Influence of Weather Parameters on the Fertility of Horse Mares (Equus Caballus)

Tierhaltung und Haltungsbiologie

Justus-Liebig-Universität Gießen

Thesis submitted in fulfilment of the requirements for the degree of
Dr. agr. (Agrarwissenschaften/Agriculture)
im Fachbereich Agrarwissenschaften der Justus-Liebig-Universität Gießen

vorgelegt von

ULRIKE VOIGT

aus Köln

Gießen, 2020

Declaration of Author

Erklärung gemäß der Promotionsordnung des Fachbereichs 09 vom 07. Juli 2004 § 17 (2)

„Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.“

U. Voigt

06.08.2020

Unterschrift

Datum

Table of Contents

Abstract

In temperate climates, mares show seasonal poly-oestrus with a change between sexually active ovulatory sexual cycles in spring / summer and reduced reproductive activity during winter months. Ambient temperature can influence the reproductive activity in horses. Numerous studies in livestock animals have shown that the combination of different weather parameters influenced the reproductive cycle. In particular, air temperature in combination with humidity as a good approximation for heat load on the animal) influences certain reproductive parameters. An established concept for measuring the influence of these two weather parameters on livestock is the Temperature-Humidity Index (THI). In horses, multiple publications have shown that ambient temperatures within the thermoneutral zone (TNZ) have a positive effect on fertility. The aim of this study was to investigate the influence and / or interaction between weather parameters (temperature, humidity, radiation, and sunshine) and their effects on reproductive factors, in particular the gestational outcome/pregnancy result. Over a period of 4 years, 19 horse mares were induced to oestrus hormonally, and subsequently were inseminated with sperm of different stallions (n = 26). On day 14, they were flushed (or pregnancy confirmed via sonography) and in case of pregnancy, seven days later the pregnancy was terminated with the use of prostaglandin. Oestrus was again induced after termination of prior pregnancy (n = 246 year-round distributed inseminations in total). The mares were kept in indoor boxes in Bad Saarow with daily access (>6 hours) to pasture or paddock. In two of the four observation years, a light program (<8 hours of darkness / day during the months of September to April) was implemented in the barn. Data on weather parameters (minimum, maximum and daily average for temperature, humidity, temperature-humidity index (THI), air pressure, longwave downward radiation, diffuse solar radiation, incoming solar radiation and minutes of sunshine per day) were collected from a weather station nearby (at a straight-line distance of 8 km from the testing site). The influence of reproductive and weather parameters (individually and in combination, continuously and partially additionally subdivided into classes) on the pregnancy result, taking into account the light program, were calculated utilizing SAS 9.4's generalized linear mixed model (procedure GLIMMIX) with a binary output (pregnant / not pregnant). Unbalanced data structure (more observations from some horses than others) and repeated data (multiple observations per horse) could thus be adequately considered. The individual combination of mare and stallion was taken into account as a random effect. The effects of the weather parameters were analysed for each biological phase of the mare (Follicular maturation, ovulation, oviductal Phase and uterine phase) separately. As expected, the timing of artificial insemination (AI) relative to ovulation had a significant impact ($p<0.05$) on the gestational outcome. The data showed that AI within 48 hours prior to ovulation had the highest chance for pregnancy, followed by AI six hours after ovulation. The lowest pregnancy rates were generated when AI was performed more than 48 hours before ovulation. While the majority of the observed weather parameters and their combinations had no significant influence on the pregnancy outcome at any stage of the reproductive cycle, a moderate diffuse solar radiation (<1000 J/cm^2) during ovulation showed a significant positive influence ($p=0.0160$) on fertility. In the oviductal phase, a higher sunshine duration per day increased fertility ($p=0.035$). In addition, higher rates of pregnancy could be achieved with weaker longwave downward radiation (<2500 J/cm^2) during follicular maturation ($p=0.0146$). In the analysis (without including data from the light program), when weather parameters were subdivided into classes, the positive influence on fertility in the uterine phase ($p=0.0122$) was shown. In addition, the ambient average temperature had a positive ($p=0.0345$) effect on the pregnancy result when inseminating within 48 hours prior to ovulation. In summary, the influence of the examined weather parameters on pregnancy rates of mares in Germany's temperate latitudes appears to be low overall. This is likely due to the fact that the horses' TNZ (TNZ = 5 °C – 25 °C) and comfort zones with respect to the less heat-load relevant weather parameters (humidity and THI), were rarely exceeded (e.g. 13% of days >25 °C).

Acknowledgements

Thank you to my supervisors, colleagues and friends…

I consider myself extremely lucky to have had amazing supervisors throughout this doctoral thesis journey. I cannot thank Prof. Uta König von Borstel and Prof. Johannes Handler enough for their mentorship, wisdom and patience on this endeavour. Their bright ideas and critical thought have challenged the status quo and contributed to research in reproduction of the mare. Many thanks also to Stefanie Neuhauser for collecting and providing the reproductive data of the mares.

Thank you to my family…

Thank you to my little Luca for being so sweet, allowing me to finish my writing. Your patience and love has carried me through this doctoral thesis.

Last but not least, thank you to all of the horses and ponies…

The kindness of horses never fails to amaze me – they are such gentle and sentient beings. I dedicate this thesis to Laila, my gorgeous Trakehner horse: Her photo next to my computer kept me writing.

Ulrike Voigt

List of Abbreviations

AHL	Accumulated Heat Load
AI	Artificial Insemination
BCS	Body Condition Score
BMEL	Bundesministerium für Ernährung und Landwirtschaft
DF	Dominant Follicle
ECM	Energy Corrected Milk
GAMP	Guide to Agricultural Meteorological Practices
HLI	Heat Loss Load Index
LCT	Lower Critical Temperature
TCZ	Thermal Comfort Zone
THI	Temperature-Humidity-Index
THIV	Temperature-Humidity Velocity Index
TNZ	Thermoneutral Zone
UCT	Upper Critical Temperature
USDA	U.S. Department of Agriculture

List of Tables

List of Figures

1 The Seasonal Reproductive Cycle of the Horse Mare

The mare is a seasonal breeder with a change between the sexually active ovulatory sexual cycles in spring and summer (breeding season) and reduced activity of sexual phases in the winter months (anoestrus) (Ginther, 1974, Morel, 2015). In the northern hemisphere, the spring and late autumn form more irregular periods of oestrous cycle activity being representing the transitions between two extremes (transitional phases). Minimum ovarian activity was reported to occur at the end of January and February (Palmer et al., 1983). The first ovulation in spring marks the beginning of the breeding season (Burkhardt, 2009). The biological breeding season of horses located in the northern hemisphere is from March through September.

The seasonal reproduction of mares is mainly driven by **daylight length** and not surprisingly, where the most research was conducted thus far. Short-wavelength blue light was reported to effectively regulate equine biological rhythms and reproductive parameters (timing of ovulation and parturition) (Murphy, 2019). Light is primarily received by the retina of the eye and distributed to the pineal gland which is known to be a transformer synthesising the hormone melatonin (Palmer et al., 1992). The information is obviously distributed through neural pathways other than the eyes (vision), as blind mares also express seasonal reproductive activity (Aurich, 2009). However, no research was identified to explain why the mares were blind (directly at birth or due to a disease such as Equine Recurrent Uveitis) and furthermore how the blindness affected reproductive activity. Conversely, to this statement, in one study the researchers applied low intensity blue light to a single eye of a group of mares. Not all mares went into oestrus with this treatment (7.5% less) compared to the group of mares treated with a common light program along with barn lighting (250 lux), however, this result was not statistically significant ($p=0.335$) (Murphy et al., 2014). Mares on light programmes under constant daily lights continued to have a change of reproductive activity between periods of increased and reduced reproductive activity. The authors concluded mares not exposed to daylight had periods of increased and reduced reproductive activities that took place between the breeding season and anovulatory stages (Palmer et al., 1982). These mares were no longer in synchrony with the seasons, nor with the animals in the herd (Van Dierendonck et al., 2004). This was confirmed by other authors for both long days and short days and was referred to as refractoriness, which can best be described as a failure of continued response to the current type of photoperiod (Kooistra et al., 1975). Removing the pineal gland or the ganglion cervical superior did not influence the seasonal reproduction, but further light influence of the reproductive cycle of those mares was not attainable (Grubaugh et al., 1982). Even today, it remains questionable as to which organ remains to be the main pathway in terms of light to influence reproductive cycle of the mare or if other environmental and weather factors are more influential. In addition, a seasonal variation of different blood parameters/serum concentrations of prolactin, thyroxine and triiodothyronine relative to season and the oestrous cycle could be shown (Irvine, 1967, Johnson, 1986). An endogenous rhythm is the basis for the seasonal reproductive activity of the mare. Steadily increasing day length results in an onset of ovulatory cycling, which typically occurs during March in the Northern hemisphere. Therefore, reproductive activity is highest with long days and short nights. In winter, lighting conditions are therefore not beneficial for an active reproduction in the mare due to short days and long nights resulting in a reduced reproductive activity; the hypothalamus pituitary and the ovaries have reduced activity and reported to finally suspend completely (Nagy et al., 2000). Foals are naturally born in the spring and summer, a season that has environmental conditions favourable for survival. In this context, one interesting study described when days were artificially short, ovarian failure increased immediately (Palmer et al., 1983). Furthermore, it was presented that mares with a foal had complete ovarian activity in May or winter when exposed to two months of artificially long days (Palmer et al., 1983).

Younger mares aged up to five years had a reduced breeding season (Palmer et al., 1983) and were earlier in cycle in the autumn than older mares (Carnevale et al., 1995). The cycle in the spring in mares older than 15 years began later than in younger mares (Carnevale et al., 1992). However, in all breeds ovulatory problems can be observed during the so-called transitional phase in the spring (Pycock, 2000, Panzani et al., 2011). The very first oestrus in a mare is generally extended and does not necessarily result in ovulation (Mari et al., 2009).

The duration of anovulatory phase, the period of the year with no cyclic activity, was influenced by external factors such as feeding and stabling (Allen et al., 2000). Mares kept outside in paddocks or on pasture year-round usually had a later onset of ovulatory activity than mares kept in stables (McDonnell, 2000). In addition, often a more customized support of individual animals in stable conditions is possible. It was further believed that indoor stabling can have a positive impact on ovarian activity due to higher temperatures when due to lower energy requirements were needed. In contrast, ambient temperatures did not have a positive effect on the reproductive cycle (Guerin et al., 1994, Zeller, 2000). One study indicated an earlier onset of oestrus in mares housed in stables at a much higher temperature compared to a control group in outdoor stables with temperatures below zero degrees Celsius (°C) (Tucker et al., 1980). However, the mares housed outside lost weight likely having an effect on the onset of oestrus, as proper nutrition and mares in a well-fed condition tended to have a higher chance for a positive pregnancy result (Fradinho et al., 2014). In well-fed mares, the ovulatory cycle started earlier in the year (Cymbaluk et al., 1990) compared to mares in a negative energy balance (Meyer et al., 2001). It could not be clarified whether an earlier start of ovulatory cycles in the year was due to access to pasture and fresh grass (and its ingredients), or only influenced by an enhanced nutritional status (Carnevale et al., 1997).

2 Effect of Weather Parameters on the Reproductive Cycle of Horse Mares´

Where are we today in equine research regarding the influence of weather parameters on the reproductive cycle? In a very limited amount of presented studies, an influence of certain weather parameters on the reproductive parameters of a horse mare was shown to exist.

Limited research was conducted on **temperature** and the effect on mares. However, a common concept regarding the influence of temperature on the horse is known as the ‘thermoneutral zone’ (TNZ). The TNZ was defined for the first time in 1985 as between -15 °C and 10 °C in still air conditions (McBride et al., 1985). However, ten years earlier temperatures ranging from 0 °C to 5°C were reported to be the lower critical temperature (LCT) in which the metabolic rate increases in order to retain deep-body temperature (Young et al., 1973, Mount, 1974). A few years later another study described the TNZ as the range of temperatures in which the horse does not need any additional energy expenditure to hold the body temperature (Christopherson et al., 1986). Based on the definition of those previous studies ten years later Morgan et al. (1997, 1998) presented different studies and established the range of the TNZ ranging from 5 °C to 25 °C (Morgan, 1997, 1998). Temperatures >30 °C were defined as the upper critical temperature (UCT), where the metabolic rate as well as the evaporative heat loss increases and the tissue thermal insulance is minimal. Peripheral vasodilation is maximal (i.e. minimal thermal insulance of the tissue) (Morgan, 1998). Particularly a very limited number of studies exist on the effect of temperature in terms of reproduction. Two studies described the onset of the first ovulation in relation to ambient temperature. Dini et. al. (2019) measured ambient temperature inside the barn and the outside temperature as a measurement of comfort and considered the temperature differences when examining the onset of ovarian activity in mares. Mares were exposed to artificial light before sunrise (overhead lighting as fluorescent tubes during two hours from 3:00am to 5:00am) and direct sunlight during the day. However, no effect of increased ambient temperatures was identified that significantly increased the time of return to oestrus (Dini et al., 2019). The authors concluded that mares under artificial photoperiod and exposed to more intense daylight had a shorter interval to first ovulation. Two authors looked at the outside temperature and obtained a strong correlation to maximum (correlation coefficient $r=0.56$; $p=0.09$) and minimum ($r=-0.67$; $p<0.01$) environmental temperatures (Guerin et al., 1994). However, the authors’ observations were based on circumstantial environmental conditions that appeared to occur together with an early onset of the natural breeding season. Similarly, results of another study evaluated the endogenous hormonal control of the mare's oestrus cycle in Thoroughbred mares (based in the UK) and observed the transition of anoestrus to oestrus following winter was slower with abrupt cold temperatures (Allen, 1987). Unfortunately, Allen did not further define the term temperatures and the mares evaluated were likely not in the thermoneutral zone (TNZ). Environmental temperature appeared to be a crucial variable influencing the oestrous cycle in mares revealing that length of oestrus was longer in certain months due to temperature and minutes of sunshine per day (Ju et al., 2002). Unfortunately, the study did not quantify temperatures and it can be assumed that temperatures were not the only parameter triggering heat stress in the mares, as the average temperature per month was taken as the basis.

An even earlier study looked at the effect of **temperature and a light program** for 120 days in two groups of pony mares (Sharp et al., 1975). The authors revealed that treated animals went into oestrus behaviour when having a larger size of dominant follicles compared to the control group. Based on the results, a differentiation between the effect of temperature and light could not be concluded.

Regarding **humidity**, most studies were performed in the training segment and here high humidity (which has a negative effect on horses), was defined as being more exceeding 75% relative humidity (Groenendyk et al., 1988, Foreman et al., 1996, Mills et al., 1996, Marlin et al., 1999, Geor et al., 2000).

However, defining different ranges of temperature and humidity that affect horses are a generalization and individuals might have distinct deviations. An embryo transfer study evaluated the effect of heat stress on the recovery of embryos and identified nearly a 50% reduction in embryo recovery rate in mares exercised under hot and humid conditions (Campbell, 2014). Zeller (2000) discovered that the duration of service period decreased with increased temperature (horses in TNZ) and lower air moisture (<~85% relative humidity) (Zeller, 2000).

One study took into account a **Temperature-Humidity Index (THI)** (THI = 0.7* wet bulb temperature + 0.3* dry bulb temperature) and the effect of mares´ thermoregulatory responses during summertime on road transport and stall confinement (Green et al., 2007). The study indicated that core body temperature of individual horses had a tendency to increase with increased THI; unfortunately, the study did not evaluate reproductive parameters. Only one study could be identified that evaluated THI and the effect on reproduction in mares: The study evaluated the effects of THI during the month of foaling on the duration of gestation of Thoroughbred mares (Scalco et al., 2018). Pregnancy data from mares housed in two different stud farms - one in sub-tropical climate (236 pregnancies of 161 mares) and one in tropical climate (201 pregnancies of 118 mares) were collected and analysed to calculate the effect of THI on gestation length. Reportedly, the management, as well as nutritional status and housing of the mares was similar. Age was the only factor identified to be different between groups of enrolled mares. No significant effect was observed between location, foal sex, parity and age of the mare. The conclusion of the study was that higher THI (THI>66) had a negative effect on the length of gestation.

Two studies were identified evaluating the effect of **solar radiation** on horses, but unfortunately, neither study evaluated solar radiation in the context of reproduction. However, horses do appear to be affected by solar radiation, as it was discovered that horses actively seek shade when solar radiation is high (Snoeks et al., 2015). Previously published data indicated shelter seeking was higher (7.1% more observations) before and during peak solar radiation (Holcomb et al., 2014). However, the reason why horses seek shelter could be different depending on each particular situation and was not clearly confirmed. When trying to find the answer why shelter was searched for on hot sunny days, studies bared different outputs. One study reported a thirty-minute shelter use had no effect on rectal and skin temperatures (p>0.05) (Hartmann et al., 2015), whereas other authors reported an effect on rectal and skin temperature, as well as respiration rate in the horses in the shade (Holcomb et al., 2013). However, horses do seek shelter for other reasons which are likely due to environmental factors such as disturbance by biting insects (King et al., 2010). Nevertheless, if solar radiation has an effect on a horse's physiological homeostasis, a corresponding effect in reproduction could be possible.

Weather is closely related to the **month and season**. Therefore it is no surprise that certain studies did not focus on weather parameters as particularly, but focused on the month/season when prominent weather conditions occurred in the evaluated month/season. One survey evaluated the reproductive success of Arabian mares in Turkey and retrospectively analysed data on 2,189 mares collected over 30 years (1976-2007) with the target to investigate, if and to what amount, environmental factors affected reproductive parameters (Cilek, 2009). The study indicated that heritability of gestation period for Arabian mares was low (12%) and that 88% of total (phenotypic) variance was determined through environmental factors. Conversely, the study only looked at the month and did not differentiate any of the two environmental parameters, temperature and humidity, that were reported to influence first service period, foaling interval, oestrus cycle per pregnancy, and number of inseminations per conception. Similarly, earlier studies taking the same approach mainly looked at the month as environmental factor and the relationship to gestation length as the reproductive parameter (Howell et al., 1951, Hintz et al., 1992, Meliani et al., 2011). A study conducted in Taiwan identified a correlation between month and reproductive parameters such as oestrus, pregnancy, parturition, as well as nursing period (Ju et al., 2002). In another study in tropical conditions, in Pakistan it was revealed that conception rates were higher in mares bred during winter (horse in TNZ) compared to summer months (horse not in TNZ/horse in heat stress) (Warriach et al., 2014). Four years later, a study conducted in Brazil demonstrated significant effects on of year, season and temperature regarding the recovery rate of the embryos ($p<0.05$), but no significant effects were observed on parameters such as year, season, hormone treatment, rainfall and photoperiod, regarding the pregnancy rate ($p>0.05$) (Rua et al., 2018). However, the authors confirmed that time and duration of the reproductive phases were different depending on horse breed. One author stated that less domesticated species such as pony breeds express a strong seasonal reproductive behaviour with long anovulatory phases during winter (Aurich, 2011). In some mares ovulatory cycles stop for a few weeks, while other mares cycle year round with reduced and/or occasional follicle growth (Donadeu et al., 2007) and stages with complete arresting ovarian activity were extremely rare (Ferreira-Dias et al., 2005). One study conducted in Taiwan, where the climate was humid with mild winter and sweltering summer, reported the distribution of the mares' oestrus over the year was bimodal (Ju et al., 2002). This reproductive occurrence may be unique for mares in Taiwan. In addition, Arabian mares based in the Kingdom of Saudi Arabia remained in oestrus the entire year (Ali et al., 2014) and this same phenomenon was observed for Arabian mares in Pakistan (Warriach et al., 2014). Another study in Egypt in Arabian mares reported mares were in oestrus the whole year, but oestrus was less frequent in summer than in spring and autumn (El-Wishy et al., 1990). It was discovered that in a subtropical environment (the study was conducted in India) postpartum reproduction and ovarian activity may be delayed (Sharma et al., 2010). A study conducted in Venezuela indicated that the length of oestrus was longer in tropical climate, where the authors related this difference more to photoperiod, than temperature (Verde et al., 2018).

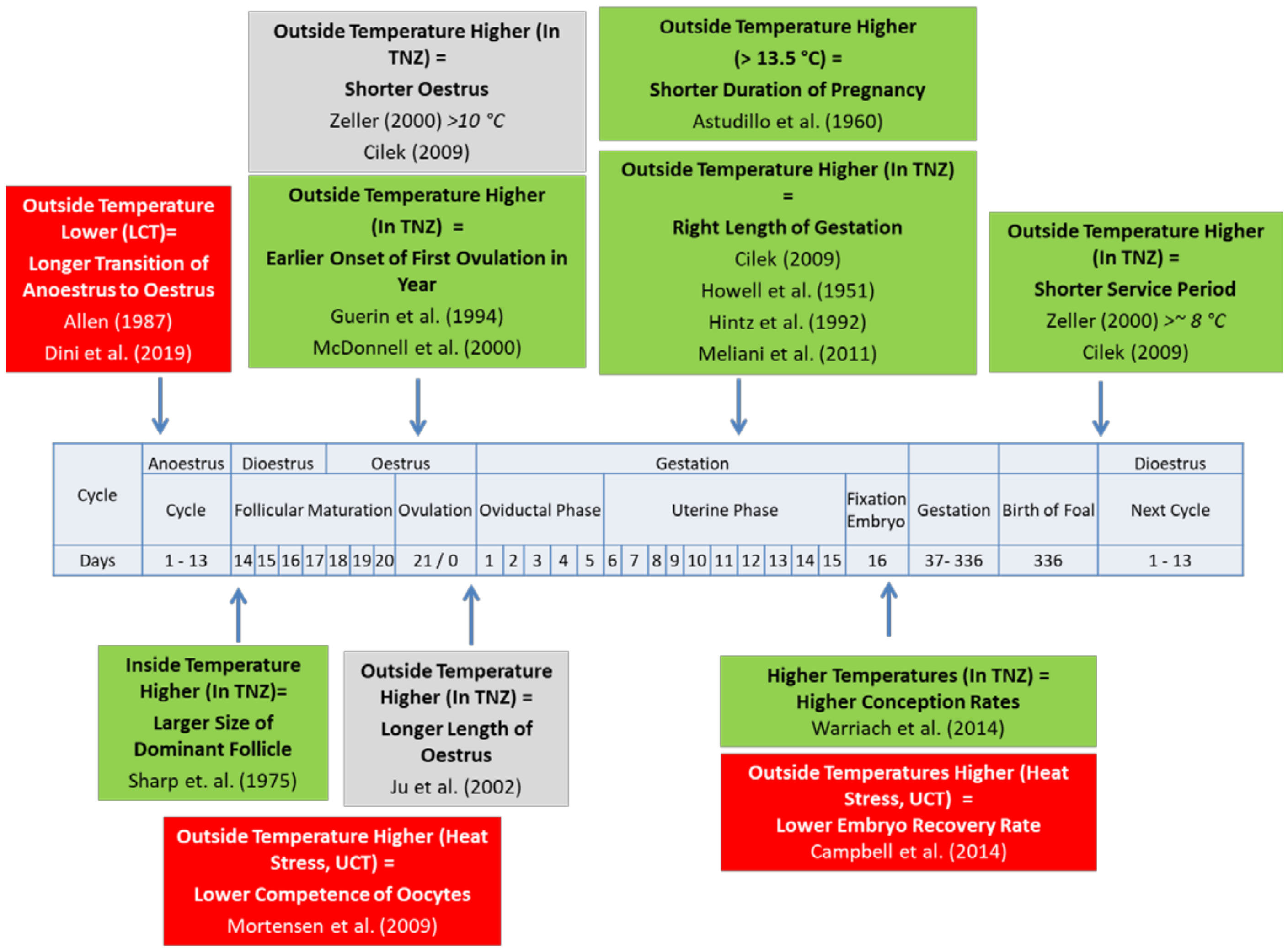
One study from the 1960s analysed 2,107 gestations and evaluated correlations between environmental **temperatures, hours of sunshine, rainfall and relative humidity** to the season of mating**.** The authors reported the most significant variables were temperature and hours of sunshine and both were highly correlated to the season of mating. Furthermore, they discovered that higher temperatures (>13.5 °C) during the season of mating resulted in a shorter duration of pregnancy (Astudillo et al., 1960).

3 The Effect of Weather Parameters on Fertility of the Horse Mare - Summary of Studies

Published studies describing the effect of weather factors on reproductive parameters in the horse mare can be summarized as follows:

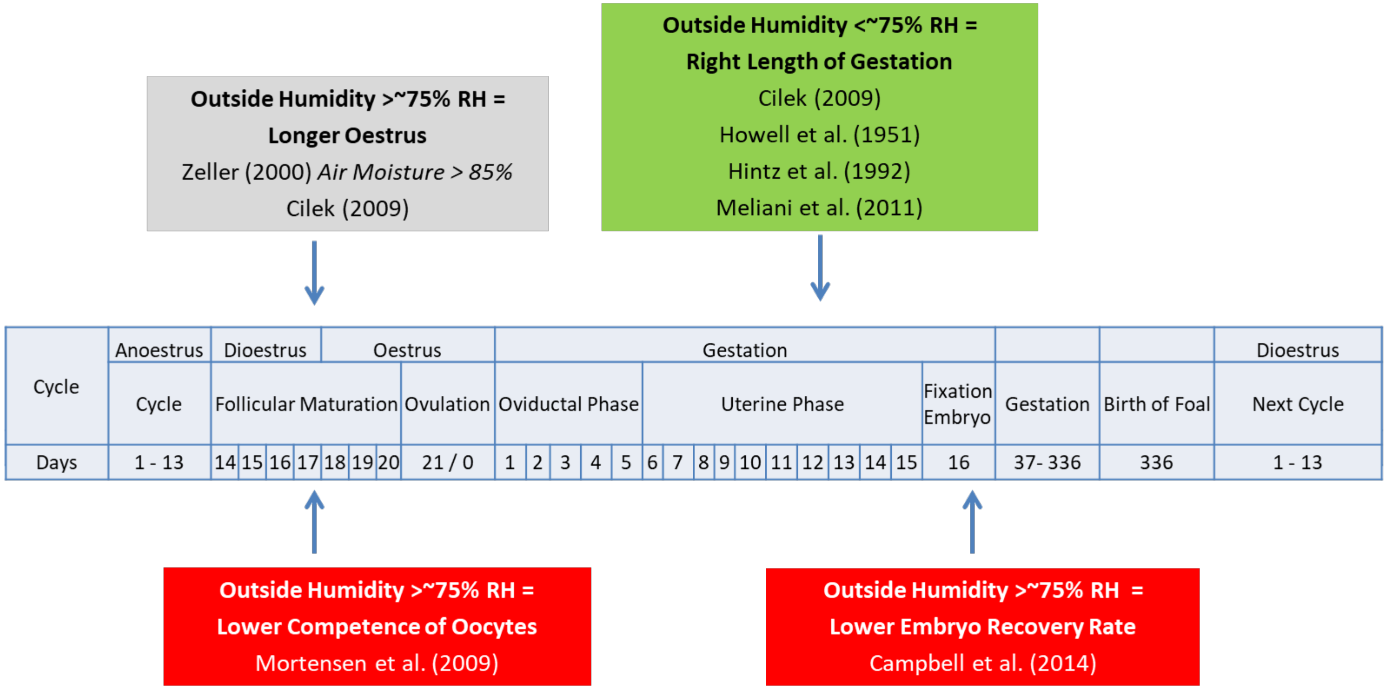
- Allen (1987) reported the endogenous hormonal control of the mare's oestrus cycle in Thoroughbred mares based in the UK observed that the transition of anoestrus to oestrus after the winter was slower with abrupt cold temperatures (not further defined).
- Sharp et al. (1975) indicated that mares exposed to higher temperatures (horses in TNZ) and a light program had larger dominant follicles compared to the control group.
- Mortensen et al. (2010) reported an association that in mares in a hot (horse not in TNZ/horse in heat stress) and humid (relative humidity >~75%) environment affected the developmental competence of their oocytes.
- Astudillo et al. (1960) presented that ambient temperature and hours of sunshine were highly correlated to the season of mating; higher temperatures (>13.5 °C) during the season of mating resulted in a shorter-duration of pregnancy.
- Dini et al. (2019) reported higher ambient temperatures during anoestrous did not have an effect on the interval to first ovulation in the spring; but mares under artificial photoperiod and exposed to sunlight during the day had a shorter interval to first ovulation.
- In addition, Guerin et al. (1994) published that the onset of the first ovulation which starts the breeding season of a mare every year, was in strong correlation to the outside maximum temperature (correlation coefficient $r=0.56$; $p=0.09$) and minimum temperature ($r= -0.67$; $p<0.01$); horses were in TNZ.
- McDonnell et al. (2000) demonstrated that mares housed outside year round had a later onset of ovulatory activity compared to mares housed inside stables.
- Ju et al. (2002) indicated that length of oestrus was longer in certain months due to temperature (horses in TNZ) and minutes of sunshine per day.
- Scalco et al. (2018) revealed that a high THI had a negative effect on the length of gestation.
- Cilek (2009) reported temperature and humidity influenced gestation length up to 88% compared to genetic factors. The study did not define specific temperatures but only reported that appropriate temperatures (horse in TNZ) were important for the proper length of gestation. Other authors findings supported these two environmental factors (temperature and humidity) influenced gestation length (Howell et al., 1951, Hintz et al., 1992, Meliani et al., 2011).
- Warriach et al. (2004) demonstrated that conception rates in mares in Pakistan were higher in mares bred during milder winter (horses in TNZ) compared to hot summer months.
- Campbell et al. (2014) published that embryo recovery rate was 50% less in mares exercised under hot (horse not in TNZ) and humid (relative humidity >~75%) conditions compared to the control group.
- Zeller (2000) and Cilek et al. (2009) both described that oestrus as well as the service period was shorter, the higher the temperature (horse in TNZ) and the lower the air moisture (relative humidity <~85%).

The graphical representations 3-1 to 3-4 show the research conducted on the effect of weather parameters display the reproductive cycle and include the study conclusions and references that support which reproductive parameters were influenced by which weather parameter, visualizing temperature, humidity, minutes of sunshine per day noting the positive or negative effect on the pregnancy result.



