J. Vet. Sci. (2002), 3(3), 145-157

JOURNAL OF
Veterinary Science

Review

Biological Rhythm in Livestock

Piccione Giuseppe* and Caola Giovanni

Dipartimento di Morfologia, Biochimica, Fisiologia e Produzioni animali Sezione di Fisiologia Veterinaria
Facoltà di Medicina Veterinaria dell'Università degli Studi di Messina Polo Universitario dell'Annunziata – 98168 – Messina (Italia)

Received July 2, 2002 / Accepted August 30, 2002

Abstract

The animal time structure is a basic fact of life, no matter if one wants to study it or not. The time-dependent, mostly rhythmic, and thus to a certain degree predictable, variations of biochemical and physiological functions and of sensitivity and resistance to many environmental agents are often quite large and offer not only new insight into animal physiology and pathology but also diagnostic possibilities and therapeutic advantages. Chronobiology, chronophysiology and its subspecialities, like chronopharmacology and chronotherapy, will certainly play an important role in the clinical medicine of the future. Successful application of chronobiology to veterinary clinical medicine, however, depends critically on a thorough knowledge of its basic principles.

Key words : biological rhythm; chronophysiology; domestic animals.

Introduction

Living matter and the evolving organisms were exposed to the earth's revolution around the sun with its periodicity of day and night, of light and darkness, with the periodic changes in the length of the daily light and dark span with the climatic changes of seasons. Many periodic functions, ranging in the length of their cycle from milliseconds (as in the activity of single neurons) to seconds (such as the heart and respiration rate) and to months (such as the oestrus cycles in mammals) have no known environmental counterpart. Three examples of biological rhythms are illustrated in figure 1. Some biochemical and biophysical mechanisms creating or maintaining periodic functions at the cellular level are related to the genetic material in nuclear DNA, while others are apparently functioning apart from nuclear material in relation to membranes or to metabolic processes in the cytoplasm (23, 67).

* Corresponding author: Giuseppe Piccione
Tel : +3990357221, Fax : +3990356951
E-mail : giupiccione@iol.it

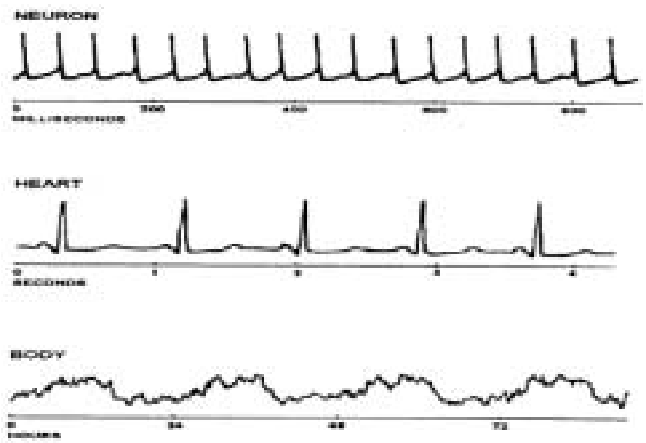

Fig. 1. Examples of biological rhythms (Refinetti, 2000)

A variety of biological variables oscillate in organisms, including behaviour, physiological functions, and biochemical factors. If any event within a biological system recurs at approximately regular intervals, we talk about a biological rhythm. The time interval after which the event recurs is called a period of the rhythm.

The predominant rhythms in nature are daily rhythms, e. g., those in rest and activity, in body temperature, in concentration of many hormones in bloods and ions in urine, etc. The rhythms are not merely passive responses to the daily alternation of light and darkness as they persist even in non periodic environment, e. g., in constant darkness. The nature of the rhythms is thus endogenous and innate and they are driven by an endogenous clock (or a pacemaker).

Even in non periodic environments, subjective days alternate with subjective nights, and the rhythms free run with a period of approximately but not precisely 24 h (6).

Such self-sustained oscillations with a period of about one day are called "circadian rhythms".

Periods of the free-running rhythms in continuous darkness, determined genetically, are dispersed within a range of 22-26 h and are species specific, e. g., the mean period is 23.4 h for house mice, about 24.0 h for Syrian hamsters and 25.0 h for humans.

In nature, circadian rhythms usually don't free run, as they are entrained to the 24 h day by external periodic factors (cycles). The relations between endogenous apparently genetically controlled biologic rhythms and environmental factors were explored in the early 1950s, and it became obvious that environmental factors were capable of determining the timing of circadian rhythms and could act as synchronizer (38), entraining agent (114) or zeitgeber (5). These terms are now used synonymously. It has to be understood that synchronizers do not create rhythms, but do determine their placement in time.

The most important and universal entraining agent is the light-dark cycle generated by the earth's rotation, but other cycles may serve as zeitgebers as well, e. g., the daily temperature cycle, timing of meals, or social cues.

Circadian clock in mammals: the pacemakers.

Pacemakers are primary oscillators, which show a genetically determined self-sustained oscillation, without external time cues, which provide timing signals to the organism that synchronize a multitude of rhythms in the same frequency range.

One of the major breakthroughs in modern neuroscience (77, 136) was the demonstration that the mammalian clock is well localized to a region of the hypothalamus just dorsal to the optic chiasm and slightly lateral to the third ventricle, the paired suprachiasmatic nuclei (SCN), a structure comprising 16000 neurons and associated glia (71, 127).

Elimination of the circadian rhythms of activity, drinking and various other rhythms (such as body temperature, blood hormone levels, sleep and oscillations in heart rate and blood pressure) by complete lesions of the SCN (but not by lesions of other brain areas) has been repeatedly demonstrated since then in a variety of mammalian species (1, 20, 126, 129, 131, 137, 139, 147, 151).

Circadian oscillation intrinsic to the SCN has been demonstrated (56), even in individual neurons (32) and the period is apparently genetically determined (117). Electrolytic lesion of the SCN disrupts several but not all the rhythms (27, 115, 124, 130, 140). Recovery of circadian rhythm does not occur, even if the lesion is performed in neonatal rats (80).

Furthermore, as the brain structure houses the biological clock, not only should destruction of the SCN eliminate rhythmicity, but replacement of the structure should restore it. Thus, when adult hamsters rendered arrhythmic by SCN lesions received implants of hypotalamic tissue from foetal hamsters or immortalized SCN cell lines, circadian rhythmicity was restored (19, 22, 69, 125).

Therefore, as the results of transplants utilizing the t (tau) mutant hamster show (116), lesioned hamsters with an original period of 20 hours started to exhibit 24-h rhythmicity after receiving a hypotalamic implant from foetuses of the 24-h-period genotype. Furthermore, when animals received implants after having experienced only partial lesion of the SCN, they showed 20-hour and 24-hour rhythmicity at the same time (146). Based on all the experimental evidence described above, it seems justified to conclude that the suprachiasmatic nucleus is the physical substrate of the circadian pacemaker.

Synchronizers

The effect of a synchronizer upon an endogenous rhythm depends on the period when the stimulus is applied (39, 41, 42, 76, 148). Endogenous rhythms show a sensitivity to the entraining agents, e. g., pulses of bright light, which have different effects upon domestic animal circadian rhythms when applied at different circadian rhythm stages, in fact in the sheep it was studied the influence of different exogenous synchronizers on the temporal circadian pattern of some haematochemical parameters (96). The phase response to different synchronizers, the length of the free running periods and the range of periods over which entrainment can appear vary among individuals of the same species (37, 43, 46, 47, 48, 66, 83, 141-145).

The influence and importance of different environmental synchronizers like light, time of feeding, upon endogenous rhythms vary from parameter to parameter. Some entraining agents may be dominant in their effects upon certain rhythms, e. g., the time of food uptake influence the circadian rhythm in intestinal cell proliferation but not, or much less, the rhythmicity in the number of circulating lymphocytes (43, 44, 46, 64, 65). The animal time structure, therefore is not necessarily the same in subjects living under different environmental conditions and following different breeding conditions.

Any measurement of a physiologic variable is characterized by the ensamble of the rhythms of many frequencies, which this function shows. These rhythms are at any one time subject to numerous and sometimes competing environmental synchronizers. So, a multitude of factors determine at any given moment the functional state of the parameter studied and the susceptibility to environmental agents.

Therefore, external stimuli not only have the capability of inducing reactions in terms of feedback mechanisms, they also influence the endogenous oscillators. Especially in ruminants, the intake of food generally leads to dramatic alterations: because of the microbic fermentation processes in the rumen, an increase of temperature of up to 1,6°C is described (16). On the other hand, a lot of studies have shown that feeding is able to act as a synchronizer, too (2, 8, 58, 61, 73, 79, 84). In ruminants, the thermal stimulus caused by food intake should be able to release thermoregulatory processes, but it might also be able to act as a synchronizer, as some investigations on the diurnal rhythm of body temperature in cattle have shown (11, 132, 149).

