and 7).

The effect of TRH-injection on milk prolactin was studied in two non-pregnant cows in late lactation. TRH increased prolactin in milk to the levels of 16.8 and 14.0 ng/ml, which are 3.6 and 1.9 folds the daily averages in evening milk during the preceeding 7 days. Prolactin responses to TRH, were clearly slighter in milk than in blood. The cow with greater response in blood had a greater response also in milk.

TRH administration did not increase prolactin levels in urine in the two cows studied.

T3 and T4 levels in blood plasma before and after the TRH injection are presented in Figs. 5, 6 and 7. T3 and T4 levels after TRH injection differed markedly from the levels measured before treatment ($P < 0.005$).

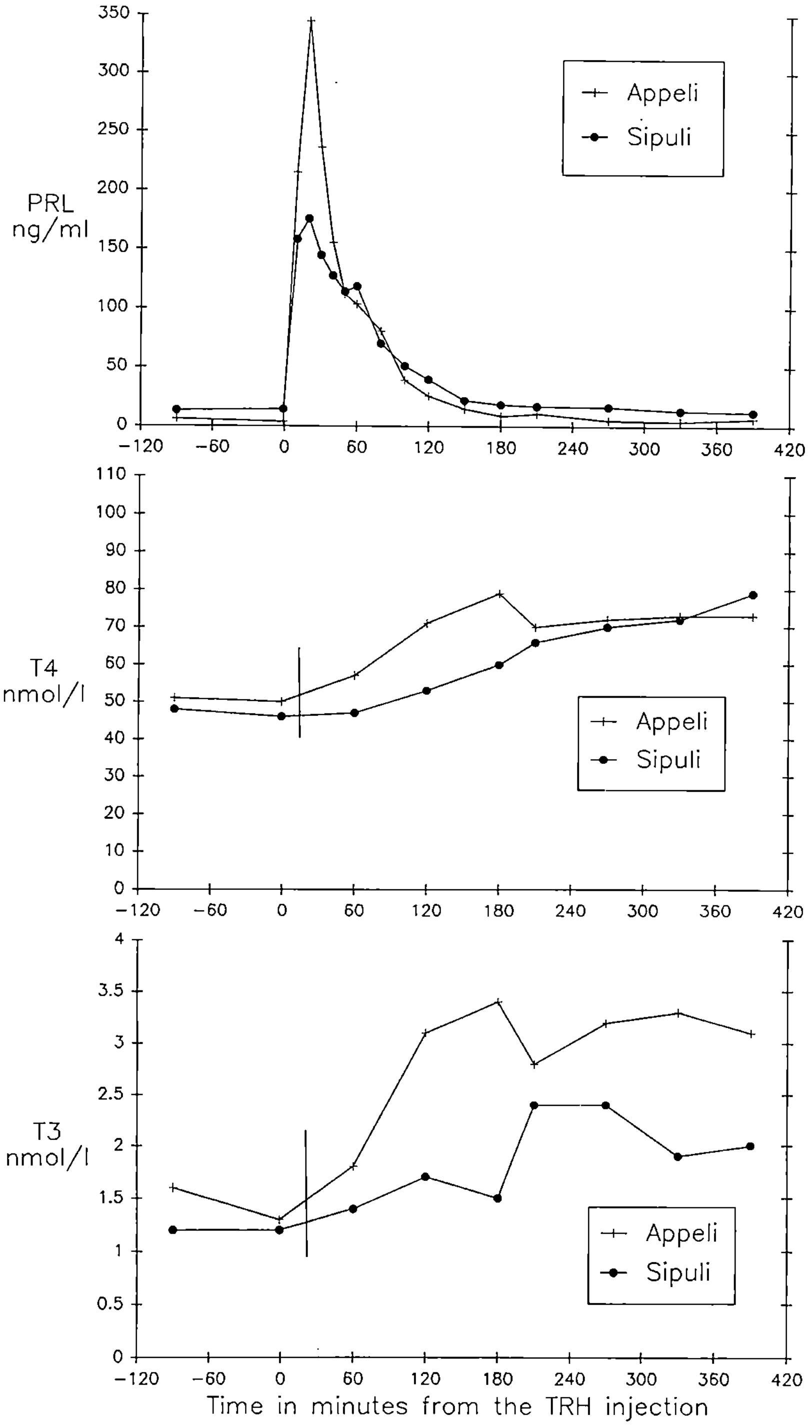

Fig. 5. Prolactin (PRL), thyroxine (T4) and triiodothyronine (T3) responses to thyrotropin releasing hormone (TRH) injection (1 µg/kg live weight, at 9.30 a.m.) in the blood plasma of two cows (Appeli and Sipuli).

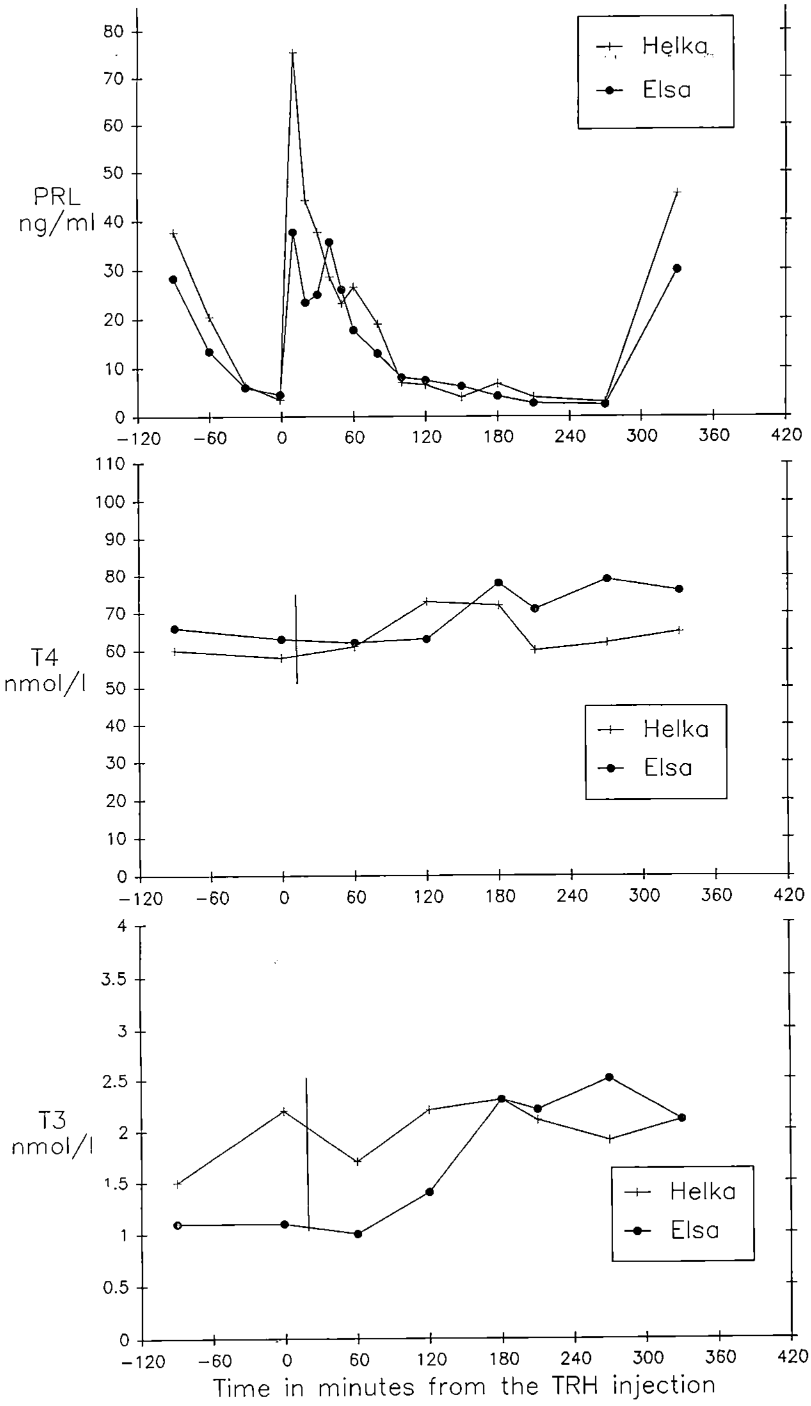

Fig. 6. Prolactin (PRL), thyroxine (T4) and triiodothyronine (T3) responses to thyrotropin releasing hormone (TRH) injection (1 µg/kg live weight, at 9.30 a.m.) in the blood plasma of identical twins (Helka and Elsa).

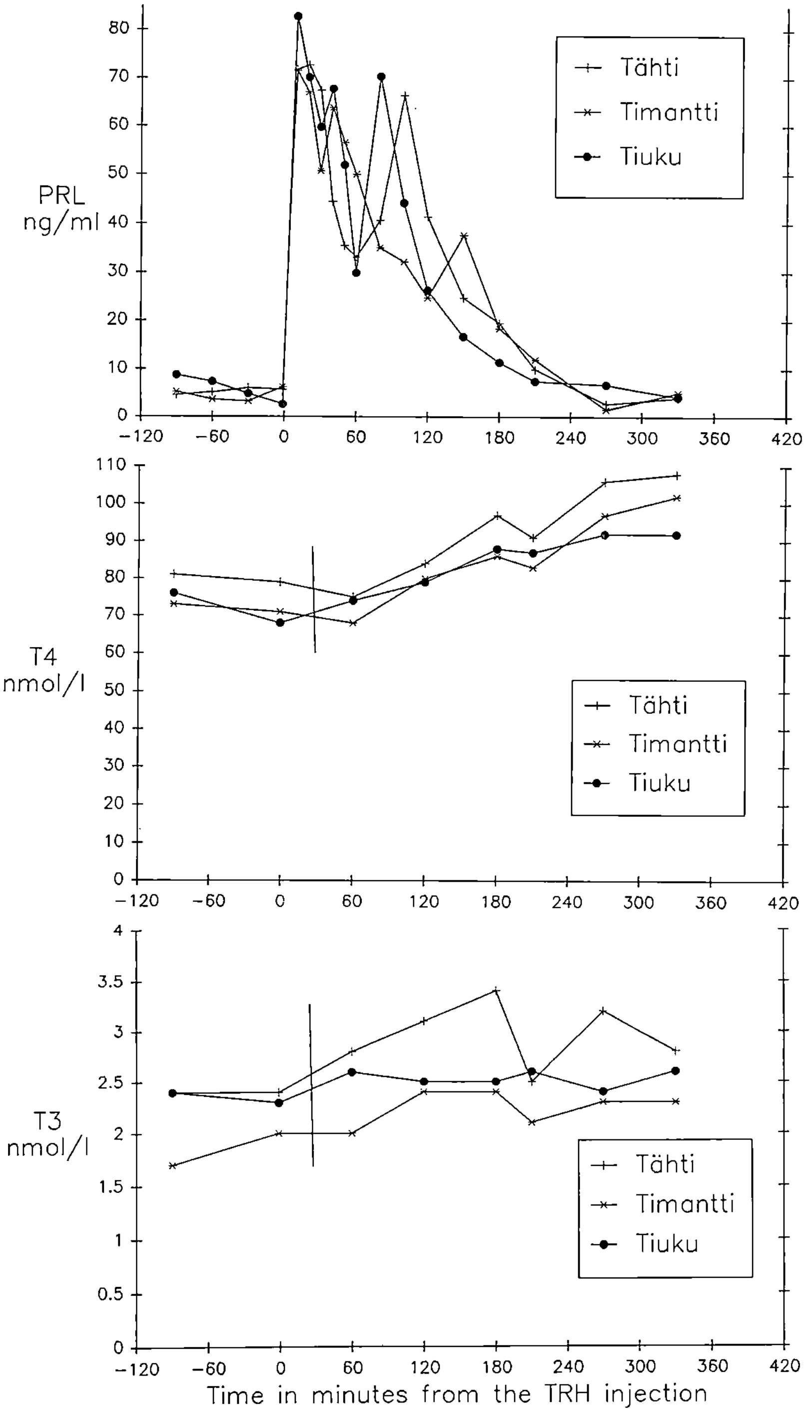

Fig. 7. Prolactin (PRL), thyroxine (T4) and triiodothyronine (T3) responses to thyrotropin releasing hormone (TRH) injection (1 µg/kg live weight, at 9.30 a.m.) in the blood plasma of heifer triplets (Tähti, Timantti and Tiuku).