Research conducted on the effect of temperature (in degrees Celsius) with TNZ= 5 °C – 25 °C, LCT= <5 °C, UCT= >30 °C following the reproductive cycle of the mare showing a positive (= green box), negative (= red box) or no (= grey box) effect on the pregnancy result

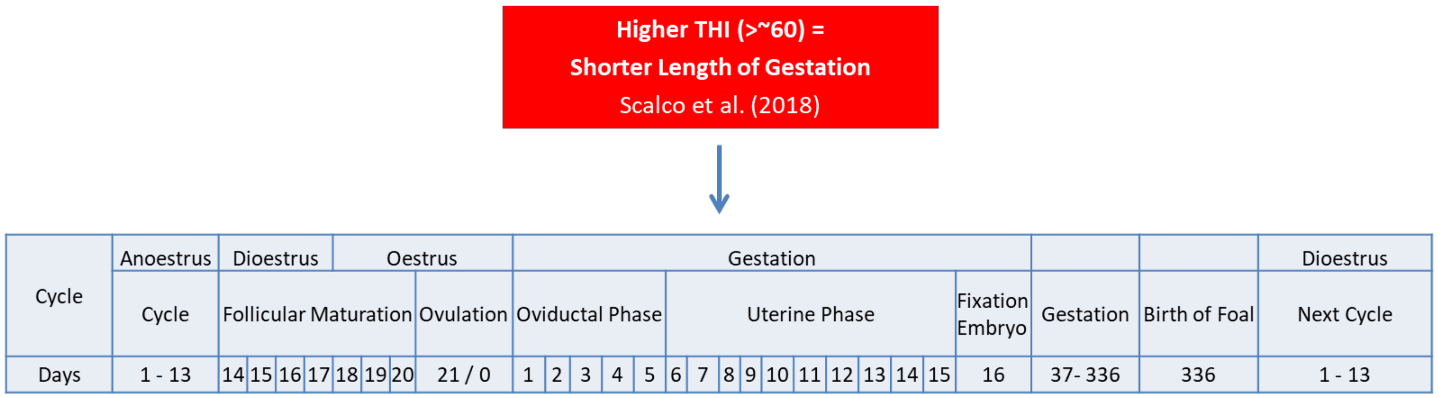
Figure 3-1: Equine research conducted on the effect of temperature



Cycle	Anoestrus	Dioestrus	Oestrus	Gestation					Dioestrus
	Cycle	Follicular Maturation	Ovulation	Oviductal Phase	Uterine Phase	Fixation Embryo	Gestation	Birth of Foal	Next Cycle
Days	1 - 13	14 15 16 17 18 19 20	21 / 0	1 2 3 4 5	6 7 8 9 10 11 12 13 14 15	16	37- 336	336	1 - 13

Research conducted on the effect of relative humidity (RH) following the reproductive cycle of the mare showing a positive (= green box), negative (= red box) or no (= grey box) effect on the pregnancy result

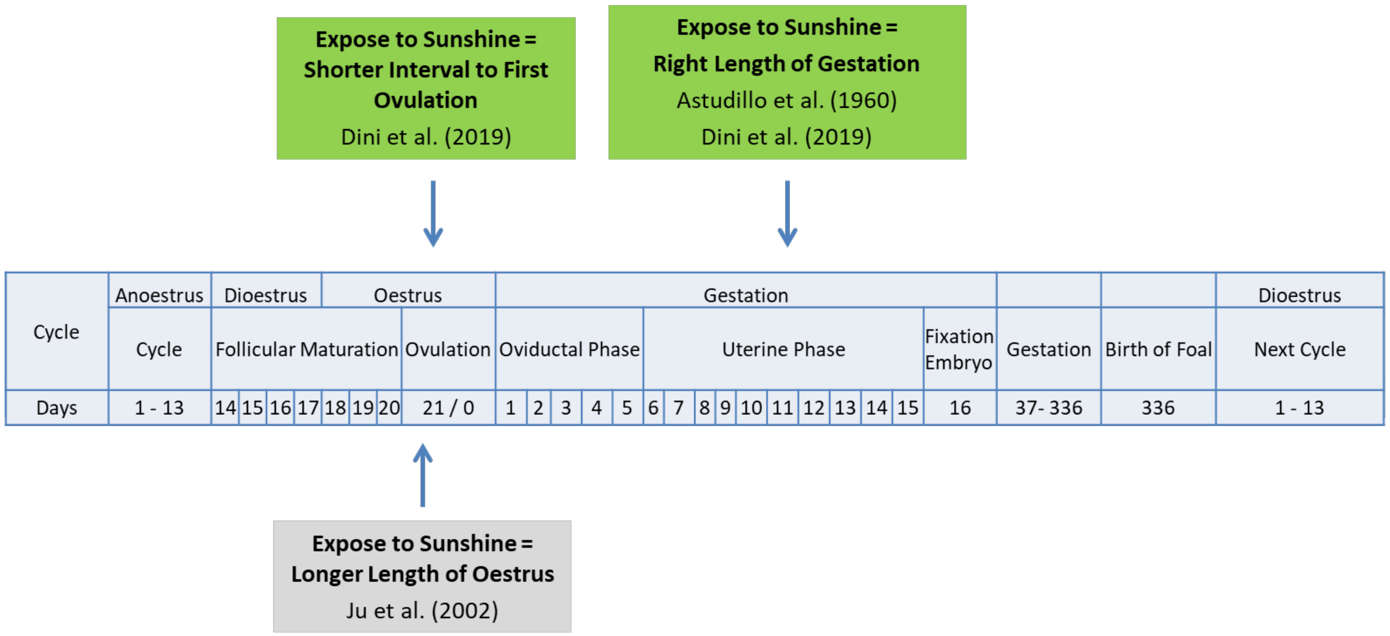
Figure 3-2: Equine research conducted on the effect of humidity



Cycle	Anoestrus	Dioestrus	Oestrus	Gestation					Dioestrus
	Cycle	Follicular Maturation	Ovulation	Oviductal Phase	Uterine Phase	Fixation Embryo	Gestation	Birth of Foal	Next Cycle
Days	1 - 13	14 15 16 17 18 19 20	21 / 0	1 2 3 4 5	6 7 8 9 10 11 12 13 14 15	16	37- 336	336	1 - 13

Research conducted on the effect of temperature and humidity (shown as THI = Temperature-Humidity Index) following the reproductive cycle of the horse mare showing a negative (= red box) effect on the pregnancy result

Figure 3-3: Equine research conducted on the effect of temperature and humidity (shown as THI)



Research conducted on the effect of sunshine following the reproductive cycle of the mare showing a positive (= green box) or no (= grey box) effect on the pregnancy result

Figure 3-4: Equine research conducted on the effect of sunshine

The figures can be summarized as follows:

- Figure 3-1 shows research conducted on the effect of temperature and demonstrates that temperatures within the TNZ have a positive impact on in every biological phase. Cold temperatures below the LCT prolong anoestrus and heat stress (UCT) before fertilization has a negative effect on the competence of oocytes. Furthermore, heat stress (UCT) does negatively affect embryo implantation and therefore has a negative effect on the pregnancy result. Temperature within the TNZ does seem to influence the length of oestrus but with no effect on the pregnancy result. Furthermore, temperature within the TNZ does shorten and therefore positively influences the service period.
- Figure 3-2 presenting research conducted on the effect of humidity shows that higher relative humidity (>75%) has an impact on the length of oestrus but not on the pregnancy result. Furthermore, higher relative humidity (>75%) negatively influences the competence of oocytes and likewise the embryo recovery rate. A lower relative humidity (<75%) positively influences the length of gestation.
- Figure 3-3 displaying /research conducted on the effect of temperature and relative humidity (shown as THI = Temperature-Humidity Index) shows that a high THI negatively influences length of gestation and therefore negatively influences the pregnancy result.
- Figure 3-4 showing research conducted on the effect of sunshine shows that exposure to sunshine in general has a positive impact on the pregnancy result in oestrus and gestation in every biological phase. It also does seem to influence the length of oestrus but without any effect on the pregnancy result.

4 Effect of Weather Parameters on the Reproductive Cycle of Livestock Animals

In comparison to horses a lot research on the influence of weather parameters on the reproductive cycle is done in livestock animals, mainly due to the economics of the food industry. Annual losses averaging 897 million USD, 369 million USD, 299 million USD, and 128 million USD for dairy, beef, swine, and poultry industries, respectively, were reported (St-Pierre et al., 2003). Pierre et al. (2003) further reported that in the USA the effects of heat stress on livestock animals can be due to location of animals and furthermore due to the fact that the animals produce more metabolic heat because they are a specific genotype. A Guide to Agricultural Meteorological Practices (GAMP) was published in 2006 summarizing the main effects of weather and climate on production/farm animals and providing guiding principles to farmers in an effort to optimize their management and reproduction strategies (Gomes Da Silva, 2006).

4.1 Large Ruminants

4.1.1 Cattle

In comparison to horses the profitability of each cow is essential to survive in the livestock business (Mee et al., 2002). This is why a lot of money is invested into research regarding cattle. Dairy cows are producing a higher amount of milk (Dillon et al., 2006), with US milk production per cow more than doubling since 1970 (NASS, 2016). And while the amount of cows in general is decreasing the herds are getting bigger (Lucy, 2001). At the same time the price for a litre of milk the farmer receives is decreasing (Mosheim et al., 2009). With the cow being more productive particularly in terms of production (van Arendonk et al., 2003) reproductive fertility is decreasing at the same time (Walsh et al., 2011). This high economic pressure is forcing farmers to increase the value per cow by ensuring the well-being of the single cow in an effort to optimize reproductive opportunities. In 1990 one study described that losses of pregnancy are costly by tracking 4,732 pregnancies followed from 2,163 cows in a 6,5 year period and even identified the different causes for abortion/death of the foetus (Thurmond et al., 1990). Fifteen years later De Vries (2006) calculated on basis of the difference of discounted future cash flows (pregnancy or no pregnancy), that the average value of a new pregnancy was 278 USD. In the year 2003 two researchers calculated the value in the same way with the result of 200 USD (Eicker et al., 2003). The difference could have been due to reproductive productive factors, as for example artificial insemination (AI). One author estimated that the value of a new pregnancy was between 253 USD and 274 USD (without the additional cost of the programmed AI breeding protocol compared with traditional breeding based on detected oestrus (Stevenson, 2001). He showed that the value of pregnancy increased with lower oestrus detection efficiency. In addition, the average cost of a pregnancy loss (abortion) was 555 USD (De Vries, 2006).

It was clearly presented that **heat stress** has a major effect on various biological and reproductive parameters (Her et al., 1988, Kadzere et al., 2002): In dairy cows heat stress was reported to occur over an upper critical temperature (UCT) of 25 °C to 26 °C independent of milk yield or acclimatization state. This study refers to body temperature and the values show that already an outside temperature that is approximately 10 °C lower then body temperature can heavily affect cows in well-being. Another study reported that the bovine thermal comfort zone (TCZ) included ambient temperatures up to 23.8 °C and was furthermore influenced by relative humidity (Campos et al., 2004). The TNZ of the cow is officially reported to be between 5 °C to 20 °C (National Research Council, 1971). A couple of studies out of the reproductive segment further reported that no interactions at ambient temperatures above 24 °C concerning temperature negatively influenced the comfort of cattle (Randall, 1993) and that ambient temperatures between 15 to 29◦C did not have any impact on growth performance (Nardone et al., 2006). Another authors reported that ambient temperatures between 10 °C to 24 °C did not change rectal temperature but increased fat-corrected milk, so the amount of energy required producing the given amount of milk, by 0.2 °C/kg in cows producing above 24 kg/day (Berman et al., 1985).

Heat stress is a major concern in bovine breeding with a majority of research done in this area (De Rensis et al., 2003). For example, it was published that heat stress leads to a reduced expression of oestrus (Younas et al., 1993, Hansen et al., 2001). Furthermore, a study revealed that there is an impact on follicular development (Wolfenson et al., 1995, Wolfenson et al., 1997, Guzeloglu et al., 2001) and on ovarian function (Wilson et al., 1998). Heat stress seems to affect productivity of follicular selection as well as dominance and size (Badinga et al., 1993). Furthermore, heat stress seems to have adverse effects on the quality of ovarian follicles (Badinga et al., 1993, Badinga et al., 1994) probably caused either by a shrinkage (Wolfenson et al., 1995) or a small follicle (2mm to 5mm in diameter) (Trout et al., 1998). In addition, it was published that follicular growth, development and luteolytic mechanisms were compromised in heat-stressed cows; as a result, luteolysis was delayed, and second wave dominant follicles did not ovulate (Wilson et al., 1998). One of the reasons might be that follicular steroid production under heat stress is different (Wolfenson et al., 1995). Various studies indicated that there is a reduced size of the first and second wave dominant follicles (Badinga et al., 1993, Wilson et al., 1998) as well as a decrease of the dominance as reflected by an increased number of large sized follicles (Badinga et al., 1993, Badinga et al., 1994, Wolfenson et al., 1995, Roth et al., 2000). Roth in 2008 discovered that there are effects of heat stress on follicular function resulting in disruption of the follicle and its enclosed oocyte (Roth, 2008). In addition, in-vivo and in-vitro studies support the view that bovine oocytes are susceptible to thermal stress at various stages of follicular development. Not only the individual ovulated oocyte, but the ovarian pool of oocytes can be damaged during heat exposure, too (Rutledge et al., 1999, Zeron et al., 2001, Al-Katanani et al., 2002). These effects were confirmed by more recent studies (Torres-Júnior et al., 2008, Souza-Cácares et al., 2019). Additionally, there is a delayed regression of subordinate follicles (Wilson et al., 1998). Such alterations may lead to the early rise of the pre-ovulatory follicles and an increased period of dominance (Wolfenson et al., 1995) both of which appear to negatively impact conception rates (Mihm et al., 1994). The effects of heat stress on follicular recruitment at this point was not studied intensively, so that there is no clear answer at this time (Wolfenson et al., 2000). However, warm months were more closely associated with lower conception rates than were cool months (33.7% compared to 40.1%) (Gwazdauskas et al., 1975, Cavestany et al., 1985). This effect had previously (Ingraham et al., 1974) and recently (Sönmez et al., 2005, Nabenishi et al., 2011) been confirmed by other authors. Along with this one study found almost the double number of incidences of retained placenta and postpartum metritis during warm months than during cold months (24.1% during the period of May through September compared to 12.2% the rest of the year) (DuBois et al., 1980). Another study presented that the pregnancy rate was significantly higher ($p<0.05$) in cooled as compared to control cows (Wise et al., 1988). The negative effect of AI with higher temperatures outside the TCZ of the cow was confirmed in other studies (Thompson et al., 1996). In addition it was revealed that conception rate was affected by heat score prior to AI (Chebel et al., 2004). Several studies with lactating dairy cows indicated late embryonic losses due to heat stress (Ealy et al., 1993, Ryan et al., 1993). Furthermore, studies showed that embryos at early developmental stages are more prone to thermal stress and become more resistant in a later developmental phase (Hansen, 2007, 2007). This might happen due to the elevation of maternal body temperature (Sakatani, 2017). Two studies indicated that high temperatures and relative humidity did not affect embryo recovery rate (Villa-Mancera et al., 2011, Pinto et al., 2017). The first study even reported that the embryo transfer success rates improved with increasing environmental temperature (odds ratio of 1.1–1.2; $p<0.003$) (Villa-Mancera et al., 2011). Other studies indicated that there is an association between mares in a hot and humid environment and changes in their ovarian follicle development and ovulation which led to a reduction in embryo recovery (Mortensen et al., 2009). In this context one study showed that cows which were exposed to heat in the summer months had reduced fertility in autumn (Hansen, 1997, Roth et al., 2001, Roth et al., 2001). Other authors confirmed the negative effect of high temperature on the embryo recovery rate and presented that in the summer a higher embryo recovery rate at 26 °C (71%) and lower one at 27 °C (51.4%) ($p<0.05$) (Oliveira et al., 2015). The same study revealed that the pregnancy result was higher at 24 °C (81.5%) and lowest (35%) at 27 °C ($p<0.05$). One early study already reported that production of embryos by superovulation and embryonic development was decreased in cows exposed to heat stress (Putney et al., 1988). Another study reported significantly lower conception rates in cows with above-normal body temperatures at the time point of insemination because of decreased fertilization and a higher incidence of embryonic deaths (Fuquay, 1981). Gwazdauskas et al. (1975) presented that an important weather parameter determining conception rate was maximum temperature on the day after insemination. Consequently, it can be summarized that various studies illustrate the influence of heat stress on oestrus, follicles, ovarian function and oocytes and can cause reductions in fertility in (lactating) dairy cows that lead to a lower pregnancy rate as well as lower amounts of milk production. However, in contradiction to this a reproductive analysis of 19266 Holstein cows from Arizona over a five year period did not find any negative effects of thermal stress on milk production and fertility (Ray et al., 1992).

Therefore, from a management perspective the target has to be to keep the cow as cool as possible (Hansen et al., 1999). As a consequence a number of studies were done assessing management practices with the target to reduce respiration rate, body temperature and improve the overall well-being of dairy cattle (Roman-Ponce et al., 1977, Blackshaw et al., 1994, Collier et al., 2006, Kendall et al., 2007).

In order to categorize heat stress in cows and take into account that temperature and humidity are very close to each other a **Temperature-Humidity Index (THI)** was defined and made a key indicator affecting performance. The THI was described for the first time in 1959 by Thom who used dry bulb temperature and wet bulb temperature in order to estimate the magnitude of heat stress in cattle (Thom, 1959). A couple of years later the THI in which horses exhibit heat stress was set to a value of 62 (Johnson et al., 1962). The THI was first used in dairy cows in the 1960s to prove the assumption that a decline in milk production could have been caused by heat/humidity. Until today various definitions of THI were set up and defined. Another study looked at eight different THI in dairy cattle with the objective to find out, if such a THI is a good indicator of heat stress in lactating dairy cows in a subtropical environment (Dikmen et al., 2009). They developed equations using meteorological variables that predicted rectal temperature. The outcome was that all THI models gave a good prediction. In most studies, the THI is based on the formula of the NRC. This THI is using means of hourly measurements of air temperature (T) and relative humidity (RH) resulting in the following formula: $THI=(1.8*T°C+32)-(0.55-0.0055\times RH\%)*(1.8*T°C-26)$ (National Research Council, 1971). The THI can be seen as an important key performance indicator and various studies in cattle were done on reproduction and lactation. For example one study tried to evaluate the relationship between the THI and the incidence of medical treatments in lactating dairy cows with a focus on fertility (looking at ovarian cysts, abnormal oestrus, endometritis, retained placenta) in the first 21 days of the breeding cycle. However, in that study, no significant effects of the THI classes and seasons on the incidence of fertility treatments were found (Sanker et al., 2013). When looking closely at the data a tendency can be found that the incidence of medical treatments was higher in THI classes $THI<50$ than in classes above this value. One study looked at the relationship between higher THIs and the expression of oestrus and showed that a THI greater 78 decreased the expression of oestrus (Sönmez et al., 2005). This effect was previously reported in other studies (Rodtian et al., 1996, Landaeta-Hernández et al., 2002). However the THI maximum threshold was not defined in detail as it was only stated that the THI was higher than 25. Other studies reported a reduced fertility and pregnancy rate with the THI being greater than $THI>72$ (Morton et al., 2007) and $THI>72.9$ (Amundson et al., 2006). Another study revealed that if the

THI was greater than 80 the first one or three days after artificial insemination the conception rate decreased from 30.6% to 23% respectively (García-Ispierto et al., 2007). However, they reported that the negative effects of heat stress on conception rate days however was already reported back in 1974 (Ingraham et al., 1974). One study looked at the effect of heat stress on ovarian follicle development, and poorer oocyte quality post-partum was obvious from a THI of 75. Other authors reported even lower thresholds (THI>73) (Schüller et al., 2014). A greater effect of THI measurements taken on the day of artificial insemination compared to THI measurements taken further days from day of insemination was confirmed in another study (Brügemann et al., 2013). In this study the THI threshold was greater than THI>60. The effect of a lower conception rate due to higher THIs on previous and concluded that a THI higher than ˜67.9 negatively influenced both parameters (Alves et al., 2014) .

In lactating cows it was recognised that milk production is reduced (Bouraoui et al., 2002) in particular when THI levels exceed 72 (in this study the THI of 72 equates e.g. to 25 °C and 50% relative humidity) (Ravagnolo et al., 2002). One study re-evaluated physiological and production parameters and specified a new THI threshold of 68 for lactating dairy cows producing more than 35 kg/day (Zimbelman et al., 2009) which was a new and contradictory result to earlier studies which stated that heat stress occurred independently from production level of milk amount . Earlier studies defined the threshold of a THI greater than 75 (McDowell et al., 1976) and a minimum of 64 (Igono et al., 1992). Similar values were published by other authors ranging from THI=35 and THI=72 where the cow is reported to be in the TCZ and THIs>78/80 affecting the biological functions of the animals (Du Preez et al., 1990, Brown-Brandl et al., 2003, Bohmanova et al., 2007). A recent study reassessed the THI in a sub-tropical region of India and stated that main physiological responses to heat stress started at THI 74 (Jeelani et al., 2019). Furthermore, the study reported that a THI of 74 to 79 led to moderate stress and THI greater than 80 induced severe heat stress. Cycling back to the concept of the TCZ one study in particular outlined a THI lower than 71 in which the cow is in the TCZ (Armstrong, 1994).

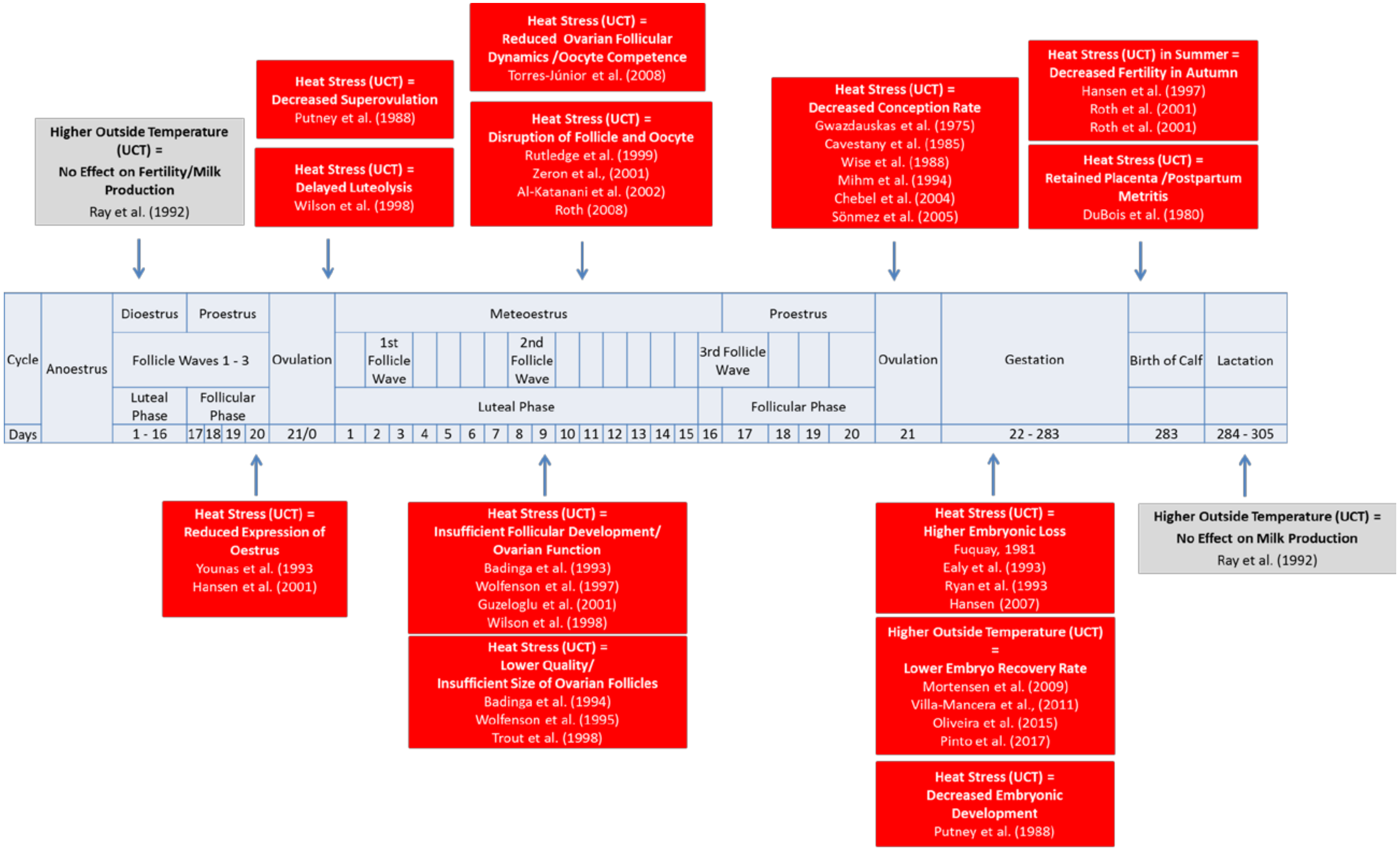
It was published that there are **genetic differences** in tolerance to heat stress (Pegorer et al., 2007) and it was presented that different genes can be turned on and off in cattle. This means that cows are able to adjust to their environment (Kondrashov, 2012) as other species (Barrett et al., 2008). Brügemann et al. (2011) investigated this topic and looked at the genetic analysis of protein yield in dairy cows. A couple of years later at the genetic background and gene expression based on the THI defined by the NRC in 1971 also known as "The Oklahoma Mesonet Cattle Heat Stress Index" looking at cattle that is mainly housed outside (Brügemann et al., 2011, Brügemann et al., 2013). In the first study they matched the collected data of nearly 155.000 Holstein cows with data from the public weather station and defined four classes of THI. They then looked at genetic correlation of test-day protein yield between the whole ranges of values for THI illustrating that additive genetic variances for daily protein yield did reduce with heat stress and were lowest at the beginning of lactation as well as at a threshold THI of 60. However, they concluded that probably no significant genotype × environment interaction for protein yield exists. In the second study they compared conception rate and somatic cell score from 5611 Holstein cows living in different housing. Even though the results did not confirm high correlations between the two parameters the authors concluded that higher THIs might have a negative effect on conception rate especially with cows located on pasture. Other research group found an interaction not only between genetics, milk composition and amount (Bernabucci et al., 2014) but similarly between genetic components and THI for example in a study done in Luxembourg and Tunisia (Hammami et al., 2008) as well as Georgia (USA) (Aguilar et al., 2009). Recently it was published that the conception rate and pregnancy rate in dairy cattle decreased with a THI above 72 (Dash et al., 2016).

The criticism regarding the THI is, however, that it does not take into account other weather parameters such as solar radiation and wind speed. **Solar radiation** seems to influence the overall well-being of cows (Da Silva et al., 2010). In the reproduction field it was revealed that follicular development and the growth of medium sized follicles both were reduced in cows exposed to direct solar radiation in the previous oestrous cycle (Roth et al., 2000). Furthermore, high radiation seems to drive cows behaviour to actively seek for shade when average ambient solar radiation is increased (Tucker et al., 2008) with the goal to decrease the radiant heat load (by 30% or more) (Blackshaw et al., 1994). In order to add in solar radiation and wind speed to the THI a **heat loss load index (HLI)** was developed which can be used as an alternative to a the THI (Gaughan et al., 2002, Gaughan et al., 2004). One study taking this HLI into account bared that shade effectively reduced rectal temperature of beef cattle when the HLI exceeded 78 (Gaughan et al., 2004). In addition to the HLI, the accumulated heat load (AHL) model was developed a couple of years later by the same group of researchers (Gaughan et al., 2008) displaying that threshold HLIs are different in different genotypes of cows. The outcome was a paper that defined seventeen genotypes (Gaughan et al., 2010). However, the HLI could not be established as a key performance indicator such as the THI.

Temperature, humidity and solar radiation are in close relationship to the **daily hours of sunshine** (Wilks et al., 1999). Light duration and intensity are critical for the level of melatonin secretion and therefore for its influence on the reproductive axis. This is because in mammalian species melatonin secreted by the pineal gland is the neuromodulator which then mediates the influence of light on the hypothalamic-pituitary-ovarian axis. And, the effect of daylight influences young cattle early: One study indicated that increased daylight enhanced growth until the onset of sexual maturity (Hauser et al., 1983). And although cows are not seasonal breeders, some results demonstrate that season of birth and season of attainment of puberty do influence the age of puberty in heifers. So for this reason season may have influenced age of puberty by affecting serum concentrations of luteinizing hormone and serum prolactin or growth rate (Schillo et al., 1983). One study conducted in Israel looked at the effect of month (including day length and the outcome of milk production) and demonstrated a negative effect of temperature on milk production; in particular the mutual effect of the environmental average temperature and day length was responsible for 0.96 of the variability in average milk production during lactation and 0.93 of that in average protein production during lactation (Barash et al., 2001). It was confirmed in several studies that increased day light leads to higher amount of milk (Hjalmarsson et al., 2014, Crawford et al., 2015). This is reported to be mediated through the IGF-1 Receptor as a higher blood IGF-1 concentration in cows is reported to lead to increased milk yields (Dahl et al., 2003). In cows raised under increased photoperiod duration with 16 hours of light and eight hours darkness a 1.,8 times higher level of prolactin resulting from the thyrotropin releasing hormone was reported (Peters et al., 1981). Some studies disclosed that keeping the cow with longer periods of light during the dry period could increase the effectiveness of dairy cattle farming during the next lactation and lead to an increased milk production (Miller et al., 2000, Auchtung et al., 2005, Velasco et al., 2008). Furthermore, it was presented that different parts of the light spectrum revealed various effects on the neuromuscular parts with maximum effects being achieved with red light and minimum effects with blue or violet light (Yurkov, 1980, Penev et al., 2014).

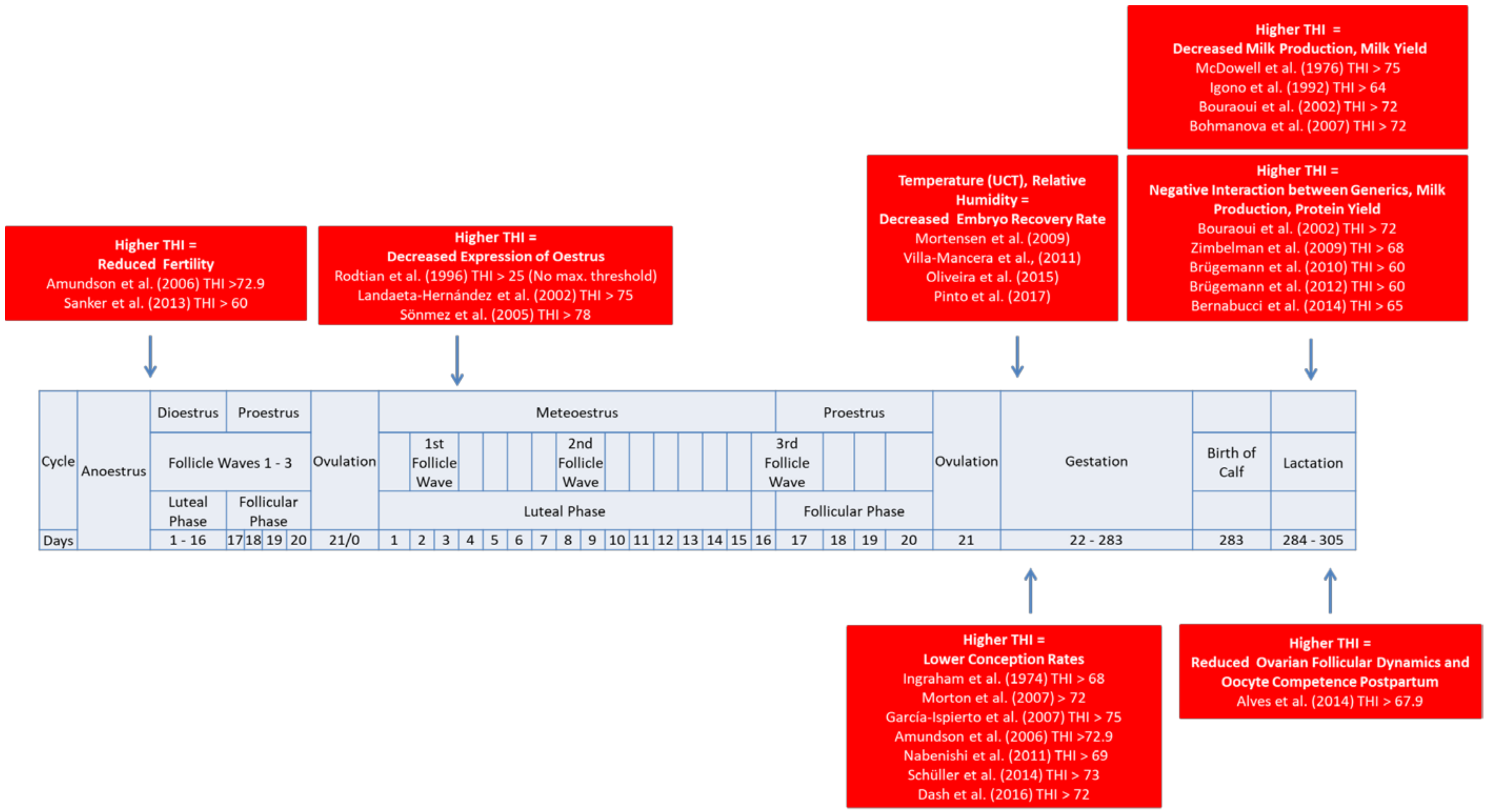
In **summary** various research was done focussing on weather parameters such as high temperatures, humidity, solar radiation, sunshine or a combination of weather parameters such as the THI or HLI. All these weather parameters are contributing to heat stress in cows and negatively impacting their comfort level. These weather parameters have negative effects on reproductive parameters such as oestrus, follicles and follicular development, ovarian function and oocytes leading to reduced fertility, a lower pregnancy rate and decreased milk production in dairy cows. Therefore, in cattle the selection for heat-tolerant animals is one possible option to mitigate the risks of heat stress (Nguyen et al., 2017). Physiological or genetic manipulation of the cow to improve embryonic resistance to elevated temperature is a promising approach for enhancing fertility of lactating dairy cows (Hansen, 2007). The overall management objective besides these genetic and reproductive options is to keep the cow in their TCZ by looking at weather parameters and key performance indicators such as the THI in order to adjust management techniques such as housing.

The graphical representations 4-1 to 4-3 display the reproductive cycle of the cow and for which aspects studies showed which reproductive parameters are influenced by which weather parameter.