Definitions and basic principles of biological rhythms

Periods of biological rhythms

The periodic variations with shorter periods (higher frequencies) than circadian, the so-called ultradian rhythms are superimposed upon the circadian rhythms of the same parameter. The circadian rhythms in turn are superimposed upon rhythms with longer periods (or lower frequencies), the so-called infradian rhythms, which include, among others, rhythms with a period of about 1 week (circaseptan rhythms) rhythms with a period of about 30 days (circatrigintan rhythms) and rhythms with a period of about 1 year (circannual rhythms and/or seasonal variations) (40, 82, 122) table 1.

Table 1. Frequency ranger frequently encountered in biologic rhythms.

Domain	Range
Ultradian	t < 20
Circadian	20h ≤ t ≤ 28h
Infradian	t > 28h
Circaseptan	t = 7±3days
Circadiseptan	t = 14±3days
Circavigintan	t = 21±3days
Circatrigintan	t = 30±3days
Circannual	t = 1years±3monts

t = period

Midline Estimating Statistic of Rhythm-MESOR

The mean value of a rhythm would ideally be represented by the mean of all instantaneous values of the oscillating variable within one period. However, in biologic time series, i. e., in clinical medicine, quasi-continuous measurements will seldom be feasible. If the rhythm under study can be approximated and defined by a mathematical model, e. g., a cosine curve, a rhythm-adjusted mean or Midline Estimating Statistic of Rhythm, a so-called MESOR (35), may be preferable. The MESOR then represents the value midway between the highest and the lowest values of the (sinusoidal or other) function used to approximate the rhythm. In fitting of a sinusoidal model the MESOR will be equal to the arithmetic mean of the data only if the data were obtained at equal intervals over an entire cycle of the rhythm.

Amplitude

The extent of an oscillation may be expressed by its range, that is the difference between the maximum and the minimal value within one period or, if a rhythm can be defined by a sinusoidal mathematical model, by the amplitude. The amplitude is defined as one-half the difference between the highest and the lowest point of the mathematical model and can be considerably different from the overall range of the data. This difference may be due to *nonsinusoidality* of the variable measured (in which case the fitting of a sinusoidal model may be inappropriate) or due to outliers in the measurements in which case the amplitude of the model may be more representative of the rhythm than the apparent peak-trough difference in the data set, i.e., if the measurements are limited to a single cycle.

Acrophase

The location in time of the rhythm is defined by the highest point−acrophase−or the lowest point− bathyphase − of the fitted model in relation to a phase reference chosen by the investigator. The timing of the phase (e. g., the acrophase) of the rhythm in relation to the phase reference is called the phase angle and is expressed in units of time or in angular degrees (one period = 360°) in a clockwise direction as lag from zero time (0° = the phase reference) (fig. 2)

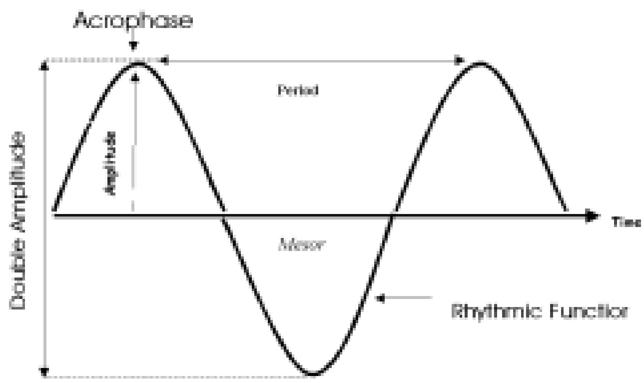

Fig. 2. Definition of parameters of a rhythmic function (e.g., by a cosine curve) fitted to the data

Cosinor analysis

Cosinor analysis (81) is probably the most enthusiastically promoted method for the analysis of rhythms, and statistical packages that deal with it are now available. It enables a set of data of a known period to be described in terms of its mesor (mean value of the fitted cosine curve), its amplitude and its acrophase (time of peak of fitted curve).

From a statistical view point, cosinor analysis simply investigates whether the data are better described by a cosine curve than by a straight line. A significant fit is taken as one for which the change that the data are fitted as well by a horizontal line as by the cosine curve is less than 5% (that is, it is one in which the amplitude of the fitted curve is significantly different from zero).

Now, the interpretation of this method is difficult, because, at first, the lack of "significant" fit does not indicate necessarily that no rhythm exists. This method is based on the assumption that the data are approximately sinusoidal in shape, but this assumption is sometimes untrue. Circadian rhythms vary greatly in their shape, which may be quite distinctive for a particular variable. We can do a lot of examples, such as plasma cortisol and urea nitrogen concentration. Given the wide variety in shapes of measured rhythms, one legitimate question is if the results obtained by cosinor analysis are valid when the shape of the data differs from that of a perfect sinusoid. As the data differs by being sinusoidal, the acrophase and the amplitude will be not true. The deviations are greater if the data are asymmetrical (fig. 3)

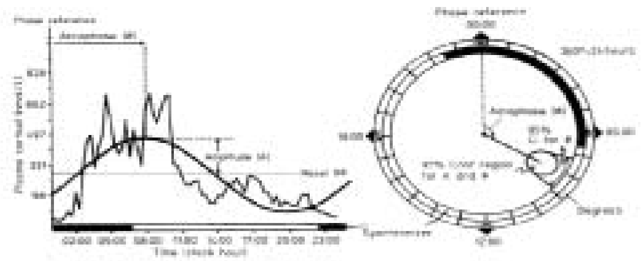

Fig. 3. Description of rhythm parameters by "Cosinor" procedure (Nelson et al., 1979) in E. Haus and Y. Touitou 1994.

Biologic rhythm in laboratory veterinary medicine

The multifrequency animal time structure represents a challenge for sampling and interpreting laboratory measurements. However, it also represents an opportunity for refining the diagnostic value of the measurement of many variables showing high amplitude rhythms and opens a new field in laboratory diagnosis. Statistically quantified rhythm parameters, and their relation to astronomic time and to each other, can serve as new end points in defining normality and in recognizing deviations from time-qualified reference values. Most laboratory parameters are subject to rhythmic variations in not one but several frequency ranges and the time of sampling and the interpretation of the results have to be adjusted accordingly. A physiologic measurement which represents the results of a spot-check, a blood drawing, may in part be determined by the interaction of several biologic rhythms in different frequencies, by trends occurring during a lifetime, and by the effects of random and non-random environmental stimuli acting all upon the same parameter. The result of a measurement obtained at one astronomic time may, therefore, represent an entirely different functional state of the animal organism than an identical result of the same parameter obtained at another time, depending on the stage of one or more rhythmic functions at the moment of sampling.(50)

In the establishment of chronobiologic reference values, numerous factors of biologic and environmental variation have to be considered which are similar to those for reference values in laboratory medicine in general (86, 134). Some of this factors are especially important in regard to chronobiologic investigations since they may alter the rhythm under study.

Laboratory measurements for chronobiologic observations have to be obtained either at a certain defined biologic time of the individual or, preferably, have to be adequate in sample density and length of the sampling span to provide statistically meaningful rhythm characteristics and their variance estimate (45). After these laboratory measurements have been obtained, they have to be compared with pertinent chronobiologic reference values derived from clinically healthy subjects comparable in their population characteristics with the subjects to be evaluated and obtained under comparable conditions. Time-qualified reference ranges, called chronodesms (36), can be developed for single individuals by repeated measurement of the same subject over numerous periods or they can be determined for groups of subjects by measurements over a single or a limited number of periods. In using peer groups for the establishment of a group chronodesm, the choice of the peer population and the conditions of the study will determine the validity of the chronodesm for a given individual or a group of subjects.

Sampling for chronobiologic studies

Statistical rhythmometry can evaluate and quantify the physiologic information obtained and, in the noisy time series obtained in clinical medicine, can help to separate the rhythmic variables from the noise of a biologic time series (21). Rhythmometry requires an adequate amount of properly collected data. The experimental design to explore rhythmic functions should be based on as much chronobiologic information as can be obtained which is related to a given problem (e. g., on the frequencies and stages anticipated).

Sampling schedules for chronobiologic investigations have to consider the "right time" (e. g., clock hour, day of the week or month of the year) for sample collection; it may vary both with the biologic timing of the subjects and with the specific problem we have to investigate.