DISCUSSION AND CONCLUSIONS

1. The long term follow up of the triplets, and especially the twins, shows that the circulating level of PRL varies with the physiological state of the animals and that identical genotypes in the same physiological states and environmental conditions respond in a very similar pattern.

2. The within-day profiles of blood PRL level elucidate the effect of milking and feeding. PRL levels are lowest in the morning and increase towards the afternoon. The results agree with the results of SCHAMS (1974) and MOLLETT and MALVEN (1982). MOLLETT and MALVEN observed the 24 h rhythm in circulating PRL levels and found that the level was the lowest between 6.00 am. and 9.00 am. In relation to blood PRL levels, the milk PRL levels in this study were low compared to the results published by BECK et al. (1978).

3. PRL response to TRH administration in this study agrees with the results obtained by other authors (SCHAMS 1974, CONVEY et al. 1973, BECK et al. 1979). BECK et al. (1979) found that PRL release after TRH injection is greater in early than in late lactation. The present material is too limited for this type of study. The result that identical genotypes responded very similarly is interesting, although not sufficient for any further conclusions.

In general, the effect of TRH injection on thyroid hormone levels in the present study is very similar to the result achieved with heifers by TVEIT et al. (1990 a). Probably the increase in T3 and T4 levels was not entirely caused by the TRH injection, as T3 and T4 levels tend to increase towards the afternoon. This was shown in non-lactating, pregnant heifers (BITMAN et al. 1984). Basal T3 and T4 levels as well as TRH response vary according to the physiological state of the animal. Lower basal levels have been measured in late compared to early and mid-lactation (AKASHA et al. 1987). In heifers thyroid hormone levels decrease after parturition, and especially T4 response to TRH administration is lower near parturition (TVEIT et al. 1990 b). The low T4 response of identical heifer twins 5—6 weeks after first parturition is in agreement with this finding.

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SELOSTUS

Prolaktiini Suomen nautakarjassa. 1

Hiehojen ja lypsylehmien veren prolaktiinipitoisuuksien pitkäaikainen ja päivittäinen vaihtelu sekä TRH lisäyksen vaikutukset veren prolaktiinin ja kilpirauhashormonien tasoihin

Vappu Kossila, Ritva Mäkelä-Kurtto ja Anu Osva

Maatalouden tutkimuskeskus

Veren prolaktiinitason pitkäaikaista ja päivittäistä vaihtelua sekä TRH-injektion vaikutusta prolaktiinin ja kilpirauhashormonien (T3 ja T4) erittymiseen tutkittiin identtisillä hiehokaksosilla, hiehokolmosilla (pitkäaikainen vaihtelu ja TRH-vaste) ja kahdella lehmällä (päivittäinen vaihtelu ja TRH-vaste). Koe-eläimiä oli yhtensä seitsemän. Hormonianalyysit suoritettiin radioimmunologista menetelmää (RIA) käyttäen.

Prolaktiinin perustaso koe-eläinten veren seerumissa ja/tai plasmassa vaihteli 4—20 ng/ml. Kolmosvasikoiden (hiehoja) ja identtisten hiehokaksosten pitkäaikaisessa seurannassa veren prolaktiinitaso vaihteli eläinten fysiologisen tilan mukaan. Näitä tekijöitä olivat ikä, sukukypsyyden saavuttaminen sekä tiineyden ja laktaation vaihe. Prolaktiinitason vaihtelu geneettisesti identtisillä kaksosilla oli samankaltaista. Kolmosista kahden prolaktiiniarvot korreloivat keskenään, mutta kolmannen arvot olivat epäsäännöllisiä.

Prolaktiinitason vaihtelussa todettiin päivärytmi siten, että aamulla taso oli alhaisimmillaan kohoten iltapäivää kohden. Prolaktiinitaso vaihteli samalla eläimellä vähiten klo 8—10 ja klo 14—15 otetuissa näytteissä. Lypsy kohotti veren prolaktiinipitoisuudet 2—9 kertaisiksi perustasoon verrattuna. TRH-injektion (1 µg/elopaino kg) jälkeen plasman prolaktiinitaso vaihteli maksimissaan 38—345 ng/ml. Nousu oli 6—38 kertainen perustasoon nähden. Kahden, ei-tiineen, myöhäisessä laktaation vaiheessa olevan lehmän arvot kohosivat eniten. Kaksoset keskenään ja kolmoset keskenään reagoivat TRH-injektioon samalla tavoin ($P < 0.005$). TRH-injektio nosti myös veren kilpirauhashormonien tasoa.

Maidon prolaktiinitaso oli noin 27 % veren tasosta. TRH-injektion prolaktiinin eritystä stimuloiva vaikutus näkyi injektiota seuranneen lypsykerran maidon prolaktiinipitoisuuden lievänä nousuna. Virtsan prolaktiinipitoisuus oli hyvin alhainen, eikä TRH-vastetta voitu havaita.

ANNALES AGRICULTURAE FENNIAE, VOL. 30: 57—62 (1991)

Seria ANIMALIA DOMESTICA N. 100 — Sarja KOTIELÄIMET n:o 100

PROLACTIN IN FINNISH DAIRY CATTLE. 2

Prolactin, thyroid hormones, selected enzymes and minerals in the blood of heifers and lactating cows before and after TRH-injection

RITVA MÄKELÄ-KURTTO, VAPPU KOSSILA and ANU OSVA

MÄKELÄ-KURTTO, R., KOSSILA, V. & OSVA, A. 1991. **Prolactin in Finnish dairy cattle. 2. Prolactin, thyroid hormones, selected enzymes and minerals in blood of heifers and lactating cows before and after TRH-injection.** Ann. Agric. Fenn. 30: 57—62. (Agric. Res. Centre of Finland, Inst. Anim. Prod., 31600 Jokioinen, Finland.)

Prolactin (PRL), triiodothyronine (T3) and thyroxine (T4) responses to an intravenous administration of thyrotropin releasing hormone (TRH) was investigated in Finnish dairy cattle in an one-day experiment. The seven animals studied were heifer triplets, identical cow twins and two single cows (not related). The number of blood samples collected per animal during the experiment was 17—18. PRL as well as T3 and T4 were determined radioimmunologically. In addition, blood enzymes (ALP, AST, ALT, CPK, GGT and LDH) and blood minerals (Ca, P and Mg) as well as some other blood parameters (cholesterol, creatinine, total protein and albumin) were analyzed.

The TRH injection (1 µg/kg live weight) clearly increased plasma prolactin to the level of 38—345 ng/ml, which was 6—38 folds the animals' basal level. The TRH injection slightly increased the levels of plasma thyroid hormones (T3 and T4), too, but it had no effect on blood enzymes, minerals or other blood parameters. The data, although limited, suggest that high basal PRL levels may be associated with low CPK values. In addition, high ALP and low AST values seemed to be connected with high T3 levels and high ALP, low AST, high creatinine and low total protein levels with high T4 levels.

Index words: heifers, lactating cows, prolactin, thyroid hormones, TRH response, minerals, enzymes, cholesterol, creatinine, total protein, albumin.

INTRODUCTION

The thyrotropin releasing hormone (TRH) releases the thyroid stimulating hormone (TSH) from the adenohypophysis into the blood circulation. TSH, in tern, stimulates the thyroid to secrete the thyroid hormones thyroxine (T4) and triiodothyronine (T3). These hormones mainly regulate the rate of basal metabolism in the body. In addition, TRH is involved in PRL release from the adenohypophysis into the blood.

The purpose of this investigation was to study simultaneous effects of TRH administration not only on the hormones, but also on the enzymes, minerals and some other blood parameters in Finnish dairy cattle.

MATERIAL AND METHODS

The TRH stimulation test was performed on seven animals: identical cow twins (Ayrshire), heifer triplets (Finncattle-Friesian crosses) and two single cows (Ayrshire). The twins (Elsa and Helka), were in early lactation. The triplets (Tähti, Timantti and Tiuku), were in their first pregnancy and the two single cows (Appeli and Sipuli) were non pregnant and in the late stage of lactation (9th month).

The TRH stimulation test was carried out by injecting 1 μg of synthetic TRH (Thyrefact, Hoechst AG)/kg live weight into the vena jugularis. All blood samples were taken via catheter from the vena jugularis. Two to four blood samples were obtained from each animal before the TRH injection. After the TRH injection, samples were collected first at 10 min intervals, then at 20 min, 30 min, and finally at one hour intervals. The total number of samples per animal was 18 for the triplets and twins, as well as 17 for the two single cows. T3 and T4 levels were determined from the samples taken before the TRH injection and after the injection at one hour intervals.

PRL was determined from heparinized blood plasma by a radioimmunoassay (RIA) method developed by MÄKELÄ and KOSSILA (1976). T3 and T4 analyses were carried out also by RIA at the Laboratory of Minerva Foundation. Blood enzymes, minerals and other blood parameters were analyzed by the standard methods.

Enzyme activities determined in blood were:

ALP (alkaline phosphatase): found particularly in bone (osteoplasts), the liver and intestinal wall.

AST (aspartateaminotransferase): found particularly in skeletal muscle, cardiac muscle, the liver and erythrocytes.

ALT (alanineaminotransferase): muscle enzyme in large animals.

CPK (creatinephosphokinase): involved in high energy metabolism. A slightly raised CPK activity is typical of hypothyroidism as CPK catabolism is apparently promoted by thyroxine.

GGT (gamma-glutamyltranspeptidase): found especially in the liver and the kidneys and is used to examine liver conditions.

LDH (lactatedehydrogenase): a large number of isoenzymes. LDH(1) is associated with cardiac muscle, the kidneys and erythrocytes and LDH(5) with the liver. Other isoenzymes are associated with skeletal muscle and the lungs.

The blood minerals analyzed in this investigation were: calcium (Ca), phosphorus (P) and magnesium (Mg). Other blood parameters studied were: cholesterol, creatinine, albumin and total protein.