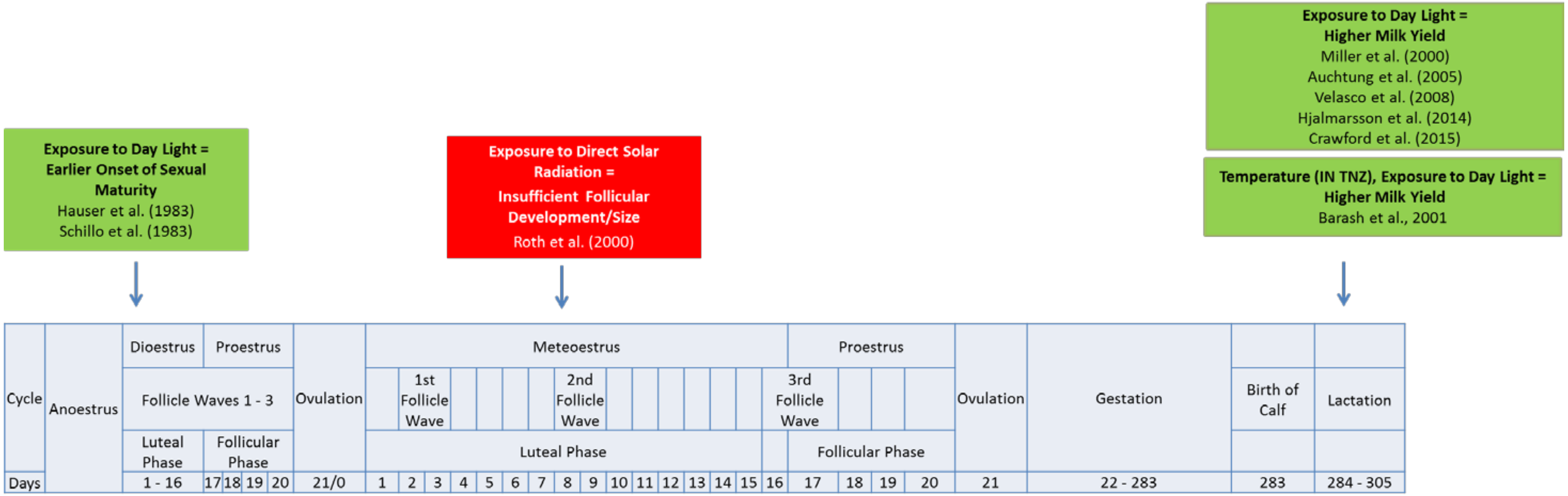
Research conducted on the effect of temperature (in degrees Celsius) with UCT=> 23.8 °C following the reproductive cycle of the cow showing negative (= red box) or no (= grey box) effect on the pregnancy result

Figure 4-1: Bovine research conducted on the effect of temperature



Research conducted on the effect of temperature and humidity (shown as THI = Temperature-Humidity Index) following the reproductive cycle of the cow showing a negative (= red box) effect on the pregnancy result

Figure 4-2: Bovine research conducted on the effect of temperature and humidity (shown as THI)



Research conducted on the effect of sunshine, day light and solar radiation following the reproductive cycle of the cow showing a positive (= green box) or negative (= red box) effect on the pregnancy result

Figure 4-3: Bovine studies conducted on the effect of sunshine, day light and solar radiation

The figures can be summarized as follows:

- Figure 4-1 showing research conducted on the effect of temperature demonstrates that heat stress (UCT) has a negative effect on the pregnancy result after the luteal phase in all biological phases, including the reduced expression of oestrus, the development of sufficient follicles as well as the disruption of ovarian function and oocytes. This results in decreased conception rate, negatively influencing the embryo implantation. Furthermore, heat stress (UCT) does not seem to affect fertility in dioestrus and milk production in lactation phase.
- Figure 4-2 presenting research conducted on the effect of temperature and humidity (shown as THI = Temperature-Humidity Index) shows that a high THI (>60) leads to reduced fertility and decreases the expression of oestrus after ovulation (THI>75). Furthermore, a higher THI decreases the embryo recovery rate and leads to lower conception rates (THI>68). In addition, the figure shows that a THI greater than 60 negatively influences milk production as well as reduced follicular and oocyte activity after giving birth.
- Figure 4-3 showing research conducted on the effect of sunshine, daylight and solar radiation shows that exposure to daylight sunshine in general has a positive impact on the onset of sexual activity and on milk yield in lactation. Direct solar radiation however has a negative influence on the development of sufficient follicles.

4.2 Buffalos

A couple of studies regarding weather parameters and reproduction were done in the domestic buffalo (Bubalus bubalis) regarding weather parameters and reproduction. Although the domestic buffalo is known to be a short day breeder it was presented that in tropical areas near the equator buffalos show a poly-oestrus (Vale, 2007). In buffalos that live in high temperature regions it was further published that approximately 50% of animals are not severely affected by heat stress in pregnancy, but buffalos that are not pregnant in the summer season go into silent heat and/or anoestrus (Singh, 2000). In the domestic buffalo it was further described that the calving season is regulated by the accessibility of pasture (Vale, 2007) which further indicates that buffalo reproduction is influenced by season. Another study indicated that there seems to be a negative correlation between reproductive performance and high THI (THI>75) in the domestic buffalo (Vale, 2007). Recently it was again revealed that a significant decline in reproductive performances of buffaloes was observed above threshold THI 75 (Dash et al., 2016).

4.3 Small Ruminants

4.3.1 Sheep

The attitude of sheep has always had a shaping and nurturing influence on the landscape. Here, the sheep grazing can contribute to maintaining the dry grassland (Benthien et al., 2018). However, landscape management has become an independent branch of the industry and is the main economic driver in the sheep business. Other economic drivers are the production of meat production and milk (Kosgey et al., 2003, Kosgey et al., 2004).

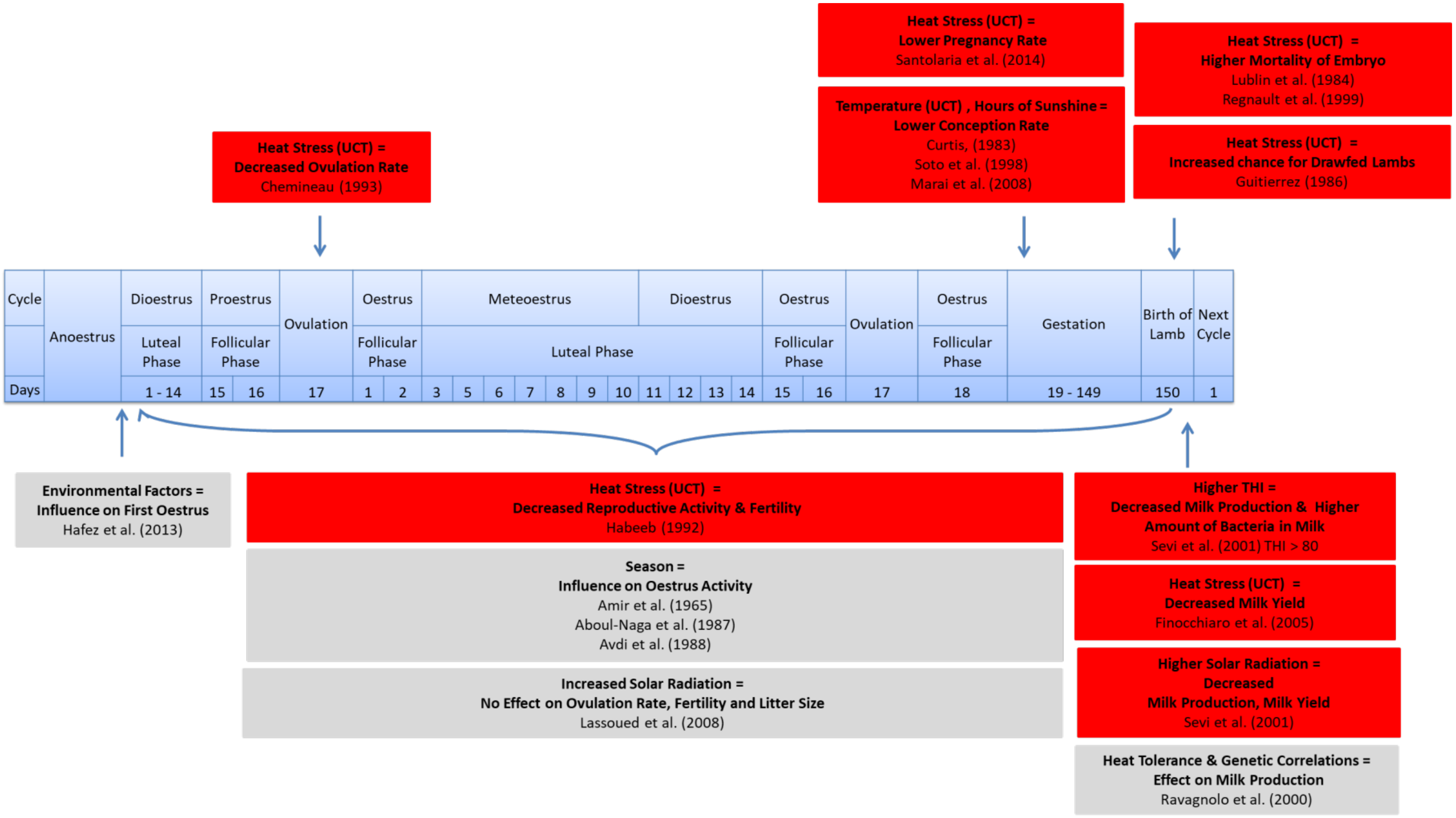
Sheep in general seem to cope better with heat stress than cattle when fed the right way (Silanikove, 2000). Heat stress (UCT) in sheep is defined as temperatures greater than 35/36 °C and around 50% humidity (Johnson, 1987, Shafie et al., 1994) The thermoneutral zones were described as being between 18 to 20 °C with 30% humidity (Marai et al., 2007). It was demonstrated that **high temperature** and **high humidity** have a high negative influence in general on biological functions sheep. The functions are reported to be feed intake, live body weight and reproductive performance (Rich et al., 1970, Marai et al., 2007, Marai et al., 2008). Another author presented that sheep experience disturbances in body metabolism (blood, water, protein, energy, minerals, enzymes hormones) and in reproductive activity and fertility due to heat stress (Habeeb, 1992). Sheep therefore do respond physically and productivity wise to heat; however the response is different if these sheep are pregnant or lactating (Abdalla et al., 1993). The first oestrus is influenced by genetic factors such as breed, time of birth and nutrition as well as environmental factors (Hafez et al., 2013). It was confirmed that oestrus, ovulation and fertilization as well as conception rate and embryonic mortality are affected by temperature and length of day by various studies. For example a decrease in oestrus activity was observed during spring in various breeds of sheep in different geographic locations ranging from the Awassi sheep in Israel (Amir et al., 1965) to the Chios sheep in Greece (Avdi et al., 1988). The best environments for reproductive activities in sheep are reported to be a medium temperature as well as a medium day length; both are present in the autumn months. Ovulation rate in ewes is at a maximum (without breeding of ewes) in the autumn and at a minimum in spring (Aboul-Naga et al., 1987) with the highest effect of heat stress on fertility in the first three days prior and post ovulation (Chemineau, 1993). Conception rate seems to be lowest in summer and negatively correlated with high temperatures and long hours of sunshine (Marai et al., 2008). The greatest effect of heat stress on conception rate was described to be at around mating (Curtis, 1983) as well as during the first seventeen days of pregnancy (Soto et al., 1998). Furthermore, it was shown that heat stress leads to a higher mortality of the embryos, probably caused by decreased supply of blood to the ovaries (Lublin et al., 1984) as well as low circulating placental hormone concentrations (Regnault et al., 1999). One study showed that in the first month of the sheep breeding season the maximum temperature before artificial insemination and pregnancy rate were negatively correlated (Santolaria et al., 2014). However, the embryo loss rate of 12% for mono-ovular sheep is much lower compared to cattle (Diskin et al., 2008). The exposure of ewes to high temperatures during early pregnancy may be accompanied by the production of dwarfed lambs, which are usually or often characterized by a hairy appearance. This effect was identified as a huge economic problem as these lambs are very small and do not fulfil the expected amounts of wool and/or milk yield (Guitierrez, 1986). Milk yield was found to be negatively correlated with heat tolerance, when defining the influence of milk production of the Mediterranean dairy sheep and heat stress (Finocchiaro et al., 2005). The researchers looked at genetic correlations between the general additive effect of milk production and the additive effect of heat tolerance copying the study on the genetic component of heat stress in dairy cattle that had the target to develop a heat index function (Ravagnolo et al., 2000). The main focus was on defining **THI** thresholds for sheep based on weather data. They found that the genetic correlations between the general additive effect of milk production and the additive effect of heat tolerance were negative for both daily milk and fat-plus-protein yields on all days. Based on this they defined a THI threshold of 23 which will result in heat stress (Finocchiaro et al., 2005). It was also shown that milk production decreases in lactating ewes when they are exposed to a THI greater than 80% (Sevi et al., 2001). And even though the THI is not defined as a key figure in sheep the way it is in cattle (because still not commonly used) it can be said that the THI is often used as an indicator to estimate the severity of heat stress (Srikandakumar et al., 2003, Fisher et al., 2005, Marai et al., 2007, Bernabucci et al., 2009, McManus et al., 2009).

A couple of studies have looked at a possible effect of **solar radiation** on sheep. One study examined a possible effect of solar radiation on milk production; however no effects on milk yield and milk composition were found (Sevi et al., 2001). Another study looked at weight gain in female lambs that were exposed to solar radiation and showed that there was a negative correlation (Nardone et al., 1991). Conversely, a study including many more animals did not reveal any impact of solar radiation on litter size. This study looked at 112 ewes and ten rams keeping half of the animals indoors and the other group outdoors. They did show an increase in respiratory and heart rates (respectively 52.7+/-8.5 vs. 100+30 and 83.3+/-5.5 vs. 92.7+/-2.5

for indoors and outdoors), but did not show any effects of solar radiation on any other reproductive parameters such as percentage of females in oestrus or the return rate (95% vs. 100% and 21% vs. 22% respectively for indoors and outdoors groups) (Lassoued et al., 2008).

As in other animals, in sheep the seasonal reproductive cycle is primary underlying a circannual endogenous rhythm which is strongly influenced by external environmental factors such as **photoperiod** (Woodfill et al., 1989). Therefore, in sheep **light programs** are part of the breeding program routine in order to increase reproductive performance as in other animals (Dankó, 2003). Woodfill et al. (1989) showed that ovariectomized, oestrogen-implanted ewes maintained in continuous lighting conditions for five years do continue to have circannual changes in luteinizing hormone secretion. However, at the same time it was shown that a development of photo-refractoriness does happen and therefore light programs should be changed at regular intervals; for example from 16 to 12 hours of light then from 12 to eight hours of light (Malpaux et al., 1988).

In sheep, clear recommendations on management practice to keep the sheep cool and to retain them in their thermal comfort zone were formulated (Sölkner et al., 1998, Kahi et al., 2004, Gizaw et al., 2009). These recommendations are physical, physiological and nutritional. Clear guidance on how to manage the mating of sheep in order to produce the best possible offspring in certain weather conditions exists. The THI can be seen as an indicator to aid in recognizing and classifying sheep in heat stress. Most of the cited studies and the knowledge of the conducted research conclude in a recent paper summarizing that weather conditions do influence pregnancy rates in sheep, suggesting that the usage of meteorological data can be very helpful to schedule the date and time of artificial insemination (Palacios et al., 2015).



Research conducted on the effect of temperature (in degrees Celsius) with UCT= >36 °C, the effect of temperature and humidity (shown as THI = Temperature-Humidity Index), the effect of sunshine and the effect of solar radiation following the reproductive cycle of the ewe showing a negative (= red box) or no (= grey box) effect on the pregnancy result

Figure 4-4: Sheep research conducted on the effect of weather parameters

Figure 4-4 showing research conducted on the effect of temperature and humidity (shown as THI = Temperature-Humidity Index) the effect of sunshine and the effect of solar radiation. The data demonstrates that heat stress (UTC) has a negative impact on fertility and pregnancy result at all biological phases, starting with a decreased ovulation rate leading to a reduced pregnancy rate and a direct effect on the offspring (e.g. increased chance for dwarfed lambs) and lower milk yield. Environmental factors including season appear to affect oestrus activity but with no effect on the pregnancy result.

4.3.2 Goats

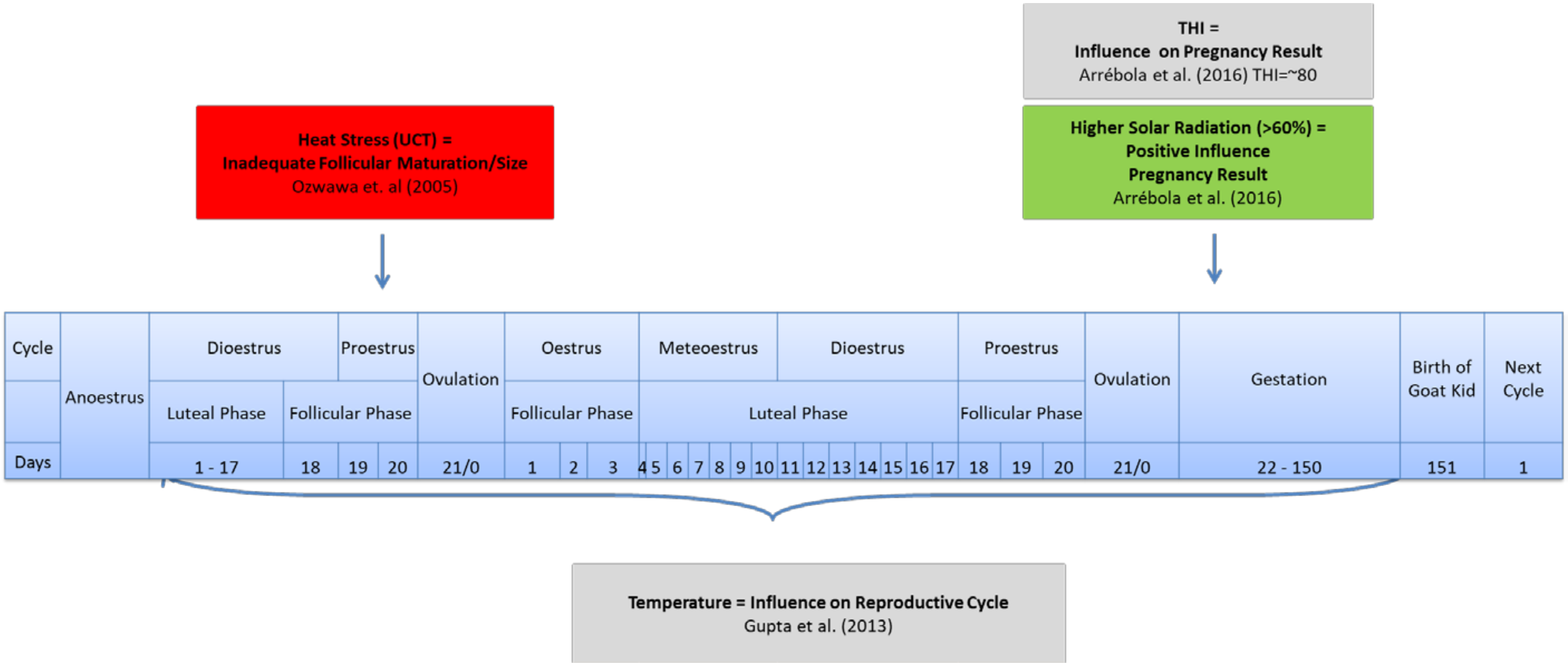
Goats are reported to be the fourth largest livestock group with 677 million goats in the world in 1999 (Morand-Fehr et al., 1999) and 90% of those goats being located in developed markets (Glimp, 1995).

Research was done looking at the effect of **temperature** as goats are often located in Mediterranean areas (Boyazoglu et al., 2001) and high temperatures affect goats and their reproductive cycle (Gupta et al., 2013). Upper critical temperatures for goats in maintenance were described to be 25 °C to 30 °C (Lu, 1989), however, pregnant goats can be affected by heat stress at lower temperatures (<20 °C) (Olsson et al., 1995). The recognized thermoneutral zone (TNZ) for goats ranges from 12 °C to 24 °C (Nikitchenko et al., 1988). Nevertheless, goats seem to be able to better adapt to heat than other animals such as cattle and sheep (Devendra, 1989). Goats can probably cope so well with thermal stress due to a higher sweating rate via a water saving ability (Shkolnik et al., 1972) as well as a lower basal metabolic rate and respiratory rate, higher skin temperature and a constant heart rate with cardiac output (Shkolnik et al., 1981). In addition, low metabolic requirements combined with the ability to reduce metabolism and the efficiency of the utilization of a high forage diet (Silanikove, 2000) further support the ability to better deal with high temperatures. One study looked at the effect of high temperatures and relative humidity on follicular maturation and exposed goats to heat of 25 °C and 50% relative humidity with a 12 hour 'light' and 12 hour 'dark' period for two days and then looked at the growth of their follicles. They found out that follicles exposed to heat stress during follicular development do not develop to an adequate size to ovulate (Ozawa et al., 2005). But the number of follicles that were observed during the experiment was not different compared to the control group.

One ten year study (2005–2014) of Florida goats based in Spain calculated a **Temperature-Humidity Index (THI)** based on the following formula: $THI = T + 0.36 \times RH + 41.2$, where T = air temperature (°C) and RH = relative humidity (Arrébola et al., 2016). The authors performed a logistic regression analysis with the positive pregnancy result after artificial insemination as a binary dependent variable including 48088 artificial inseminations. They indicated that general management and food were important factors affecting fertility especially as they revealed that month and year were correlated to weather and pregnancy rates. Furthermore, they reported that the odds ratio indicated that mean temperature was beneficial, and minimum temperature was disadvantageous to achieving a positive pregnancy rate. They did further report the THI (which was not significant) and other parameters, but did not report any effects on pregnancy rates towards these detailed weather parameters. A previous study by the same authors demonstrated that higher temperatures, solar radiation but lower relative humidity and rainfall had a negative effect on pregnancy rates (Arrébola et al., 2016). However, in conclusion weather was mostly related to fertility in goats after artificial insemination in spring which is in line with the natural reproductive cycle of goats (Guillen-Muñoz et al., 2018).

A couple of studies are available on the effect of solar radiation on small ruminants. One study exposing goats to direct **solar radiation** showed an increase in respiratory rate (133%) and heart rate (12%) resulting in higher rectal temperatures (1.5%) compared to goats kept in shaded areas (Al-Tamimi, 2007). These goats required up to two weeks to adapt to thermal stress through physiological modifications such as respiratory rate.

In goats the manipulation of the reproductive cycle via a **light program** is interesting for management reasons such as the synchronisation of female goats and offspring (Oliveira et al., 2001) or for genetic reasons such as the distribution of better-quality genotypes (Notter, 2012). However, as mentioned before, goats are often located in subtropical regions which is why the treatment of the female goat with a light program in order to increase fertility in terms of achieving pregnancy is not necessary in most cases (Delgadillo et al., 2004). Here light programs are used to increase the rate of kiddings as shown by Chemineau et al. (1996) so that 75% of pregnancies have two goat kids (Chemineau et al., 1996) or to improve milk yield (Garcia-Hernandez et al., 2007). Light programs in goats are often combined with the subcutaneous application of melatonin implants to mimic the real breeding season (Malpaux et al., 1995).



Research conducted on the effect of temperature (in degrees Celsius) with UCT= >25 °C, the effect of temperature and humidity (shown as THI = Temperature-Humidity Index) and the effect of solar radiation following the reproductive cycle of the goat showing a positive (= green box), negative (= red box) or no (= grey box) effect on the pregnancy result

Figure 4-5: Goat research conducted on the effect of weather parameters

Figure 4-5 showing research conducted on the effect of temperature and humidity (shown as THI), the effect of sunshine and the effect of solar radiation. The data demonstrates that temperature does have an effect on reproductive activity at all biological phases but this does not influence the pregnancy result, particularly if the THI is greater than 80. Conversely, it was shown that heat stress in follicular maturation negatively influences the follicles and the pregnancy result. A higher solar radiation (>60%) positively influences the pregnancy result.

According to the studies reviewed in this dissertation it can be said that in small ruminants such as sheep and goats weather parameters seem to effect reproductive performance and that the manipulation of reproduction in female goats is commonly done to increase fertility of the animals. Research was conducted on the effect of weather parameters and data from weather stations is used for goats in order to make use of the optimal reproductive environment.

4.3.3 Pig/Swine

Reproductive problems in the domestic pig are recognized as a potential economic loss to the pig industry. Most of this loss seems to be related to heat stress as estimated by St-Pierre et al. (2003) by specifying the monetary value to be around 299 Million USD per year. In terms of reproductive success the main target in swine reproduction is to influence the size of piglets when born (Renaudeau et al., 2003) as well as achieving a high growth rate of the piglets in the first weeks after birth (time period was defined as puberty) (Flowers et al., 1989). Another important target reported is to get the sows pregnant as fast as possible again after weaning (Gourdine et al., 2016).

When analysing the effect of weather on swine it should be kept in mind that in many countries swine are in general kept inside stables around the year and therefore the effect of weather is limited in those pigs. For reasons of cost reduction and work efficiency, new sow barns are generally planned today for at least 300 to 400 animals and fattening houses for at least 2,000 to 3,000 animals. In a lot of countries, such as the European Union, regulations and laws regulate how stables for pigs must be designed. In Germany, these regulations are implemented in the “Tierschutz-Nutztierhaltungsverordnung” with the relevant section on keeping pigs (BMEL, 2016). It includes how much light/daylight must be present in the stables. Therefore, when reviewing the published data and outlining possible effects of weather parameters on reproduction it should be investigated how the pigs are housed and which effect weather parameters could actually have.

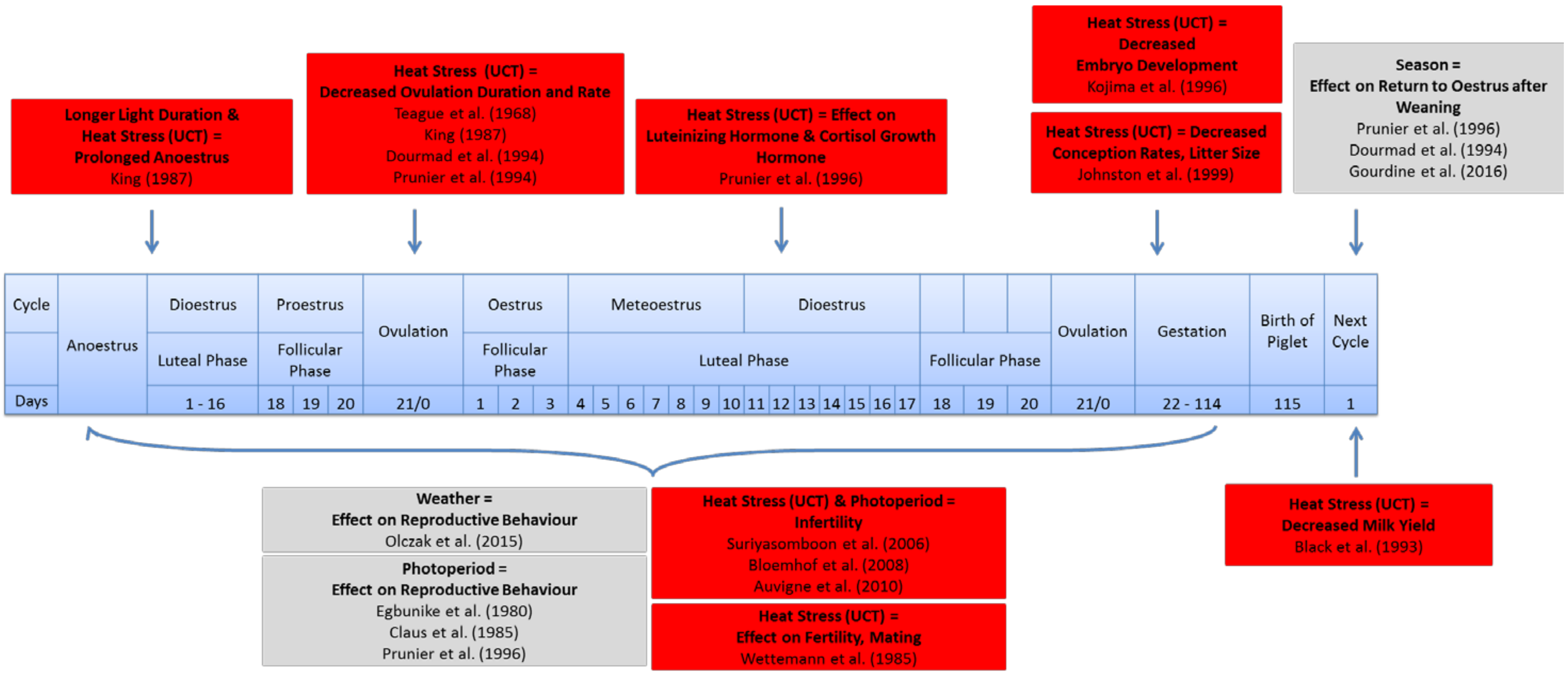
Weather is recognized to influence the overall behaviour of pigs especially temperature (Hahn et al., 1987, Geers et al., 1989) as pigs cannot sweat. It is described that thermoneutral zone (TNZ) of pigs is around 15 °C to 21 °C (Coffey et al., 1995) and that heat stress in pigs occurs with ambient temperatures above ~27 °C (UCT), both depending on age, weight, housing and feeding (Fraser, 1985, Brown-Brandl et al., 2004, Huynh et al., 2004). Therefore, higher temperatures affect their reproductive behaviour of pigs (Olczak et al., 2015). Particularly, the combination of **temperature** and **light/photoperiod** (Egbunike et al., 1980, Claus et al., 1985, Prunier et al., 1996) was reported to be influential regarding reproduction of pigs. A five year study (2003 to 2007) in France including 266 indoor farms, 22.773 batches and 610.117 sows had the objective of investigating the relative roles of high temperature and photoperiod as environmental factors of seasonal infertility in swine (Auvigne et al., 2010). In this study the seasonal infertility was defined as the relative difference between the fertility rate in summer and winter of the same year. The outcome of the study was that regarding the seasonal infertility in particular the year was statistically significant ($p<0.001$) and temperature was significantly higher in the year 2003 than in the other years; this however did not show any statistical differences. Even the authors could not show a direct statistical effect between seasonal infertility and temperature, they assumed that heat stress does influence the seasonal fertility in a negative way as shown in other larger studies (Suriyasomboon et al., 2006, Bloemhof et al., 2008). The other effect that was taken into consideration in this large study by Auvigne et al. (2010) was photoperiod. However, at the end of the study the authors concluded that no effect of photoperiod on fertility could be determined as all animals were under the same photoperiod due to the design of the study (Auvigne et al., 2010).

It is known that pigs are seasonal breeders (Hurtgen et al., 1980) and that the duration and intensity of light controls reproduction through the diurnal variations in melatonin secretion by the pineal gland and subsequent adaptations in gonadotrophin secretion (Erlich et al., 1985). However, light duration does not seem to influence gonadotrophin secretion in sows (Kermabon et al., 1995). Under heat stress the flow secretion of the luteinizing hormone and circulating concentrations of cortisol are decreased whereas those of growth hormone are increased (Prunier et al., 1996). Just recently a study analysed cortisol levels of sows in two consecutive reproductive cycles via hair cortisol and stated that physiological phases did have a significant effect on the cortisol concentration ($p<0.00001$) showing an increased cortisol concentration during late pregnancy and lactation (Bacci et al., 2014). Furthermore, the authors showed that the season of the year had a significant effect ($p<0.005$), with the lowest cortisol concentration documented during the hot season. This explains why long light duration and more obviously high ambient temperature play a role in a prolonged anoestrus (King, 1987) and that ovulation rate can be lower in summer and the duration of oestrus longer in summer than in late autumn and winter. In consequence the interval from weaning to oestrus is prolonged in summer (King, 1987, Prunier et al., 1994). Furthermore, one study indicated that an increase in light duration results in a decrease in loss of live weight during lactation when ambient temperatures are >25 °C but not with temperatures lower than <25 °C. The cause however might be a reduction in appetite as litter growth did not change. One study confirmed that energy intake is lower at higher temperatures (18 °C to 28 °C) which did result in greater heat production (20%), decline in milk yield (25%) and a less intake of food (40%) (Black et al., 1993). However, as reproductive activity and milk production are depending on mainly only nutrition there could be an indirect effect on reproduction and the time of the return to oestrus (Dourmad et al., 1994). One early study looked at intensive pig breeding units and monitored those over a period of 2.5 years (930 days). It was recognized that the main period of least reproductive activity were the warm summer months of July and August (Stork, 1979). As in other animals' genetic differences in tolerance to heat stress was shown (Renaudeau et al., 2007, Zumbach et al., 2008). This shows that **temperature and heat stress** in pig seem to have an effect on multiple reproductive factors regarding pre- and post-mating (Xue et al., 1994). It was shown that increased environmental temperatures decease ovulation rate (Teague et al., 1968) and that heat stress in sows is correlated with decreased dry matter intake as well as milk yield (Black et al., 1993). Furthermore, heat stress leads to a decrease in body weight in the lactating sow (Black et al., 1993). It is however not clear if high ambient temperatures correspondingly affect the return of oestrus after weaning. While one study did confirm a significant effect (Prunier et al., 1996) other authors such as Gourdine et al. (2016) found that there was no effect of the weaning-to-oestrus interval by season (Gourdine et al., 2016). One study published that heat stress has an influence on fertility of the sow up to five weeks before and after mating (Wettemann et al., 1985). They moreover discovered that reduced conception rates and reduced litter size are the result of sows under heat stress between zeros to 16 days after mating. However, other authors did not find any correlation with litter size in sows under heat stress (Johnston et al., 1999). Heat stress however does seem to have a negative effect on embryo development (Kojima et al., 1996). One study looked in particular at heat stress in young and older animals and found that younger animals are not as susceptible to high temperatures as younger animals that have not entered the breeding season (Flowers et al., 1989). One study looked at **minimum temperatures** for pigs defining the animal's lower critical temperature (LCT) that affects the pig. The LCT of the pig depends on body weight and age (Baker, 2004) and is effective when temperature falls below 12 degrees Celsius (Baker, 2004). Likewise, it can be concluded that low temperature has a negative effect on pig reproduction (Hafez, 1964).