Biologic rhythms in haematology

The number of circulating formed elements in the peripheral blood shows circadian rhythms in all cell lines. Circadian rhythms of red cells and of their related parameters are of low amplitude, while those of circulating lymphocytes and granulocytes are of high amplitude and they may in certain instances be of diagnostic importance. The circadian rhythms of the blood elements are of a complex nature. In addition to distribution between compartments, other factors, such as marrow release and cell removal may be involved. Therefore, extrapolation from the circadian rhythm in the peripheral blood to that in the bone marrow has to be approached with much caution.

Circadian rhythms and seasonal variations (or endogenous circannual rhythms) have been documented for several end points either of or related to cell proliferation in the human and animal bone marrow (133). Many authors pointed out the importance of chronobiology for laboratory haematology (49, 133).

The great variability in the number of circulating formed elements in the peripheral blood has been noted since techniques for counting these structures became available during the second half of the last century. It was soon recognized that some of these variations do not occur at random, but are the expression of regularly recurring rhythmic events (57, 128). With improvements in the accuracy and precision of the haematological methods of investigation, it became apparent that some of these periodic variations, especially in the circadian range, are highly reproducible and predictable in their timing and, in some instances, are large enough to be of clinical interest (49).

In the study of haematological parameters in the peripheral blood, rhythmic events have been described in the frequency range of a few hours (109), in the prominent circadian range in the horse (31, 63, 109, 150) and in the dog (106). These rhythmic variations may be superimposed upon rhythms with periods between 15 and 30 days (109) including circavigintan and circatrigintan rhythms in the horse and pig (93, 101, 109) and seasonal and circannual variations (28, 29, 30).

The recognition of a multifrequency time structure in the number and functions of the circulating corpuscular elements in the peripheral blood and in haematopoietic organs is essential for the scientific and clinical exploration of haematological parameters. Circadian acrophase maps of hematologic variables and information on the extent of their circadian variations have become available. The high amplitude variations of some parameters, e. g., the number of circulating neutrophils and lymphocytes, may have diagnostic implications in clinical medicine. Time-qualified reference ranges are of importance in functions with high amplitude rhythm.

In clinical medicine, chronobiology leads to a redefinition of the usual ranges for certain high amplitude parameters and adds new end points, such as the rhythm parameters of MESOR, amplitude, and acrophase, for the description of normalcy. Alterations in the organism's time structure may be of importance for the early recognition of abnormal function, often before structural disease can be identified. Chronotherapeutic interventions with and without rhythm manipulations are expected to provide a more effective approach to the treatment of haematological disorders.

Biological rhythms in haematochemical parameters

In clinical chemistry, circadian, circaseptan, circavigintan, circatrigintan and circannual rhythms of plasma and urinary solutes have been described in domestic animals. In most functions the circadian and the circannual rhythms show the highest amplitudes. Only in a few parameters, however is the amplitude of the rhythms of plasma solutes large enough to pose diagnostic problems, Age, sex, species, race, feeding and production may modulate the biological rhythms in domestic animals.

In the study of hematochemical parameters, rhythmic events have been described in the frequency range of ultradian, circadian, circatrigintan and circannual rhythm in the horse and foal (87, 88, 92) and in the cow and calf (14, 85, 89, 91), in the sheep and goats (12, 13, 96, 97, 100), in the pig (101) and in the rabbit (90). In the study of biological rhythm of some chemical-physical urinary parameters has been described in the frequency range of ultradian and circadian rhythm in the cow (95, 99) and horse (111).

The future application of biological rhythm in laboratory medicine will depend critically on advances in the field of data collection and data analysis. The technology for automated sample collection including in vivo measurements of hematochemical, haematological end points as such is available but has not been refined and miniaturized for routine chronobiologic sampling in biology and medicine.

Biological rhythm of body temperature in livestock

The body temperature shows a distinct cyclic variation throughout the solar day. It is often used as a marker rhythm because of its ease of measurement and large endogenous component. There are other rhythms which can be attributed directly to changes in body temperature. The temperature of biological tissues affects their metabolic rate according to the Q_{10} value. For example, a Q_{10} of 2, means that the rate of metabolism is doubled for every 10 ℃ rise in temperature.

For thermoregulatory purposes, the body may be divided into a central part or core and a peripheral part or shell. The temperature of the core is relatively constant and its daily range of oscillation is about 0.6-1.0 ℃. Core temperature is usually indicated by measurement of the rectal temperature in the domestic animals.

The relative stability of core temperature is maintained despite changes in environmental conditions. The temperature of the body's shell is more variable and responsive to the ambient temperature. Normally, there is a 4 ℃ gradient between the core and mean skin temperatures, with a further gradient to the environment. This allows heat exchange between the organism and the environment: without a facility for losing heat to the environment, the heat gained due to metabolic processes would cause a fatal rise in core temperature within an hour. This process is accelerated during exercise when up to 80% of the energy used in muscle contractions may be dissipated as heat within the tissues.

The body temperature is regulated by clusters of cells within the hypothalamus deep within the brain. There is a heat-loss centre, which activates mechanisms for losing heat when the body temperature is rising, and a heat-gain centre, which stimulates mechanisms that protect against the cold. Increased blood flow to the skin and secretion of sweat on the skin surface promote heat loss, while heat-conserving mechanisms include vasoconstriction (to reduce skin blood flow) and elevated metabolic rate. Clearly, the thermal state of the body represents a balance between heat-gain and heat-loss mechanisms. There is a neural link between the hypothalamic area and cells of the SCN (suprachiasmatic nucleus), which is thought to be the site of a biological clock.

Chronobiological studies of thermoregulation have entailed cooling or heating the body at different times of the day and monitoring responses. The body's thermal state can be altered rapidly by immersing, usually only part of the body, in water, or exposing the individual to an environmental chamber. In warming up after experimental cold-immersion, the blood flow to the skin is greater in the morning than in the afternoon. The peak of adrenergic activity, which would cause increased vasoconstriction and promote a rise in core temperature, occurs about midday or early in the afternoon. The threshold for onset of sweating and forearm blood flow has been reported to be higher at 16:00 and 20:00 compared to 24:00 and 04:00 (138). These observations are consistent with the conclusion that it is the control of body temperature rather than the loss or gain of heat that varies in a circadian cycle (121).

The research on biological rhythms of body temperature is an especially important topic in physiological research because it involves the integration of effort of two large groups of researchers: those interested in the regulation of body temperature and those interested in the mechanisms of biological timing. The homeostatic perspective of thermal physiologists is enriched by insights on the temporal organization of physiological functions, while the biological timing perspective of those who study biological clocks is enriched by empirical knowledge of an important effectors mechanism through which the clock performs its functions (119). This is confirmed by a relative abundance of reviews on biological rhythms of body temperature in domestic animals (3, 10, 11, 17, 18, 33, 34, 51, 52, 53, 59, 68, 70, 72, 74, 75, 98, 102, 107, 110, 112, 113, 118). Daily variation of the body temperature of endothermic animals is influenced by changes in their physical activity and metabolic level (135), but is synchronized to the daily changes in light intensity, temperature, and perhaps other factors of the environment (17). Thermal conductance of many mammals and birds also varies in circadian manner, being higher during the active phase than the inactive phase of the day (7). this variations facilitates heat loss when animals are active, and heat conservation when they are sleeping.

Photoperiod and annual reproductive rhythms

Annual reproductive cycles are characteristic of most temperate zone mammals (24). For many species the time of year during which environmental conditions are most conducive to survival of the young remains essentially invariant from year to year. Among such mammals, adaptive timing of the annual breeding season in often largely dependent on photoperiodic control (24). Despite the importance of photoperiod as the principal synchronizer for annual reproductive rhythms, few studies have considered the physiological basis of photoperiodic time measurements in mammals (24).

It was demonstrated a converse control mechanism in the sheep: in this autumnal breeder oestrus was induced out of season by reducing the daily photoperiod, whereas increasing the photoperiod prolonged anoestrus (fig. 4). Following these pioneering investigations extensive experimental work has resulted in confirmation of these findings and the demonstration of parallel control mechanisms in both males and females of other species. In autumn breeders such as the goat and ram short days are necessary for the induction and maintenance of spermatogenesis, whereas in spring breeders such as the vole, snowshoe hare and ferret testicular function is stimulated by long days (24) (fig. 5).