The variance analyses were carried out using methods suitable for the data with unequal subclass numbers. To describe the data the following model was used:

$$Y_{ijk} = \mu + a_i + b_j + e_{ijk}$$

where, Y_{ijk} = the ijk'th observation
μ = LS-mean
a_i = effect of the i'th animal (i = 1 . . 7)
b_j = effect of j'th time class (j = 1. . .3, 1:before, 2:0—2 hours, 3:over 2 hours from the TRH injection)
e_{ijk} = residual term with variance δ_e^2

All the effects were regarded as fixed. Animal × time class interaction term was excluded from the model as no interaction seemed to exist between these effects.

RESULTS AND DISCUSSION

Results on the blood hormone, enzyme, mineral and other parameter levels during the TRH stimulation test are presented in Table 1. The research results obtained on the prolactin, T3 and T4 responses to TRH have been reported in more detail by the authors (KOSSILA et al. 1991).

The most marked response occurred in the PRL level after the intravenous TRH administration. The maximum PRL level varied between 38—345 ng/ml in blood plasma depending on the animal. The values were 6—38 fold the animals' basal levels. Also according to SCHAMS (1974), prolactin response to TRH injection was clearly observable in dairy cattle.

In addition, slight increases in T3 and T4 levels could be found after TRH administration (Table 1). These results were in good agreement with those presented by TVEIT et al. (1990 a, 1990 b) on cattle studies. However, T3 and T4 responses were slower than PRL responses (Table 1).

Only small changes in the blood enzymes, minerals or the other parameters studied could be seen after TRH treatment (Table 1). However, the variation of all these parameters during the TRH stimulation test was within the normal range (KERR 1989). The results obtained indicate that TRH administration had no effect on these parameters.

Table 1. Levels of PRL, T3, T4 and other blood parameters before and after TRH administration. (PRL = prolactin, T3 = triiodothyronine, T4 = thyroxine, Ca = calcium, P = phosphorus, Mg = magnesium, ALP = alkaline phosphatase, ALT = alanineaminotransferase, AST = aspartateaminotransferase, CK = creatinophosphokinase, GGT = gammaglutamyltranspeptidase, LDH = lactohydrogenase).

Variable	LS-Means								Unit
	Before	s.e.	0—2 h after TRH inj.	s.e.	>2 h after TRH inj.	s.e.		Overall	
N (animals)	7		7		7			7	
n (samples)	14		14		30			58	
T3	1.74	0.13	2.12	0.13	2.56	0.09	***	2.14	nmol/l
T4	63.6	1.7	67.6	1.7	80.9	1.1	***	70.7	nmol/l
N (animals)	7		7		7			7	
n (samples)	24		63		37			124	
PRL	14.7	8.0	66.7	4.9	10.1	6.4	***	30.5	ng/ml
Ca	2.4	0.04	2.3	0.02	2.4	0.03		2.4	mmol/l
P	1.5	0.03	1.5	0.02	1.5	0.03		1.5	»
Mg	2.4	0.01	2.3	0.01	2.4	0.01		2.4	»
ALP	92.2	1.3	88.2	0.8	90.6	1.1	*	90.4	iu/l
ALT	13.9	0.5	14.3	0.3	14.9	0.4		14.3	»
AST	55.2	0.6	54.0	0.4	54.1	0.5		54.4	»
CPK	101.5	1.6	96.7	1.0	97.1	1.3	*	98.4	»
GGT	18.5	0.4	18.0	0.2	18.0	0.3		18.2	»
LDH	244	2.8	243	1.7	245	2.2		244	»
Cholesterol	3.9	0.04	3.8	0.02	3.9	0.03		3.9	mmol/l
Creatinine	124	1.4	120	0.9	122	1.1		122	µmol/l
Total protein	75.0	0.6	73.5	0.4	74.4	0.5		74.3	g/l
Albumin	39.4	0.4	38.5	0.2	38.9	0.3		39.0	g/l

s.e. = standard error
F-test: time classes differ significantly
* $P<0.05$
*** $P<0.005$

If the levels of the enzymes measured in this study are compared with those published by TANHUANPÄÄ et al. (1979), it seems that APT, ALT and LDH levels are higher in AI-bulls than in heifers and cows. The levels of other enzymes and other parameters in the blood of heifers and cows in the present study, were of the same magnitude as those of the AI-bulls (TANHUANPÄÄ et al. 1979).

Simple correlations between basal PRL (eg. average level of circulating PRL not affected by TRH or milking), T3, T4 and parameters studied are presented in Table 2. The material is very limited both in number and genetic structure for correlation estimation. However, the material suggests that low CPK values are associated with high basal PRL levels, high ALP and low AST values with high T3 levels, high ALP, low AST, high creatinine and low total protein levels with high T4 levels (Table 2). More reliable correlation coefficients based on bull material will be reported later.

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Table 2. Simple correlations between different blood parameters and basal prolactin, T3 and T4 levels. (PRL = prolactin, T3 = triiodothyronine, T4 = thyroxine, Ca = calcium, P = phosphorus, Mg = magnesium, ALP = alkaline phosphatase, ALT = alanineaminotransferase, AST = aspartateaminotransferase, CK = creatinophosphokinase, GGT = gammaglutamyltranspeptidase, LDH = lactohydrogenase).

	PRL	T3	T4
Ca	−0.21	0.50	0.45
P	−0.60	0.70	0.74
Mg	−0.38	−0.15	0.17
ALP	−0.29	0.80*	0.88**
ALT	−0.57	0.31	0.44
AST	0.12	−0.83*	−0.80*
CPK	−0.86*	0.08	0.50
GGT	0.64	−0.19	−0.59
LDH	−0.35	0.52	0.53
Cholesterol	−0.25	−0.21	−0.50
Creatinine	−0.56	0.47	0.78*
Total protein	0.03	−0.64	−0.84*
Albumin	−0.29	−0.33	−0.48

F-test: * $P<0.05$, ** $P<0.01$

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SELOSTUS

Prolaktiini Suomen nautakarjassa. 2

Hiehojen ja lehmien veren prolaktiinin, kilpirauhashormonien, eräiden entsyymien ja mineraalien pitoisuudet ennen ja jälkeen TRH lisäyksen

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Maatalouden tutkimuskeskus

Veren prolaktiinin (PRL) ja kilpirauhashormonien (T3 ja T4) pitoisuuksia tutkittiin suomalaisella lypsykarjalla kokeessa, jossa tyrotropiinia vapauttavaa hormonia (TRH) lisättiin (1 µg/elopaino kg) seitsemän eläimen verenkiertoon. Tutkitut eläimet olivat hiehokolmosia, identtisiä lehmäkaksosia sekä kaksi yksittäistä lehmää, jotka eivät olleet sukua toisilleen. Kokeen aikana kustakin eläimestä kerättiin 17—18 verinäytettä. PRL- sekä T3- ja T4-hormonit määritettiin radioimmunologisesti. Tämän lisäksi verinäytteistä analysoitiin entsyymejä (ALP, AST, ALT, CPK, GGT ja LDH), kivennäisaineita (Ca, P ja Mg) sekä eräitä muita veren ainesosia (kolesteroli, kreatiniini, kokonaisvalkuainen ja albumiini).

TRH-lisäyksen seurauksena plasman prolaktiinipitoisuudet kohosivat huomattavasti, arvoihin 38—345 ng/ml, jotka olivat 6—38 kertaisia perustasoon verrattuna. Lisäyksen johdosta myös T2- ja T4-tasot nousivat lievästi. TRH-lisäyksellä ei ollut vaikutusta veren entsyymien, kivennäisaineiden tai muiden tutkittujen ainesosien pitoisuuksiin. Aineisto, joskin hyvin rajoittunut, viittasi siihen, että alhaisiin CPK arvoihin liittyisi korkea prolaktiinin perustaso. Lisäksi on viitteitä siitä, että korkeisiin T3 arvoihin saattaisi liittyä korkeampia ALP:n ja matalampia AST:n arvoja ja korkeisiin T4 tasoihin korkeampia ALP:n, matalampia AST:n, korkeampia kreatiniinin ja matalampia kokonaisvalkuaisen arvoja.

Appendix. Time class means by animal.

Variable	Before	0—2 h after TRH inj.	>2 h after TRH inj.	Normal values
Prolactin				
Appeli	4.8	145.9	8.3	
Sipuli	13.6	111.1	16.5	
Elsa	13.1	21.6	9.1	
Helka	17.1	29.8	12.7	
Tähti	5.3	52.6	12.2	
Timantti	4.6	50.2	15.0	
Tiuku	5.9	55.9	9.4	
T3				
Appeli	1.5	2.5	3.2	
Sipuli	1.2	1.6	2.1	
Elsa	1.1	1.2	2.3	
Helka	1.9	2.0	2.1	
Tähti	2.4	3.0	3.5	
Timantti	1.9	2.2	2.3	
Tiuku	2.4	2.6	2.5	
T4				
Appeli	51	64	73	
Sipuli	47	50	69	
Elsa	65	63	76	
Helka	59	67	65	
Tähti	80	80	101	
Timantti	72	74	92	
Tiuku	72	77	90	
Ca				
Appeli	2.5	2.4	2.5	2—3 mmol/l
Sipuli	2.2	2.0	2.1	
Elsa	2.3	2.4	2.5	
Helka	2.5	2.3	2.4	
Tähti	2.6	2.7	3.0	
Timantti	2.2	1.9	1.9	
Tiuku	2.5	2.5	2.2	
P				
Appeli	1.3	1.5	1.5	1—2.5 mmol/l
Sipuli	0.9	1.0	1.0	
Elsa	1.4	1.3	1.5	
Helka	1.3	1.3	1.5	
Tähti	1.7	1.6	1.4	
Timantti	1.8	1.8	1.8	
Tiuku	2.1	2.0	2.0	
Mg				
Appeli	0.82	0.85	0.87	1—2 mmol/l
Sipuli	0.77	0.72	0.77	
Elsa	1.03	1.02	1.03	
Helka	0.95	0.95	1.00	
Tähti	0.98	0.99	0.92	
Timantti	0.82	0.83	0.77	
Tiuku	0.66	0.77	0.78	
ALP				
Appeli	45	43	48	up to 300 iu/l
Sipuli	42	39	36	
Elsa	61	58	61	
Helka	75	75	76	
Tähti	135	123	130	
Timantti	155	148	150	
Tiuku	131	132	133	

ALT				
Appeli	22	22	22	
Sipuli	5	8	5	
Elsa	12	14	15	< 100 iu/l
Helka	8	10	10	
Tähti	17	14	15	
Timantti	20	18	19	
Tiuku	14	15	19	
AST				
Appeli	55	56	56	
Sipuli	71	72	70	
Elsa	71	69	69	< 100 iu/l
Helka	70	66	66	
Tähti	38	33	34	
Timantti	41	40	42	
Tiuku	40	41	41	
CPK				
Appeli	80	76	73	
Sipuli	56	57	54	
Elsa	141	141	140	around 100 iu/l
Helka	132	112	110	
Tähti	99	88	96	
Timantti	98	98	103	
Tiuku	104	104	104	
GGT				
Appeli	21	23	21	
Sipuli	24	23	23	
Elsa	14	13	14	< 60 iu/l
Helka	16	15	16	
Tähti	17	14	16	
Timantti	20	19	19	
Tiuku	19	19	18	
LDH				
Appeli	198	201	200	
Sipuli	201	240	235	
Elsa	240	234	236	
Helka	237	228	230	200—300 iu/l
Tähti	234	222	231	
Timantti	276	271	273	
Tiuku	306	307	308	