Furthermore, **humidity** seems to play a key role in the reproduction of pigs (Teague et al., 1968, Suriyasomboon et al., 2006). Pigs are often exposed to high humidity when stabled indoors (Kunavongkrit et al., 2000). It was recognized that humidity can go up to 80% depending on the floor of the pig stable (Randall, 1983). Nevertheless, it was presented that humidity seems to have very little effect on feed efficiency, rectal temperature and skin temperature (Morrison et al., 1969, Hahn et al., 1987). An early study by the same author showed that there is a decrease in average daily gain with an increase in relative humidity from 45% to 95% (Morrison et al., 1966). The Temperature-Humidity-Index (THI) is a common tool to define heat stress in pigs (Lucas et al., 2000). Furthermore, in one study on the economic losses of livestock a Temperature-Humidity-Index (THI) for sows was used: The threshold is 74 (St-Pierre et al., 2003). Looking at the studies it becomes clear that high humidity has an impact on pigs but besides very low humidity in addition to high temperatures leads to heat stress in pigs (Egbunike et al., 1980).

Only one study looked at the effect of **radiation heat loss** defined as the loss of heat through the air between areas of different temperatures, but did not take reproduction into account unfortunately (Baker, 2004).

The graphical representation 4-6 displays the reproductive cycle of the sow and for which aspects studies showed which reproductive parameters are influenced by which weather parameter.



Research conducted on the effect of temperature (in degrees Celsius) with UCT= >27 °C as well as the effect of daylight and season following the reproductive cycle of the sow showing a negative (= red box) or no (= grey box) effect on the pregnancy result

Figure 4-6: Swine research conducted on the effect of weather parameters

Figure 4-6 showing studies/research conducted on the effect of temperature, the effect of sunshine and the effect of season demonstrates that heat stress (UTC) does have a negative effect on reproductive activity at all biological phases and therefore on the pregnancy result. Heat stress (UCT) prolongs the onset of oestrus, decreases ovulation rate, negatively influences luteinizing hormone as well as cortisol growth hormone and leads to lower conception rates and embryonic development. Furthermore, heat stress (UCT) leads to a decreased litter size and less milk yield. Weather, season and exposure to sunlight influence the reproductive activity but with no effect on the pregnancy result.

Several studies on management recommendations in terms of creating the optimal comfort zone are available and commonly used by farmers. Recommendations are available for: indoor housed pigs (Jackson et al., 2018), ventilation (Morsing et al., 1997), sprinkling (Hsia et al., 1974, Barbari et al., 2009) systems as well as nutrition (McGlone et al., 1988, Close et al., 2007). In the future, the swine industries want to obtain more data on the microclimate within the barns. However, it is probably difficult to access information in a large number of herds. Therefore another approach was proposed to collect data about management routines used by farmers to identify weather and seasonal effects and to analyse their effectiveness (Auvigne et al., 2010).

4.4 Avian Species

4.4.1 Poultry

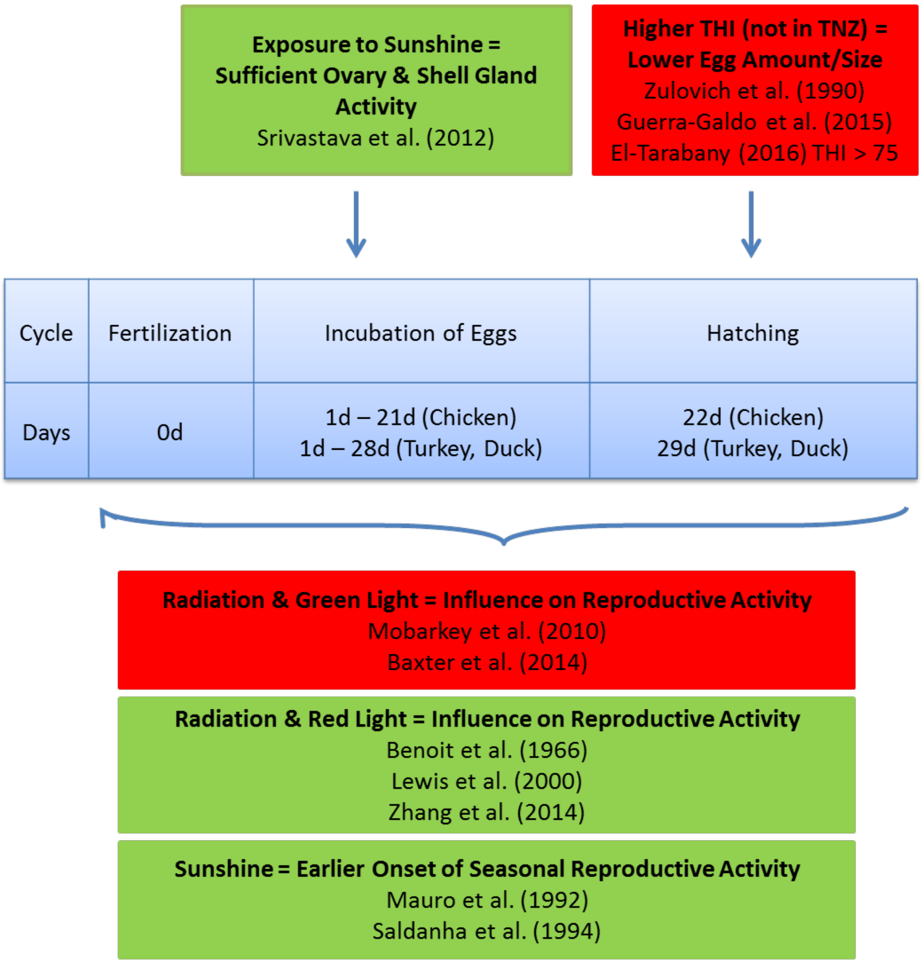
Research was conducted in poultry and in particular on temperature and humidity. It was determined that the thermoneutral zone (TNZ) in poultry is around 18 °C to 22 °C and that temperature and humidity together have a high influence on production efficiency and live performance, as reported in a range of studies summarized in the following paragraph. A number of studies in livestock animals have used a Temperature-Humidity-Index (THI) as thermal comfort index as it integrates the effects of temperature and humidity and may offer certain principles to predict the effects of thermal conditions on performance. One study on heavy broilers looking at different temperature and humidity illustrated that THI has a a high influence in broiler chickens (Purswell et al., 2012). This studies used dry-bulb temperature (15 °C, 21 °C, and 27 °C) and relative humidity (50%, 65%, and 80%), and calculated the THIs (formula: $THI = 0.85 * Tdb + 0.15 * Twb$). THIs were in the range of 14.8 to 26.9 measuring live performances parameters such as body (gain) weight, body feed intake as well as feed conversion ratio. The body temperature was taken in three birds from each treatment group. The results indicated that as the THI reaches approximately 21 °C, the performance significantly decreased and body temperature increased up to 1.7 °C above normal body temperature for broilers (41 °C). However, the variability in the individual animals was very high so that the regression analysis only displayed a quadratic relationship between THI and the measured performance. Another interesting study using a temperature humidity index ($THI=0.6tdb+ 0.4twb$) in chickens demonstrated that the number of eggs produced, as well as the egg size declined significantly with rising THIs (Zulovich et al., 1990). This was confirmed in another study where Japanese quails (Coturnix japonica) were exposed to heat stress (THI>75) and showed decreased fertility indices and egg quality traits (El-Tarabany, 2016). In addition, it was recently published that poultry typically require a high ventilation rate to maintain thermal comfort and production efficiency (Guerra-Galdo et al., 2015). This work was based on the description of a Temperature-Humidity Velocity Index (THIV) for market-size broilers (Tao et al., 2003). The THVI has the form of $THVI= (tdb + twb)*V$ (where tdb= dry-bulb air temperature and twb=wet-bulb temperature and V=Velocity). Although a THI reveals relative importance of temperature and humidity on animals it does not integrate velocity which is important to be taken into account in tunnel-ventilated housing which is especially relevant in broiler production.

However, other large birds are observed to feel better in mild to moderate thermal environments, indicating that the upper critical temperature (UCT) may be lower at larger body weights. Regarding the physiological responses of tom turkeys the level of stress at different ages was elevated (Brown-Brandl et al., 1997). Eight different THI groupings were evaluated (23 °C to 40 °C and 40% to 90% relative humidity). In very young turkeys up to 6 weeks of age, there were no effects regarding temperature or humidity on the stress level, and in the older turkeys (>ten weeks of age) temperature could not initiate any stress but humidity was more important. The authors concluded that this might be an important factor to maintain internal body temperature. Another study ten years later used the THI as well as long-term hourly summer weather data collected over 30 years from 238 locations in the USA, resulted in a Building Thermal Model for housing poultry. The model gives specific guidelines and recommendations on where to build a poultry farm and which tools, such as cooling systems, could be necessary in order to achieve the ideal housing environment (Green et al., 2007).

In addition, sunshine and length of day are reported to be important environmental factors for timing the onset of seasonal reproductive activity in avian species (Saldanha et al., 1994). One study tried to explain the effect of photoperiod on the onset of reproductive activity by looking at the vasoactive intestinal peptide in female turkeys which appeared to be a physiologically relevant prolactin releasing factor during the course of the reproductive cycle (Mauro et al., 1992). Another study investigated the effect of sunshine and length of day and looked at the role of neurohypophysial peptide arginine vasotocin within the variation in the ovary and shell gland activity of the Japanese quail (Coturnix japonica) (Srivastava et al., 2012). The results indicated that diverse peptides and hormones are involved in the reproductive cycle of avian species as in other species, which is now generally accepted. In general it can be concluded that diverse peptides and hormones are involved in the reproductive cycle of avian species as in other species.

Several studies indicated that ducks (Benoit et al., 1966), poultry (Lewis et al., 2000) and chicken (Zhang et al., 2014) respond to radiation. All studies concluded that these avian species are sensitive to red light longwave radiation (around 630/640nm). This happens through the hypothalamic extra-retinal photoreceptors, which are receptive to this range of long-wave radiation, and positively affects reproductive performance such as an increased number of eggs produced. The green light (radiation) activates the retinal photoreceptors in a way, which decreases reproductive performance (Mobarkey et al., 2010, Baxter et al., 2014).

The graphical representation 4-7 displays the reproductive cycle of poultry and for which aspects studies showed which reproductive parameters are influenced by which weather parameter.



Research conducted on the effect of temperature (in degrees Celsius) with the TNZ from 18 °C to 22 °C, the effect of temperature and humidity (shown as THI = Temperature-Humidity Index) and the effect of radiation and light/sunshine following the reproductive cycle of poultry showing a positive (= green box) or negative (= red box) effect on the pregnancy result

Figure 4-7: Poultry research conducted on the effect of weather parameters

Figure 4-7 showing research conducted on the effect of temperature, temperature and humidity (shown as THI = Temperature-Humidity Index) and the effect of radiation and light/sunshine displays that radiation and green light have a negative influence on the pregnancy result at every biological phase. Radiation and red light however do have a positive influence on the pregnancy result at every biological phase as well as sunshine. Sunshine also stimulates ovary and shell gland activity after fertilization. The figure furthermore shows that a higher THI (>75) leads to a reduced amount of eggs and egg size.

4.4.2 Wildlife Birds

Because of their routine 24-hour behaviours, (same pattern of behaviour every day regarding eating, sleeping, flying) and small body size, wildlife birds are reported to be severely affected by environmental factors such as air temperature, solar radiation and wind speed, (McKechnie et al., 2010) including corresponding impact on endocrine responses (Wingfield, 1984). Conversely, it is reported that Wrynecks Jynx torquilla, which are small birds (35g) and belong to genus woodpecker, are probably not strongly influenced by temperature and rainfall (Geiser et al., 2008). However, environmental factors together define a particular microclimate which does have a certain influence on water and energy budgets of small birds (Wolf et al., 1996). These microclimates have an effect on reproduction in terms of rate of egg laying, egg weight, clutch sizes shell quality but moreover patterns of nestling growth and parental patterns (Hafez, 1964, Wingfield, 1984). In a variety of birds it was confirmed that weather conditions (Dawson et al., 2005) and in particular radiation influences the way the nests are built, ensuring that eggs have the best conditions to be brood (Walsberg, 1981). One study in the United Kingdom showed that insectivorous birds rely on a short period of insect richness to feed their offspring and therefore these birds have to time their reproductive activities accordingly (Charmantier et al., 2008). This adaption of behaviour to weather conditions was previously revealed in other studies. (Krijgsveld et al., 2003, Dawson et al., 2005). Wild bird species such as the Black Headed Bunting respond to radiation (Malik et al., 2004). Many more studies exist on wild bird species, but are not reviewed in this dissertation.

4.5 Other Animals

Limited research was conducted in **mice**, and it was presented that heat stress does impact some reproductive parameters. Aroyo et. al demonstrated in one study that not only the individual ovulated oocyte, but moreover the ovarian pool of oocytes can be damaged during heat exposure (Aroyo et al., 2007) and that embryonic losses due to heat stress can occur (Ozawa et al., 2002). Similar effects of heat stress on follicular development in Pregnant Mare Serum Gonadotropin treated immature rats confirmed that the number of ovulated oocytes was significantly lower in animals exposed to heat than in the control group (Shimizu et al., 2000). However, Aroyo et al. (2007) revealed in a second study that offspring developed from heat-stressed mice did not differ from control groups that were not exposed to heat in their learning potential or episodic memory (Aroyo et al., 2007). Even though early reproductive stages in mice could be influenced by heat. Furthermore heat stress in pregnancy does not seem to affect the later offspring. A possible explanation might be that mice are able to switch on and off different genes depending on temperature and humidity (Cammack et al., 2006).

Rabbits appear to be significantly affected by heat, humidity and light. The start of the breeding season was shown to be dependent on the weather (Wight et al., 1961) and certain reproductive parameters are influenced especially by heat stress. In female rabbits it was presented that gestation length (Mahrose, 2000), conception rate (Bassuny, 1999), litter size (Ayyat et al., 1998), weight (Habeeb et al., 1999) and milk production (Nasr, 1994) decline with heat stress. Marai et al. (2001) calculated a **Temperature-Humidity Index** (THI) for rabbits where db °C = dry bulb temperature in degrees Celsius and RH = relative humidity in percentage. The results were then classified as follows: THI<27.8=absence of heat stress, THI 27.8 - 28.9 = moderate heat stress, THI 28.9 – 30 = severe heat stress and THI>30 = very severe heat stress (Marai et al., 2001). It can be summarized that hyperthermia with a THI>30 has a strong negative effect on reproduction in rabbits (Marai et al., 2002).

One interesting study which is worth mentioning looked at **antelope** and indicated that solar ultraviolet radiation triggers a positive reproductive impulse in this species (Shelford, 1954). Unfortunately, the authors did not specify which reproductive parameters are affected in detail.

Additional studies looked at the influence of weather on the reproductive performance of **marine animals** and indicated that reproduction and breeding might be correlated with temperature (Kinne, 1964, Olive, 1995, Orton, 2009) and photoperiod (Pearse et al., 1982, Pearse et al., 1986, McClintock et al., 1990).

5 Effects of Weather Parameters on Pregnancy Rate in Farmed Species around Days of Pre and Post Artificial Insemination

As stated in the text above the pregnancy rates are reported to be affected by the weather parameters such as temperature (heat stress) and Temperature-Humidity-Index (THI) the days around insemination. Published studies are summarized in the table 5-1.

Species	Days	Weather Parameter	Reproductive Parameter	Author, Year
Cattle	-2d	THI	Pregnancy rate	Ingraham et al., 1974
Cattle	-2d, -5d, +5d,	THI	Non-return rate	Ravagnolo et al. 2002
Cattle	+1d to +7d	Temperature, Heat Stress	Embryonic development	Putney et al., 1988
Cattle	Day of AI	Temperature, Heat Stress	Low fertilization, embryonic death	Fuquay, 1981
Cattle	+1d	Temperature, Heat Stress	Pregnancy Rate	Gwazdauskas et al., 1975
Cattle	-3d Day of AI	THI	Pregnancy Rate	García-Ispierto et al., 2007
Cattle	Day of AI	THI	Pregnancy Rate	Brügemann et al., 2013
Pig	-35d to +35d	Temperature, Heat Stress	Pregnancy Rate	Wettemann et al., 1985
Pig	Day of AI +16d	Temperature, Heat Stress	Pregnancy Rate, decreased litter size	Wettemann et al., 1985
Sheep	-3d to +3d	Temperature, Heat Stress	Fertility, decreased ovulation rate, increased embryonic death, lower birth weight of the lambs	Chemineau, 1993
Sheep	Around AI/Mating	Temperature, Heat Stress	Pregnancy rate	Curtis, 1983
Sheep	First month before AI and first 17 days of pregnancy	Temperature, Heat Stress	Pregnancy rate	Santolaria et al., 2014
Sheep	First 17 days of pregnancy	Temperature, Heat Stress	Pregnancy rate	Soto et al., 1998
Sheep	Heat exposure earlier in gestation	Heat Stress	Embryonic Death	Regnault et al., 1999
Rabbit	Day 3-5	Heat Stress	Embryonic Death	Lublin et al., 1984

Table 5-1: Overview of studies reporting an influence of weather parameters on specific days in the reproductive cycle and specific reproductive parameters

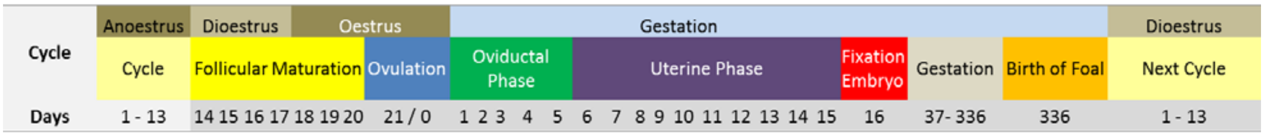
6 Weather and Meteorological Data

A detailed analysis showed that weather is expected to be extreme in the coming years in Europe and in particular in Spain, southern France and Italy (Segnalini et al., 2013). Earlier reports expected a climate change in terms of global warming (Klinedinst et al., 1993). A scenario in that study showed that it is expected that Temperature-Humidity-Indices (THIs) will increase between 3 and 4 units in the years 2041 to 2050. The analysis was based on the daily mean values of the temperature and relative humidity outputs which had been previously measured for a different research study (Istituto Nazionale di Geofisica e Vulcanologia, 2007) and only the mean values of temperature and humidity were assed. THIs were calculated for four decades 2011–2020, 2021–2030, 2031–2040 and 2041–2050 and relative to the entire study area but the change of THIs was considered to be the highest in the years 2041 – 2050 as described above. Earlier reports expected a climate change in terms of global warming (Klinedinst et al., 1993). Meteorological data from public weather stations are used to create a detailed report of environmental conditions on animal facilities even if these weather stations are kilometres away (Colston et al., 2018). However, other studies criticise the use of meteorological data from weather stations as there can be massive deviations from the site the animals are housed at, especially if they are stabled in barns (Schüller et al., 2013).

7 Material and Methods

Data were collected in the years 2013 to 2016 in Germany, Bad Saarow (GPS: 52° 16' 27.466" N 14° 3' 16.764" E) in the Equine Centre for Reproduction, University Berlin, Germany. Here 19 horse mares were located. The mares were aged from two to 23 years (median: 11 years) and were housed in stables with regular daily turn out to pasture in the summer months (Mai to October). The mares were from different breeds: There were nine Haflinger, five Shetland ponies, three Standardbreds, one trotter and one Thoroughbred mare. All mares received regular health checks, were dewormed and vaccinated on a regular basis and were sex-healthy and fertile from a reproductive perspective. All mares had a Body Condition Score (BCS) of five (5 = moderate) or six (6 = moderate fleshy) (Henneke et al., 1983). The mares were fed pellets according to their body weight/BCS and had hay ad libitum. The 19 horse mares were artificially inseminated various times with sperm from different stallions. In sum, there were 246 year round artificial inseminations. The data on reproductive parameters of these horse mares was collected over a period of four years. The defined reproductive parameters were chosen to be the follicular characteristics/size and the structure of the uterus/uterine edema. The overall objective was to achieve pregnancy. Pregnancy was confirmed via sonography or the mares were flushed around day seven. In the overall study period, three foals were born (Mare 7 in the years 2012 and 2016, mare 19 in the year 2016). All other pregnancies were terminated with prostaglandin after sonography.

In this time period of four years' data on weather parameters was collected from the closest local weather station located in Lindenberg, Germany (GPS: 52°12'29.8"N 14°07'11.1"E). This weather station is 8 km air-line distance away from the Equine Centre for Reproduction, University Berlin, Germany. The weather station measures a value every hour for certain weather parameters such as temperature, humidity and radiation (longwave downward radiation, diffuse solar radiation and solar incoming radiation). The values were measured 24 hours/day. The minutes of sunshine per day were only measured every hour in the time from 3am to 5pm. For all parameters, a daily average was calculated such as for temperature, humidity and air pressure. For all radiation data and for sunshine the hourly measurements were added together to capture the daily total load in joule per cm^2 for radiation as well as minutes of sunshine. The result was one value per day which was either an average or a sum depending on data. Reproductive data and weather parameters were equally analysed for each biological phase of the reproductive cycle (Figure 7-1). For every day in every biological phase the average of each weather parameter was calculated. For example, the phase follicular maturation has five days. For every day, the average was calculated as described in the paragraph with a result of one value for this phase.



Cycle	Anoestrus	Dioestrus	Oestrus	Gestation					Dioestrus
	Cycle	Follicular Maturation	Ovulation	Oviductal Phase	Uterine Phase	Fixation Embryo	Gestation	Birth of Foal	Next Cycle
Days	1 - 13	14 15 16 17 18 19 20	21 / 0	1 2 3 4 5	6 7 8 9 10 11 12 13 14 15	16	37- 336	336	1 - 13

Figure 7-1: Reproductive cycle of the mare

In the next step an average value was calculated per biological phase. This result was one value per biological phase. As there were 19 mares, which were artificially inseminated over repeated cycles for four years, and every mare having four biological phases the result was in sum 968 single biological phases each having one value per weather parameter. Ergo for every biological phase the above named weather parameters were calculated. The dependent variable therefore was the pregnancy result. Every weather parameter was analysed in every biological phase to determine if pregnancy was achieved or not.

The hypotheses which were based on the literature review are the following:

- Certain weather parameters influence fertility and pregnancy result
- Temperature has an effect on fertility and pregnancy result
- Humidity has an effect on fertility and pregnancy result
- THI has an effect on fertility and pregnancy result
- Air pressure has an effect on fertility and pregnancy result
- Light (sunshine, daylight or artificial light) has an effect on fertility and pregnancy result
- Radiation has an effect on fertility and pregnancy rate

7.1.1 Weather Parameters

Weather parameters that were analysed are temperature, humidity, Temperature-Humidity Index (THI), air pressure, longwave downward radiation, diffuse solar radiation, solar incoming radiation and minutes of sunshine per day. All data is numerical continuous that can take any value within a range.

The THI was calculated in addition based on Brügemann et al. (2013) and the NRC (1971) with means of hourly measurements of air temperature (T) and relative humidity (RH) resulting in the formula:

$$\mathrm{THI} = \frac{(1.8 \times T^{\circ}\mathrm{C} + 32) - (0.55 - 0.0055 \times \mathrm{RH}\%)}{(1.8 \times T^{\circ}\mathrm{C} - 26)}$$

Because of hourly data per 24 hours for a period of four years an average value was calculated per biological phase which resulted in four values per horse due to four biological phases. The table 7-1 displays an example of how the weather parameters were reported per biological phase.

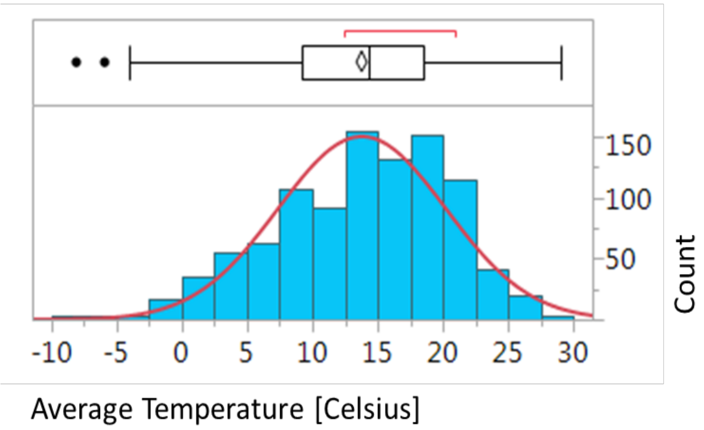
Mare	Biological Phase	Duration in days	Average of Temperature [in °C]
Mare 1	Follicular Maturation	7	8.5
	Ovulation	1	5.1
	Oviductal Phase	5	9.4
	Uterine Phase	10	10.5

Table 7-1: Example of the weather parameter of average temperature [in ° C] per biological phase per mare

Histograms were used to visualize the distribution as graphical methods are very useful for examining frequency distributions and to check if data was from a normal distribution. Summary statistics were calculated for every weather parameter including mean, standard deviation, standard error and confidence interval as well as minimum and maximum value, the median and the range. There was a different amount of data n for the weather parameters. This is because the weather parameters were recorded by public weather stations to different time points per day and sometimes values were not recorded due to failure of measurement. The distribution and summary statistics of the weather parameters were summarized and are shown in the histograms 7-3 to 7-10.

7.1.2 Distribution of Average Temperature

The distribution of average temperature was measured over four years in degrees Celsius [°C] and resulted in 968 values in total. The histogram below shows that the distribution is roughly symmetrical about is the median of 14.3 °C and has the frequency concentrated around the mean of 13.8 °C. The median is only 0.5 °C away from the mean. If the distribution is symmetrical the mean and the median will be about the same which leads to the point that the tails of the histogram are roughly symmetrical. Note the range is very high with 37.2 °C and provides an indication of statistical dispersion. This is also important for the estimation of the scatter of the data. The 95% confidence interval is 13.4 °C - 14.2 °C so we can expect 2/3 of the observations to lie within the one standard deviation of the mean and 95% to lie within two standard deviations of the mean.

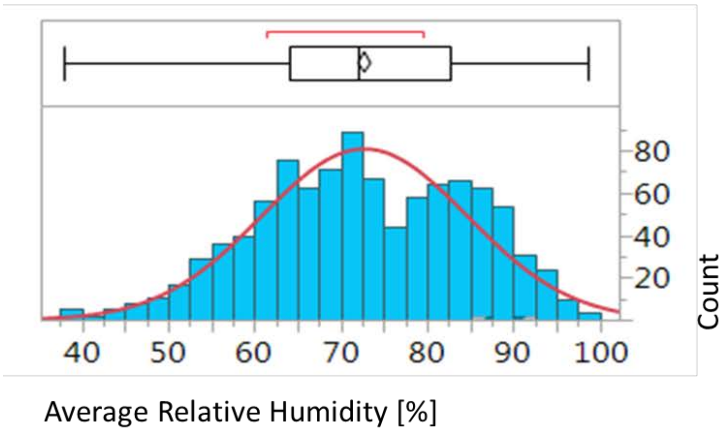


Mean	13.8
Std Dev	6.4
Std Err Mean	0.2
95% CI for Mean	13.4-14.2
Minimum	-8.1
Maximum	29.1
Median	14.3
Range	37.2
N	968

Figure 7-2: Distribution of average temperature [Celsius = °C]

7.1.3 Distribution of Average Relative Humidity

The distribution of average relative humidity measured in % over four years and resulted in 968 values in total. The relative humidity is defined as the ratio of the partial pressure of water vapour on the balance of water vapour pressure at a given temperature. It is the proportion of water vapour, which is contained in the air. The histogram below shows that the distribution is roughly symmetrical about is the median of 72.0% and has the frequency concentrated around the mean of 72.8%. The median is only 0.8% away from the mean which confirms a fairly symmetrical distribution. The range of 60.7% and the low standard deviation of 0.4% provide an indication of statistical dispersion. This is also important to notice when estimating the scatter of the data. Our 95% confidence interval is 72.0% - 73.5% so we can expect 2/3 of the observations to lie within the one standard deviation of the mean and 95% to lie within two standard deviations of the mean.

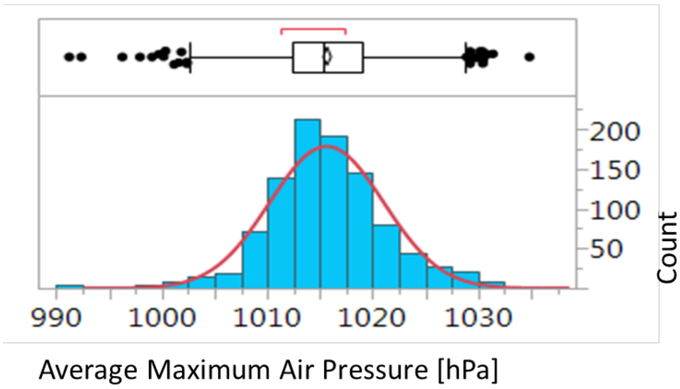


Mean	72.8
Std Dev	12.0
Std Err Mean	0.4
95% CI for Mean	72.0-73.5
Minimum	38.0
Maximum	98.6
Median	72.0
Range	60.7
N	968

Figure 7-3: Distribution of average relative humidity [%]

7.1.4 Distribution of Average Air Pressure Maximum

The distribution of average air pressure maximum is measured in Hektopascal (hPa) over four years and resulted in 968 values in total. Air pressure has a daily periodical rotation, which has two maximum and two minimum values per day. It is influenced by the daily fluctuations in the air temperature. The maximum value can, in general, be found at 10 am and 22 pm, the minimum value at 4 am and 4pm (local time, solar time). The highest air pressure measured on the 23rd January 1907 in Greifswald, Germany was 1060.8 hPa which is ~200km air-distance away from the Equine Centre for Reproduction, University Berlin in Bad Saarow (DWD, 2019). The histogram below shows that the distribution is symmetrical about is the median of 1015.3 hPa and has the frequency concentrated around the mean of 1015.6 hPa. The median is only 0.3 hPa away from the mean that confirms a symmetrical distribution. The range of 43.7 hPa provides an indication of a high statistical dispersion. This is also important when estimating the scatter of the data. Our 95% confidence interval is 10.15.3 hPa - 1016.0 hPa so we can expect 2/3 of the observations to lie within the one standard deviation of the mean and 95% to lie within two standard deviations of the mean.

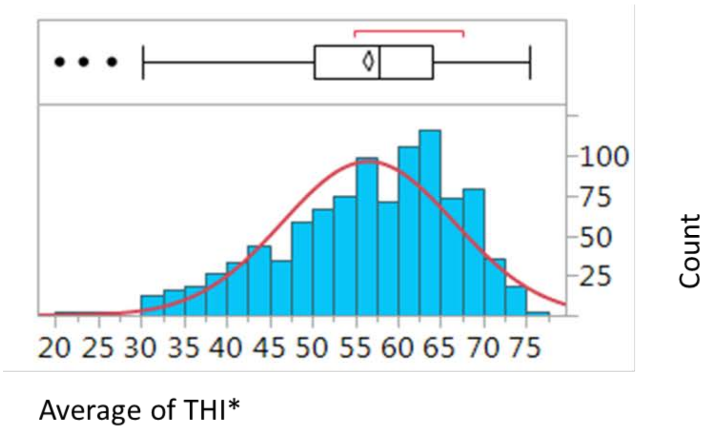


Mean	1015.6
Std Dev	5.4
Std Err Mean	0.2
95% CI for Mean	1015.3-1016.0
Minimum	991.2
Maximum	1035.0
Median	1015.3
Range	43.7
N	968

Figure 7-4: Distribution of average air pressure maximum [in hPa]

7.1.5 Distribution of Average THI

The Temperature-Humidity Index (THI) was calculated in addition based on Brügemann et al. (2013) and the NRC (1971) with means of hourly measurements of air temperature (T) and relative humidity (RH) as explained above. The distribution is based on 968 values in total. The histogram below shows that the distribution is skewed to the left, so the left tail is longer; the mass of the distribution is concentrated more on the right of the histogram. However, the most of the values are also concentrated about is the median of 57.7 and the frequency is concentrated around the mean of 56.6. The median is 0.9 points away from the mean. If the distribution is symmetrical the mean and the median will be about the same which confirms the skewness of this curve as explained above. Note the range is very high with 55 and provides an indication of statistical dispersion. This is also important when estimating the scatter of the data. The 95% confidence interval is 56.0 - 57.2 so we can expect 2/3 of the observations to lie within the one standard deviation of the mean and 95% to lie within two standard deviations of the mean.