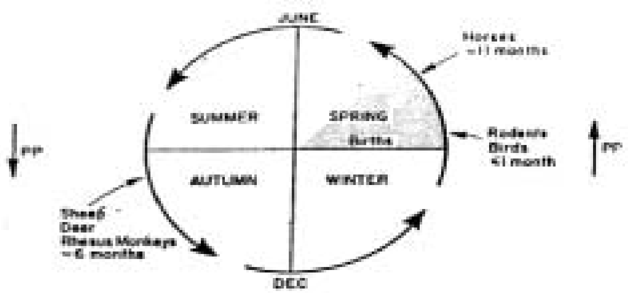

Fig. 4. Seasonal reproduction in mammals (Karsch et al., 1984)

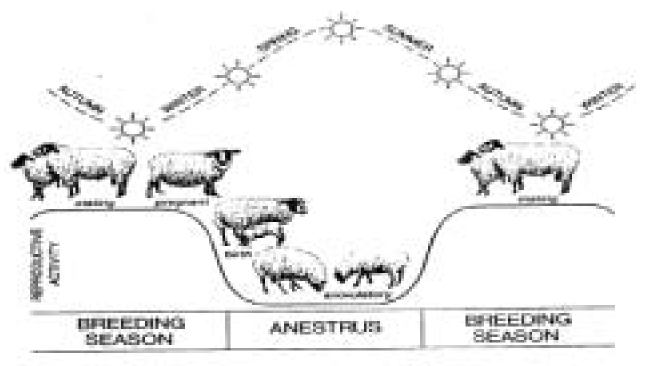

Fig. 5. Circannual reproductive cycles in the ewe (Foster et al., 1986)

In rats and Djungarian hamsters, the rhythm in melatonin production is driven by the pineal rhythm in Nacetyltransferase, which forms the melatonin precursor Nacetylserotonin (54, 62). The Nacetyltransferase rhythm is controlled by a circadian pacemaker located in the suprachiasmatic nuclei of the hypothalamus, similar to the locomotor activity rhythm (62, 78). There is the hypothesis of a two-component pacemaking system controlling the Nacetyltransferase-rhythm (54, 55), such as was proposed originally for the locomotor activity rhythms in nocturnal rodents (114). An evening component controls the evening Nacetyltransferase rise and is the primary responder to evening light. A morning component controls the morning Nacetyltransferase decline and is the primary responder to morning light. Both components are mutually coupled in a complex pacemaker and interact with each other. A phase marker of the evening component is the time of the Nacetyltransferase rise; a phase marker of the morning component is the time of the morning Nacetyltransferase decline. The phase relationship between both components, given by the phase relationship between the rise and the decline, determines the duration of elevated nocturnal Nacetyltransferase activity in the rat and hence the duration of high melatonin production. When rats are maintained on long days, light intruding into the late evening hours phase delays the evening Nacetyltransferase rise and eventually the evening component of the pacemaker. However, light intruding into the early morning hours phase advances the morning Nacetyltransferase decline and eventually the morning component of the pacemaker (54, 55). Consequently, the phase relationship between the evening Nacetyltransferase rise and the morning decline is compressed on long days but decompressed on short days.

Similarly, in all mammalian species the duration of high night melatonin production and concentration is longer in short than in long days (54). The duration of nocturnal melatonin pulse appears to be the signal-transducing information on day length, i. e., on photoperiod, in organisms (15, 54, 123). Information on photoperiod is very important as it allows the organism to prepare in advance for the time to come, e. g., to time reproduction in such a way that the growth of the offspring occurs when environmental conditions are most favourable. So, the circadian system forms not only the innate temporal program of organisms but may also serve as an internal calendar. When rats or Djungarian hamsters are transferred from long to short days, it takes some time before the animals recognize the full shortening of the photoperiod (54). Memory of long days may be stored in the pacemaker, namely in the phase relationship between the evening and the morning components.

It appears that, in considering resetting of the circadian clocks, it is necessary to take into account not only the time when light is present but a photoperiod and probably the state of the pacemaker as well.

Circadian components of Physiology and Athletic Performance

Most physiological functions exhibit circadian rhythmicity: maximum and minimum function occur at specific times of day. In the mammals circadian rhythms are expressed as oscillations in physiological processes (e. g., body temperature, heart rate, hormone levels) which are responsive either to internal (e. g., neurotransmitters, electrolytes, or metabolic substrates) or external (e. g., environmental factors, food or stressors) stimuli. It is now widely accepted that most, if not all, parameters when examined with high sample frequency will show rhythmicity.

The majority of the resting physiological variables are thought to influence athletic horse's performance. For example, when body temperature, circulating levels of hormones and metabolic functions are manipulated artificially prior to exercise (e. g., by pharmacological agents), performance is affected. Endogenous circadian changes in these resting parameters might mediate parallel changes in both performance and physiological responses to exercise over a 24-hour period.

The chronobiology of sport and exercise has been researched in three ways which can be ordered in terms of scientific validity. First, the times of day when athletic horses perform the best (or worst) in actual sports events have been examined. Second, performances in simulated competitions or time-trials have been investigated at different times of the day. Finally, the responses to recognized laboratory tests of performance have been examined in various experimental conditions.

As above mentioned, many organic functions, such as heart rate, arterial blood pressure, serum electrolytes and body temperature show cyclically fluctuating values. From a practical viewpoint, knowledge of the fluctuations in the various haematic parameters can determine the best moment (of the day, night, week or month) to take blood samples for evaluation of those physiological parameters which are important from a diagnostic, therapeutic and, last but not least, a medical viewpoint. In fact, chronobiological research applied to sport physiology in humans, has enabled identification of the range of performance variability during different temporal periods (day, month and year). The study of chronophysiological responses to physical activity, which involve the athlete's whole organism, is a complex matter, as athletic performance is a multifactor entity. Therefore, interpretation and chronophysiological evaluation require knowledge of the rhythms of the different functional systems involved.

In particular, rhythmic variations in arterial blood pressure and some blood-gas and electrocardiographical parameters in the athletic horse have been studied. (4, 9, 25, 94, 103-105, 108, 109). These parameters, which are used for the definition of athletic performance, influence the planning of the training process and of competitive activity and can be especially useful in deciding the type, intensity and duration of daily training.

Conclusions

The chronobiological study are extremely important for veterinary medicine, not only for the application of better therapy and for the more reliable interpretation of experimental results, but also for a controlled and economic development of livestock productivity.

Naturally the problem is made more complex by the fact that every animal species is characterized by its own rhythms. Examples of this are the differences between species in the rhythms of the oestrus cycles, in fertility percentage and in the vitality indices of spermatozoa at various times of the day, in rhythms of the cycloid type which we see in the figures for bovine fertility, and those of the hatching of hen's eggs in the course of a year, in the seasonal and daily frequencies in the serum content of ceruloplasmin in pigs and other species of domestic animals, in the circadecadic cycles in the growth rhythms of chickens, in the circadian variations in the α, β and γ casein content of the milk from the various species of mammals, in the complex and multiple metabolic activity developed from the extensive bacterial and infusorial symbiosis in the rumen, and in many other phenomena already described but not yet formulated and interpreted according to the concepts of modern chronobiology.

This modern science could contribute greatly to veterinary medicine and aid the solution of many problems connected with fertility, the tabulation of the rhythms of ovulation, the diseases occurring in racing and breeding animals following their transport to different time zones, the economics of growth in relation to the consumption and utilization of food-stuffs, and all the cast problems of mass medicine and the genetic selection of animal populations.

The method of temporal evaluation of fluctuating systems gives us another view point of a phenomenon, perceiving within it rhythmic variations which, once differentiated allow us to formulate more precise forecasts and to take decisions outside the probabilities. The study of biological rhythms takes on considerable importance, since they are correlated to the state of health of the single animal and of the population as a whole. The living organism is characterized by extreme variability: in fact the majority of physiological variables follow an oscillating rhythmic pattern. The determination of the spectrum of frequencies typical of biological rhythms has made it possible to give a specific foundation to the dynamic concept of well-being. Furthermore, the study of the modification produced in such rhythms as a result of given stimuli caused by environmental factors makes it possible to study the capacity of reaction and of adaptation of animals to the environment, as also the pathological reactions, and hence to improve their output by intervening upon the environment and the breeding techniques.