Cholesterol				
Appeli	4.3	4.2	4.2	
Sipuli	3.9	3.9	3.8	
Elsa	3.9	3.8	3.8	2—3 mmol/l
Helka	4.0	3.8	4.0	
Tähti	3.1	3.0	3.3	
Timantti	4.0	3.9	4.0	
Tiuku	4.0	4.1	4.4	
Creatinine				
Appeli	104	94	95	
Sipuli	101	99	100	
Elsa	130	129	128	< 150 µmol/l
Helka	127	118	122	
Tähti	127	123	126	
Timantti	145	145	146	
Tiuku	135	133	133	
Total protein				
Appeli	84	82	81	
Sipuli	86	83	82	
Elsa	77	76	76	60—80 g/l
Helka	81	76	78	
Tähti	53	51	54	
Timantti	73	71	74	
Tiuku	73	74	75	
Albumin				
Appeli	42	42	41	
Sipuli	40	39	39	
Elsa	40	39	39	25—35 g/l
Helka	41	38	39	(higher in large
Tähti	32	29	30	animals)
Timantti	41	40	42	
Tiuku	42	42	42	

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Seria ANIMALIA DOMESTICA, N. 101 — Sarja KOTIELÄIMET n:o 101

PROLACTIN IN FINNISH DAIRY CATTLE. 3

Variation, repeatability and heritability of prolactin levels in the blood of artificial insemination (A. I.) bulls and correlation with individual and progeny performance

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On three bull stations and on one performance testing station were 405 blood samples from 300 A.I. bulls collected in winter during 1974—1978. The samples were analysed for prolactin level (PRL) by a radioimmunoassay method. The objectives were to analyse variations in blood PRL levels and evaluate the value of a single PRL measurement as a marker for one or several characteristics for which A.I. bulls are progeny tested.

PRL levels in the whole material ranged 0.1—260 ng/ml the mean being 10.4 ± 12.6 ng/ml after eliminating the most extreme values. Differences in the PRL levels between stations and/or years were significant ($0.001 < P < 0.01$). PRL levels also decreased with the age of the bulls ($0.05 < P < 0.10$), which varied from 8 to 47 months. Repeatablity within 35 minutes (30 bulls) was 0.73 ± 0.08 and repeatability over three years (three single measurements) was 0.21 ± 0.25 in nine bulls. A moderate heritability of 0.25 ± 0.17 was estimated for the basal PRL level. The data consisted of 19 sires with 3—40 bull progeny. Phenotypic correlations between basal PRL level and the non-return rate and growth index were 0.05 (n.s.) and 0.15 ($P < 0.05$), respectively. Genetic correlations were calculated as the simple correlation between PRL level and breeding value in milk yield (absolute yield, kg), fat content (%), protein content (%), fertility index, calf mortality, mastitis frequency and acetone frequency. Correlation coefficients were —0.07 ($n = 174$), 0.06 ($n = 174$), 0.13 ($n = 174$), —0.11 ($n = 140$), —0.16 (n 140), —0.07 ($n = 28$) and —0.31 ($n = 28$), respectively. The estimates of phenotypic and genetic correlations differed and usually showed a higher association, when calculated separately for growing and mature bulls. It was concluded that despite the moderate heritability and the associations of PRL with lactation (especially protein and fat synthesis), growth and possibly with energy metabolism found, research is still needed to sufficiently clarify the picture regarding practical applications.

Index words: dairy cattle, bull, prolactin, variation, repeatablity, heritability, milk production, growth, fertility, calf mortality, acetone, mastitis.

INTRODUCTION

The hormone prolactin is secreted by acidophilic cells (mammotrophs) of the anterior pituitary. In synergism with other hormones, it participates in the physiological control of mammary growth, lactogenesis and lactation (for a review see TUCKER 1981). During lactation, prolactin is known to be essential in milk protein and fat synthesis on the cellular level (CHOI et al. 1988, SHAMAY et al. 1989). Prolactin is also thought to be involved in mineral metabolism and in the partitioning of energy between maintenance, body reserves and mammary gland and/or growth (for a review see BAUMAN and CURRIE 1980, BAUMAN and McCUTCHEON 1985).

Although the important role of prolactin in the physiology of lactation has been clearly established, very little attention has been paid to the genetic basis of prolactin secretion. In this study, the methods of quantitative genetics and animal breeding theory have been applied to prolactin research data on A.I. bulls with the main emphasis being on basal prolactin level. The objectives were: 1) to analyze variation in the blood prolactin levels of A.I. bulls, and 2) to evaluate the value of a single prolactin measurement as a marker for one or several characteristics for which A.I. bulls are progeny tested.

MATERIAL AND METHODS

At three bull stations 405 blood samples were collected from bulls owned by the Salapausselkä A.I. Centre (Salpausselkä Bull Station), the Pirkanmaa A.I. Centre (Pirkkala Bull Station) and the Breeding Service (Rauhalinna Bull Station) and from the Performance Testing Station (Humppila) owned by the Finnish Animal Breeders' Association. The samples were collected in 1974—78 during October, November, December and January, i.e. during the darkest period of the year. The station, number of samples and sampling times were:

Station	Date	n	
Salpausselkä	Nov 1974	30	(S-74)
— » —	Dec 1975	87	(S-75)
— » —	Dec 1976	60	(S-76)
— » —	Nov 1977	60	(S-77)
Pirkkala	Dec 1977	48	(P-77)
Rauhalinna	Jan 1978	60	(R-78)
Humppila	Oct 1976	60	(H-76)
		405	

Eleven bulls had three samples, 57 had two and 232 bulls had one sample analysed for PRL level. In the case that a bull had several samples, the samples were then taken at one-year intervals. Breed distribution in the whole material was: 284 Ayrshire, 15 Friesian and 1 Finncattle bulls. The bulls of the Performance Testing Station were 8—12 months old. The age of A.I. bulls varied between 12 and 47 months and differences in the mean age of the bulls between station-year subclasses were not statistically significant ($P > 0.10$).

A radioimmunoassay method for bovine prolactin determinations developed by MÄKELÄ and KOSSILA (1976) was employed for the PRL analyses. Mean RIA sensitivity for PRL was 0.1 ng/ml and the inter-assay and intra-assay coefficients of variation were 14 % and 8 %, respectively.

Samples were taken by a needle from the vena jugularis. Serum or heparinized plasma was used for prolactin determinations. The majority of the analyses were made on serum, and heparinized plasma was used only in samples obtained from the Salpausselkä Bull Station in 1974.

Additional data employing different sampling techniques was collected on 30 bulls at the Salpausselkä Bull Station. Each bull was sampled 2—4 times. Samples were taken with a needle or catheter from the ear vein or the vena jugularis. The time between samplings was either 1—2 minutes or 30 minutes. As no differences between the different techniques were observed ($P > 0.10$), and as the order of blood sampling had no effect on PRL levels ($P > 0.10$), this material was used for the repeatability estimation.

Progeny testing results were provided by the Finnish Animal Breeders' Association for the following characteristics: fat percentage of milk, protein percentage of milk, milk yield (305 days, kg), fertility index (calving interval and number of services per pregnancy), calf mortality, mastitis frequency and acetone frequency (number of registered cases diagnosed by a veterinarian). Progeny tests were calculated in 1989. The average number of daughters per bull was 233, ranging from 21 to 3474. 88 % of bulls had less than 350 progeny, on which the progeny test was based. In addition, there was information on the bulls' own growth index, weight at the age of one year and non-return rate in A.I. use.

As described by SCHAMS (1974), DAVIS et al. (1979) and KLINDT (1988) blood PRL levels (in bulls or rams) vary within a day, showing sharp, short term increases. The number of secretory peaks in bulls between 8 a.m. and 4 p.m. has been reported to average one, ranging from 0 to 3 (KLINDT 1988). During secretory peaks, blood PRL levels average 3—4 fold the basal level (KLINDT 1988). In this study, the exceptionally high PRL levels were interpreted to have been measured during a peak, or else they were regarded as "abnormal" for other reasons. After a preliminary analysis, which showed significant differences between the station-year subclasses in PRL levels, the data was selected using different criteria for different station-year subclasses. The upper limit is based on the distribution of PRL levels on each station and year in a way that discarding intensity becames the same:

Station-year

S-74	bull's PRL level should be	<45 ng/ml
S-75	— » —	<36 ng/ml
S-76	— » —	<10 ng/ml
S-77	— » —	<39 ng/ml
P-77	— » —	<76 ng/ml
R-78	— » —	<51 ng/ml
H-76	— » —	<58 ng/ml

In case the bull had several PRL measurements, only one measurement was randomly chosen for all other analysis, except for the repeatability estimation.

The data was analyzed using the least square analysis of variance with computational methods suitable for data with unequal subclass numbers. Prolactin levels were transformed into a natural logarithmic scale. The Hendersson III method was employed for variance component estimation.

Fixed effects were analysed using three different models:

$$Y_{ij} = \mu + a_i + b_1x_{ij} + b_2x_{ij} + e_{ij} \qquad \text{(Model 1)}$$

Y_{ij} = the ij'th observation
μ = LS mean
a_i = effect of i'th station-year (i = S-74, S-75, S-76, S-77, P-77, R-78, H-76)
b_1 = regression on age (in months)
b_2 = regression on weight at the age of one year
e_{ij} = residual term with variance δ_e^2

$$Y_{ij} = \mu + a_i + b_1x_{ij} + e_{ij} \qquad \text{(Model 2)}$$

Y_{ij} = the ij'th observation, (H-76 material only)
a_i = effect of i'th age class (i = 8, 9, 10, 11, 12 month)
b_1 = regression on weight at the age of one year

$$Y_{ijk} = \mu + a_i + b_j + e_{ijk} \qquad \text{(Model 3)}$$

Y_{ijk} = the ijk'th observation
a_i = effect of i'th station-year (i = P-77, R-78)
b_j = effect of j'th breed (j = Ay, Fr)

The repeatability of PRL level in 2—4 samples taken within 35 minutes was estimated using the following statistical model:

$Y_{ij} = \mu + B_j + e_{ij}$ (Model 4)

Y_{ij} = the ij'th observation (unselected S-74 material)

B_j = effect of j'th bull (regarded as random)

Repeatability of a bull's PRL level over the three subsequent years was estimated using the following statistical model:

$Y_{ijk} = \mu + a_i + B_j + e_{ijk}$ (Model 5)

Y_{ijk} = the ijk'th observation (S-74, S-75, S-76, S-77)

a_i = effect of i'th year (i = -74, -75, -76, -77)

B_j = effect of j'th bull (regarded as random)

Heritability was estimated on half-sib correlation. The statistical model to describe the data was:

$Y_{ijk} = \mu + a_i + B_j + e_{ijk}$ (Model 6)

Y_{ijk} = value of ijk'th observation

a_i = effect of i'th station-year (i = S-74, S-75, S-76, S-77, P-77, R-78, H-76)

B_j = effect of j'th sire (regarded as random)

The minimum number of bull progeny the sire should have to be accepted in the heritability estimation was limited to 3 or 8.