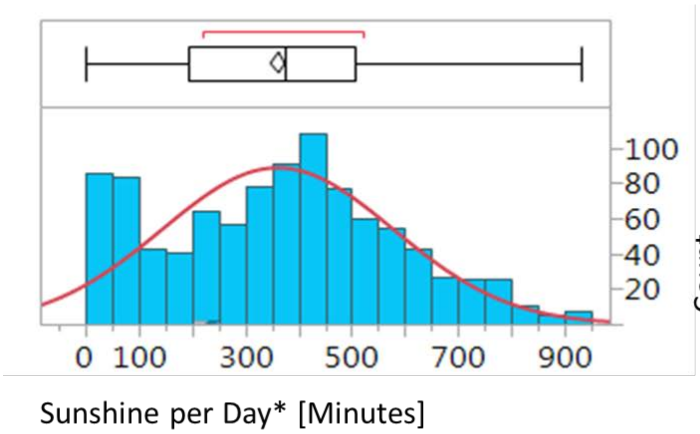
*THI calculated with maximum values per biological phase

Mean	56.6
Std Dev	10.0
Std Err Mean	0.3
95% CI for Mean	56.0-57.2
Minimum	20.5
Maximum	75.5
Median	57.7
Range	55.0
N	968

Figure 7-5: Distribution of average THI

7.1.6 Distribution of Sunshine per Day [in Minutes]

The distribution of sunshine per day was measured over four years in minutes and resulted in 967 values in total. One value was not measured due to a failure in measurement by the public weather station. The distribution is not symmetrical. The histogram has a binominal distribution because there are 40 days without sunshine (Minutes of sunshine per day = 0). Therefore, it is leptokurtic in nature. In sum there were 311 values in the range of 0 to 250 minutes of sunshine per day. As shown in the histogram the median at 375.7 minutes of sunshine per day and has the frequency concentrated around the mean of 361.0 minutes of sunshine per day. The median is not too far away from the mean (14.7 minutes). Note the range is very high with 934 minutes and provides an indication of statistical dispersion. This is also important when estimating the scatter of the data. Our 95% confidence interval is 347.2 to 374.7 minutes of sunshine per day so we can expect 2/3 of the observations to lie within the one standard deviation of the mean and 95% to lie within two standard deviations of the mean.



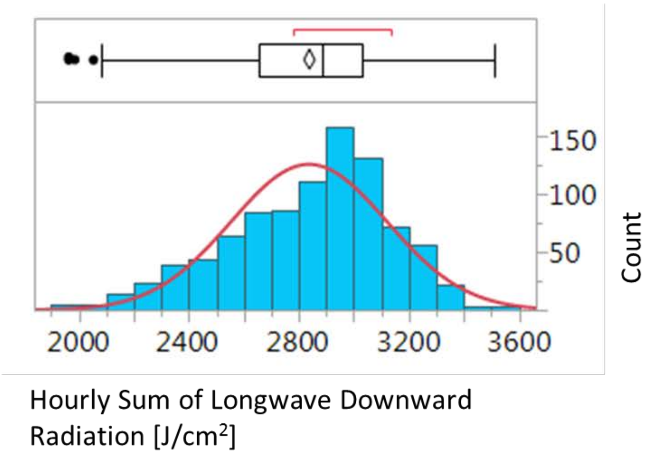
*Day = 3am – 8pm

Mean	361.0
Std Dev	217.8
Std Err Mean	7.0
95% CI for Mean	347.2-374.7
Minimum	0.0
Maximum	934.0
Median	375.7
Range	934.0
N	967

Figure 7-6: Distribution sunshine per day [in Minutes]

7.1.7 Distribution of Hourly Sum of Longwave Downward Radiation [J/cm^2]

The distribution of longwave downward radiation was measured in Joule per cm^2 [J/cm^2] over four years as hourly sum and resulted in 902 values in total. The reason for the lower amount of data is that there were a lot of missing values (MCAR = missing completely at random) due to failure in measurement by the public weather station. The histogram below shows that the distribution is roughly symmetrical about is the median of 2888.8 J/cm^2 and has the frequency concentrated around the mean of 2838.1 J/cm^2. The range is very high with 1551.0 J/cm^2 and provides an indication of statistical dispersion. This is also important when estimating the scatter of the data. Our 95% confidence interval is 2819.7 J/cm^2 - 2856.7 J/cm^2 so we can expect 2/3 of the observations to lie within the one standard deviation of the mean and 95% to lie within two standard deviations of the mean.

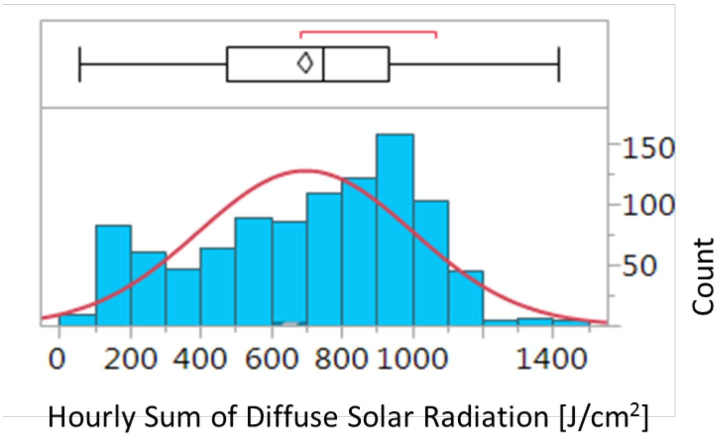


Mean	2838.2
Std Dev	283.2
Std Err Mean	9.4
95% CI for Mean	2819.7-2856.7
Minimum	1963.0
Maximum	3514.0
Median	2888.8
Range	1551.0
N	902
MCAR	968-902= 66

Figure 7-7: Distribution of hourly sum of longwave downward radiation [in J/cm^2]

7.1.8 Distribution of Hourly Sum of Diffuse Solar Radiation [J/cm^2]

The distribution of diffuse solar radiation was measured over four years as hourly sum in Joule per cm^2 [J/cm^2] and resulted in 968 values in total. Diffuse radiation is received at the surface from the sky vault after scattering and inter-reflection within the atmosphere including reflection from clouds. The distribution is not symmetrical. The median is 746.0 J/cm^2 and the mean is 699.1 J/cm^2, both values are not close to each other. The range is very high with 1364.0 J/cm^2 and provides an indication of statistical dispersion. This is also important when estimating the scatter of the data. Our 95% confidence interval is 680.0 J/cm^2 - 718.3 J/cm^2.

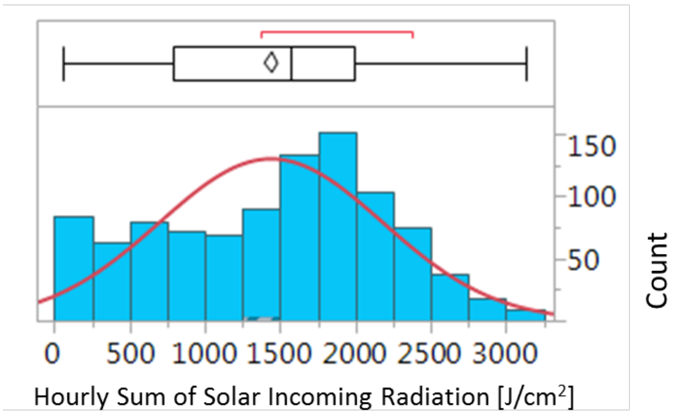


Mean	699.1
Std Dev	302.6
Std Err Mean	9.7
95% CI for Mean	680.0-718.3
Minimum	53.0
Maximum	1417.0
Median	746.0
Range	1364.0
N	968

Figure 7-8: Distribution of hourly sum of diffuse solar radiation [in J/cm^2]

7.1.9 Distribution of Hourly Sum of Solar Incoming Radiation [J/cm^2]

The distribution of solar incoming radiation was measured over four years as hourly sum in Joule per cm^2 [J/cm^2] and resulted in 968 values in total. The solar incoming radiation includes the direct and the diffuse part of the solar radiation with respect to the horizontal plane. The distribution is not symmetrical. The median is 1568.9 J/cm^2 and the mean is 1439.4 J/cm^2, so both values are not close to each other. Note the range as being the size of the smallest interval which contains all the data is very high with 3083.0 J/cm^2 and provides an indication of statistical dispersion. This is important when estimating the scatter of the data. Our 95% confidence interval is 1392.5 J/cm^2 - 1486.2 J/cm^2 so we can expect 2/3 of the observations to lie within the one standard deviation of the mean and 95% to lie within two standard deviations of the mean.



Mean	1439.4
Std Dev	742.4
Std Err Mean	23.9
95% CI for Mean	1392.5-1486.2
Minimum	54.0
Maximum	3137.0
Median	1568.9
Range	3083.0
N	968

Figure 7-9: Distribution of hourly sum of solar incoming radiation [in J/cm^2]

7.2 Light Program

A light program was conducted in two years. The first light program was implemented in the year 2014. The light program started in September 2014 and was carried out until April 2015. The second light program started in September 2015 and ended in April 2016. During the light program the light in the stable was kept on from sunset until 10:00pm and again switched on at 06:00am so that the mares had a maximum of eight hours of darkness per night. From the 968 available time points in the four years the study was conducted, 315 (33%) time points had a light program and 653 (64%) records did not have a light program. The figure 7-10 displays the data on the light program.

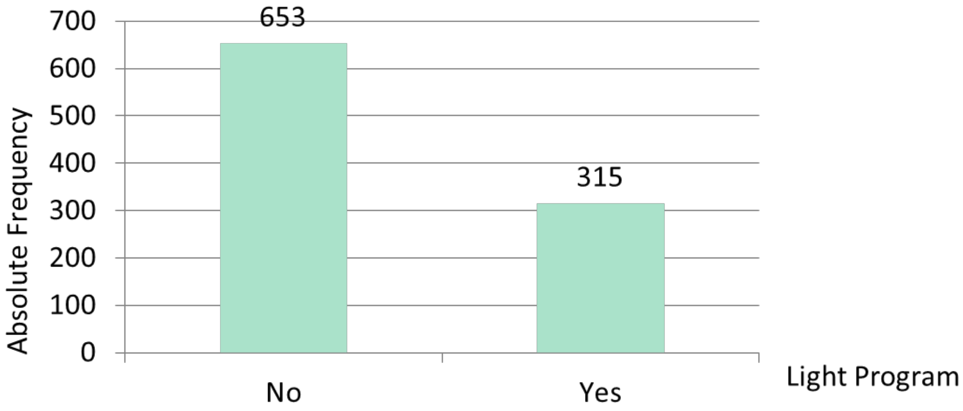


Figure 7-10: Absolute frequency of data on light program in all four years

Visualising the two years the light program was done in the respective months the following data was accessible as shown in figure 7-11. Here in sum 425 records were available of which 133 (31%) were without light program and 292 (69%) were with light program.

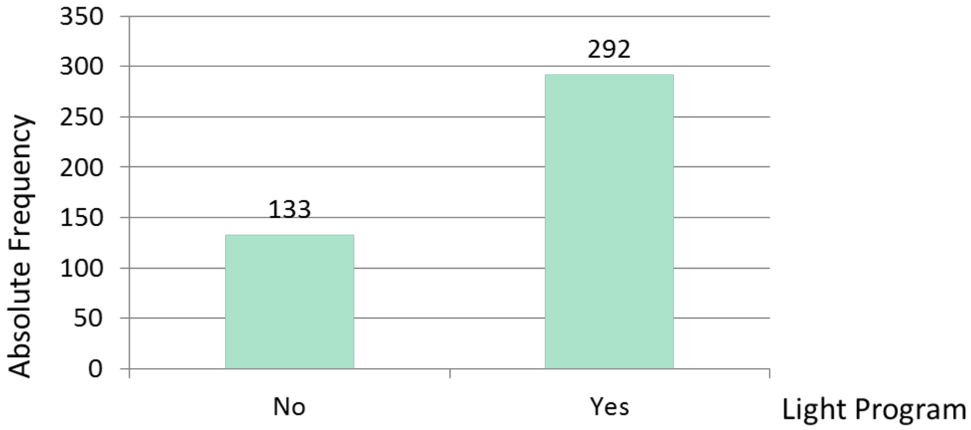


Figure 7-11: Absolute frequency of data on light program in years of light program

During the day the mares had daylight according to the Deutscher Wetterdienst (DWD, 2019) the times of sunrise and sunset as shown in the table 7-2.

Year 2014/2015

Date	Sunrise	Sunset	Length of Daylight in Hours
1-Sep-14	6:15 AM	7:50 PM	13:34
1-Oct-14	7:05 AM	6:40 PM	11:35
1-Nov-14	6:59 AM	4:34 PM	9:34
1-Dec-14	7:51 AM	3:54 PM	8:02
1-Jan-15	8:14 AM	4:00 PM	7:46
1-Feb-15	7:45 AM	4:49 PM	9:03
1-Mar-15	6:51 AM	5:41 PM	10:49
1-Apr-15	6:40 AM	7:36 PM	12:55

Year 2015/2016

Date	Sunrise	Sunset	Length of Daylight in Hours
1-Sep-15	6:15 AM	7:51 PM	13:35
1-Oct-15	7:04 AM	6:41 PM	11:36
1-Nov-15	6:59 AM	4:34 PM	9:35
1-Dec-15	7:50 AM	3:54 PM	8:03
1-Jan-16	8:14 AM	4:00 PM	7:46
1-Feb-16	7:46 AM	4:48 PM	9:02
1-Mar-16	6:50 AM	5:42 PM	10:52
1-Apr-16	6:38 AM	7:37 PM	12:58

Table 7-2: Sunrise and sunset in Bad Saarow in month with light program

7.3 Description of Horse Mares and Stallions

7.3.1 Mares

All mares were healthy, regularly dewormed and vaccinated. All mares had a Body Condition Score (BCS) of five (5 = moderate) or six (6 = moderate fleshy) (Henneke et al., 1983). The mares were fed pellets according to their body weight/BCS. Also from a reproductive perspective all mares were healthy and fertile.

7.3.1.1 Age of Mares

The mares were aged from two years to 23 years (median: 11 years) in the year 2013 when the study started. The age of all mares in the year 2013 is presented in the figure 7-12.

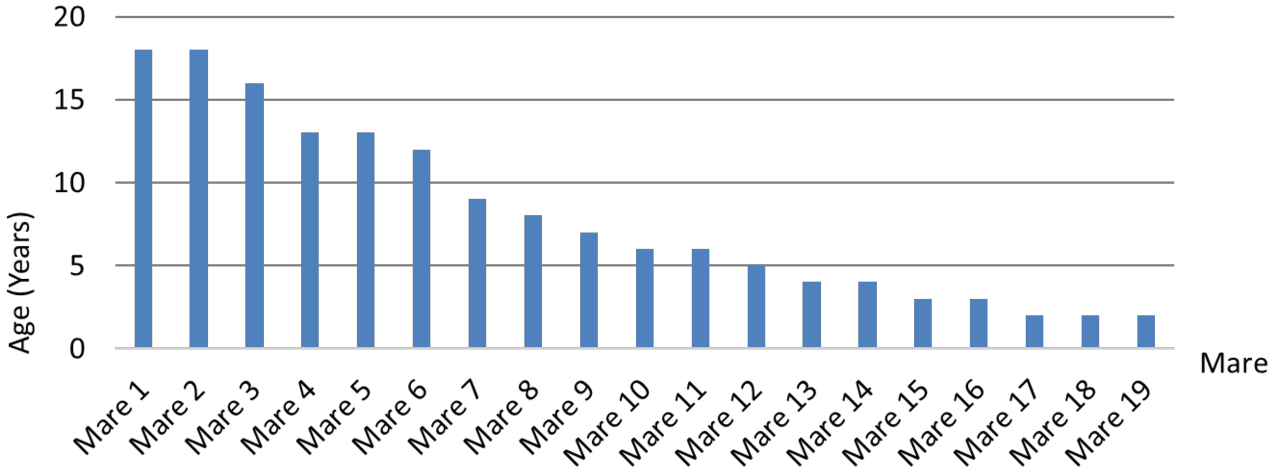


Figure 7-12: Age of mares in the year 2013

7.3.1.2 Breed of Horse Mares

Five different breeds were represented by the mares as shown in the figure 7-13.

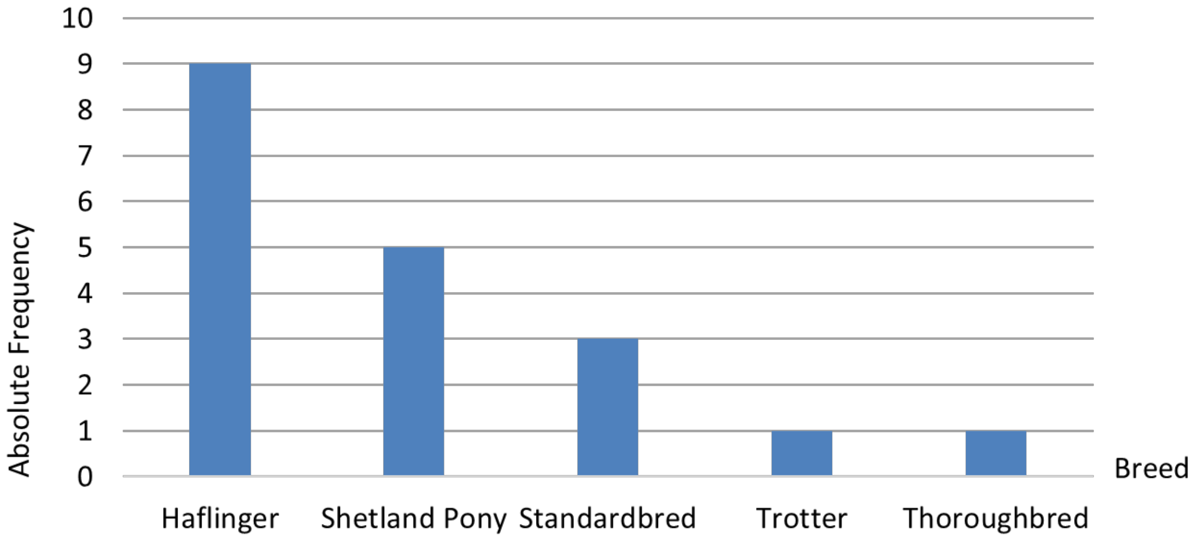


Figure 7-13: Breed of mares

7.3.1.3 Husbandry and Exercise of Horse Mares

The mares were kept in inside stables in Bad Saarow with a window to the outside and daily access (>6 hours) to pasture or paddock for a couple of hours. They were fed hay ad libitum and concentrate feed according to their bodyweight. The bedding was straw and boxes were cleaned every day.

Figure 7-14: Picture of stable mares where kept in

Figure 7-15: Picture of stable mares where kept in

7.3.2 Stallions

All Stallions were healthy, regularly dewormed and vaccinated. Also from a reproductive perspective all mares were healthy. Not much information on the Stallions was available for this dissertation. Few of them were based in the Reproduction Center in Bad Saarow.

7.4 Reproductive Parameters

Reproductive parameters analysed were the size of the dominant follicle and the structure of the uterus/uterine edema as characteristic parameters to specify fertility in horse mares.

7.4.1 Dominant Follicle

The data on the size of the dominant follicle is data numerical continuous data that can take any value within a range. The dominant follicle is shown as area of the length and the width of the follicle calculated with the formula:

$$\left(\frac{\text{Dominant Follicle Length [in mm]}}{2} * \frac{\text{Dominant Follicle Width [in mm]}}{2}\right) * 3.14159$$

7.4.2 Structure of Uterus/Uterine Edema

The data on the structure of the uterus/uterine edema is ordinal data which is a categorical, statistical data type where the variables have natural, ordered categories and the distances between the categories are not known. The ordinal scale is distinguished from the nominal scale by having a ranking. The ranking/scoring system is shown in the table 7-3.

Grade of Structure of Uterus/Uterine Edema	Assessment/Characterisation
0	No edema is characterized and there is a homogenous echotexture as well as the presence of an active corpus luteum
1	The difference in echotexture with grade 0 is evident but no individual folds are identifiable
2	First sign of uterine edema in the mare; cervix visualized ultrasonographically as a fishbone appearance
3	The uterine edema is identifiable and a cart wheel pattern is visible
4	Maximal grade of uterine edema with a true cart wheel shape

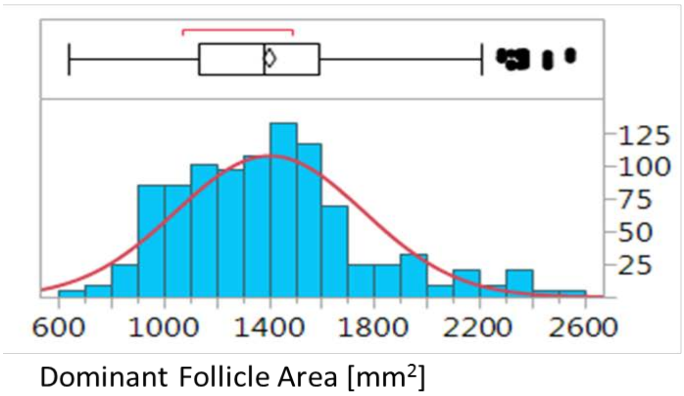
Table 7-3: Scoring system for structure of uterus/uterine edema

Here data of every horse mare over a period of four years per biological phase was collected which resulted in four values per mare and cycle due to four biological phases.

A histogram was used to look at the distribution as graphical methods are very useful for examining frequency distributions. For the dominant follicle area, a histogram was used to prove if the data is from a normal distribution. Summary statistics were calculated including mean, standard deviation, standard error and confidence interval as well as minimum and maximum value, the median and the range. For the structure of the uterus/uterine edema as this is ordinal data the scale was shown.

7.4.3 Distribution of Area of Dominant Follicle

The distribution of the area of the dominant follicle was measured in square millimetre [mm^2] over four years in all 19 mares and resulted in 968 values in total. The distribution is roughly symmetrical around the median of 1382.3mm^2 and has the frequency concentrated around the mean of 1402.8mm^2. The median is only 20.5mm^2 away from the mean. Note the range as being the size of the smallest interval which contains all the data is high with 1916.4mm^2 and provides an indication of statistical dispersion. This is important when estimating the scatter of the data. Our 95% confidence interval is 1380.3mm^2 - 1425.4mm^2. In order to lead to ovulation the dominant follicle should exceed a size from more than 40mm in diameter (Hinrichs, 1998) which is an area of 1257.0mm^2. If we compare this with the mean and the median we are slightly above this value. According to this 82% of data showed a sufficiently large enough follicle that leads to ovulation.



Mean	1402.8
Std Dev	357.3
Std Err Mean	11.5
95% CI for Mean	1380.3-1425.4
Minimum	632.2
Maximum	2548.6
Median	1382.3
Range	1916.4
N	968

Figure 7-16: Distribution dominant follicle area [mm^2]

7.4.4 Distribution of Structure of Uterus/Uterine Edema

The distribution of the structure of uterus/uterine edema was analysed by the number of frequency per structure as this is ordinal data. The figure 7-17 shows that nearly 92% of the data (960 = 108 + 500 + 352 out of 968 = 91.7%) showed a good (2 to 4) structure of the uterus/uterine edema so presumably the mares could have gotten pregnant.

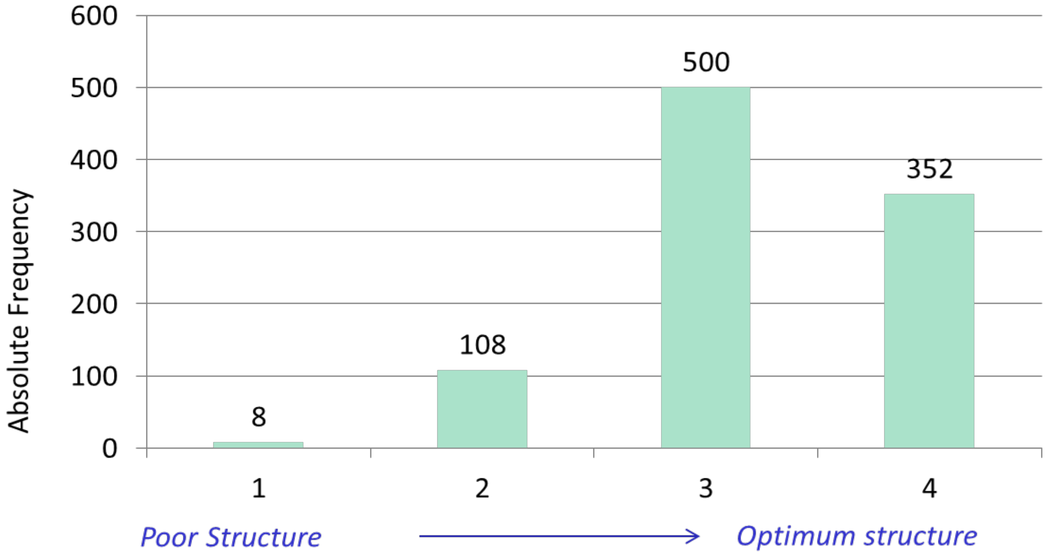


Figure 7-17: Distribution of structure of uterus/uterine edema

7.4.5 Distribution of other Reproductive Parameters

Other reproductive parameters were the age of mares, the number of cycle per mare and the time point of artificial insemination.

7.4.6 Number of Cycles per Mare

The cycles were numbered to monitor of the individual mare over the period of four years. It was not expected that the number of cycle had an effect on the pregnancy result. However, as it is part of the raw data it helped to get on overview of how often each mare was artificially inseminated over the four-year period. The mean is 38.7, but 77% (748 from 968) of the data include the first 13 cycles. The following figure 7-18 visualizes this data.

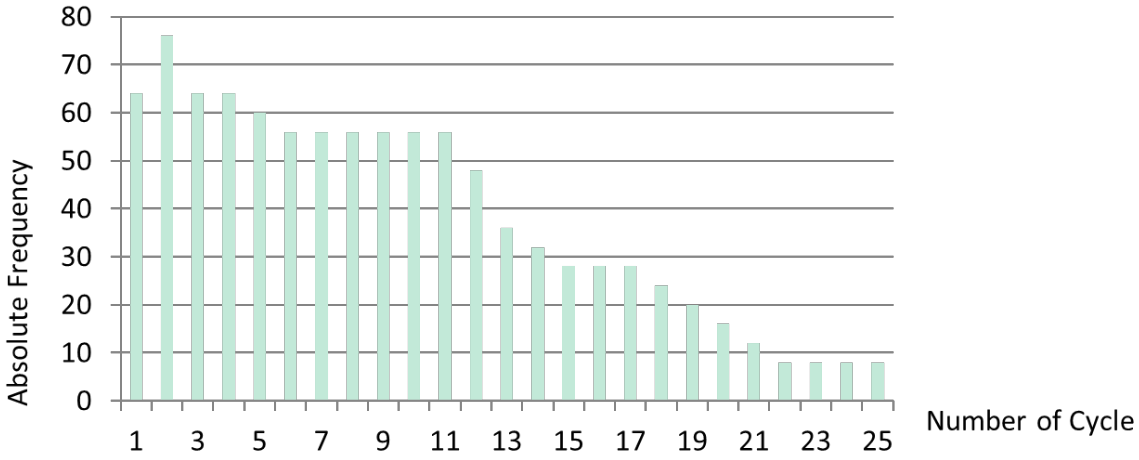


Figure 7-18: Distribution of number of cycle

7.4.7 Time Point of Artificial Insemination

The mares were artificially inseminated at different time points. Three time points were used to artificially inseminate the mares as shown in the table 7-4.

Time Points of Artificial Insemination before and after Ovulation
>48 hours before Ovulation
<48 hours before Ovulation
<6 hours after Ovulation

Table 7-4: Time points of artificial insemination

The distribution shows that nearly 80% (728) of the mares were artificially inseminated within <48 hours before ovulation. The figure 7-19 shows the distribution of time points.

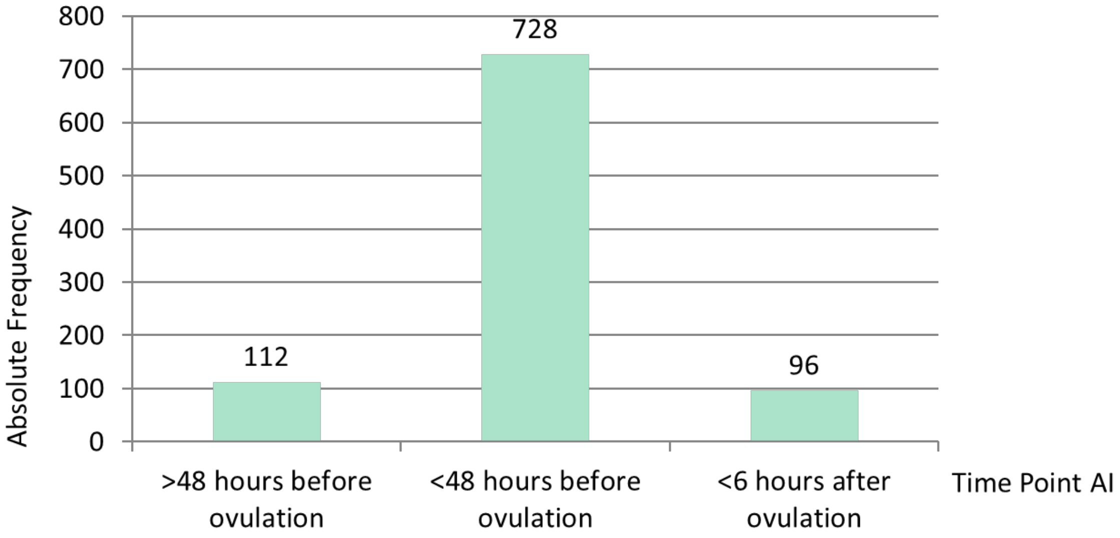


Figure 7-19: Distribution of time point of artificial insemination (AI)

7.4.8 Method of Confirmation of Pregnancy Result

Pregnancy was confirmed via sonography or the mares were flushed around day seven. In case the mares should not have a foal pregnancy was terminated with Prostaglandin after sonography. The distribution of the method used to confirm pregnancy was analysed by the number of frequency, as this is ordinal data. The figure shows that in 65% of cases sonography was used to confirm pregnancy. Note, that two foals were born and in all other mares, the pregnancy was terminated after sonography. Mares were flushed in only 35% of cases.

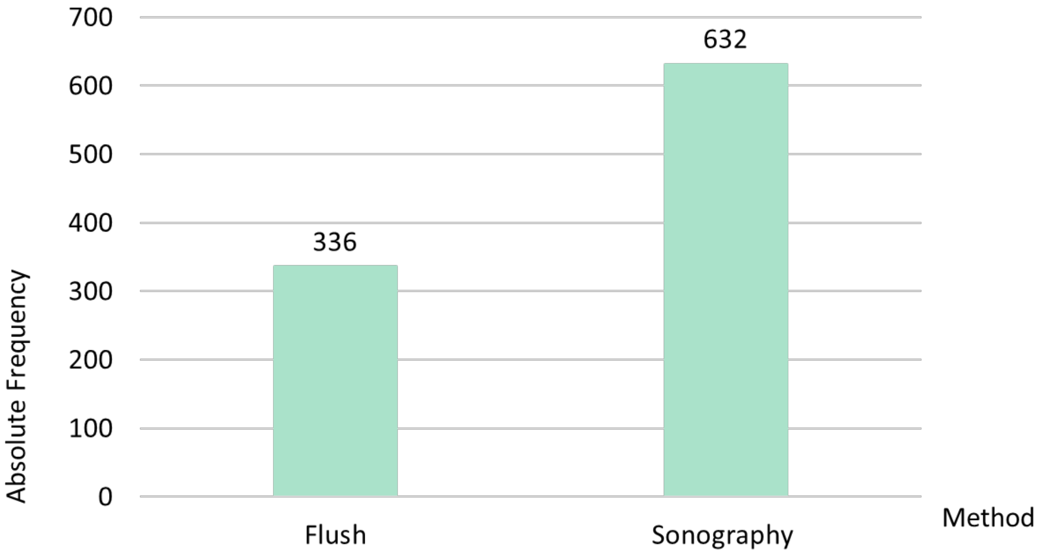


Figure 7-20: Method of confirmation of pregnancy

7.5 Effect of Weather Parameters on Pregnancy Result

Regarding the possible effect of the weather parameters on the pregnancy result each weather parameter was put in relationship to the pregnancy result in every biological phase.

7.5.1 Temperature and Humidity shown as THI

It was analysed if the THI had an effect in the various biological phases of the mare. 968 measurements were divided in classes of ten resulting in five groups in order to classify appropriately based on descriptive statistics. As shown above the values are concentrated around the median of 57.7 and have the frequency concentrated around the mean of 56.6. This resulted in the middle class from 50 to 60 and then the other classes were set up around those values on in order incorporate minimum and maximum values.

Classes of THI	
1	30-40
2	40-50
3	50-60
4	60-70
5	70-80

Table 7-5: Classes of THI

Every biological phase was analysed. The figure 7-21 shows the grouping of the THI in the different biological phases. In follicular maturation the pregnancy rate was higher if the THI was between 30 and 60 as here more than 50% achieved pregnancies were presented. A THI greater than 60 showed a pregnancy rate of 55% negative. With a THI greater than 70 the results were 69% negative. In ovulation the pregnancy rate was 71% with a THI between 30 and 40 as well as equal results of pregnancy or no pregnancy (50% negative and 50% positive) with a THI between 40 and 50. Furthermore, if the THI was greater than 50 then the pregnancy rate was only 47% and even in the class 70 - 80 it was only a pregnancy rate of 37%. The oviductal phase was very similar to the follicular maturation were lower THIs (THI smaller than 50) show a positive pregnancy result (THI greater than 50%) and in the higher range (THI between 60 and 80) the pregnancy result was only 43% positive. The uterine phase was very similar to the ovulation: With a THI greater than 60 in more than 50% of the data no pregnancy was achieved. A THI greater than 60 shows 45% positive results and in the class with a THI higher than 70 to 80 the pregnancy rate was only 17%. According to this data in some classes a linear relationship and correlation could be possible anticipating that a THI greater than 60 did not lead to pregnancy. The statistical analysis therefore analysed the effect of classified THIs and the pregnancy result in every biological class.