References

1. **Aalto, J. and Kiianmaa K.** Circadian rhythms of water and alcohol intake: Effect of REM-sleep deprivation and lesion of the suprachiasmatic nucleus. Alcohol, 1984, **1**, 403-407.
2. **Abe, H. and Rusak B.** Anticipatory activity and entrainment of circadian rhythms in syrian hamsters exposed to restricted palatable diets. Am. J. Physiol. 1992, **263**, R116-R124.
3. **Araki C.T., Nakamura R.M., Kam L.W., and Clarke N.L.** Diurnal temperature patterns of early lactating cows with milking parlor cooling. J. Dairy Sci. Jun, 1985, **68 (6)**, 1496-1501.
4. **Arcigli A., Assenza A.M., Piccione G., Fazio F., and Caola G.** Circadian variability of some physiological components of athletic horse performance. European College of sport Science, Cologne, Germany, 2001, **6**, 903.
5. **Aschoff, J.** Circadian rhythms within and outside their ranges of entrainment, pp. 172-181. In: Assenmacher I, Farner DS (eds) Environmental endocrinology. Springer, Berlin Heidelberg New York, 1978.
6. **Aschoff, J.** Freerunning and entrained circadian rhythms. **4,** 81-93. Handbook of Behavioral Neurobiology. Biological Rhythms. New York, Plenum, 1981a.
7. **Aschoff, J.** Thermal conductance in mammals and birds: its dependence on body size and circadian phase. Comp. Biochem. Physiol. 1981b, **69A**, 611-619.
8. **Aschoff, J. and Von Goetz C.** Effects of feeding cycles on circadian rhythms in squirrel monkeys. J. Biol. Rhythms. 1986, **1**, 267-276.
9. **Attanzio G., Assenza A.M., Piccione G., and Caola G.** Serum electrolytes and electrocardiographic parameters: circatrigintan temporal comparison in the athletic horse. Proceedings 7th Congress World Equine Veterinary Association. 2001, 310.
10. **Ayo J.O., Oladele S.B., Ngam S., Fayomi A., and Afolayan S.B.** Diurnal fluctuations in rectal temperature of the Red Sokot goat during the harmattan season. Res. Vet. Sci. Feb, 1999, **66 (1)**, 7-9.
11. **Bitman J., Lefcourt A., Wood D.L., and Stroud B.** Circadian and ultradian temperature rhythms of lactating dairy cows. J. Dairy Sci. 1984, **67**, 1014-1023.
12. **Caola G., Assenza A.M., Fazio E., and Piccione G.** Andamento ultradiano di alcuni parametri ematochimici nella Pecora e nella Capra. Annali della Facoltà di Medicina Veterinaria di Messina, 1991, **28**, 51-63.
13. **Caola G., Fazio E., Piccione G., and Ravidà A.** Variazioni giornaliere di metaboliti ematici nella Pecora comisana al pascolo. Atti Congr. Int. S.I.P.A.O.C. 1992, **10**, 212-214.
14. **Caola G., Fazio E., Giofré F., Panzera M., and Piccione G.** Andamento annuale di alcuni metaboliti ematici nel Vitello in accrescimento. Atti Soc. It. Buiatria. 1993, **25**, 549-554.
15. **Carter, D.S. and Goldman B.D.** Antigonadal effects of timed melatonin infusion in pinealectomized male Djungarian hamsters (Phodopus sungorus sungorus): duration is the critical parameter. Endocrinology. 1983, **113**, 1261-1267.
16. **Dale H.E., Stewart R.E., and Brody S.** Rumen temperature I. Temperature gradients during feeding

and fasting. Cornell Vet. 1954, **44**, 368-374.
17. **DaSilva, R.G. and Minomo F.R.** Circadian and seasonal variation of the body temperature of sheep in a tropical environment. Int. J. Biometeorol. 1995, **39**, 69-73.
18. **Davidson, T.L. and Fewell J.E.** Ontogeny of a circadian rhythm in body temperature in newborn lambs reared independently of material time cues. J. Dev. Physiol. Feb, 1993, **19 (2)**, 51-56.
19. **DeCoursey, P.J. and Buggy J.** Restoration of circadian locomotor activity in arrhythmic hamsters by fetal SCN transplants. Comparative Endocrinology. 1988, **4**, 49-54.
20. **DeCoursey P.J., Krulas J.R., Mele G., and Holley D.C.** Circadian performance of suprachiasmatic nuclei (SCN)-lesioned antelope ground squirrels in a desert enclosure. Physiology and Behavior. 1997, **62**, 1099-1108.
21. **De Prins, J. and Hecquet B.** Data Processing in Chronobiological Studies, pp. 90-113. In: Biologic Rhythms in Clinical and Laboratory Medicine. Touitou and Haus (eds), Springer-Verlag Berlin Heidelberg, 1992.
22. **Earnest D.J., Liang F.Q., Ratcliff M., and Cassone V.M.** Immortal time: Circadian clock properties of rat suprachiasmatic cell lines. Science. 1999, **283**, 693-695.
23. **Edmunds, L.N. jr** Cellular and Molecular Aspects of Circadian Oscillators: Models and Mechanisms for Biological Timekeeping, pp. 35-54. In: Biologic Rhythms in Clinical and Laboratory Medicine. Touitou and Haus (eds), Springer-Verlag Berlin Heidelberg, 1992.
24. **Elliott, J.A.** Circadian rhythms and photoperiodic time measurement in mammals. Federation proceedings. 1976, **35**, 2339-2346.
25. **Fazio F., Piccione G., and Caola G.** Cronoritmicità della pressione arteriosa nel Cavallo saltatore durante un allenamento pre-agonistico. Atti Soc. It. Sci. Vet. 1999, **53**, 63-64.
26. **Foster D.L., Karsch F.J., Olster D.H., Ryan K.D., and Yelon S.M.** Determination of puberty in a seasonal breeder. Rec. Prog. Horm. Res. 1986, **42**, 331-334.
27. **Fuller C.A., Lydic R., Sulzman F.M., Albers E.H., Tepper B., and Moore Ede M.C.** Circadian rhythm of body temperature persists after suprachiasmatic lesions in the squirrel monkey. Am. J. Physiol. 1981, **241** (Regulatory Integrative Comp. Physiol. 10), R385-R391.
28. **Gill J., Szwarocka-Priebe T., Krupska U., and Peplowska Z.** Seasonal Changes in Hematological Indices, Protein and Glycoprotein Levels and in Activity of Some Enzymes in Arabian Horses. Bulletin de l'Academie Polonaise des Sciences. Série des sciences biologiques Cl. V. 1978, **26(10)**, 719-723.
29. **Gill J., Kompanowska-Jezierska E., Jakubow K., Kott A., and Szumska D.** Seasonal changes in the white blood cell system, lyzozyme activity and cortisol level in arabian brood mares and their foals. Comp. Biochem. Physiol. 1985, **81A (3)**, 511-523.
30. **Gill, J. and Kompanowska-Jezierska E.** Seasonal changes in the red blood cell indices in arabian brood mares and their foals. Comp. Biochem. Physiol. 1986, **83A (4)**, 643-651.
31. **Gill, J. and Rastawicka M.** Diurnal changes in the hematological indices in the blood of racing Arabian horses. Polskie Arch. Wet. 1986, **26 (1/2)**, 169-179.
32. **Green, D.J. and Gillette R.** Circadian rhythm of firing rate recorded for single cells in the rat suprachiasmatic brain slice. Brain Research. 1982, **245**, 198-200.
33. **Greig, W.A. and McIntyre W.I.** Diurnal variation in rectal temperature of N'dama cattle in the Gambia. Br. Vet. J. mar-apr, 1979, **135 (2)**, 113-118.
34. **Hahn, J.L.** Body temperature rhythms in farm animals: a review and assessment relative to environmental influences, pp. 325-337. Proceedings 11th International Congress Biometereology, West Lafayette, USA, Sep, Driscoll D and Box EO (eds). SPB Academic Publishing, The Hague, Netherlands, 1987.
35. **Halberg F., Carandente F., Cornelissen G., and Katinas G.S.** Glossary of chronobiology. Chronobiologia (suppl.). 1977, **4**, 1-189.
36. **Halberg F., Lee J.K., and Nelson W.L.** Time-qualified reference intervals chronodesms. Experientia. 1978, **34**, 713-716.
37. **Halberg F., Cornelissen G., Sothern R.B., Wallach L.A., Halberg E., Ahlgren A., Kuzel M., Radke A., Barbosa J., Goetz F., Buckley J., Mandel J., Schuman L., Haus E., Lakatua D., Sackett L., Berg H., Wendt H.W., Kawasaki T., Ueno M., Uezono K., Matsuoka M., Omae T., Tarquini B., Cagnoni M., Garcia Sainz M., Perez Vega E., Wilson D., Griffiths K., Donati L., Tatti P., Vasta M., Locatelli J., Camagna A., Lauro R., Tritsch G., and Wetterberg L.** International geographic studies of oncological interest on chronobiological variables, pp. 553-596. In: Kaiser H (ed) Neoplasms-comparative pathology of growth in animals, plants and man. Williams and Wilkins, Baltimore, 1981.
38. **Halberg, F.** Chronobiologic engineering, pp. 263-297. In: Ensminger WD, Selam JL (eds) Infusion systems in medicine. Futura, Mount Kisco, 1989.
39. **Haus, E.** Periodicity in response and susceptibility to environmental stimuli. Ann. NY Acad. Sci. 1964, **117**, 281-291.
40. **Haus, E. and Halberg F.** Circannual rhythm in level and timing of serum corticosterone in standardized inbred mature C-mice. Environ. Res. 1970, **3**, 81-106.
41. **Haus E., Halberg F., Kuhl J.F.W., and Lakatua D.J.** Chronopharmacology in animals. Chronobiologia (suppl. 1). 1974a, **1**, 122-156.
42. **Haus E., Halberg F., and Loken M.K.** Circadian susceptibility-resistance cycle of bone marrow.. Cells to whole body x-irradiation on Balb/C mice, pp. 115-122. In: Scheving LE, Halberg F, Pauly JE (eds) Chronobiology.