Genetic correlations were calculated as simple correlations between PRL level and breeding value. Phenotypic correlations were calculated between the bulls' PRL and individual merit in growth and non-return rate. As the PRL level was strongly affected by factors associated with station-years, the two most extreme subclasses were excluded from the correlation analyses. To be accepted for genetic correlation estimation, a bull should have a minimum of 50 daughters on which the progeny test was based. This was necessary to avoid the effect by the correction of the breeding values towards the population mean.

RESULTS

Variation

The range of PRL levels in the whole material was 0.1—260.0 ng/ml. After employing different selection criteria for different station-years the mean PRL level was 10.4 ng/ml in serum (n = 291) with a standard error of 12.6 ng/ml. The distribution of PRL levels after eliminating extremely high PRL levels is presented in Fig. 1.

The mean PRL levels of bulls measured from different **stations** and **years** are presented in Table 1. PRL levels were strongly affected by the factors associated with station-years. The differences were statistically significant ($0.001 < P < 0.01$, Model 1). They might have been caused by differences in daily routines, feeds, etc. of the stations (bull station effect), differences in feeds and light and temperature conditions between the years (year effect) and possible differences in sampling routines.

The results of this study suggest that prolactin levels tended to decrease as the animals **aged** ($0.05 < P < 0.10$, Model 1) (Fig. 2). Instead, the **live weight** of the bull at age one year

Table 1. Mean prolactin levels of A.I. bulls measured for different stations and years.

Station-year	PRL mean ng/ml	n	Standard error ng/ml	Range ng/ml
S-74	10.6	27	10.2	3.0—39.5
S-75	9.5	76	8.5	1.0—31.9
S-76	1.6	48	1.6	0.1— 7.5
S-77	7.6	55	6.4	0.7—34.9
P-77	22.6	44	20.3	1.4—75.0
R-78	7.0	52	8.9	0.1—32.9
H-76	13.7	57	12.9	0.7—52.2

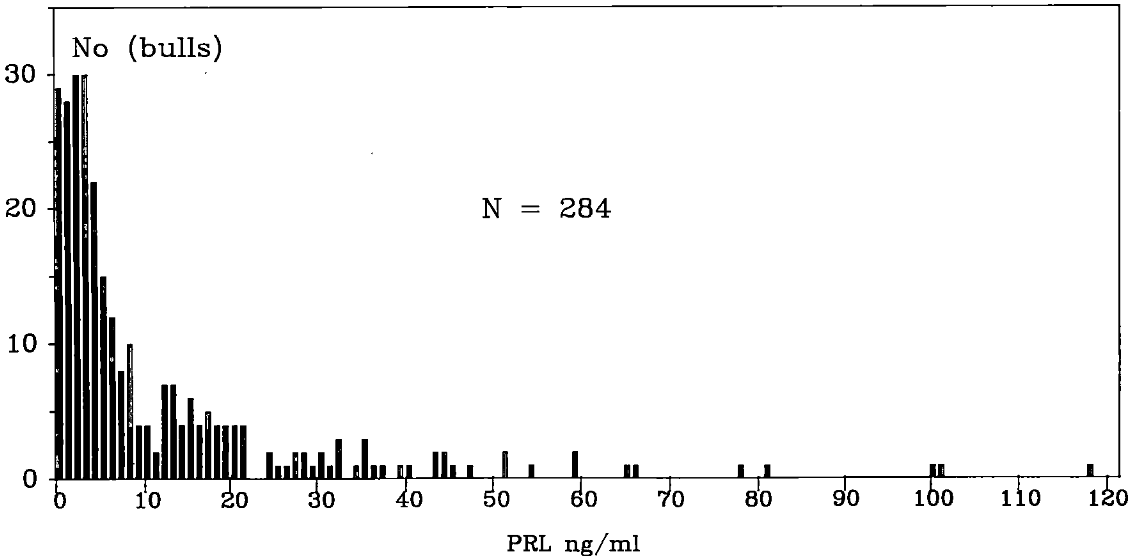

Fig. 1. Distribution of blood PRL levels in A.I. bulls.

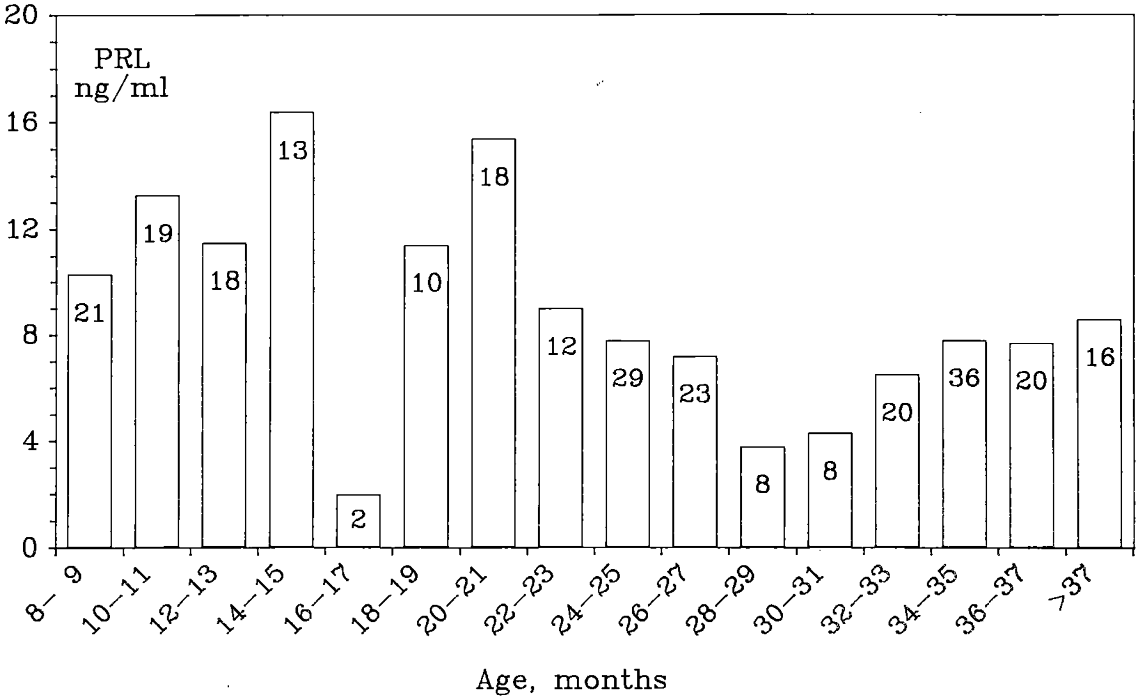

Fig. 2. Effect of age of the bull on blood PRL level in Finnish Ayrshire bulls measured during winter months. (One observation per bull, extremely high PRL values excluded).

seemed not to be associated with the basal PRL level in blood ($P > 0.10$, Model 1).

Among the **young bulls** at the Performance Testing Station (H-76) neither the **age** of the bull (from 8 to 12 months) nor its **live weight** at the age of one year had any significant effect on PRL levels ($P > 0.10$, Model 2).

The number of Friesian bulls in the material was very limited. Based on the results of 13 Friesian bulls measured at the Rauhalinna or Pirkkala Bull Stations, it seems that Friesian bulls have a higher basal PRL level ($\bar{x} = 27.2 \pm 23.4$ ng/ml compared to Ayrshire bulls ($\bar{x} = 12.4 \pm 14.5$ ng/ml). However, in the statistical analyses using a natural logarithmic scale, the difference between **breeds** was not statistically significant ($P > 0.10$, Model 3).

Repeatability

Repeatablity of PRL levels within 35 minutes was 0.73 ± 0.08 (Model 4) ($n = 30$ bulls), showing that a single measurement is relatively reliable in determing the PRL level at a given moment, and that sampling routines did not seem to cause secretory peaks.

On the basis of 9 bulls sampled 3 times at the Salpausselkä Bull Station, the repeatability of a single PRL measurement over the three subsequent years was 0.21 ± 0.25 (Model 5). It was higher compared to 0.13 in three samples collected within 10 days (TUCKER et al. 1973). VINES et al. (1976) calculated a repeatability of 0.27 for basal PRL level in prepubertal heifers during 4 days of sampling. DAVIS et al. (1979) collected blood samples at 15 min intervals for 12 hours in rams, and determined within month repeatabilities for overall PRL concentration, basal PRL level, frequency of secretory peaks and mean amplitude of secretory peaks. The repeatabilities were 0.47, 0.20, 0.0, and 0.0, respectively (DAVIS et al. 1979). The repeatability of 0.21 over three years in this study is relatively high compared to other published estimates. The results suggest that despite the remarkable within day variations in PRL levels, a bull's PRL level, in relation to the PRL levels of other animals measured at the same time, is repeatable to some extent even when measured only in a single blood sample.

Heritability

Heritability estimates and detailed information on the data they are based on, are presented in Table 2. The calculated estimates varied around 0.25 (Model 6). Excluding the S-76 material (26 observations) had no significant effect on the estimates.

TUCKER et al. (1973) could not find additive genetic variation in blood PRL levels, which probably is more a reflection of an unknown variation behind the PRL levels than a lack of genetic variation. Instead, moderate or high heritability estimates (in cattle, sheep and pigs) have been published for some other hormones such as thyroid hormones, FSH (follicle stimulating hormone) and insulin (REINECKE et al. 1986, BODIN et al. 1986, JUST et al. 1983, FLACH et al. 1985).

Correlations

The correlations between the bulls' PRL level and their individual performance in growth and non-return rate, as well as correlations between the bulls' PRL and progeny testing results are presented in Table 3. Although it is not possible to present meaningfull standard errors for genetic correlations, they probably would be quite high. The correlations were also calculated separately for H-76 and R-78 material, e.g. in young and mature bulls (Table 3).

The phenotypic correlation with growth index was positive, but low, although statistically significant ($P < 0.05$). In young animals the correlation was higher, but negative. Except for the negative correlation coefficient found in young animals, the present results agree with the research of OHLSON et al. (1987), who have shown that higher growth potential is associated with elevated characteristics in growth hormone and prolactin secretion.

Correlations with milk production characteristics were also low in general. The correlation with milk yield (absolute yield) was —0.07, which is lower and opposite to 0.16 published by KLINDT (1988). Low genetic correlation coefficients between basal PRL level and milk yield agree with the results of selection experiments. Selection for high milk yield has not changed the basal PRL level of animals in selected lines (KAZMER et al. 1986, BONCZEK et al. 1988).

The highest correlations in milk production characteristics found in this study, were calculated between PRL level and fat and protein percentages in milk, especially when PRL was measured in young animals. KLINDT (1988) has published positive correlations between bulls' basal PRL level and breeding value in milk fat and protein yields (0.34, 0.36 respectively). Experiments using tissue cultures have shown that prolactin is essential both in fat and protein synthesis on the cellular level in the mammary gland (CHOI et al. 1988, SHAMAY et al. 1989). In this respect, positive correlations between PRL secretion characteristics and protein or fat percentages in milk are logical, although the correlations need not to be linear.