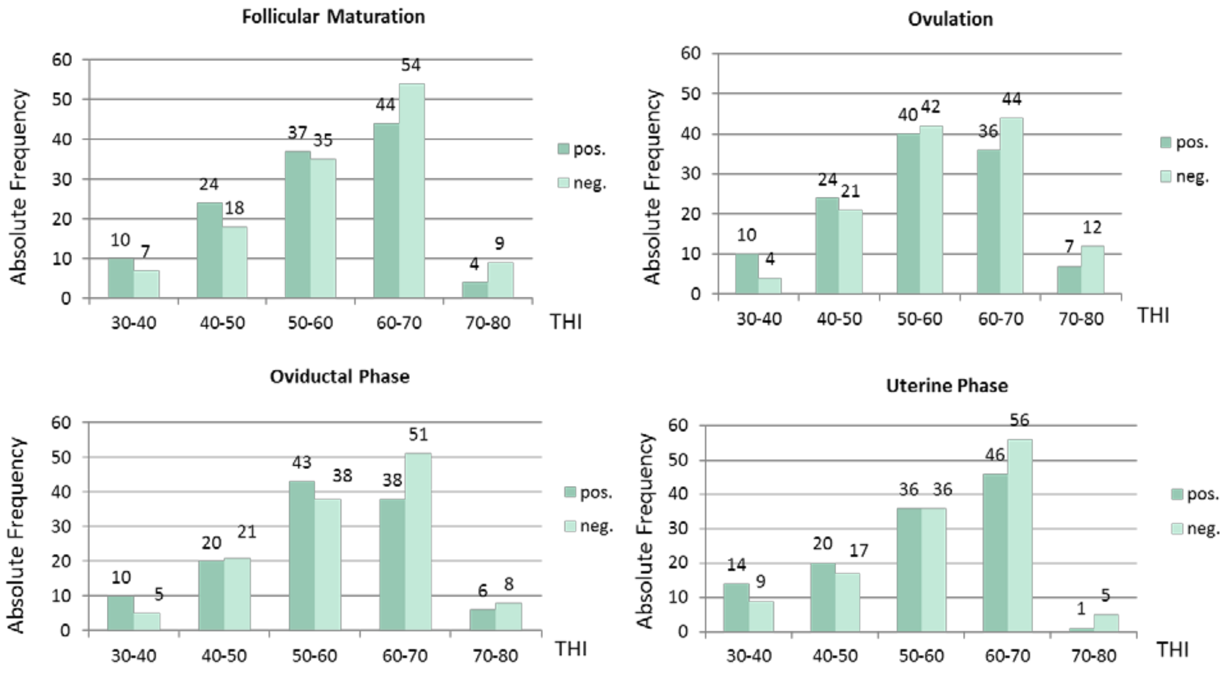


Figure 7-21: THI per biological phase and pregnancy result

7.5.2 Air Pressure

It was analysed if air pressure had an effect in the various biological phases of the mare. 968 measurements were divided in classes of 5 hPa according to descriptive statistics. The median was 1015.3 hPa and the mean was 1015.5 hPa which resulted in the middle class. In order to capture minimum and maximum values the other groups were set up around this middle class as shown in the table 7-6.

Classes of Air Pressure in hPa	
1	1000 – 1005
2	1005 – 1010
3	1010 – 1015
4	1015 – 1020
5	1020 – 1025

Table 7-6: Classes of air pressure [in hPa]

The figure 7-22 shows the grouping of the air pressure in the different phases. In follicular maturation in the classes from 1000 hPa to 1015 hPa around 50% of cases did not result in pregnancy. In the classes from 1015 hPa to 1020 hPa the data shows that 50% of cases did result in pregnancy and an air pressure greater than 1020 hPa to 1025 hPa did not result in pregnancy in 50% of the cases. In ovulation in the classes from 1000 hPa to 1005 hPa in more than 50% no pregnancy was achieved; same in the higher classes 1015 hPa to 1025 hPa 58%. In the classes from 1010 hPa to from of 1015 hPa the results showed a pregnancy in more than 55% of cases. In the oviductal phase in three of the classes (1000 hPa to1005 hPa, 1010 hPa to 1015 hPa and 1020 hPa to1025 hPa) there was no difference in the results (50% positive, 50% negative). In the class 1005 hPa to 1010 hPa nearly 60% pregnancies were achieved and in the classes from of 1015 hPa to 1020 hPa in 56% of cases no pregnancy was realized. In the class higher than 1020 hPa no real difference can be seen. However, the differences are small and no linear relationship or correlation could be identified here from this data.

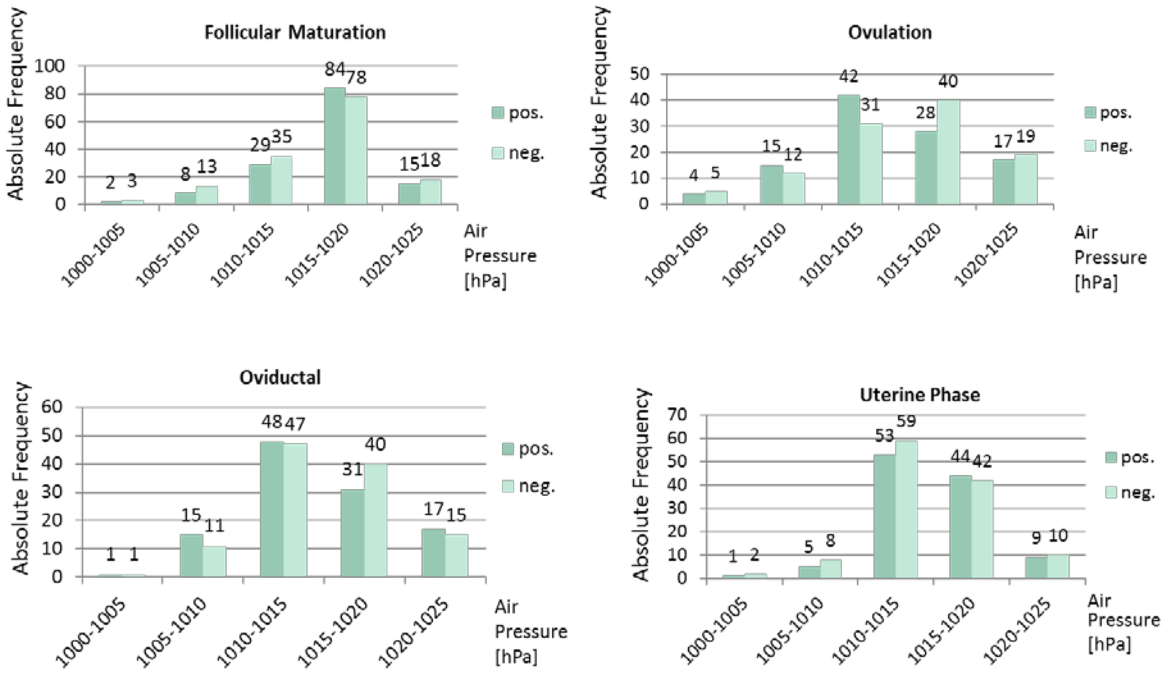


Figure 7-22: Air pressure [in hPa] per biological phase and pregnancy result

7.5.3 Minutes of Sunshine per Day

It was analysed if the amount of minutes of sunshine per day had an effect in the various biological phases of the mare. Sunshine was measured daily from 3am to 8pm. 967 measurements were divided in classes by 250 minutes. The median of 375.7 minutes of sunshine per day and has the frequency concentrated around the mean of 361.0 minutes of sunshine per day. The median was 14.7 minutes away from the mean. In order to group the data based on the descriptive statistics the following was considered: First group lay in the lower range, the second group around the median and the third group at the higher end while there was an extreme group with more than 12.5 hours of sunshine per day. The figure 7-7 shows the classes of minutes of sunshine per day.

Classes of Minutes of Sunshine per Day	
1	0 – 250
2	250 – 500
3	500 – 750
4	750 – 1000

Table 7-7: Classes of minutes of sunshine per day

In follicular maturation in almost all classes (0 to 750 minutes of sunshine per day) the results were equal: In around 50% of cases pregnancy was achieved and 50% did not get pregnant. Just in the class 750 to 1000 minutes of sunshine per day 67% of cases show a negative result. In ovulation the results showed a positive pregnancy result 59% in the class 0 to 250 minutes of sunshine per day but a negative pregnancy result of 58% in the class 250 to 500 minutes of sunshine per day. In the class with 500 to 750 minutes of sunshine per day the pregnancy rate was 52% positive. Again in the highest class of more than 750 to 1000 minutes of sunshine per day 64% of cases did not achieve pregnancy. In the oviductal phase the results in the classes did show differences: In the class from 0 to 250 minutes of sunshine per day 63% of cases were positive, in the classes 250 to 500 minutes of sunshine per day only 43% of cases were positive and in the class 500 to 750 minutes of sunshine per day only 31% of cases were positive. In the class from 750 to 1000 minutes of sunshine per day were 67% of the cases were positive. In the uterine phase the data was ~50% positive and ~50% negative in all classes from 0 to 750 minutes of sunshine per day, but in the class from 750 to 1000 minutes of sunshine per day no single pregnancy was achieved – however, only two cases fell into this class so the number is small. Nevertheless, the differences are small and no linear relationship or correlation can be seen here from this data.

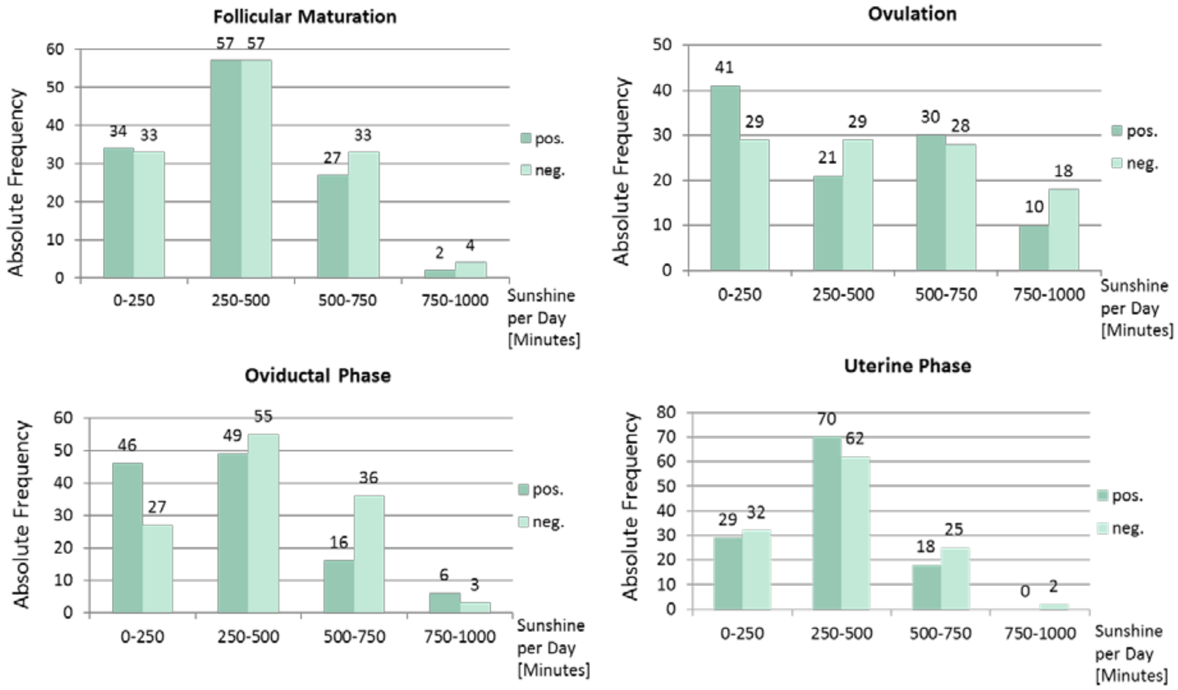


Figure 7-23: Sunshine per day [in minutes] measured from 3am to 8pm per biological phase and pregnancy result

The raw data showed that there were 40 days without any sunshine and in sum 311 values in the class from 0 to 250 minutes of sunshine per day so this data was grouped in classes as the table 7-8 shows.

Classes of Minutes of Sunshine per Day (0 to 250 Minutes)	
1	0 – 60
2	60 – 120
3	120 – 180
4	180 – 250

Table 7-8: Classes of minutes of sunshine per day (0 to 250 minutes)

The data shows that in follicular maturation the results were equal (~50% positive and ~50% negative) in most of the classes. In the class 120 to 180 minutes of sunshine per day there were 63% pregnancies achieved. In ovulation in the classes 0 to 60 minutes of sunshine per day and in the class 120 to 180 minutes of sunshine per day the results were equal (~50% positive and ~50% negative). In the class 60 to 120 minutes per day however 83% of cases were positive and in the class 180 to 250 minutes of sunshine per day 67% of cases were positive. The data shows that in the oviductal phase the results were more than 54% positive in all of the classes. In the classes 60 to 250 minutes even more than 62% of cases were positive. In the uterine phase in the class 0 to 60 minutes of sunshine per day 54% were negative and were equal (50% positive and 50% negative) in the classes 60 to 120 minutes of sunshine per day. Just in the class 120 to 180 minutes of sunshine per day there were 55% pregnancies achieved while in the class of 180 to 250 minutes per day 67% of cases were negative. However, the differences are small. And, as the light program was done in two winters (2014/2015 and 2015/2016) data of the sunlight could have been masked by the artificial light in the stable resulting in a more positive effect. The statistical analysis therefore investigated in a correlation between sunlight and light program and the effect on the pregnancy result.

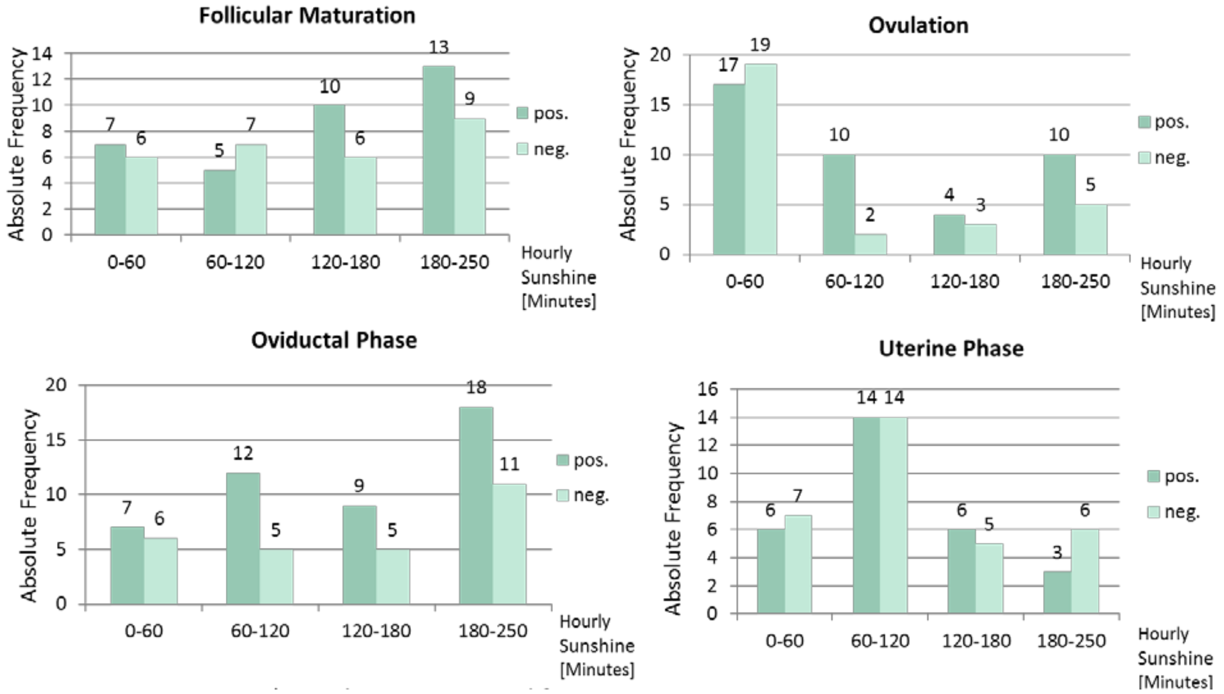


Figure 7-24: Sunshine per day [in minutes] measured from 3am to 8pm per biological phase and pregnancy result

7.5.4 Longwave Downward Radiation

The measurements of longwave downward radiation resulted in 902 values in total which are reported in Joule per cm^2 [J/cm^2]. The reason for the lower amount of data was that there were a lot of missing values (MCAR = missing completely at random) due to failure in measurement from the public weather station. The table 7-9 shows the grouping of longwave downward radiation in the different phases which are based on the descriptive statistics. The distribution is roughly symmetrical about is the median of 2888.8 J/cm^2 and has the frequency concentrated around the mean of 2838.2 J/cm^2. The minimum value of 1963.0 J/cm^2 and the maximum value of 3514.0 J/cm^2 were taken as the lower and upper level of the classes which then resulted in the classes as shown in the table 7-9

Classes of Longwave Downward Radiation in J/cm²	
1	1900 – 2500
2	2500 – 3000
3	3000 – 3500

Table 7-9: Classes of longwave downward radiation in J/cm²

In follicular maturation the results in the lowest class from 1900 J/cm² to 2500 J/cm² show that 70% of the data was positive, so a pregnancy was achieved. The middle class from 2500 J/cm² to 3000 J/cm² shows equal results (~50% positive and ~50% negative). In the class 3000 J/cm² to 3500 J/cm² there were only 35% of the results positive. In ovulation in the class 1900 J/cm² to 2500 J/cm² the pregnancy rate was 64% and in the class 2500 to 3000 the results regarding the pregnancy rate were equal (50% positive and 50% negative). In the class 3000 J/cm² to 3500 J/cm² there were only 42% pregnancies archived. Two results were measured outside of the classes showing a longwave downward radiation >3500 J/cm², but this was only two times the value 3514 J/cm² and these outliers showed one positive and one negative result. They were taken therefore out of the grouping as outliers. In the oviductal phase the results showed 67% positive cases in the low class from 1900 J/cm² to 2500 J/cm² while the results were equal (~50% positive and ~50% negative) in the other classes from 3000 J/cm² to 3500 J/cm². Similar results were presented in the uterine phase were 65% of the results were positive in the low class from 1900 J/cm² to 2500 J/cm² and the results were equal (~50% positive and ~50% negative) in all of the classes. According to the data it could have been assumed that a higher longwave downward radiation (>2500 J/cm²) led to a lower pregnancy rate (negative results) in ovulation, oviductal phase and uterine phase. Higher longwave downward solar radiation (>3000 J/cm²) did not lead to pregnancy (negative result) in any biological phases. However, the differences are small and no linear relationship or correlation was identified from this data.

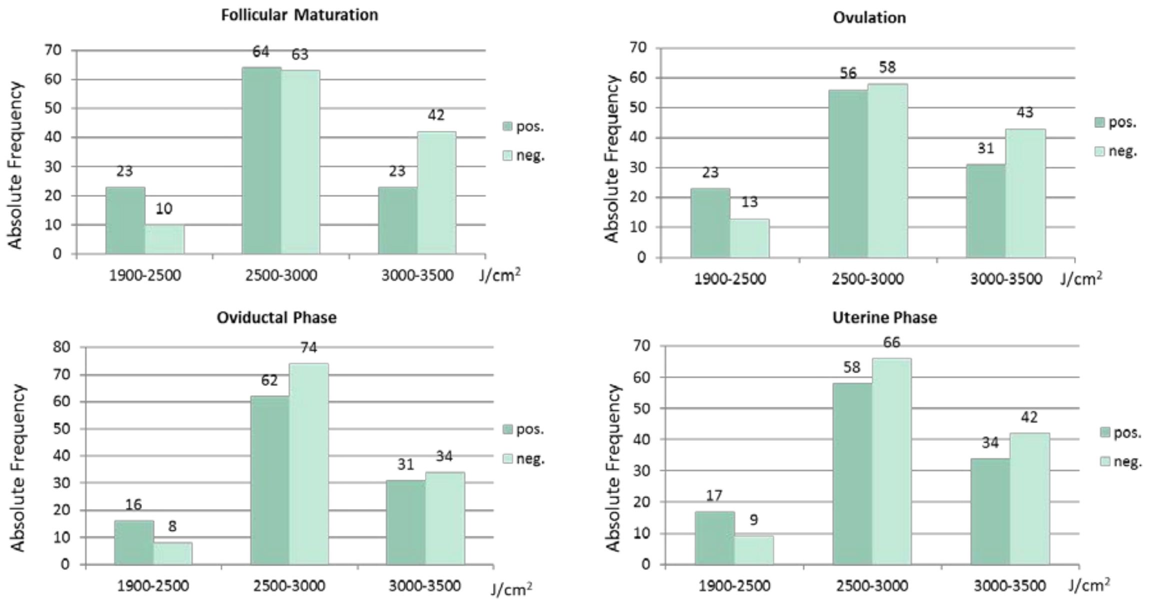


Figure 7-25: Longwave downward radiation per biological phase and pregnancy result

7.5.5 Diffuse Solar Radiation

Diffuse solar radiation is received at the surface from the sky vault after scattering and inter-reflection within the atmosphere including reflection from clouds. The measurements of diffuse solar radiation resulted in 968 values in total which are reported in Joule per cm^2 [J/cm^2]. The figure 7-10 shows the grouping of diffuse solar radiation based on descriptive statistics. Here the mean is 699.1 J/cm^2 and the median is 746.0 J/cm^2. The minimum value is 53 and the maximum value is 1417.0 J/cm^2. Based on these values the classification of diffuse solar radiation resulted in the classes shown in table 7-10.

Classes of Diffuse Solar Radiation in J/cm^2	
1	0 – 500
2	500 – 1000
3	1000 – 1500

Table 7-10: Classes of diffuse solar radiation [in J/cm^2]

In follicular maturation in the low class (0 J/cm^2 to 500 J/cm^2) 55% of the results were positive, whereas in the middle class (500 J/cm^2 to 1000 J/cm^2) 55% were negative. In the class 1000 J/cm^2 to 1500 J/cm^2 only 41% of the results were positive. In ovulation in the lower class (0 J/cm^2 to 500 J/cm^2) 49% of the results were positive and in ovulation the middle class (500 J/cm^2 to 1000 J/cm^2) 44% were positive, while in the highest class (1000 J/cm^2 to 1500 J/cm^2) 60% of cases were positive. In the oviductal phase in the class 500 J/cm^2 to 1000 J/cm^2 54% of the results were positive, though in the classes 500 J/cm^2 to 1500 J/cm^2 up to 51% of the results were negative. In the uterine phase diffuse solar radiation between 500 J/cm^2 to 1000 J/cm^2 59% resulted in pregnancy whereas in the other two classes up to 55% of the cases show a negative pregnancy result. Higher diffuse solar radiation (> 500 J/cm^2) did not lead to pregnancy (negative result) in the biological phases follicular maturation, oviductal phase, and uterine phase. In the ovulation however 60% pregnancies were achieved in the class from 1000 J/cm^2 to 1500 J/cm^2. However, the differences are small and no linear relationship or correlation could be seen here from this data.

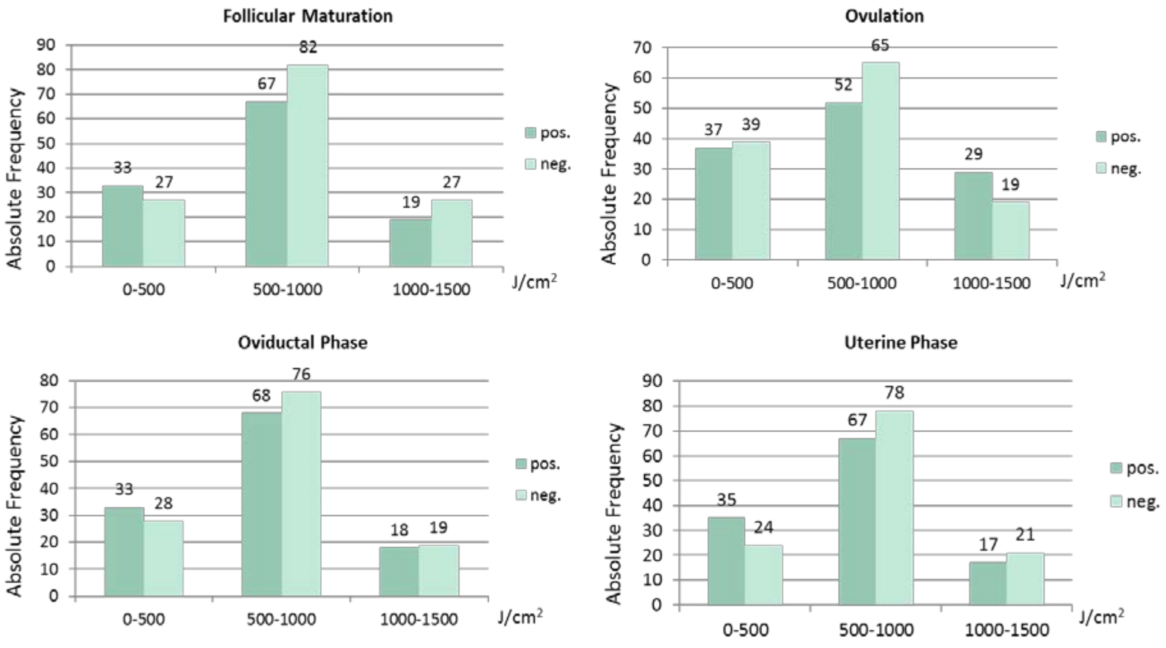


Figure 7-26: Diffuse solar radiation per biological phase and pregnancy result

7.5.6 Incoming Solar Radiation

The solar incoming radiation includes the direct and the diffuse part of the solar radiation with respect to the horizontal plane. The measurements of incoming solar radiation resulted in 968 values in total which are reported in Joule per cm^2 [J/cm^2]. Due to the descriptive statistics which showed that the mean is 1439.4 J/cm^2 and the median 1568.9 J/cm^2 with the minimum value of 54.0 J/cm^2 and the maximum value of 3137.0 J/cm^2 the classes were set for solar incoming radiation as shown in table 7-11.

Classes of Incoming Solar Radiation in J/cm^2	
1	0 – 1000
2	1000 – 2000
3	2000 – 3000
4	3000 – 4000

Table 7-11: Classes of incoming solar radiation in J/cm^2

In follicular maturation in the low class from 0 J/cm^2 to 1000 J/cm^2 56% of the results were positive whereas in the classes from 1000 J/cm^2 to 3000 J/cm^2 more than 50% of the results were negative. No data was available in the highest class (3000 J/cm^2 – 4000 J/cm^2). In ovulation, the results were ~50% positive and ~50% negative in the class 0 J/cm^2 to 1000 J/cm^2 and in the class from 1000 J/cm^2 to 2000 J/cm^2 56% of pregnancies were positive. In the class from 2000 J/cm^2 to 3000 J/cm^2 more than 50% of the data showed that no pregnancy was achieved. In the highest class from 3000 J/cm^2 to 4000 J/cm^2 86% of the results were negative; however, here only seven records from 968 were available so the number is small. In the oviductal phase in the lower class from 0 J/cm^2 to 1000 J/cm^2 in 58% of the cases pregnancy was achieved. In the class from 1000 J/cm^2 to 2000 J/cm^2 no difference was seen, the results were ~50% positive and ~50% negative. Class 2000 J/cm^2 to 3000 J/cm^2 showed a pregnancy rate of only 38%. No data was available in the highest class (3000 J/cm^2 to 4000 J/cm^2). In the uterine phase the data was slightly more than 50% positive results for the classes from 0 J/cm^2 to 1000 J/cm^2 and from 1000 J/cm^2 to 2000 J/cm^2. In the highest class from 2000 J/cm^2 to 3000 J/cm^2 60% of the cases did not result in pregnancy. No data was available in the highest class (3000 J/cm^2 to 4000 J/cm^2). Higher incoming solar radiation (1000 J/cm^2) did not lead to pregnancy (negative result) in follicular maturation and oviductal phase. Higher incoming solar radiation (>2000 J/cm^2) did not lead to pregnancy (negative result) in ovulation and uterine phase. However, the differences are small and no linear relationship was determined form this data.

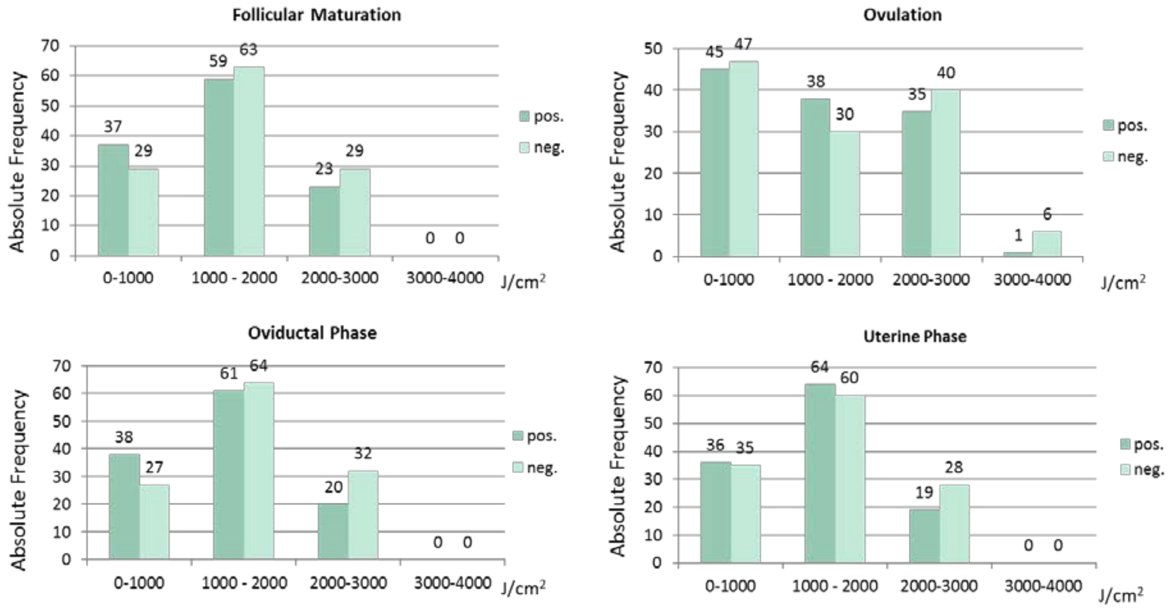


Figure 7-27: Incoming solar radiation in J/cm^2 per biological phase and pregnancy result

7.6 Stratification of Pregnancy Result by Month

Because all weather parameters relate to the season and month it was analysed how many pregnancies were achieved in which month. As the number of records and positive and negative pregnancies per biological phase was the same amount in all biological phases all data was analysed in once without considering the separate biological phases. Pregnancies started beginning of the year in January with the lowest frequency (28 records in total). The pregnancy rate this month was 57%. In February 40 records were available and 90% of all cases resulted in pregnancy. In the month up to end of June the results were similar (~50% positive and 50% negative) with an increasing amount of records. The number of records had a peak in July (149 records) but the pregnancy rate here was only 41%. The number of pregnancies decreased again with another small peak in October (66 records but pregnancy rate only 45%). Overall 55.1% records were available in the first half of the year. On average more positive pregnancies were achieved (58.3%) at the beginning of the year compared to the second half of the year (41.6%). This does reflect the natural breeding rhythm of mare on the northern hemisphere.