Igaku-Shoin, Tokyo, 1974b.
43. **Haus E., Lakatua D.J., Sackett-Lundeen L., and Swoyer J.** Chronobiology in laboratory medicine, pp. 13-82. In: Reitveld WT (ed) Clinical aspects of chronobiology. Bakker, Baarn, 1984a.
44. **Haus E., Lakatua D.J., Sackett-Lundeen L., and White M.** Circannual variation of intestinal cell proliferation in BDF_1 male mice on three lighting regimens. Chronobiol. Int. 1984b, **1**, 185-194.
45. **Haus, E.** Requirements for chronobiotechnology and chronobiologic engineering in laboratory medicine, pp. 331-372. In: Scheving L.E., Halberg F., Ehret C.F. (eds) Chronobiotechnology and chronobiological engineering. Kluwer Academic, Netherlands, (NATO ASI series, series E: applied sciences, n. 120), 1987.
46. **Haus E., Nicolau G.Y., Lakatua D., and Sackett-Lundeen L.** Reference values for chronopharmacology. Annu. Rev. Chronopharmacol. 1988, **4**, 333-424.
47. **Haus E., Nicolau G., Lakatua D.J., Sackett-Lundeen L., and Petrescu E.** Circadian rhythm parameters of endocrine functions in elderly subjects during the seventh decade to the ninth decade of life. Chronobiologia. 1989, **16**, 331-352.
48. **Haus E., Nicolau G.Y., Lakatua D.J., Sackett-Lundeen L., and Swoyer J.** Circadian rhythms in laboratory medicine, pp. 21-33. In: Fanfani M., Tarquini B. (eds) Reference values and chronobiology. Arand and Brent, Florence, 1990.
49. **Haus, E.** Chronobiology of circulating blood cells and platelets, pp. 504-526. In: Biologic Rhythms in Clinical and Laboratory Medicine. Touitou and Haus (eds), Springer-Verlag Berlin Heidelberg, 1992.
50. **Haus, E. and Touitou Y.** Chronobiology in Laboratory Medicine, pp. 673-708. In: Biologic Rhythms in Clinical and Laboratory Medicine. Touitou and Haus (eds), Springer-Verlag Berlin Heidelberg, 1992.
51. **Hertrampf B., Pfeiffer A., Pourhassan H., Schlikker P., Bredenfeld H., Wischer E., and Rosing W.** Studies on normal daily rectal temperature in various breeds of sheep. Dtsch. Tierarztl. Wochenschr. 1978, Jun 5, **85 (6)**, 249-259.
52. **Hunsaker W.G., Reiser B., and Wolynetz M.** Vaginal temperature rhythms in sheep. Int. J. Chronobiol. 1977, **4 (3)**, 151-162.
53. **Igono M.O., Molokwu E.C., and Aliu Y.O.** Seasonal variations in rectal temperature of Yankasa sheep. Vet. Res. Commun. 1983, Jun, **6 (3)**, 223-226.
54. **Illnerová, H.** Entrainment of mammalian circadian rhythms in melatonin production by light. Pineal Res. Rev. 1988, **6**, 173-217.
55. **Illnerová H., Vanêcêk J., and Hoffman K.** Different mechanisms of phase delays and phase advances of the circadian rhythm in rat pineal N-acetyltransferase activity. J. Biol. Rhythms. 1989, **4**, 187-200.
56. **Inouye, S.T. and Kawamura H.** Characteristics of a circadian pacemaker in the suprachiasmatic nucleus. J. Comp. Physiol. 1982, **146**, 153-160.
57. **Japha, A.** Die Leukozyten beim gesunden und krankewn Säugling. Jahrbuch Kinderheilk. 1900, **52**, 242-270.
58. **Jilge, B. and Stahele H.** Restricted food access and light-dark: impact of conflicting zeitgebers on circadian rhythms of the rabbit. Am. J. Physiol. 1993, **264**, R708-R715.
59. **Kanno, Y.** Experimental studies on body temperature rhythm in dogs I. Application of cosinor method to body temperature rhythm (author's transl) Nippon Juigaku Zasshi, 1977, Feb, **39 (1)**, 69-76.
60. **Karsch F.J., Bittman E.L., Oster D.L., Goodman R.L., and Legan S.J., and Robinson J.E.** Neuroendocrine basis of seasonal reproduction. Rec. Prog. Horm. Res. 1984, **40**, 185-225.
61. **Kennedy G.A., Coleman G.J., and Armstrong S.M.** Restricted feeding entrains circadian wheel-running activity rhythms of the Kowari. Am. J. Physiol. 1991, **261**, R819-R827.
62. **Klein D.C. and Moore R.J.** Pineal N-acetyltransferase and hydroxyindole-O-methyltransferase: control by the retinal hypothalamic tract and the suprachiasmatic nucleus. Brain Res. 1979, **174**, 245-262.
63. **Komosa M., Flisinska-Bojanowska A., and Gill J.** Diurnal changes in the haemoglobin level, red blood cell number and mean corpuscular haemoglobin in foals during the first 13 weeks of life and in their lactating mothers. Comp. Biochem. Physiol. 1990, **96A (1)**, 151-155.
64. **Lakatua D.J., Haus E., and Sackett-Lundeen L.** Thyroid and adrenal synchronization in Swiss-Webster female mice under the effect of "competing" synchronizers. Chronobiologia. 1983a, **10**, 137.
65. **Lakatua D.J., White M., Sackett-Lundeen L., and Haus E.** Change in phase relations of circadian rhythms in cell proliferation induced by time-limited feeding in Balb/C x $D8A/2F_1$ mice bearing a transplantable Harding-Passey tumor. Cancer Res. 1983b, **43**, 4068-4072.
66. **Lakatua D.J., Nicolau G.Y., Bogdan C., Petrescu E., Sackett-Lundeen L., Irvine P., and Haus E.** Circadian endocrine time structure in clinically healthy human subjects above 80 years of age. J. Gerontol. 1984, **6**, 648-654.
67. **Lakatua, D.J.** Molecular and Genetic Aspects of Chronobiology, pp. 65-77. In: Biologic Rhythms in Clinical and Laboratory Medicine. Touitou and Haus (eds), Springer-Verlag Berlin Heidelberg, 1992.
68. **Lefcourt A.M., Huntington J.B., Akers R.M., Wood D.L., and Bitman J.** Circadian and ultradian rhythms of body temperature and peripheral concentrations of insulin and nitrogen in lactating dairy cows. Domest. Anim. Endocrinol. 1999, Jan, **16 (1)**, 41-55.
69. **Le Sauter, J. and Silver R.** Suprachiasmatic nucleus lesions abolish and fetal grafts restore circadian

gnawing rhythms in hamsters. Restorative Neurology and Neuroscience. 1994, **6**, 135-143.