Relatively high correlations were observed between a bull's PRL level and breeding value in acetone frequency. High PRL levels were associated with low acetone frequency among

Table 2. Heritability of basal PRL level in blood of A.I. bulls. h^2 = heritability, s.e. = standard error, Range = range of number of bull progeny per sire, R^2 = multiple correlation coefficient.

h^2	s.e	n sires	n total	Range	R^2	Restrictions
0.25	0.17	19	252	3—40	0.30	min. 3 progeny/sire
0.25	0.20	8	202	8—40	0.29	min. 8 progeny/sire
0.25	0.18	19	226	3—37	0.14	min. 3 progeny/sire and S-76 excluded

Table 3. Relationship of prolactin to individual and progeny performance of A.I. bulls. r = correlation coefficient, n = number of bulls.

			PRL measured in			
			young bulls (8—12 months)		mature bulls (22—47 months)	
progeny:	r	n	r	n	r	n
milk yield (305 days, kg)	—0.07	174	—0.09	39	—0.12	46
fat% in milk	0.06	174	0.37	39	—0.06	46
protein% in milk	0.13	174	0.24	39	0.11	46
fertility index[i]	—0.11	140	—0.15	39	—0.10	46
calf mortality	—0.16	140	—0.27	39	0.23	46
mastitis frequency	—0.07	28	—0.25	9	0.09	12
acetone frequency	—0.31	28	0.58	9	—0.25	12
individual:						
non-return rate	—0.05	135	0.37**	39	—0.28*	46
growth index	0.15*	194	—0.26	39	0.16	45

* ($P < 0.05$)
** ($P < 0.01$)
[i] high values mean good fertility

daughters, except in young animals. The present material is too limited for further conclusions. However, acetone is a disease caused by disorders in energy metabolism. The observed correlations could thus be a sign of the role of PRL in energy and lipid metabolism.

The results presented in Table 3 suggest that the age of the bull is important when interpreting the basal PRL levels. High PRL levels may possibly indicate different things when measured in growing or in mature animals, as the correlations with progeny and phenotypic characteristics seem to differ at different ages.

PRL and animal breeding

As shown by other authors, the blood PRL levels of male ruminants (bulls and rams) fluctuate within a day and with several secretory peaks (SCHAMS 1974, DAVIS et al. 1979, KLINDT 1988). In order to determine the basal PRL level, a minimum of three measurements taken at one-hour intervals should be taken to avoid secretory peaks and to decrease error variation.

The results of this study indicate that heritability of basal PRL level is moderate. In this respect, the use of bulls' PRL levels in breeding programmes would be effective. Although PRL plays an important role in milk synthesis and possibly in energy metabolism, it is still very unclear as to what factor in PRL secretion is critical in milk synthesis, growth, etc. and what negative consequences would follow if PRL characteristics were altered. A better understanding of genetic and other associations between PRL secretory parameters and important production characteristics is still needed, before PRL parameters could be utilized in cattle breeding.

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SELOSTUS

Prolaktiini Suomen nautakarjassa. 3 Keinosiemennyssonnien veren prolaktiinitason vaihtelu, toistuvuus ja periytyvyys sekä yhteys sonnin ja sonnin jälkeläisten tuotanto-ominaisuuksiin

ANU OSVA, VAPPU KOSSILA ja RITVA MÄKELÄ-KURTTO

Maatalouden tutkimuskeskus

Kolmeltasadalta keinosiemennyssonnilta, jotka olivat kasvatuskoeasemalla tai sonniasemalla (kolme sonniasemaa), kerättiin talvikuukausien aikana vuosina 1974—1978 yhteensä 405 verinäytettä. Radioimmunologista menetelmää käyttäen määritettiin näytteistä prolaktiini-hormonin (PRL) taso. Tavoitteena oli tutkia veren PRL arvojen vaihtelua ja arvioida yksittäisen määrityksen ennustusarvoa suhteessa sonnin omiin tai sen jälkeläisten tuotannon tai käytön kannalta tärkeisiin ominaisuuksiin.

Koko aineistossa PRL-taso vaihteli 0.1—260 ng/ml. Keskiarvoksi, kun äärimmäiset arvot oli karsittu, saatiin 10.2 ± 12.6 ng/ml. Erot keinosiemennysasemien ja/tai vuosien välillä olivat huomattavia ($0.001 < P < 0.01$). Prolaktiiniarvot myös laskivat sonnien iän kasvaessa ($0.05 < P < 0.10$). Kolmenkymmenenviiden minuutin kuluessa otettujen näytteiden toistuvuus oli 0.73 ± 0.08 ja kolmena eri vuotena yhdeksältä sonnilta samalla sonniasemalla otetuissa näytteissä toistuvuus oli 0.21 ± 0.25. Prolaktiinitaso näyttäisi olevan kohtuullisen periytyvä. Heritabiliteetin arvoksi saatiin 0.25 ± 0.17. Aineisto käsitti 19 isää, joilla oli 3—40 sonnijälkeläistä kullakin.

Fenotyyppiset korrelaatiot sonnin PRL-tason ja ei-uusintaprosentin ja kasvuindeksin välillä olivat 0.05 (n.s.) ja 0.15 ($P < 0.05$), vastaavasti. Geneettiset korrelaatiot laskettiin PRL-tason ja jalostusarvojen välisinä yksinkertaisina korrelaatioina. PRL-tason geneettisiksi korrelaatioiksi maitotuotokseen (absoluuttinen tuotos, kg), maidon rasvapitoisuuteen, maidon valkuaispitoisuuteen, hedelmällisyysindeksiin, vasikkakuolleisuuteen, utäretulehdusfrekvenssiin ja asetonitautifrekvenssiin saatiin —0.07 ($n = 174$), 0.06 ($n = 174$), 0.13 ($n = 174$), —0.11 ($n = 140$), —0.06 ($n = 140$), —0.07 ($n = 28$) ja —0.31 ($n = 28$), vastaavasti.

Fenotyyppiset ja geneettiset korrelaatiot poikkesivat toisistaan ja viittasivat usein voimakkaampaan korrelaatioon, kun ne laskettiin erikseen kasvavien ja toisaalta täysi-ikäisten sonnien tuloksista.

Tulosten ja kirjallisuuden perusteella tultiin siihen johtopäätökseen, että huolimatta PRL-tason kohtuullisesta periytyvyydestä ja prolaktiinin yhteydestä maidontuotantoon (erityisesti valkuaisen ja rasvan synteesiin), eläimen kasvuun ja mahdollisesti energia-aineenvaihduntaan, tarvitaan vielä huomattavasti tutkimusta ennenkuin kuva on riittävän selvä käytännön sovellutuksille.

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Seria ANIMALIA DOMESTICA, N. 102 — Sarja KOTIELÄIMET n:o 102

PROLACTIN IN FINNISH DAIRY CATTLE. 4

Correlations between prolactin, selected entzymes, minerals, trace elements and some other blood parameters in artificial insemination (A. I.) bulls

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KOSSILA, V., MÄKELÄ-KURTTO, R., OSVA, A. & TANHUANPÄÄ, E. 1991. **Prolactin in Finnish dairy cattle. 4. Correlations between prolactin, selected entzymes, minerals, trace elements and some other blood parameters in artificial insemination (A. I.) bulls.** Ann. Agric. Fenn. 30: 73—80. (Agric. Res. Centre of Finland, Inst. Anim. Prod., SF-31600 Jokioinen, Finland.)

The associations between serum prolactin (PRL) and selected enzymes (ALP, ALT, AST, GGT, LDH), minerals (Ca, P, Mg, K, Na), trace elements (Fe, Cu, Zn) and some other blood parameters (blood urea nitrogen, creatinine, total protein, cholesterol) were studied in 316 A.I. bulls. Blood samples were collected at three bull stations and at one performance testing station. The age of the bulls varied between 8 and 47 months. The correlations were estimated as overall correlations (all bulls included) and separately for three age groups (12—21, 22—31 and over 31 months).

Both generally and in different age groups, ALP was correlated negatively with PRL level (overall: $r = —0.25$, $P < 0.001$; 12—21 months: $r = —0.35$, $P < 0.05$; 22—31 months: $r = —0.11$, $P > 0.05$; > 31 months: $r = —0.28$, $P < 0.05$). The overall correlations between PRL and the other variables studied, were nearly zero. In young animals (12—21 months), the correlation of PRL with the other variables studied, with the exception of BUN, total protein and cholesterol, was negative and statistically significant with ALP, AST, CPK and K ($r(AST) = —0.31$, $r(CPK) = —0.30$, $r(K) = —0.29$). Except for ALP activity, the correlations with enzymes were positive in bulls aged 22—31 months, the correlation between PRL and AST being statistically significant ($r = 0.32$). In bulls over 31 months of age, the correlation estimates were significant for ALP and P ($r(P) = 0.20$). Furthermore, cholesterol and PRL levels seemed to be related differently at different ages. The correlation coefficient was positive in young animals and negative in older animals.

According to the results, it was concluded that 1) simultaneous changes occur in ALP activities and PRL levels. 2) The negative correlation estimates found in young animals between PRL and the enzymes studied (associated with high energy metabolism and metabolism in bone, muscle and liver) and the reversal of these estimates from negative into positive in older animals, suggest that interpretation of PRL levels is dependent on factors associated with age of animals. For instance, high PRL levels found in young, growing animals possibly mean different things in metabolism than the high values detected in older animals (high levels refer to high levels within a corresponding age group).

Index words: dairy cattle, bulls, prolactin, minerals, trace elements, enzymes, cholesterol, creatinine, total protein, blood urea nitrogen.

INTRODUCTION

Prolactin (PRL) is either known or suspected to participate in many important metabolic processes such as lactogenesis, mineral and lipid metabolism, and in the partitioning of energy between different tissues in the body (revs. by BAUMAN and CURRIE 1980, BAUMAN and McCUTHEON 1985, TUCKER 1981). In recent studies, PRL has been reported to be essential in milk synthesis on the cellular level (CHOI et al. 1988, SHAMAY et al. 1989) and prolactin secretion characteristics have been found to be associated with animal growth (OHLSON et al. 1987).

The objectives of the present study were to examine the correlations between serum PRL levels and such blood parameters that are either directly or indirectly involved in growth, as well as in lipid, high energy and mineral metabolisms.

MATERIAL AND METHODS

The data consisted of 405 blood samples collected from 316 A.I. bulls at three bull stations owned by the Salpausselkä A.I. Centre (Salpausselkä Bull Station), the Pirkanmaa A.I. Centre (Pirkkala Bull Station) and the Breeding Service (Rauhalinna Bull Station) and at the Performance Testing Station (Humppila) owned by the Finnish Animal Breeders' Association. The blood samples were collected during one day:

Station	Date	n	
Salpausselkä	Nov 1974	30	(S-74)
— » —	Dec 1975	87	(S-75)
— » —	Dec 1976	60	(S-76)
— » —	Nov 1977	60	(S-77)
Pirkkala	Dec 1977	48	(P-77)
Rauhalinna	Jan 1978	60	(R-78)
Humppila	Oct 1976	60	(H-76)
		405	

Feeding was not restricted before the collection of blood samples. It is probable that this increased the error variation in the BUN and total protein values, but not in the values for enzyme activities or mineral and trace element levels.