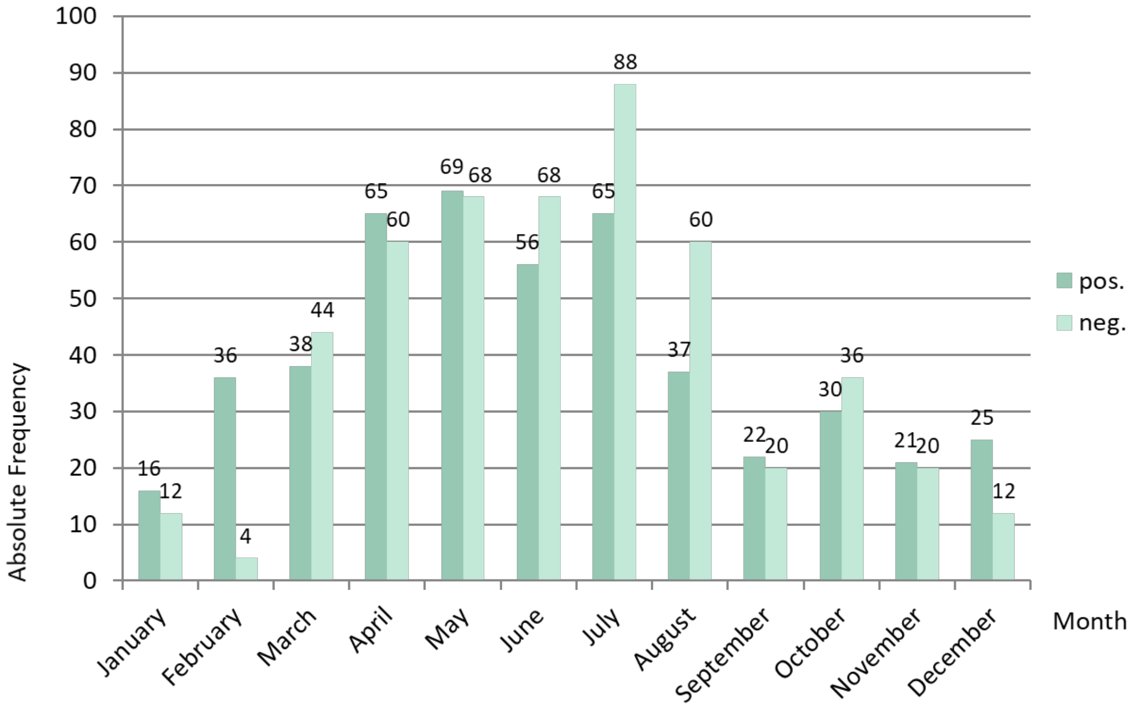


Figure 7-28: Stratification of pregnancy result by month

7.7 Stratification of Pregnancy Result by Light Program

A light program was done in two winters (2014/2015 and 2015/2016). It was analysed if there was an effect of the light program on the pregnancy result considering only at the months September to April in all years, so data without and with light program were compared directly. The graphic 7-29 displays the pregnancy result and light program in every biological phase. Follicular maturation and ovulation show the same numbers in terms of frequency and results: With no light program the pregnancy rate was 59% and with light program the pregnancy rate was 53%. However, the number of records per phase with light program was higher (74 records) then without light program (41 records). The oviductal phase shows a pregnancy rate of 60% without light program and 51% with light program. And, the uterine phase show slightly different amount of frequencies compared to the oviductal phase. However, the pregnancy rates were similar to the other phases (61% pregnancy rate without light program and 51% with light program. On the first view, it can be noted that the pregnancy rates were lower for the data with light program. However, it is unclear if the light program really did not have a positive effect on the pregnancy result. The statistical analysis questioned this effect and a respective analysis was done.

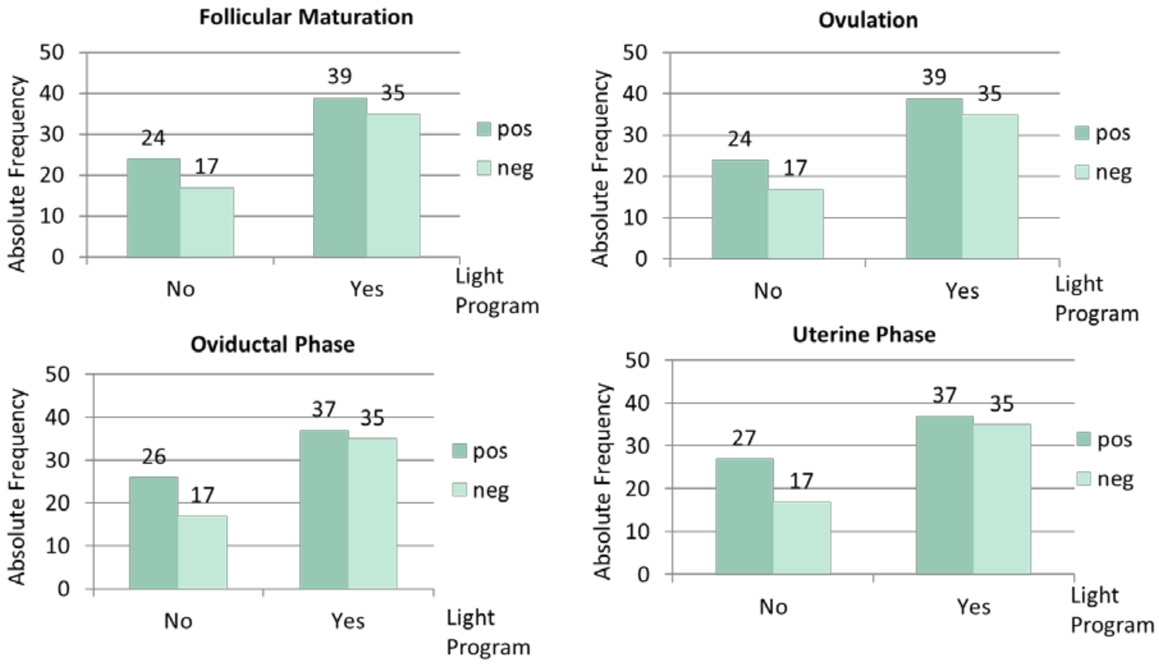


Figure 7-29: Stratification of pregnancy result by light program per biological phase

In order to analyse the effect of the light program on the pregnancy result the month as such was analysed to determine how the light program influenced the month and the related minutes of sunshine per month. Only the month in which a light program took place were therefore compared (September to April in the years 2014/2015 and 2015/2016). In September, 14 records without light program and 28 without light program were available it was seen that in the period with light program 71% of the results were positive whereas in the period without light program only 43% were positive. In October, 14 records were available with light program and 52 without light program, the data showed that without light program 71% of the results were positive while in the period without light program only 38% were positive. So, the double amount of pregnancies also in terms of absolute frequency was achieved. In November, nine records were available without light program and 42 records with light program, the data showed that nine positive pregnancies (100% pregnancy rate) were achieved in the period without light program. In the period with light program 63% of the results were positive. This was more than the double amount of pregnancies compared to the period without light program. In December, nine records were available without light program and 28 records with light program the data shows that nine pregnancies were achieved in the period without light program and no negative results (100%). In the period with light program 57% of pregnancies were positive. This was 43% more positive pregnancies then without light program. In January were four records were available without light program and 24 records with light program were available the data showed that 2/3 more pregnancies were achieved when the light program was in place. But also 50% of the results showed a negative result when using the light program. In February were 32 records were available without light program and eight records with light program the data showed that 88% of the cases were positive without light program. With light program 100% of the pregnancies were positive, but the absolute amount of pregnancies was much lower (28.6%) compared to the data when no light program was used. In March were 28 records were available in the period without light program and 54 records with light program it was recorded from the data that more than two times more pregnancies were achieved with a light program compared to no light program while the amount of no pregnancy was similar. In April were 59 records were available without light program the number of positive pregnancies was 61% and the negative results 39%. With light program 64% of pregnancies could be achieved.

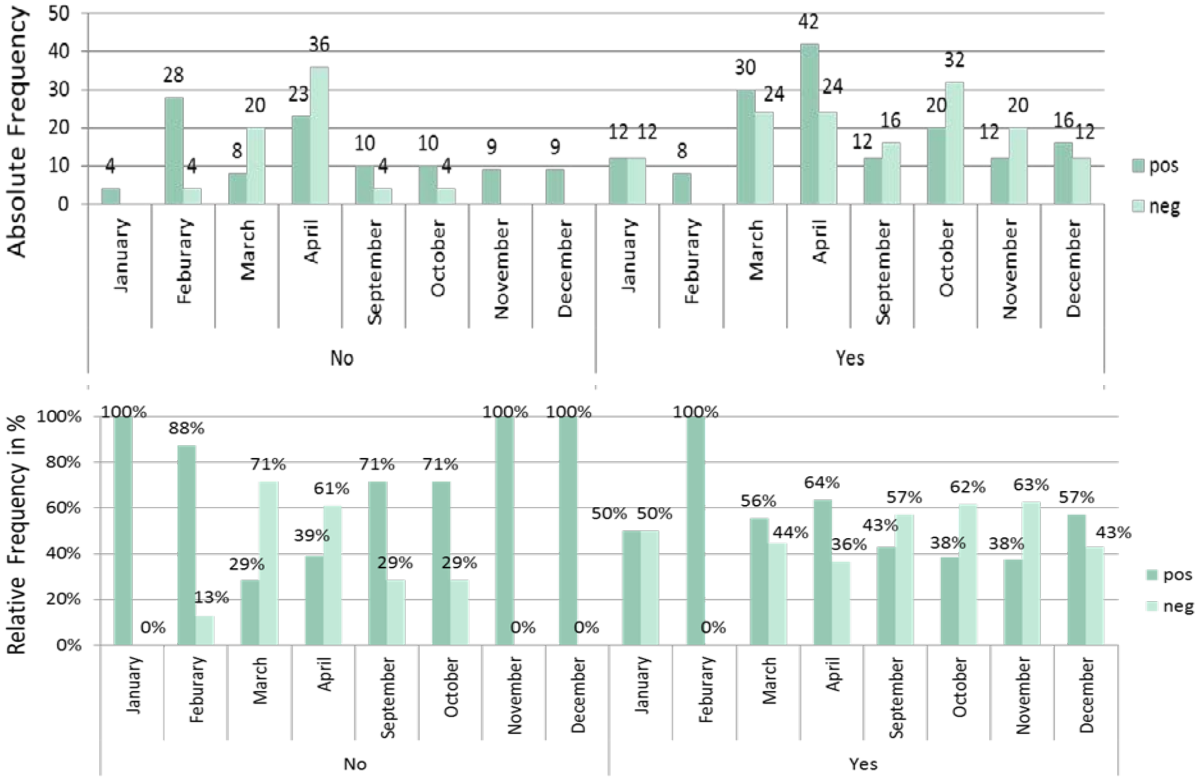


Figure 7-30: Stratification of pregnancy result by light program per biological phase per month in absolute and relative frequency

7.8 Stratification of Pregnancy Result by Minutes of Sunshine per Day, Month and Light Program

It could have been possible that minutes of sunshine, month and light program together had a stimulus on the pregnancy result because the light program was continued in the winter month mimicking summer in minutes of sunshine. Based on the descriptive statistics a scatterplot was done showing the minutes of sunshine per day which presented that most data existed in the range of 200 to 800 minutes of sunshine per day and fewer data from 0 to 200 minutes of sunshine per day as well as 800 to 1000 minutes of sunshine per day. New classes were categorized considering the number of records per range in particular on the frequency of records. The table 7-12 displays an overview of the classes.

Classes of Minutes of Sunshine per Day based on Frequency of Records	
1	0 – 200
2	200 – 300
3	300 – 400
4	400 – 500
5	500 – 600
6	600 – 700
7	700 – 800
8	800 – 1000

Table 7-12: Classes of minutes of sunshine per day

In order to better visualize where the highest amount of records lay and to define further statistics boxplots were created. The figure 7-31 shows the boxplots.

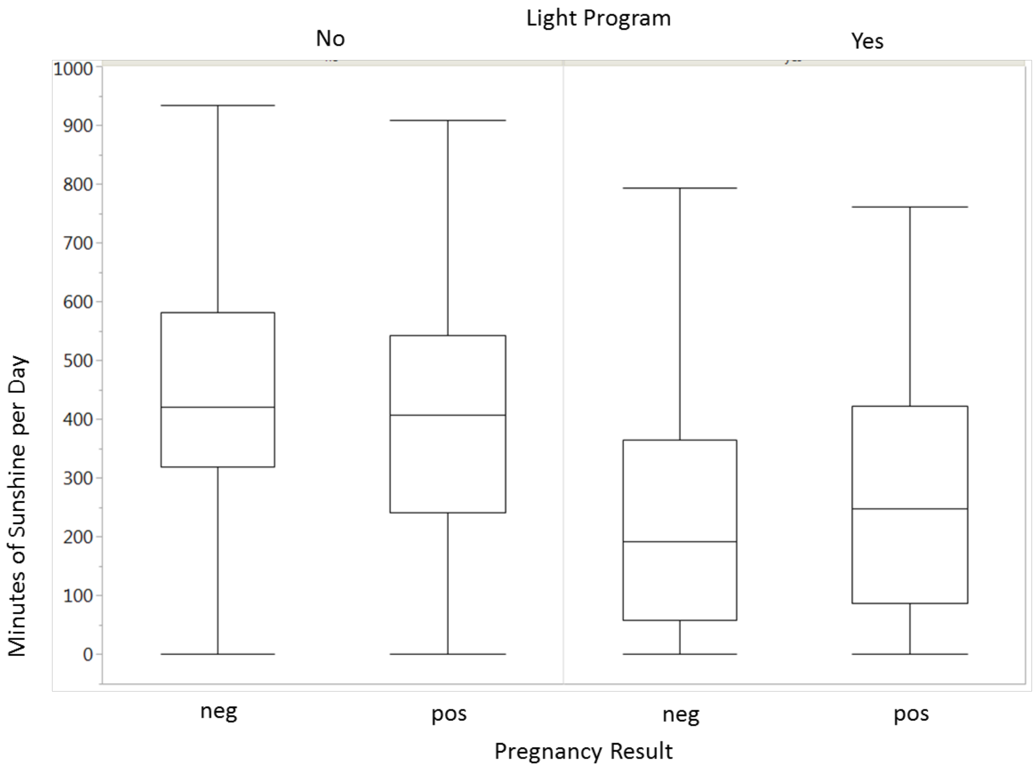


Figure 7-31: Stratification of pregnancy result by light program and sunshine per day in minutes shown as boxplot

The data without a light program and a negative result on pregnancy showed that the range is zero to 930 minutes of sunshine per day. The boxplot itself is from 310 to 590 minutes of sunshine per day with a median of 410 minutes of sunshine per day. The upper quartile is from 410 to 590 minutes of sunshine per day and lower quartile is from 320 to 410 minutes of sunshine per day. Without light program the data with a pregnancy archived shows that the range is from zero to 900 minutes of sunshine per day. The boxplot itself is from 250 to 540 minutes of sunshine per day showing the median at 405 minutes of sunshine per day. The upper quartile is from 405 to 540 minutes of sunshine per day and the lower quartile from 250 to 405 minutes of sunshine per day. The data without light program and a positive pregnancy result show that the range zero to 780 minutes of sunshine per day. The boxplot is at 90 to 410 minutes of sunshine per day and the median at 250 minutes of sunshine per day. The upper quartile is from 250 to 410 minutes of sunshine per day and the lower quartile from 90 to 250 minutes of sunshine per day. With light program the data were a pregnancy was archived shows that the range is from zero to 800 minutes of sunshine per day with the boxplot located at 60 to 360 minutes of sunshine per day. The median is at 200 minutes of sunshine per day. The upper quartile is from 200 to 360 minutes of sunshine per day and the lower quartile is from 60 to 200 minutes of sunshine per day. It, therefore, looks as if with light program a lower amount of minutes of sunshine was needed to achieve pregnancy.

Analysing the data per biological phase with a boxplot a difference could be seen in ovulation for the data with light program. The mean of the data for not achieving a pregnancy is at 25 minutes of sunshine per day and the mean for achieving pregnancy is at 100 minutes of sunshine per day. In all other biological phases no difference was visible as the figure 7-32 shows.

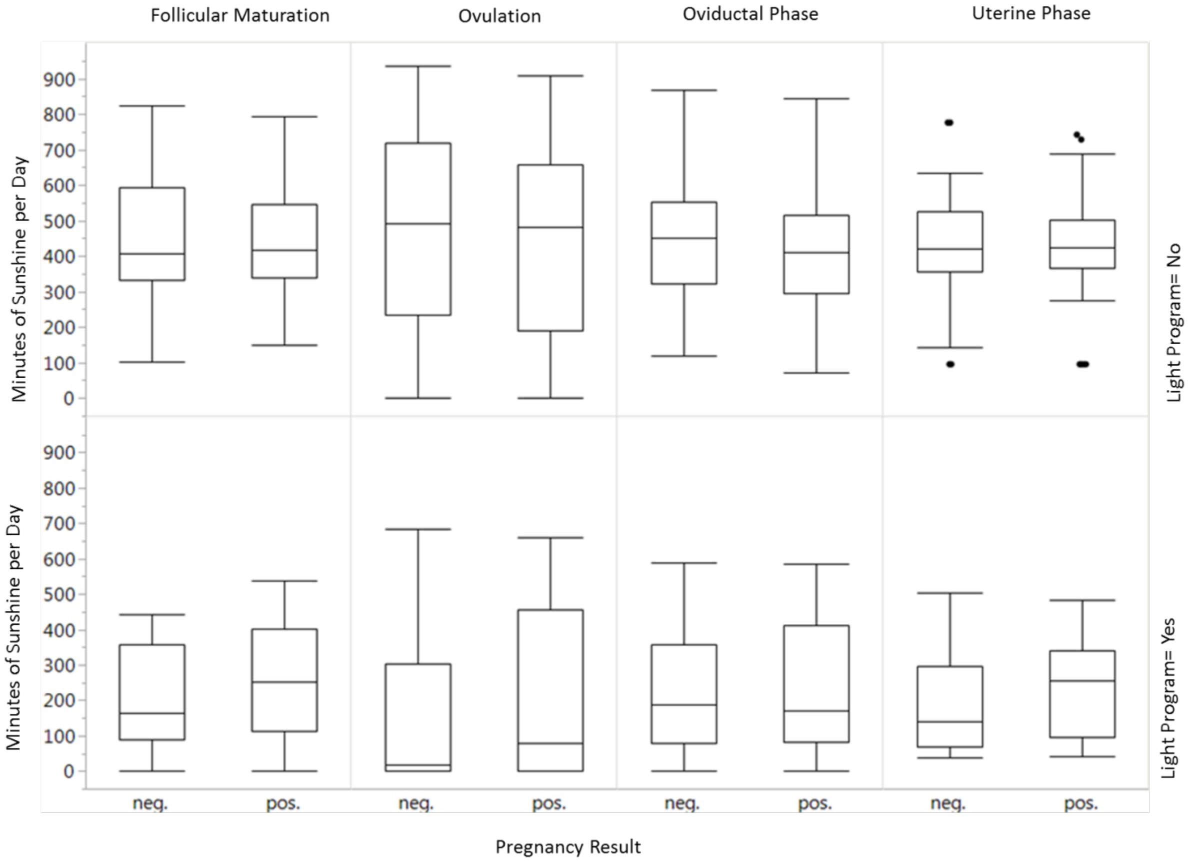


Figure 7-32: Stratification of pregnancy result by light program and sunshine per day in minutes per biological phase shown as boxplot

7.9 Effect of Reproductive Parameters on Pregnancy Result

7.9.1 Mares

The data on mares is nominal. Nominal scales are used for labelling variables without any quantitative value. All of these scales are mutually exclusive so there is no overlap and none of them have any numerical significance. The graphic 7-33 shows the absolute of pregnancies per mare over the period of the four years the study was done. Most records exist on are 14 (100 records) and Mare 15 (100 records). However, the pregnancy result of Mare 14 was 52% positive pregnancies and 48% negative pregnancies. The result for Mare 15 (Age 3 years) was 76% pregnancy result. The second-largest amount of records exist on Mare 4 (Age 18 years) (84 records and 57% positive results) and on Mare 5 (Age 9 years) (80 records and 55% positive results). 76 records exist on Mare 10 (Age 2 years) with 47% positive results. Highest rates in terms of achieving pregnancy independent from the number of pregnancies were Mare 1 (Age 16 years) with 77% and Mare 18 (Age 8 years) with 73%; Mare 2 (Age 13 years) has only one data point which was a no pregnancy. The worst result was achieved by Mare 19 (Age 4 years) with 17%. All other mares have pregnancy rates around 50%.

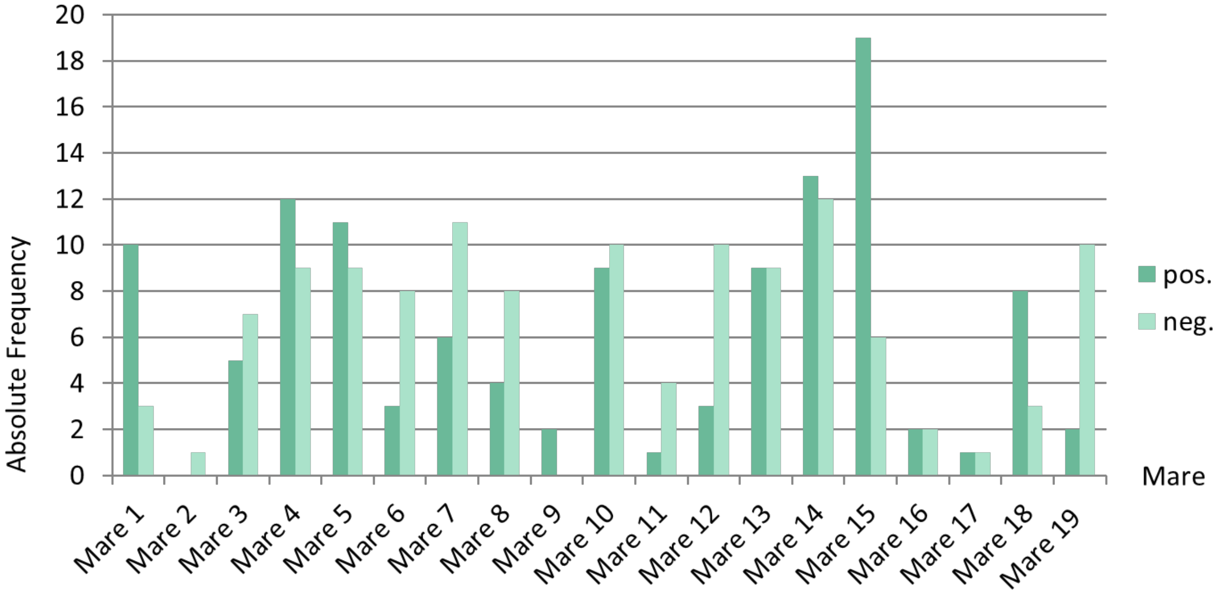


Figure 7-33: Mare and pregnancy result

7.9.2 Dominant Follicle Area

A certain size of the dominant follicle is required to artificially inseminate the mare and achieve pregnancy (Hinrichs, 1998). The measurements resulted in 968 records reported in square millimetre [mm^2] of dominant follicle area. The descriptive statistics presented the median of 1382.3mm^2 and the mean of 1402.8mm^2. The minimum value was 632.2mm^2 and the maximum value was 2548.6mm^2. The classes were based on those numbers and resulted in the classes shown in the table 7-13.

Classes of Dominant Follicle Area in mm^2	
1	0 – 1000
2	1000 – 1500
3	1500 – 2000
4	2000 – 2600

Table 7-13: Classes of dominant follicle area in mm^2

In this data which included 968 records more than half of the data (519 records which are 54%) existed on the follicle size area of 1000mm^2 to 1500mm^2. The second-largest amount of data was available on the size 1500mm^2 to 2000mm^2 (251 records which are 26%). When evaluating at the other two classes (0mm^2 to 1000mm^2 and 2000mm^2 to 3000mm^2) limited amount of data was available (63 records which are 6.5%). In follicular maturation only 30% of cases resulted in pregnancy in the first class (0mm^2 to 1000mm^2). The middle classes from 1000mm^2 to 1500mm^2 and 1500mm^2 to 2000mm^2 showed very similar results: 55% and 66% respectively of the data showed a pregnancy. In the higher class from 2000mm^2 to 2600mm^2 56% of results were negative. In ovulation the data was similar to follicular maturation; 37% of cases result in pregnancy in the class 0mm^2 to 1000mm^2. The middle classes showed very similar regarding the result 1000mm^2 to 1500mm^2 and 1500mm^2 to 2000mm^2 52% and 56% respectively of the data showed a pregnancy. In the higher class from 2000mm^2 to 2600mm^2 80% of results were negative. In the oviductal phase only 23% of cases resulted in pregnancy in the class from 0mm^2 to 1000mm^2. In all other classes in at least 52% (up to 56%) of the cases a pregnancy was achieved. In the uterine phase the results were similar with follicular maturation and ovulation; only 38% of cases resulted in pregnancy in the class 0mm^2 to 1000mm^2. The middle classes were equal regarding the result 1000mm^2 to 1500mm^2 and 1500mm^2 to 2000mm^2 both showed a pregnancy in 52% of the cases. In the higher class from 2000mm^2 to 2600mm^2 56% of results were negative. In summary, the data shows that pregnancy rates were highest with a dominant follicle size area of 1000mm^2 to 2000mm^2 due to highest pregnancy rates and largest amount of data available.

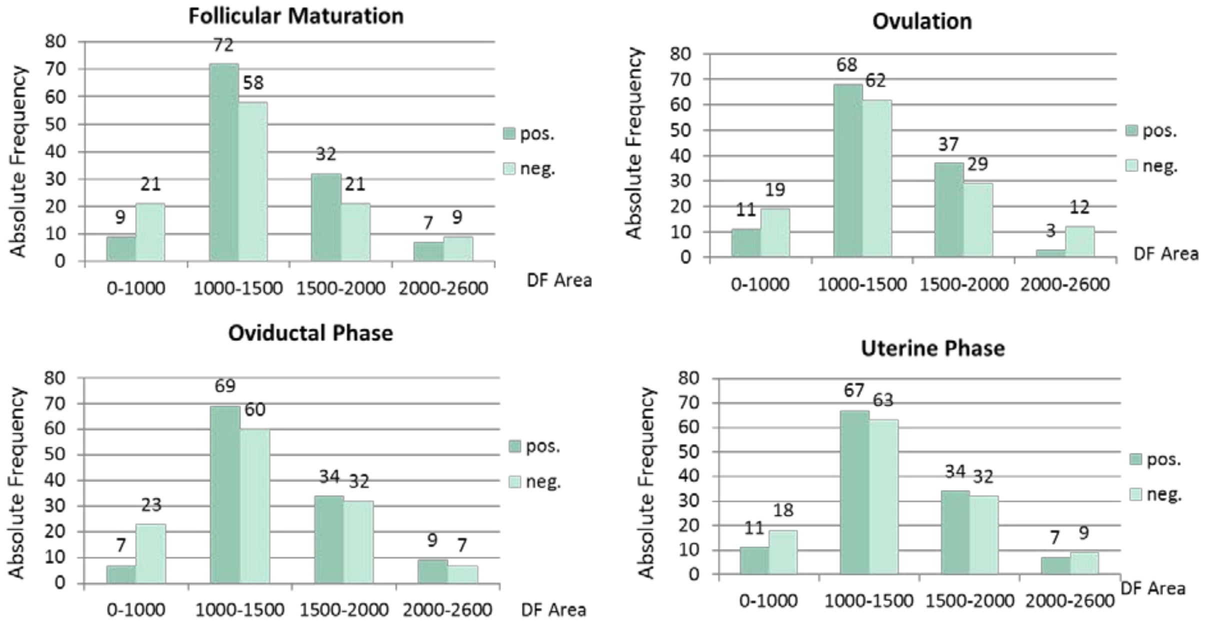
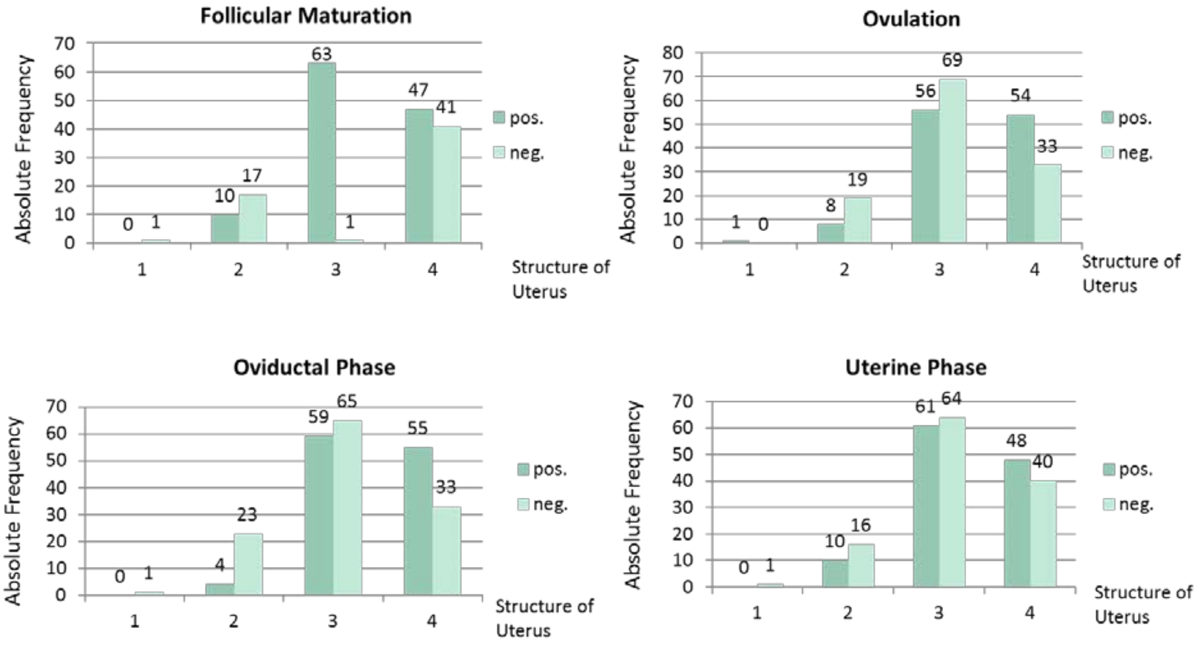


Figure 7-34: Size of dominant follicle (DF) area in mm^2 on pregnancy result per biological phase

7.9.3 Structure of Uterus/Uterine Edema

A certain structure of the uterus/uterine edema is required in which the mare is inseminated. In this data which included 968 records, the classes were defined on basis of the ordinal data which were described in the first part of this dissertation. Here it can be noted that most data existed on uterus structure classified as 3 (48.3%) and 4 (36.3%) – this applies for all phases. In follicular maturation a clear effect for a pregnancy result could be seen for uterus structure 3: 99% of the data showed a pregnancy. In the other classes no big differences could be identified (~50% positive and ~50% negative). In ovulation there was a clear effect for a lower pregnancy rate (70% of results negative) with the uterus structure 2. With the uterus structure being 3 the results for pregnancy was low (45%) and with the uterus structure being 4 the pregnancy rate was 62%. In the oviductal phase 85% of the cases show a negative result with a uterus structure of 2. 52% of the results were negative with a uterus structure of 3 and 63% pregnancies were achieved with a uterus structure of 4. In the uterine phase in class 2 only 38% pregnancies were achieved. In the other classes the results were ~50% positive and ~50% negative. In summary pregnancy rates were highest with a structure of uterus/uterine edema of 2 to 4.



Follicular Maturation

Ovulation

Oviductal Phase

Uterine Phase

Figure 7-35: Structure of uterus on pregnancy result per biological phase

7.9.4 Time Point of Artificial Insemination

A certain time point is required in which the mare is artificially inseminated. In this data which included 968 records, the classes were defined on basis of the ordinal data which was already described in the first part of this dissertation. Here it can be noted that most data existed on <48 which was artificial insemination less than 48 hours before ovulation (76%); here 56% pregnancies could be achieved. Within artificial insemination at >48 hours before ovulation the pregnancy rate was 36% (respectively 33% in follicular maturation). In the phase of artificial insemination at <6 hours after ovulation the pregnancy rate was 17%. In summary highest pregnancy rate was achieved with the artificial insemination within <48 hours before ovulation.

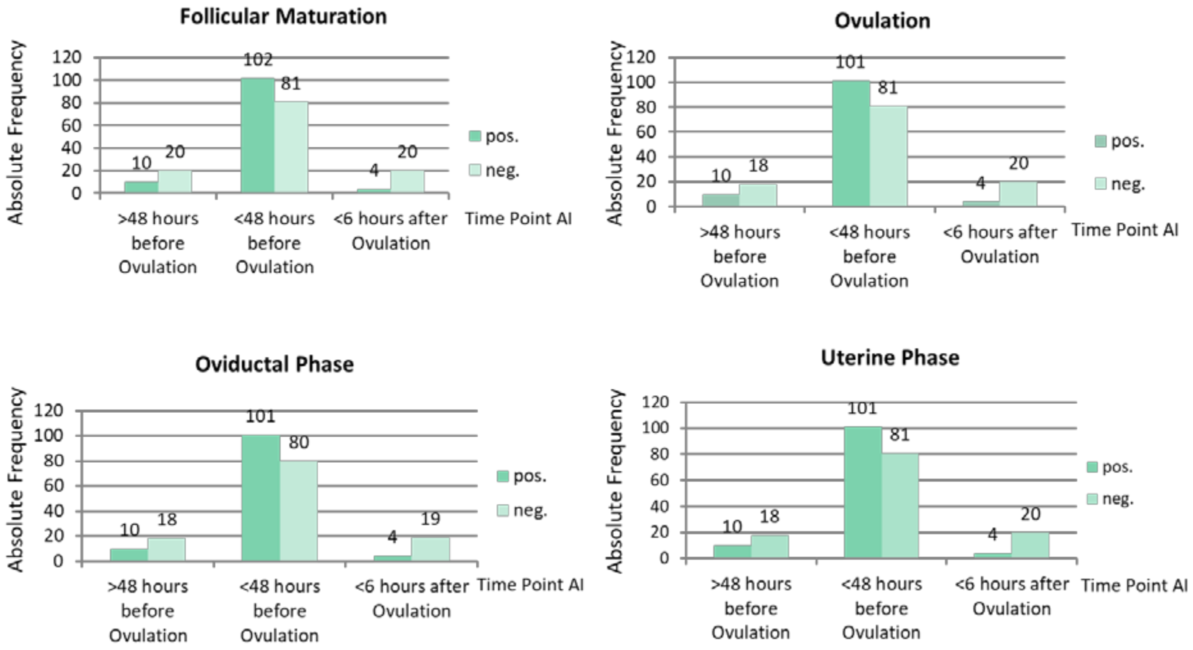


Figure 7-36: Time point of artificial insemination (AI) and pregnancy result per biological phase

7.9.5 Stallions

The data on stallion is nominal without quantitative values and mutually exclusive. 26 stallions were used for insemination of the 19 mares over a period of four years.