70. **Macaulay A.S., Hahn J.L., Clark D.H., and Sisson D.V.** Comparison of calf housing types and tympanic temperature rhythms in Holstein calves. J. Dairy Sci. 1995, Apr, **78 (4)**, 856-862.
71. **Meijer, J.H. and Rietveld W.J.** Neurophysiology of suprachiasmatic pacemaker in rodents. Physiol. Rev. 1989, **69**, 671-674.
72. **Mendel, V.E. and Raghavan G.V.** A study of diurnal temperature patterns in sheep. J Physiol. 1964, **174**, 206-216.
73. **Mistlberger R.E., Houpt T.A., and Moore-Ede M.C.** Food-anticipatory rhythms under 24-hour schedules of limited access to single macronutrients. J. Biological Rhythms. 1990, **5 (1)**, 35-46.
74. **Mohr, E.G. and Krzywanek H.** Variation of core-temperature rhythms in unrestrained sheep. Physiol. Behav. 1990, Sep, **48 (3)**, 467-473.
75. **Mohr, E.G. and Krzywanek H.** Endogenous oscillator and regulatory mechanisms of body temperature in sheep. Physiol. Behav. 1995, Feb, **57 (2)**, 339-347.
76. **Moore-Ede M.C., Czeisler C.A., and Richardson G.S.** Circadian timekeeping in health and disease. I. Basic properties of circadian pacemakers. New England J. Med. 1983, **309**, 469-476.
77. **Moore, R.Y. and Eichler V.B.** Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Research. 1972, **42**, 201-206.
78. **Moore, R.J. and Card J.P.** Neuropeptide Y and the circadian system, pp. 293-301. In: Neuropeptide Y. New York: Raven, 1989.
79. **Morimoto, Y. and Yamamura Y.** Regulation of circadian adrenocortical periodicities and of eating-fasting cycles in rats under various lighting conditions, pp. 177-188. In: Masami Suda, eds. Biological Rhythms and their Central Mechanisms. Elsevier/North-Holland: Biomedical Press, 1979.
80. **Mosko, S.S. and Moore R.Y.** Neonatal suprachiasmatic nucleus ablation: absence of functional and morphological plasticity. Proc. Natl. Acad. Sci. USA. 1978, **75**, 6243-6246.
81. **Nelson W., Tong U., Lee J., and Halberg F.** Methods for cosinor-rhythmometry. Chronobiologia. 1979, **6**, 305-323.
82. **Nicolau G.Y., Lakatua D., Sackett-Lundeen L., and Haus E.** Circadian and circannual rhythms of hormonal variables in clinically healthy elderly men and women (abstr). Chronobiologia. 1983, **10**, 144.
83. **Nicolau G.Y., Lakatua D.J., Sackett-Lundeen L., and Haus E.** Circadian and circannual rhythms of hormonal variables in clinically healthy elderly men and women. Chronobiology Int. 1984, **1**, 301-319.
84. **Ottenweller J.E., Tapp W.N., and Natelson B.H.** Phase-shifting the light-dark cycle reset the food-entrainable circadian pacemaker. Am. J. Physiol. 1990, **258**, R994-R1000.
85. **Panzera M., Fazio E., Giofré F., Piccione G., and Caola G.** Variazioni circadiane di alcuni parametri ematochimici nella Bovina Bruna Alpina a stabulazione fissa. Atti Soc. It. Buiatria. 1992, **24**, 101-111.
86. **Petit Clerc, C. and Solberg H.** Approved recommendation -1987- on the theory of reference values, part 2. Selection of individuals for the production of reference values. Report of expert panel on theory of reference values (EPTRV) of the international federation of clinical chemistry (IFCC). J. Clin. Chem. Clin. Biochem. 1987, **25**, 639-644.
87. **Piccione G., Assenza A.M., Fazio E., and Caola G.** Periodicità annuale di alcuni parametri ematochimici nel Puledro purosangue inglese in accrescimento. Annali della Facoltà di Medicina Veterinaria di Messina. 1993, **30**, 47-57.
88. **Piccione G., Assenza A.M., Fazio E., and Caola G.** Andamento circadiano di alcuni metaboliti azotati sierici e di calcio, fosforo e ferro nell'Asino di razza Ragusana. Atti A.S.Sa.V. 1994a, **1**, 160-164.
89. **Piccione G., Fazio E., Giofré F., and Caola G.** The circatrigintan progress of some hematochemical parameters in lactating stalled cows. Proceeding World Buiatrics Congress. 1994b, **18**, 919-922.
90. **Piccione G., Assenza A.M., and Caola G.** Organizzazione temporale circadiana di alcuni parametri ematochimici nel Coniglio di razza Californiana. Atti Soc. It. Sci. Vet. 1995, **49**, 285-286.
91. **Piccione G., Assenza A.M., and Caola G.** Periodicità circadiana di alcuni parametri ematochimici in bovine e vitelli di razza Brown. Atti Soc. It. Buiatria. 1996a, **28**, 415-422.
92. **Piccione G., Assenza A.M., Giofré F., and Caola G.** Andamento circannuale di alcuni metaboliti azotati, della glicemia e di calcio e fosforo in cavalli di razza Murgese. Atti Soc. It. Ippologia. 1996b, **13**, 61-68.
93. **Piccione G., Assenza A.M., Scribano P., and Caola G.** Organizzazione temporale circasemidiana di alcuni parametri ematochimici, ematologici e della temperatura rettale e cutanea nel Cavallo Purosangue inglese. Atti A.S.Sa.V. 1996c, **3**, 17-23.
94. **Piccione G., Castaldo C., Carta S., and Caola G.** Study on the circadian rhythmicity of some electrocard-iographical parameters in the horse. Proceedings World Equine Veterinary Association. 1997a, **5**, 160-163.
95. **Piccione G., Morgante M., Assenza A.M., and Caola G.** Studio preliminare sulla periodicità giornaliera di alcuni parametri chimico-fisici urinari nella Bovina Frisona. Atti Soc. It. Buiatria. 1997b, **29**, 333-339.
96. **Piccione G., Attanzio G., and Fazio F.** The influence of the different exogenous synchronizers on the temporal circadian pattern of some hematochemical parameters in the Sheep. Proceedings of the 6th Congress of Fe.Me.S.P. Rum. 1998a, 14-16 May, Postojna (Slovenia), 469-472.
97. **Piccione G., D'Anna M., and Fazio F.** Pattern

temporale ematochimico ed ematologico nella Capra di razza Maltese. Atti S.I.P.A.O.C. 1998b, **13**, 368-372.

98. **Piccione G., Fazio F., Scribano P., and Caola G.** La temperatura corporea nel Cavallo Sanfratellano: pattern temporale circasemidiano, circadiano, circatrigintano e circannuale. Atti Soc. It. Sci. Vet. 1998c, **52**, 73-74.
99. **Piccione G., Filippini G., Morgante M., and Scribano P.** Andamento circasemidiano diurno di alcuni parametri sierici e urinari nella bovina. Atti Soc. It. Buiatria. 1998d, **30**, 305-312.
100. **Piccione G., Giofré F., Scribano P., and Caola G.** Circatrigintan temporal progress of some hematochemical parameters in the Sheep and in the Goat. Proceedings of the 6th Congress of Fe.Me.S.P.Rum. 1998e, 14-16 May, Postojna (Slovenia), 473-477.
101. **Piccione G., Giudice E., Niutta P.P., and Caola G.** Pattern temporale circatrigintano di alcuni parametri ematochimici ed ematologici nel Suino. Atti S.I.P.A.S. 1998f, **24**, 229-235.
102. **Piccione G., Attanzio G., Fazio F., and Caola G.** The circasemidian, circadian, circatrigintan and circannual pattern of the body temperature in the sheep and in the lamb. Proceedings of the 7th Congress of Fe.Me.S.P. Rum. 1999a, 403-406.
103. **Piccione G., Fazio F., Assenza A.M., and Caola G.** Struttura temporale circadiana di alcuni parametri emogasanalitici nel Cavallo saltatore. Atti Soc. It. Ippologia. 1999b, **14**, 33-34.
104. **Piccione G., Fazio F., and Caola G.** Circasemidian ans circadian oscillation of the arterial blood pressure and heart rate of the atlete horse. Journal of Equine Veterinary Science. 1999c, **19**, 574.
105. **Piccione G., Fazio F., and Caola G.** Cronoperformance del Cavallo saltatore: andamento circatrigintano di alcuni parametri emogasanalitici. Atti Soc. It. di Fisiologia Veterinaria. 1999d, **3**, 44-48.
106. **Piccione G., Giudice E., and Niutta P.P.** La cronobiologia nelle indagini di laboratorio: pattern temporale circadiano di alcuni parametri ematochimici ed ematologici nel cane. Atti Soc. It. Diagnostica di Laboratorio Veterinaria. 1999e, **2**, in press.
107. **Piccione G., Attanzio G., Assenza A.M., Calagna G., and Caola G.** The temporal pattern of body temperature in the adult cow and calf. Proceedings of the 8th Congress of Fe.Me.S.P.Rum. 2000, 406-411.
108. **Piccione G., Assenza A.M., Attanzio G., Fazio F., and Caola G.** Chronophysiology of arterial blood pressure and heart rate in athletic horses. Slovenian Vet. Res. 2001a, **38** **(3)**, 243-248.
109. **Piccione G., Assenza A.M., Fazio F., Giudice E., and Caola G.** Different Periodicities of Some Haematological Parameters in Exercise-Loaded Athletic Horses and Sedentary Horses. J. Equine Sci. 2001b, **12** **(1)**, 17-23.
110. **Piccione G., Celona B., Russo M., and Assenza A.M.** Il ritmo circadiano della temperatura corporea nel cane mantenuto in ambiente climatico controllato e in ambiente naturale. Atti Soc. It. di Fisiologia Veterinaria. 2001c, **4**, 50-53.
111. **Piccione G., Morgante M., Celona B., and Fazio F.** Chronophysiological evaluation of urinary excretion in the horse by analysis of fractional excretion of electrolytes. SISVet Annual Meeting selected abstracts. 2001d, **3**, 67-68.
112. **Piccione G., Caola G., and Refinetti R.** Maturation of the daily body temperature rhythm in sheep and horse. Journal of Thermal Biology. 2002a, **27**, 175-178.
113. **Piccione G., Caola G., and Refinetti R.** The circadian rhythm of body temperature of the horse. Biological Rhythm Research. 2002b, **33**, 113-119.
114. **Pittendrigh, C.S.** Circadian systems: entrainment. Handbook of Behavioral Neurobiology. Biological rhythms. New York: Plenum, 1981, **4**, 95-123.
115. **Prosser R., Kittrell E.M.W., and Satinoff E.** Circadian body temperature rhythms in rats with suprachiasmatic nuclear lesions, pp. 67-70. In: Thermal Physiology, edited by JRS Hales. New York: Raven, 1984.
116. **Ralph, M.R. and Menaker M.** A mutation of the circadian system in golden hamsters. Science. 1989, **241**, 1125-1127.
117. **Ralph M.R., Foster R.G., Davis F.C., and Menaker M.** Transplanted suprachiasmatic nucleus determines circadian period. Science. 1990, **247**, 975-978.
118. **Recabarren S.E., Vergara M., Llanos A.J., and Seron-Ferre M.** Circadian variation of rectal temperature in newborn sheep. J. Dev. Physiol. 1987, Oct, **(5)**, 399-408.
119. **Refinetti, R. and Menaker M.** The Circadian Rhythm of Body Temperature. Physiology & Behavior. 1992, **51**, 613-637.
120. **Refinetti, R.** Biological Rhythms, p. 20, In: Circadian Physiology. CRC Press, Boca Raton, Lonndon, New York, Washington, D.C., 2000.
121. **Reilly T., Atkinson G., and Waterhouse.** Physiological rhythms at rest, pp. 15-17. In: Biological Rhythms & Exercise, Oxford, 1997.
122. **Reinberg, A.** Aspects of circannual rhythms in man, p. 423. In: Pengelley E.T. (ed) Circannual clocks annual biologic rhythms. Academic, New York, 1974.
123. **Reiter, R.J.** The pineal gland: an important link to the environment. News Physiol. Sci. 1986, **1**, 202-205.
124. **Reppert S.M., Perlow M.J., Ungarleider L.G., Mishkin M., Tamarkio L., Orloff D.G., Hoffman H.J., and Klein D.C.** Effects of damage to the suprachiasmatic area of the anterior hypothalamus on daily melatonin and cortisol rhythms in the rhesus monkey. J. Neurosci. 1981, **1**, 1414-1425.
125. **Romero M.T., Lehman M.N., and Silver R.** Age of donor influences ability of suprachiasmatic nucleus grafts to restore circadian rhythmicity. Developmental