The breed distribution was 300 Ayrshire, 15 Friesian and 1 Finncattle bulls. The data has been previously described in more detail by OSVA et al. (1991).

All samples were determined for phosphorus (P) (not S-74), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), copper (Cu) and zinc (Zn). The samples of the S-77, P-77 and R-78 materials, were also determined for alkaline phosphatase (ALP), aspartateaminotransferase (AST), alanineaminotransferase (ALT), creatinephosphokinase (CPK), gamma-glutamyltranspeptidase (GGT), lactatedehydrogenase (LDH), creatinine, blood urea nitrogen (BUN), total protein and cholesterol.

A radioimmunoassay method for bovine prolactin determinations developed by MÄKELÄ and KOSSILA (1976) was used for the PRL analyses. The mean RIA sensitivity for PRL was 0.1 ng/ml and inter-assay and intra-assay coefficients of variation were 14 % and 8 %, respectively. Enzyme activities were determined according to the recommendations of the Committee on Enzymes of the Scandinavian Society for Clinical Physiology (1974, 1976) with the modification that CPK activity was measured without preincubation at 37 °C. Mineral and trace elements were determined at the Agricultural Research Centre of Finland, Department of Animal Production, and enzymes and other blood parameters at the Central Laboratory of the College of Veterinary Medicine, Helsinki.

Blood samples were taken from the vena jugularis. Serum or heparinized plasma was used for the prolactin determinations. The majority of the PRL analyses were performed on serum, and heparinized plasma was used only in the samples from the Salpausselkä Bull Station taken in 1974. Enzyme activities, minerals, trace elements, creatinine, cholesterol, BUN and total protein were determined in plasma.

Exceptionally high PRL levels were excluded from the statistical analyses. As the station-year subclasses differed significantly in mean PRL level, different selection criteria were used for different station-year subclasses (see OSVA et al. 1991).

In order to avoid errors in the statistical analyses due to single exceptional values in the variables studied, the distribution of each variable was checked. Exceptional values were excluded from the statistical analysis applying the following criteria: CPK $<$ 300 iu/l, GGT $<$ 75 iu/l, LDH $<$ 500 iu/l, 1.6 mmol/l $<$ BUN $<$ 7.0 mmol/l, creatinine $<$ 220 µmol/l, K $<$ 25.0 and Zn $>$ 20.0 µg%.

The data was analyzed using the least square analysis of variance with computational methods suitable for data with unequal subclass numbers. Prolactin levels were transformed into a natural logarithmic scale. If a bull had several samples analyzed, only one of these was randomly chosen.

First, all of the variables were analysed for the significance of three fixed effects, eg. effect of station-year, effect of age and effect of live weight of the bull at age one year. Second, the correlation coefficients between PRL level and each variable studied, were calculated employing different statistical models based on these results (Table 2). In all cases, the model included the station-year factor. Whenever the effect of age and/or weight was statistically significant ($P < 0.01$ = **), these were included in the model as regression variables.

The correlations between PRL and other blood parameters were calculated for three age groups, 12—21 months, 22—31 months and $>$ 31-month-old bulls. In these analyses the age of the bull was not included in the model.

RESULTS AND DISCUSSION

The means, standard deviations and range of values for each variable studied are given in Table 1. Exceptionally high enzyme activities and creatinine levels were found in some animals. The normal values are shown in Table 1 (KERR et al. 1989, ANON. 1991). After eliminating the most exceptional values, some values which were higher than the reference values still remained in the data.

A summary of the results of the variance analysis examining the fixed effects are given in Table 2. The differences between station-year subclasses were highly significant in all other cases except ALT, AST, GGT and creatinine. Most variables seemed to change with age (ALP, LDH, creatinine, BUN, total protein, P, Na, Fe, Cu and Zn). The effect of live weight at the age of one year (eg. growth rate + sexual maturity) was significant for AST, GGT and LDH levels.

The overall correlations between blood PRL level and the other blood parameters were about zero and not statistically significant (last column in Table 3). The only exception was ALP activity, which seems to be negatively correlated with PRL level. ALP is involved in mineral metabolism and a high level of activities (within the normal range) usually correspond with active transport of minerals from the gut and between blood and bone tissues. In mineral deficient diets, ALP activities increase and may exceed the reference values (Kossila et al. 1977). The statistically significant negative correlation between PRL and ALP, after eliminating the effect of age, indicate that higher PRL levels are found when ALP activities are low. The overall correlations between PRL and ALT, CPK, GGT, LDH and creatinine presented here differ significantly from estimates in cow material (Mäkelä-Kurtto et al. 1991). For ALP and AST, the estimates were of same order as those found in cows (Mäkelä-Kurtto et al. 1991).

When the correlations were calculated separately for different age groups the overall picture changed. Correlations with enzymes, creatinine, minerals and trace elements were negative in young animals (12—21 months), being statistically significant ($P < 0.05$) for ALP, AST, CPK and K (Table 3). In bulls aged 22—31 months, the correlation coefficients between PRL and enzymes and creatinine changed to positive (not with ALP) and were higher compared to the estimates calculated for bulls aged 32 months or more (Table 3). These results suggest that interpretation of PRL levels is dependent on factors associated with age of animals. For instance, high PRL levels found in young, growing animals possibly mean different things in metabolism than the high values detected in older animals (high levels refer to high levels within a corresponding age group).

Likewise, the correlation of PRL level with the bulls' own growth index was negative at young age, changing to positive in older animals (Osva et al. 1991).

In general, minerals and trace elements showed a weak association with PRL level, and no systematic change from negative to positive correlation estimates with age could be detect-

Table 1. Means, standard deviations and range of variation in blood PRL and mineral levels, enzyme activities and in some other blood parameters among Finnish A.I. bulls. Mean age of the bulls as well as mean weight at the age of one year are also given.

	Mean	Unit	s.d.	Range**	n	Reference values
PRL	10.6	ng/ml	12.7	0.1 — 75.0	291	
ALP	193.4	iu/l	80.3	57 —437	166	< 300[k]
ALT	31.3	iu/l	7.7	12 — 64	167	< 100[k]
AST	64.6	iu/l	19.5	32 —152	168	< 138[a]
CPK	111.7	iu/l	50.5	37 —395	166	< 348[a]
GGT	24.8	iu/l	10.6	10 — 90	167	< 36[a]
LDH	290.1	iu/l	77.7	143 —856	155	200—300[k]
Phosphorus	3.53	mg%	0.88	1.60— 5.14	296	
Calcium	10.2	mg%	5.77	8.33— 13.33	315	
Magnesium	2.22	mg%	0.28	1.50— 3.53	316	
Potassium	19.1	mg%	1.49	15.4 — 23.6	269	
Sodium	310.7	mg%	27.5	266 —457	269	
Iron	177.0	ug%	71.2	64 —522	307	
Copper	117.7	ug%	26.2	65 —264	313	
Zinc	103.0	ug%	29.3	18 —314	314	
Creatinine	150.5	µmol/l	41.9	83 —566	164	< 150[k]
BUN	3.56	mmol/l	0.82	1.6 — 7.4	161	
Total protein	80.7	g/l	9.2	63 —123	168	
Cholesterol	3.99	mmol/l	0.91	2.3 — 7.0	120	
Live weight*	456	kg	30.1	397 —593	299	
Age	24.2	months	9.5	8 — 47	315	

* live weight at the age of one year

** All observations (one sample per bull) except in PRL levels, where the most extreme values are excluded

[a] = Anon. 1991

[k] = Kerr 1989

Table 2. Statistical significance of certain fixed effects on the blood parameters studied.

	Station-year	Age of the bull	Live weight at the age of one year
ALP	***	***	n.s.
		b(ALP × Age) = —5.5 iu/l	
ALT	n.s.	n.s.	n.s.
AST	n.s.	n.s.	**
		b(AST × Weight) = —0.12 iu/l	
CPK	***	n.s.	n.s.
GGT	*	n.s.	*
LDH	***	***	***
		b(LDH × Age) = 1.78 iu/l	
		b(LDH × Weight) = —0.44 iu/l	
Phosphorus	***	***	n.s.
		b(P × Age) = —0.02 mg%	
Magnesium	***	n.s.	n.s.
Calcium	***	n.s.	n.s.
Potassium	***	n.s.	n.s.
Sodium	***	*	n.s.
Iron	***	***	n.s.
		b(Fe × Age) = 2.3 µg%	
Copper	***	***	n.s.
		b(Cu × Age) = 0.68 µg%	
Zinc	***	**	n.s.
		b(Zn × Age) = 0.48 µg%	
Creatinine	*	***	n.s.
		b(Creat × Age) = 1.11 µmol/l	
BUN	***	***	n.s.
		b(BUN × Age) = 0.03 mmol/l	
Total protein	***	*	n.s.
Cholesterol	***	n.s.	n.s.

Table 3. Correlation between blood PRL level and selected enzymes, minerals, trace elements and some other blood parameters in A.I. bulls.

	Age of the bulls							
	12—21 months	n	22—31 months	n	> 31 months	n	ALL	n
ALP	—0.35*	33	—0.11	52	—0.28*	63	—0.25***	148
ALT	—0.25	33	0.15	53	—0.08	63	0.01	150
AST	—0.31*	32	0.32*	49	0.01	61	0.06	143
CPK	—0.30*	33	0.07	53	0.16	62	—0.02	148
GGT	—0.22	33	0.22	52	0.07	62	0.12	148
LDH	—0.10	32	0.24	48	—0.09	50	0.11	130
P	—0.11	55	—0.03	80	0.20*	83	0.05	218
Ca	—0.11	58	—0.13	85	0.09	97	0.00	241
Mg	—0.15	58	—0.04	85	0.09	97	0.01	241
Na	—0.16	39	—0.15	77	0.12	80	—0.07	197
K	—0.29*	39	0.07	77	0.09	80	0.04	197
Fe	—0.02	58	0.04	85	0.15	92	0.06	235
Cu	—0.05	58	—0.10	85	—0.15	92	—0.09	235
Zn	—0.19	58	—0.13	85	—0.04	92	—0.10	235
Creatinine	—0.12	30	0.19	54	0.06	62	0.02	147
BUN	0.13	28	—0.05	50	0.09	63	0.06	141
Total protein	0.24	33	0.10	54	—0.04	63	0.06	150
Cholesterol	0.31	13	0.11	46	—0.24	47	0.02	107

(the statistical model varies according to variables, station-year effect is always included)
* ($P < 0.05$), *** ($P < 0.001$)

Table 4. Correlation estimates between selected enzymes, minerals, trace elements, creatinine, BUN and total protein in 80 A.I. bulls at three bull stations (the statistical model included the station-year effect).