Brain Research. 1993, **71**, 45-52.
126. **Ruby N.F., Ibuka N., Barnes B.M., and Zucker I.** Suprachiasmatic nuclei influence torpor and circadian temperature rhythms in hamsters. American Journal of Physiology. 1989, **257**, R210-R215.
127. **Rusak, B.** The mammalian circadian system: models and physiology. J. Biol. Rhythms. 1989, **4**, 121-134.
128. **Sabin F.R., Cunningham R.S., Doan C.A., and Kindwale J.A.** The normal rhythm of white blood cells. Bull Johns Hopkins Hosp. 1927, **37**, 14-67.
129. **Sano H., Hayashi H., Makino M., Takezawa H., Hirai M., Saito H., and Ebihara S.** Effects of suprachiasmatic lesions on circadian rhythms of blood pressure, heart rate and locomotor activity in the rat. Japanese Circulation Journal. 1995, **59**, 565-573.
130. **Satinoff, E. and Prosser R.A.** Suprachiasmatic nuclear lesions eliminate circadian rhythms of drinking and activity, but not of body temperature in male rats. J. Biol. Rhythms. 1988, **3**, 1-22.
131. **Sato, T. and Kawamura H.** Effects of bilateral suprachiasmatic nucleus lesions on the circadian rhythms in a diurnal rodent, the Siberian chipmunk. Journal of Comparative Physiology A. 1984, **155**, 745-752.
132. **Simmons K.R., Dracy A.E., and Essler W.O.** Diurnal temperature patterns in unrestrained cows. J. Dairy Sci. 1965, **48**, 1490-1493.
133. **Smaaland, R. and Laerum O.D.** Chronobiology of human bone marrow, pp. 527-546. In: Biologic Rhythms in Clinical and Laboratory Medicine. Touitou and Haus (eds), Springer-Verlag Berlin Heidelberg, 1992.
134. **Solberg, H.E.** Approved recommendation -1986- on the theory of reference values, part 1. The concept of reference values. Report of expert panel on theory of reference values (EPTRV) of the international federation of clinical chemistry (IFCC). Clin. Chim. Acta. 1987, **165**, 111-118.
135. **Stanier M.W., Mount L.E., and Bligh J.** Energy balance and temperature regulation. Cambridge University Press, Cambridge, 1984.
136. **Stephan, F.K. and Zucker I.** Circadian rhythms in drinking behavior and locomotor activity are eliminated by suprachiasmatic lesions. Proc. Natl. Acad. Sci. USA. 1972, **54**, 1521-1527.
137. **Stephan, F.K. and Nunez A.A.** Elimination of circadian rhythms in drinking, activity, sleep and temperature by isolation of the suprachiasmatic nuclei. Behavioral Biology. 1977, **20**, 1-16.
138. **Stephenson L.A., Winger C.B., O'Donovan B.H., and Nadel E.R.** Circadian rhythms in sweating and cutaneous blood flow. Amer. J. Physiol. 1984, **246**, R321-R324.
139. **Stetson, M.H. and M. Watson-Whitmyre.** Nucleus suprachiasmaticus: The biological clock in the hamster. Science. 1976, **191**, 197-199.
140. **Szafarczyk A., Ixart G., Malaval F., Nouguier-Soule J., and Assenmacher I.** Effects of lesions of the suprachiasmatic nuclei and of p-chlorophenylalanine on the circadian rhythms of adrenocorticotrophic hormone and corticosterone in the plasma, and on locomotor activity of rats. J. Endocrinol. 1979, **83**, 1-16.
141. **Touitou Y., Fevre M., Lagoguey M., Carayon A., Bogdan A., Reinberg A., Beck H., Cesselin F., and Touitou C.** Age and mental health-related circadian rhythm of plasma levels of melatonin, prolactin, luteinizing hormone and follicle-stimulating hormone in man. J. Endocrinol. 1981, **91**, 467-475.
142. **Touitou Y., Sulon J., Bogdan A., Touitou C., Reinberg A., Beck H., Sodoyez J.C., and van Cauwenberge H.** Adrenal circadian system in young and elderly human subjects: a comparative study. J. Endocrinol. 1982, **93**, 201-210.
143. **Touitou Y., Lagoguey M., and Bogdan A.** Seasonal rhythms of plasma gonadotropins: their persistence in elderly men and women. J. Endocrinol. 1983a, **96**, 15-21.
144. **Touitou Y., Sulon J., Bogdan A., Reinberg A., Sodoyez J.C., and Demey-Ponsart E.** Adrenocortical hormones, ageing and mental condition: seasonal and circadian rhythms of plasma 18-hydroxy-11-deoxycorticosterone, total and free cortisol and urinary corticosteroids. J. Endocrinol. 1983b, **96**, 53-64.
145. **Touitou Y., Touitou C., Bogdan A., Reinberg A., Motohashi Y., Auzeby A., and Beck H.** Circadian and seasonal variations of electrolytes in aging humans. Clin. Chim. Acta. 1989, **180**, 245-254.
146. **Vogelbaum, M.A. and Menaker M.** Temporal chimeras produced by hypothalamic transplants. Journal of Neuroscience. 1992, **12**, 3619-3627.
147. **Warren W.S., Champney T.H., and Cassone V.M.** The suprachiasmatic nucleus controls the circadian rhythm of heart rate via the sympathetic nervous system. Physiology and Behavior. 1994, **55**, 1091-1099.
148. **Wever, R.** Bright light affects human circadian rhythm. Pflügers Arch. 1983, **396**, 85-87.
149. **Wrenn T.R., Bitman J., and Sykes J.F.** Diurnal patterns of bovine body temperature. J. Dairy Sci. 1961, **44**, 2077-2080.
150. **Yashiki K., Kusunose R., and Takagi S.** Diurnal variations of blood constituents in young thoroughbred horses. J. Equine Sci. 1995, **6 (3)**, 91-97.
151. **Zucker I., Boshes M., and Dark J.** Suprachiasmatic nuclei influence circannual and circadian rhythms of ground squirrels. American Journal of Physiology. 1983, **244**, R472-R480.