ALP	ALT	AST	CPK	GGT	LDH	BUN	Creatinine	Total protein	Cholesterol	Ca	P	Mg	K	Na	Fe	Cu	Zn
ALP	—.19*	—.08	.21*	—.08	—.14	—.30**	—.07	.08	—.07	.12	.37***	.06	.18	—.08	—.16	—.30**	—.03
	ALT	.15	.06	—0.7	.11	.16	.09	—.11	.05	—.23*	.18	—.02	—.02	.18	—.16	—.07	.05
		AST	.29**	.38***	.45***	.05	.21*	.24*	.04	.09	.01	.05	—.07	.05	.36***	.40***	.22*
			CPK	—.06	.08	—.08	.07	.34***	—.15	.06	.08	—.08	—.02	.08	.06	—.08	—.05
				GGT	.37***	.17	.03	—.01	.22*	—.03	—.19*	—.07	—.10	.00	.36***	.35***	.07
					LDH	.15	.05	.06	—.01	—.08	.03	.14	—.05	—.03	.24*	.28**	.10
						BUN	.14	—.20*	.26**	—.03	.12	.20*	.11	.00	.14	.03	.13
							Creatinine	—.04	.18	.01	—.08	—.02	.02	—.19*	—.02	.08	—.01
								Total protein	—.07	—.10	—.06	—.18	—.19*	.05	—.04	.26**	.15
									Cholesterol	—.20*	.10	.22*	.23*	.01	—.14	.23*	.14
										Ca	.00	.22*	.28**	—.07	.24*	—.11	.03
											P	.25*	.41***	.14	—.14	—.31**	.12
												Mg	.45***	—.02	.13	—.18	.29**
													K	.14	—.11	—.09	.18
														Na	—.08	.07	.11
															Fe	.10	.00
																Cu	.18

ed. The only significant correlations observed, were the negative correlation with potassium in young animals and a positive correlation with phosphorus in bulls aged 32 months or more.

The correlations between PRL and blood BUN and total protein were not statistically significant and usually around zero. The correlation between PRL and cholesterol seemed to depend on the age of the animals. It was positive at a young age and negative in older animals. The correlation estimates between enzymes, minerals, trace elements, creatinine, BUN and total protein are given in Table 4 as additional information.

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SELOSTUS

Prolaktiini Suomen nautakarjassa. 4
Keinosiemennyssonnien veren prolaktiinitason yhteys eräisiin veren entsyymeihin, kivennäis- ja hivenaineisiin sekä eräisiin muihin veren ainesosiin

Vappu Kossila, Ritva Mäkelä-Kurtto, Anu Osva ja Eero Tanhuanpää

Maatalouden tutkimuskeskus ja Helsingin yliopisto

Veren seerumin prolaktiinitason yhteyksiä plasmasta mitattujen entsyymien (ALP, ALT, AST, GGT, LDH) aktiivisuustasoihin, mineraali- ja hivenainetasoihin (Ca, P, Mg, K, Na, Fe, Cu, Zn) sekä eräiden muiden plasman ainesosien pitoisuuksiin (ureatyppi, kreatiniini, valkuainen, kolesteroli) tutkittiin 316 keinosiemennyssonnilla. Verinäytteet kerättiin kolmen keinosiemennysaseman ja Humppilan kasvatuskoeaseman sonneista. Sonnien ikä vaihteli kahdeksasta 47:ään kuukauteen. Muuttujien väliset korrelaatiot arvioitiin koko aineistosta, sekä erikseen kolmessa ikäryhmässä (12—21, 22—31 ja > 31 kk vanhat sonnit).

Yleisesti sekä eri ikäkausina ALP korreloitui negatiivisesti prolaktiinitasoon (kaikki sonnit: r = —0.25, P < 0.001; 12—21 kk: r = —0.35, P < 0.05; 22—31 kk: r = —0.11, P > 0.05; > 31 kk: r = —0.28, P < 0.05). Muutoin koko aineistosta lasketut korrelaatiokertoimet olivat lähellä nollaa. Nuorilla eläimillä prolaktiinitason korrelaatiot tutkittuihin ominaisuuksiin lukuunottamatta ureatyppeä, valkuaista ja kolesterolia olivat negatiivisia. Tilastollisesti merkitsevät korrelaatiot saatiin prolaktiinin ja ALP-, AST- ja CPK-aktiivisuuksien sekä kaliumpitoisuuden välille (AST: r = —0.31; CPK: r = —0.30; K: r = —0.29). Lukuunottamatta alkaalista fosfataasia (ALP) korrelaatiot entsyymiaktiivisuuksiin muuttuivat positiivisiksi ikävälillä 22—31 kk. Näistä prolaktiinin ja AST:n välinen korrelaatio oli tilastollisesti merkitsevä (AST: r = 0.32). Yli 31 kk vanhoilla sonneilla saatiin merkitsevä korrelaatio ALP:n lisäksi plasman fosforipitoisuuteen (P: r = 0.20). Myös kolesterolin korrelaatio prolaktiinitasoon näytti olevan sidoksissa eläimen ikään. Nuorella iällä korrelaatiokerroin oli positiivinen muuttuen negatiiviseksi vanhemmilla eläimillä.

Johtopäätöksinä voidaan todeta että, 1) tulokset viittaavat siihen, että prolaktiinitaso ja ALP-aktiivisuus korreloivat negatiivisesti keskenään, 2) Negatiiviset korrelaatiot prolaktiinitasoon ja energia-aineenvaihduntaan sekä luuston, lihasten ja maksan aineenvaihduntaan liittyvien entsyymien välillä nuorilla, kasvavilla eläimillä. Näiden korrelaatioiden muuttuminen positiivisiksi eläinten vanhetessa viittaisi siihen, että prolaktiinitasojen fysiologinen tulkinta olisi sidoksissa eläimen ikään. Tällöin esimerkiksi nuorten eläinten korkeat arvot saattaisivat merkitä ainakin osittain eri asioita kuin korkeat arvot vanhemmilla eläimillä (korkealla arvolla tarkoitetaan korkeata arvoa verrattuna ao. ikäryhmän keskiarvoon).

ANNALES AGRICULTURAE FENNIAE VOL. 30: 81—123 (1991)

LIITE — APPENDIX

LUETTELO VUONNA 1990 JULKAISTUISTA MAATALOUSALAN KIRJOITUKSISTA

List of agricultural papers published in 1990 in Finland

MAATALOUDEN TUTKIMUSKESKUS

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1012	08	Mariehamn
	12	Bomarsund
2311	09	Liinamaa
	10	Lapuan asema
	12	Kauhava
1142	06	Ouran saaristo
	08 + 05	Santee
	09	Köörtilä
	11	Ahlainen
	12	Lankoski
1143	06	Palus
	08	Kullaa
	09	Tuurujärvi
	10	Kynsikangas
	11	Sääksjärvi
	12	Kivijärvenmaa
2124	07	Lempiäniemi
	08	Länsi-Teisko
	09	Parkkuu
	10	Kämmenniemi
	11	Teisko
	12	Murole
1242	09	Petalax
	10	Pörtom
	12	Övermalax
1334	04	Maxmo
	08	Oravais
1244	01	Niemenkylä
	02	Sarvijoki
1021	07	Saltvik
	10	Sund
1141	08	Lankoori
	10	Luvia
	11	Viasvesi
2132	01	Kalvola
	02	Sääksmäki
	03	Valkeakoski
	04	Leteensuo
	05	Tyrväntö
	06	Laitikkala
	07	Hattula
	08	Alvettula
	09	Ilmoila
	10	Eteläinen
3713	09	Sodankylä
2311	06	Ylihärmä
	07	Lapua
	08	Hellanmaa
	11	Ojutjärvi
2323	07	Veteli
	08	Tastula
2311	04	Malkamäki
	05	Kosola
	04	Jurva
	06	Kyläinpää
	07	Nopankylä
	10	Koskenkorva
	11	Huissinkylä
1333	10 + 2311 01	Ylistaro
	11 02	Lehmäjoki
	12 02	Kaurajärvi
2042	01	Karisjärvi
	02	Vuotinainen
2141	04	Pälkäne
	05	Sahalahti
	06	Ponsa
	07	Aitoo
	08	Pakkala
	09	Uiherla
	10	Luopioinen
	11	Rautajärvi
	12	Kuhmalahti
2233	02	Kerte
	03	Roosinpohja
	04	Nytkyme
	06	Koskenpää
	12	Moksi
3142	01	Otava
	02	Vuolinko
	03	Harjunmaa
	04	Porrassalmi
	05	Mikkeli
	06	Hiirola
3211	01	Kouhinsalo
	04	Putkilahti
	05	Oittila
	06	Ristinselkä
3241	09	Mäkrämäki
	10	Sorsakoski
	12	Oravikoski
3243	01	Unnukka
3321	10	Maaselänlahti
	11	Vuohtomäki

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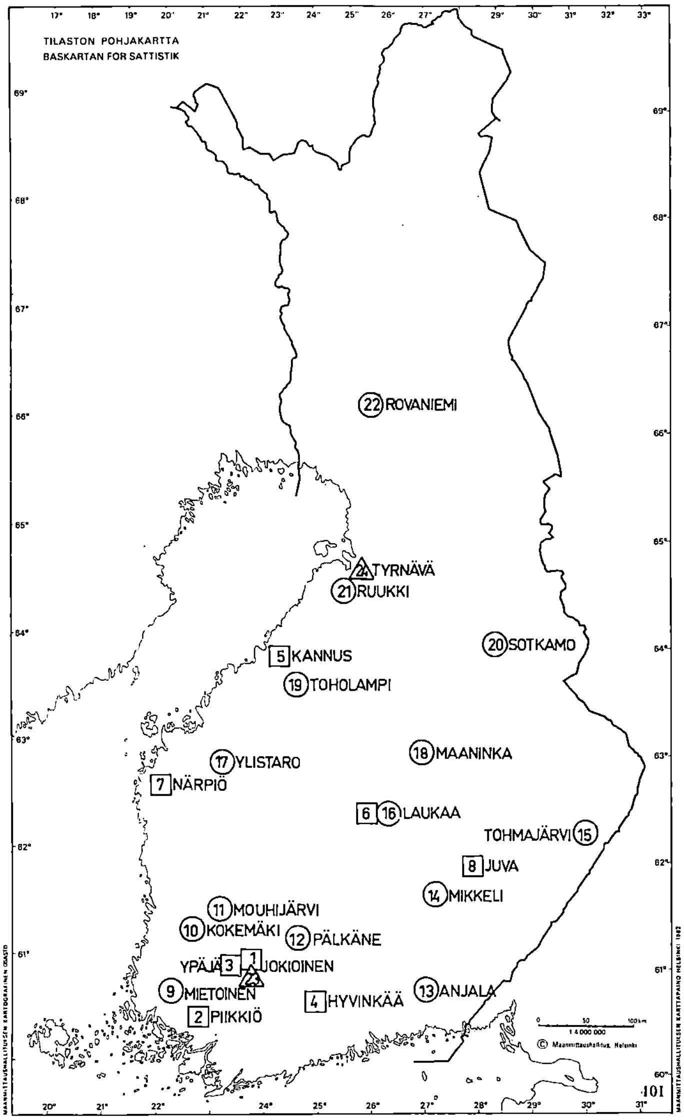

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