The figure 7-37 shows the different stallions and the effect on the pregnancy result in the horse mare.

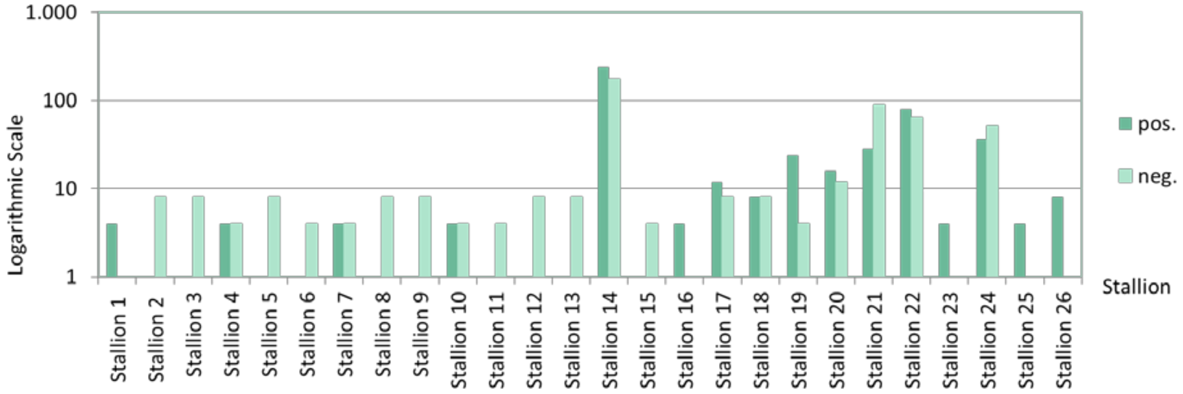


Figure 7-37: Stallions and pregnancy result shown in a logarithmic scale

The data presented that only a couple of stallions were used. Stallion 14 was used in 43% of artificial inseminations, Stallion 22 in 15% of artificial inseminations, Stallion 21 in 12% and Stallion 24 in 10% of artificial inseminations. Four stallions were used in 79% of artificial inseminations. Stallion 21 had a high amount of negative results (76% negative). Stallion 14 showed a high amount of positive pregnancy results (57% positive). In all other stallions, no marked difference in the pregnancy result could be identified.

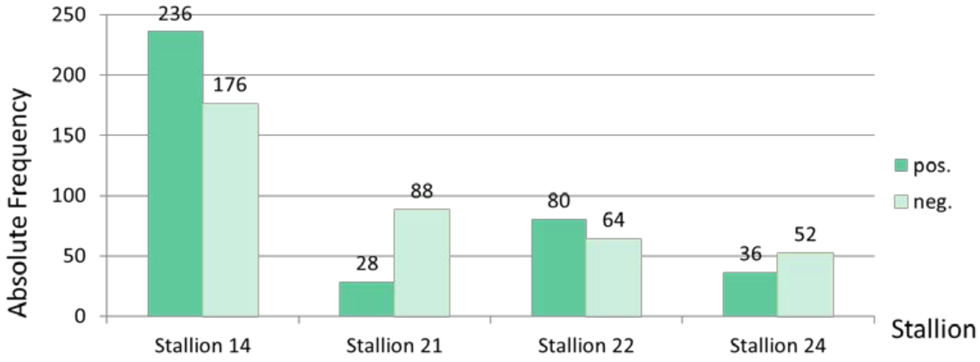


Figure 7-38: Four most used (79%) stallions and pregnancy result

7.10 Summary of Descriptive Statistics and Decision on Statistical Methods

Analysing the data there was continuous, ordinal and nominal data accessible. According to the descriptive statistics the data appeared to influence each other in various ways and there seemed to be an effect of weather on the pregnancy result dependent on various reproductive parameters.

The core assumptions and hypotheses based on the descriptive analyses are shown in the table 7-14. These were further investigated in the statistical analysis.

Parameter	Hypothesis
Temperature and Humidity were shown as THI	• A THI (>60) in any biological phase does not lead to pregnancy (negative result) • A linear relationship could be possible
Air Pressure	• Pregnancy rates are lower at higher air pressure (>1020 hPa) • No linear relationship does exist
Minutes of Sunshine per Day	• There is no effect of minutes of sunshine per day on the pregnancy result • No linear relationship does exist • Sunlight and light program interact and effect the pregnancy result • A lower amount of minutes of sunshine is needed to achieve pregnancy if horses are exposed to a light program
Longwave Downward Radiation	• Longwave downward radiation (>2500J/cm^2) does not lead to pregnancy (negative result) in ovulation, oviductal phase, and uterine phase • Higher longwave downward radiation (>3000J/cm^2) does not lead to pregnancy (negative result) in all biological phases • No linear relationship does exist
Diffuse Solar Radiation	• Diffuse solar radiation (>500J/cm^2) does not lead to pregnancy (negative result) in follicular maturation, oviductal phase, and uterine phase • No linear relationship does exist
Incoming Solar Radiation	• Incoming solar radiation (>1000J/cm^2) does not lead to pregnancy (negative result) in follicular maturation and oviductal phase • Incoming solar radiation (>2000J/cm^2) does not lead to pregnancy (negative result) in ovulation and uterine phase • No linear relationship does exist
Light Program	• The pregnancy rates were lower for the data with light program • With light program a lower amount of minutes of sunshine is needed to achieve pregnancy; an interaction does exist between minutes of sunshine per day and light program • No linear relationship does exist
Month	• Overall 55.1% records were available in the first half of the year and 44.9% on the second half of the year • At the beginning of the year, more pregnancies were achieved (58.3%) compared to the second half of the year (41.6%)
Dominant Follicle Area	• Pregnancy rates were highest with a dominant follicle size area of 1000mm^2 to 2000mm^2

Parameter	Hypothesis
Structure of Uterus/Uterine Edema	• The structure of the uterus/uterine edema being 2 to 4 increases pregnancy rates
Time Point of Artificial Insemination before and after ovulation	• In summary, the highest pregnancy rate is achieved with artificial insemination within 48 hours before ovulation as well as 6 hours after ovulation
Mare	• There is an effect on pregnancy result according to which mare is used
Stallion	• There is an effect on pregnancy result according to which stallion is used

Table 7-14: Core statements and hypotheses derived from descriptive statistics

8 Statistical Methods

The previously mentioned data was analysed with a generalized linear mixed model, in detail a logistic regression with a binary outcome which was the pregnancy result (positive or negative) as the dependent variable. Unbalanced data (more observations from one horse than another) and repeated data (several observations per horse) were accounted for in the logistic regression. The hypotheses summarized in the table at the end of the descriptive part built the basis of the logistic regression. For the statistical analysis SAS 9.4 was used, in particular the GLIMMIX procedure as it enables to specify a generalized linear mixed model and to perform confirmatory inference in such models. The influence of each weather parameter (temperature, humidity, Temperature-Humidity Index (THI), air pressure, longwave downward radiation, diffuse solar radiation, solar incoming radiation and minutes of sunshine per day) and each reproduction-related parameter (size of the dominant follicle and the structure of the uterus/uterine edema) was analysed separately for every biological phase of the mare (i.e. the influence of average temperature during follicular maturation on pregnancy result, the influence of average temperature during ovulation on pregnancy result, the influence of average temperature during oviductal phase on pregnancy result, the influence of average temperature during uterine phase on pregnancy result and same analysis for every weather parameter stated above). In addition, the weather and reproductive parameters over all phases were analysed, if no difference was identified between the biological phases in the descriptive statistics.

These analyses are based on the model.

$$\boxed{\text{Model: } y = r + w + j + m*s + e}$$

With:

y = pregnancy result 1/0

r = one/several reproductive parameter

w = one/several weather parameters

j = year (2013, 2014, 2015, 2016)

m*s = effect of the individual combination of mare and stallion

e = random error

All analysis and models are described in detail in the chapters 2.1 and 2.2.

8.1 Logistic Regression with Random Effects – Linear (Glimmix in SAS 9.4) – Analysis 1 to3

Due to the results from the descriptive statistics, three analyses were done in SAS 9.4 to show if a linear relationship existed between the parameters using the proc Glimmix in SAS 9.4.

1. All Parameters (Weather and Reproductive) per Biological Phase	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameters: Temperature-Humidity Index (THI), Air Pressure, Minutes of Sunshine per Day, Longwave Downward Radiation, Diffuse Solar Radiation, Solar Incoming Radiation 2. Reproductive Parameters: Time Point of Artificial Insemination, Dominant Follicle Area, Uterus Structure/Uterine Edema
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-1: All parameters (weather and reproductive) per biological phase

2. All Parameters (Weather and Reproductive) per Biological Phase and Year	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameters: Temperature-Humidity Index (THI), Air Pressure, Minutes of Sunshine per Day, Longwave Downward Radiation, Diffuse Solar Radiation, Solar Incoming Radiation 2. Reproductive Parameters: Time Point of Artificial Insemination, Dominant Follicle Area, Uterus Structure/Uterine Edema 3. Year
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-2: All parameters (weather and reproductive) per biological phase and year

3. All Parameters (Weather and Reproductive)	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameters: Temperature-Humidity Index (THI), Air Pressure, Minutes of Sunshine per Day, Longwave Downward Radiation, Diffuse Solar Radiation, Solar Incoming Radiation 2. Reproductive Parameters: Time Point of Artificial Insemination, Dominant Follicle Area, Uterus Structure/Uterine Edema
Random Effects	Mare and Stallion
Biological Phase	All Biological Phases in one analysis

Table 8-3: All Parameters (weather and reproductive)

8.2 Generalized linear mixed model with Random Effects with Weather Parameters subdivided into Classes (Glimmix in SAS 9.4) -Analysis 4

The weather parameters were subdivided into classes according to the results from the descriptive statistics and it was analysed if a correlation existed in a certain class of the parameters. The time point of artificial insemination was kept in the analysis. Correspondingly, the random effect of the individual combination of mare and stallion was kept in the analysis.

In this analysis (4a to 4g) seven statistical analyses were done investigating every single weather parameter in a separate analysis. The single weather parameters are summarized in the table 8-4.

Weather Parameter	Classes
a. THI Divided into the same classes as done in the descriptive analysis.	1. 30 - 40 2. 40 - 50 3. 50 - 60 4. 60 - 70 5. 70 – 80
b. Air Pressure (in hPa) Divided into the same classes as done in the descriptive statistics.	1. 1000 - 1005 2. 1005 - 1010 3. 1010 - 1015 4. 1015 - 1020 5. 1020 – 1025
c. Sunshine (0 - 1000 Minutes per Day) Divided into the same classes as done in the descriptive statistics and letters were dedicated to every class. Classes are A to D available.	A = 0 - 250 Minutes B = 250 - 500 Minutes C = 500 - 750 Minutes D = 750 - 1000 Minutes
d. Sunshine (0 - 250 Minutes per Day) Divided into smaller classes and letters were dedicated to every class. The classes A to D are available.	A = 0 - 60 Minutes B = 60 - 120 Minutes C = 120 - 180 Minutes D = 180 - 250 Minutes
e. Longwave Downward Radiation [in J/m^2] Divided into the same classes as done in the descriptive statistics.	A = 0 - 1500 J/m^2 B =1500 - 2000 J/m^2 C.= 2000 - 2500 J/m^2 D. = 2500 - 3000 J/m^2 E. = 3000 - 3500 J/m^2
f. Diffuse Solar Radiation [in J/m^2] Divided into the same classes as done in the descriptive analysis and letters were dedicated to every class. Classes A to C are available.	A = 0 - 500 J/m^2 B = 500 - 1000 J/m^2 C = 1000 - 1500 J/m^2
g. Solar Incoming Radiation [in J/m^2] Divided into the same classes as done in the descriptive analysis and letters were dedicated to every class. Classes A to D are available.	A = 0 - 1000 J/m^2 B = 1000 - 2000 J/m^2 C = 2000 - 3000 J/m^2 D = 3000 - 4000 J/m^2

Table 8-4: Analysis of logistic regression with random effects with weather parameters sub-divided in classes

In the different analyses with one single weather parameter furthermore, all reproductive parameters (time point of artificial insemination, dominant follicle area, uterus structure/uterine edema in the analysis per biological phase) were kept in the analysis. This led to the analysis as shown in the table 8-5 .

4. Seven Statistical Analysis investigating every Weather Parameter and Reproductive Parameters in a separate Analyses as shown in the previous table per Biological Phase	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Single Weather Parameter a. Temperature-Humidity Index (THI) b. Air Pressure c. Minutes of Sunshine per Day with Classes (0-1000 Minutes of Sunshine per Day) d. Minutes of Sunshine per Day with Classes (250-1000 Minutes of Sunshine per Day) e. Longwave Downward Radiation f. Diffuse Solar Radiation g. Solar Incoming Radiation 2. Reproductive Parameters all included in every analysis: Time Point of Artificial Insemination, Dominant Follicle Area, Uterus Structure/Uterine Edema
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-5: Seven statistical analysis investigating every weather parameter and reproductive parameters in a separate analyses as shown in the previous table per biological phase

8.3 Influence of Light Program and Minutes of Sunshine per Day on Pregnancy Result via the Logistic Regression with Random Effects with Weather Parameters subdivided into Classes (Glimmix in SAS 9.4) – Analysis 5

As shown in the descriptive statistics, it might have been possible that sunshine and light program together had an influence on the pregnancy result, because the light program was continued in the winter month mimicking summer in minutes of sunshine per day. In order to further analyse the effect of the light program and identify a possible interaction to minutes of sunshine per day the data was separated in this analysis (number 5a to 5j) investigating the data for which no a light program was implemented and secondly taking only the data collected from times when a light program was done. In addition, the different classes of sunshine regarding the number of minutes of sunshine per day were analysed. The time point of artificial insemination was kept in the analysis. Correspondingly, the random effect of the individual combination of mare and stallion was kept in the analysis. Ten analyses were done assessing all biological phases together as well as the individual biological phases. The classes of sunshine minutes per day (0 - 250 sunshine of minutes per day and 0 - 1000 minutes of sunshine per day) were analysed. Furthermore, two analyses were done using the classes according to the frequency of records per class, again without and with light program. The tables 8-6 to 8-15 give an overview of the analyses and which independent variables were used.

a. Only Data without Light Program in all Biological Phases	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Sunshine in Minutes per Day b. Light Program = No 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed including all Biological Phases

Table 8-6: Only data without light program all biological phases

b. Only Data without Light Program per Biological Phase	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Sunshine in Minutes per Day b. Light Program = No 2. Reproductive Parameter: Time of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-7: Only data without light program per biological phase

c. Only Data without Light Program per Biological Phase in Classes of Minutes of Sunshine per day (0-250 Minutes)	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Sunshine in Minutes per Day b. Light Program = No 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Non (as data did not converge when leaving the random effect in the analysis)
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-8: Only data without light program all biological phases in classes of minutes of sunshine per day (0-250 minutes)

d. Only Data without Light Program per Biological Phase in Classes of Minutes of Sunshine per day (250 – 1000 Minutes)	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Sunshine in Minutes per Day b. Light Program = No 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-9: Only data without light program all biological phases in classes of minutes of sunshine per day (250 - 1000 minutes)

e. Only data with Light Program per Biological Phases in Classes of Sunshine of Minutes per Day based on the frequency of records (0 - 1000 Minutes)	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Sunshine in Minutes per Day b. Light Program = Yes 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-10: Only data with light program in all biological phases in classes of sunshine of minutes per day based on the frequency of records (0 - 1000 minutes of sunshine per day)

f. Only Data with Light Program in all Biological Phases	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Sunshine in Minutes per Day b. Light Program = Yes 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed including all Biological Phases

Table 8-11: Only data with light program all biological phases

g. Only Data with Light Program per Biological Phase	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Sunshine in Minutes per Day b. Light Program = Yes 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-12: Only data with light program per biological phase

h. Only Data with Light Program per Biological Phase in Classes of Minutes of Sunshine per Day (0 – 250 Minutes)	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Sunshine in Minutes per Day b. Light Program = Yes 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-13: Only data with light program all biological phases in classes of minutes of sunshine per day (0 – 250 minutes)

i. Only Data with Light Program per Biological Phase in Classes of Minutes of Sunshine per Day (250 - 1000 Minutes)	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Sunshine in Minutes per Day b. Light Program = Yes 2. Reproductive Parameter: Time of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase (without random effect as data did not converge)

Table 8-14: Only data with light program all biological phases in classes of minutes of sunshine per day (250 - 1000 minutes)

j. Only Data With Light Program per Biological Phase in Classes of Minutes of Sunshine per Day based on Frequency of Records (0 - 1000 Minutes)	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Sunshine in Minutes per Day b. Light Program = Yes 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-15: Only data with light program in all biological phases in classes of minutes of sunshine per day based on frequency of records (0 - 1000 minutes)

8.4 Influence of Light Program and Longwave Downward Radiation on Pregnancy Result via the Logistic Regression with Random Effects with Weather Parameters subdivided into Classes (Glimmix in SAS 9.4) – Analysis 6

As the analysis 4e showed a significant effect in longwave downward radiation in follicular maturation (p=0.0146), it will, therefore, be further analysed (in analysis 6a to 6f) if there was also an interaction with the light program and if both together had an influence on the pregnancy result. The data was separated in these analyses investigating the data where no light program was done and secondly taking only the data where a light program was done. In addition, the different classes of longwave downward radiation were analysed. The time point of artificial insemination was kept in the analysis. Correspondingly, the random effect of the individual combination of mare and stallion was kept in the analysis. Ten analyses were done assessing all biological phases together as well as the individual biological phase. The three classes of longwave downward radiation were analysed (1500 - 3500 J/m^2). The tables 8-16 to 8-21 give an overview on the analyses and which independent variables were used.

a. Only Data without Light Program all Biological Phases	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Longwave Downward Radiation b. Light Program = No 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed including all Biological Phases

Table 8-16: Only data without light program all biological phases

b. Only Data without Light Program per Biological Phase	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Longwave Downward Radiation b. Light Program = No 2. Reproductive Parameter: Time of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-17: Only data without light program per biological phase

c. Only data without Light Program per Biological Phase in Classes of Longwave Downward Radiation (1500 - 3500 J/m^2)	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Longwave Downward Radiation b. Light program = No 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-18: Only data without light program in all biological phases in classes of longwave downward radiation were analysed (1500 - 3500 J/m^2

d. Only Data with Light Program in all Biological Phases	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	b. Weather Parameter: a. Longwave Downward Radiation b. Light Program = Yes c. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed including all Biological Phases

Table 8-19: Only data with light program all biological phases

e. Only Data with Light Program per Biological Phase	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Longwave Downward Radiation b. Light Program = Yes 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-20: Only data with light program per biological phase

f. Only Data with Light Program per Biological Phase in Classes of Longwave Downward Radiation (1500 - 3500 J/m^2)	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: a. Longwave Downward Radiation b. Light Program = Yes 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-21: Only data with light program all biological phases in classes of longwave downward radiation (1500 - 3500 J/m^2)

8.5 Influence of Weather Parameters around Artificial Insemination via the Logistic Regression with Random Effects (Glimmix in SAS 9.4) – Analysis 7

Because literature reported a high influence of temperature and Temperature-Humidity-Index (THI) on the days around artificial insemination (AI) on fertility, it was analysed if there was any effect detectable in the present data. In this analysis the data five days prior and 5 days after ovulation will be analysed. The time point of artificial insemination was kept in the analysis. Correspondingly, the random effect of the individual combination of mare and stallion was kept in the analysis.

7. Analysis on Influence of Weather Parameters around Artificial Insemination	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: Maximum Temperature, Average Temperature, THI 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Time Point of Artificial Insemination • 5 days pre AI • 5 days post AI

Table 8-22: Analysis on influence of weather parameters around AI

8.6 Influence of Weather Parameters around Artificial Insemination via the Logistic Regression with Random Effects subdivided in Classes (Glimmix in SAS 9.4) – Analysis 8

In addition, the different classes of maximum temperature, average temperature and Temperature-Humidity Index (THI) were analysed. The time point of artificial insemination was kept in the analysis. Correspondingly, the random effect of the individual combination of mare and stallion was kept in the analysis.

For maximum temperature, the classes were divided in steps of five degrees Celsius [°C] starting at -10 °C up to 40 °C. For average temperature, it was done equally with the highest class of 35 °C. For THI the same classes as shown in the descriptive part were used.

8. Analysis on Influence of Weather Parameters around Artificial Insemination divided in Classes	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameter: Maximum Temperature, Average Temperature, THI 2. Reproductive Parameter: Time Point of Artificial Insemination
Random Effects	Mare and Stallion
Biological Phase	Data analysed per Time Point of Artificial Insemination • 5 days pre AI • 5 days post AI

Table 8-23: Analysis on influence of weather parameters around Artificial Insemination divided in classes

8.7 Influence of Flush and Sonography on the Pregnancy Result in the Next Cycle via the Logistic Regression with Random Effects (Glimmix in SAS 9.4) – Analysis 9 a, b

It was further analysed if the method of confirmation of pregnancy (Flush or Sonography) had an effect on the pregnancy result within the next cycle of insemination including and the weather parameters. As the flush was one at different time points (Day 6, 7, 7,5 and 8) in the first step all time points of flush were analysed separately and in a second step the time points were put into one groups so that less groups were available in the analysis. As for sonography no different time points were available this method was not further grouped. The time point of artificial insemination was kept in the analysis. Correspondingly, the random effect of the individual combination of mare and stallion was kept in the analysis.

9 a. Analysis on Influence of Flush done at different Time Points and Sonography on the Pregnancy Result in the Next Cycle	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameters: Temperature-Humidity Index (THI), Air Pressure, Minutes of Sunshine per Day, Longwave Downward Radiation, Diffuse Solar Radiation, Solar Incoming Radiation 2. Reproductive Parameters: Method of Confirmation of Pregnancy (all days of flush analysed separately = Day 6, 7, 7,5 and 8), Time Point of Artificial Insemination, Number of Cycle
Random Effects	Non (as data did not converge when leaving the random effect in the analysis)
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-24: Analysis on influence of flush done at different time points and sonography on the pregnancy result in the next cycle

9 b. Analysis on Influence of Flush (all in one group) and Sonography on the Pregnancy Result in the Next Cycle	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameters: Temperature-Humidity Index (THI), Air Pressure, Minutes of Sunshine per Day, Longwave Downward Radiation, Diffuse Solar Radiation, Solar Incoming Radiation 2. Reproductive Parameters: Method of Confirmation of Pregnancy (all days of flush in one group), Time Point of Artificial Insemination, Number of Cycle
Random Effects	Non (as data did not converge when leaving the random effect in the analysis)
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-25: Analysis on influence of flush (all in one group) and sonography on the pregnancy result in the next cycle

8.8 Influence of Flush and on the Pregnancy Result in the Next Cycle via the Logistic Regression with Random Effects subdivided in Classes (Glimmix in SAS 9.4) – Analysis 10 a, b

To further analyse a possible effect of the method of confirmation of pregnancy (Flush or Sonography) on the pregnancy result within the next cycle of insemination including and the weather parameters. As the flush was one at different time points (Day 6, 7, 7,5 and 8) in the first step all time points of flush were analysed separately and in a second step the time points were put into one groups so that less groups were available in the analysis. As for sonography no different time points were available this method was not further grouped. Weather parameters were divided in the classes as described in the descriptive part: Minutes of sunshine per day, longwave downward radiation, and diffuse solar radiation. The time point of artificial insemination was kept in the analysis. Correspondingly, the random effect of the individual combination of mare and stallion was kept in the analysis.

10 a. Analysis on Influence of Flush done at different Time Points and Sonography on the Pregnancy Result in the Next Cycle divided in Classes	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameters: a. Minutes of Sunshine per Day with Classes (0-1000 Minutes of Sunshine per Day) b. Longwave Downward Radiation c. Diffuse Solar Radiation 2. Reproductive Parameters: Method of Confirmation of Pregnancy (all days of flush analysed separately = Day 6, 7, 7,5 and 8), Time Point of Artificial Insemination, Number of Cycle
Random Effects	Non (as data did not converge when leaving the random effect in the analysis)
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

Table 8-26: Analysis on influence of flush done at different time points and sonography on the pregnancy result in the next cycle divided in classes

10 b. Analysis on Influence of Flush (all in one group) and Sonography on the Pregnancy Result in the Next Cycle divided in Classes	
Dependent Variable	Pregnancy Result – positive (pos) and negative (neg)
Independent Variables	1. Weather Parameters: a. Minutes of Sunshine per Day with Classes (0-1000 Minutes of Sunshine per Day) b. Longwave Downward Radiation c. Diffuse Solar Radiation 2. Reproductive Parameters: Method of Confirmation of Pregnancy (all in one group), Time Point of Artificial Insemination, Number of Cycle
Random Effects	Non (as data did not converge when leaving the random effect in the analysis)
Biological Phase	Data analysed per Biological Phase • Follicular Maturation • Ovulation • Oviductal Phase • Uterine Phase

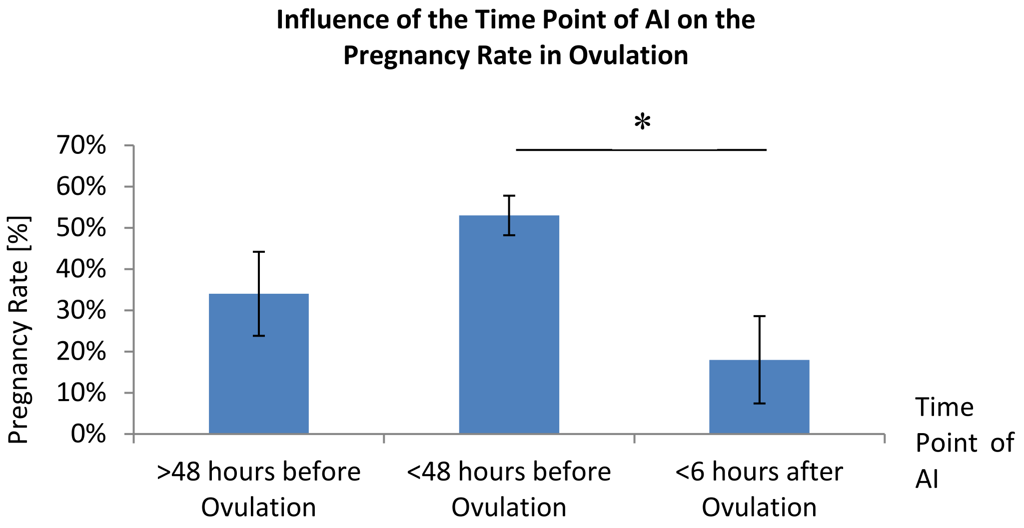
Table 8-27: Analysis on influence of flush (all in one group) and sonography on the pregnancy result in the next cycle divided in classes

9 Results

9.1 Results of the Logistic Regression with Random Effects Linear (Glimmix in SAS 9.4) – Analysis 1 to 3

9.1.1 Analysis 1: All Parameters (Weather and Reproductive) per Biological Phase

Analysing all weather and reproductive parameters with the random effect of the individual combination of mare and stallion, the results showed that in all phases the time point of artificial insemination (AI) had a significant influence (Follicular maturation: p=0.0177, ovulation: p=0.0313, oviductal phase p=0.0284, uterine phase p=0.0118) on the pregnancy result. In all phases a significant difference could be seen between the AI <48 hours before ovulation and <6hours after ovulation. The figure 9.1 represents the back-transformed least-square means and standard errors as well as the significant differences between the time points of AI in ovulation.



* p ≤ 0.05: significant (error probability less than 5%)

Figure 9-1: Influence of the time point of AI on the pregnancy rate [%] in ovulation (back-transformed least-square means and standard errors)

9.1.2 Analysis 2: All Parameters (Weather and Reproductive) per Biological Phase and Year

Analysing all weather and reproductive parameters with the random effect of the individual combination of mare and stallion and taking the year into account in follicular maturation the time point of artificial insemination (AI) had a significant influence (p=0.046) on the pregnancy result but after correction for multiple comparisons there were no significant differences between the time points of AI.

In ovulation diffuse solar radiation had a significant effect (p=0.0160) on the pregnancy result. The odd ratio of 1.002 (estimate of 0.002226) showed that the presence of diffuse solar radiation increased the probability of a positive pregnancy result. However, there is no causality from the odds ratio as multiple weather parameters and the years were analysed in this analysis. Furthermore, when dividing diffuse solar radiation in the classes (1500 - 3500 J/m^2) as outlined in the descriptive part that none of the classes were significant.

In the oviductal phase, the minutes of sunshine per day had a positive and significant influence (p=0.035) on the pregnancy result. Ergo, with more sunshine the chance is higher to achieve pregnancy. Due to the possible interaction of sunshine and light program, more analysis on the effect of minutes of sunshine per day and the division into classes was done in separate analysis in chapter.

In the uterine phase the time point of AI had a significant effect (p=0.0321) on the pregnancy result but after correction for multiple comparisons there were no significant differences between the time points of AI.

9.1.3 Analysis 3: All Parameters (Weather and Reproductive)

Analysing all weather and reproductive parameters and taking into account all phases in one analysis with the random effect of the individual combination of mare and stallion the only parameter that had a significant influence (p=0.0027) in all phases on the pregnancy result was the time point of artificial insemination (AI). The figure 9-2 represents the back-transformed least-square means and standard errors as well as the significant differences between the time points of AI.

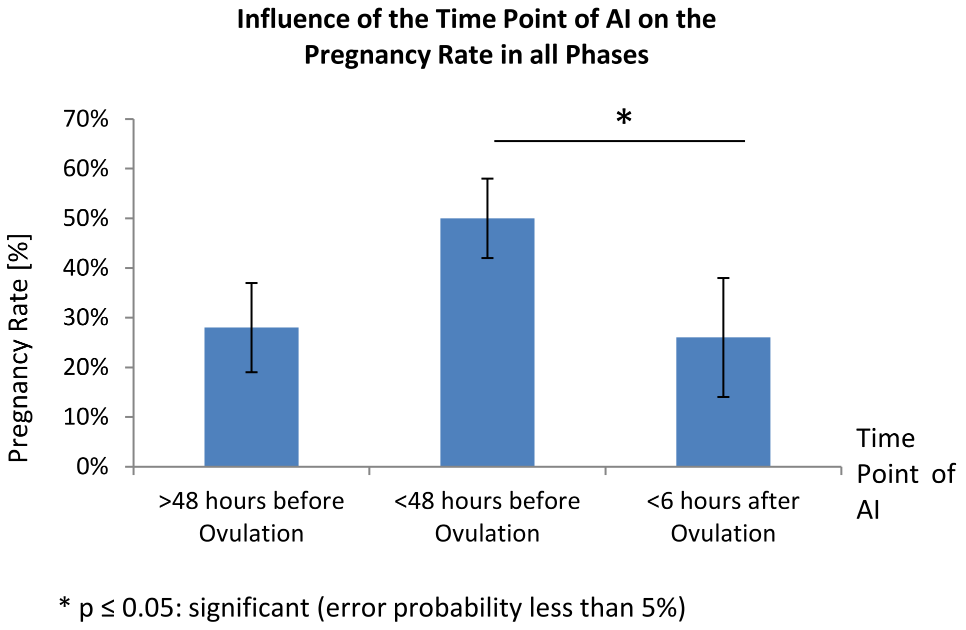


Figure 9-2: Influence of the time point of AI on the pregnancy rate [%] in all phases (back-transformed least-square means and standard errors)

9.2 Results of the Logistic Regression with Random Effects with Weather Parameters subdivided into Classes (Glimmix in SAS 9.4) – Analysis 4

In order to prove the interaction between minutes of sunshine per day and the use of a light program the statistical analysis 4a to 4g was used and showed the results.

9.2.1 Analysis: 4a. Temperature-Humidity Index (THI)

No significant effects (p>0.05) of the THI divided into classes at any of the time points in the mares' cycle) on the probability of a mare to get pregnant were seen.

9.2.2 Analysis 4b: Air Pressure

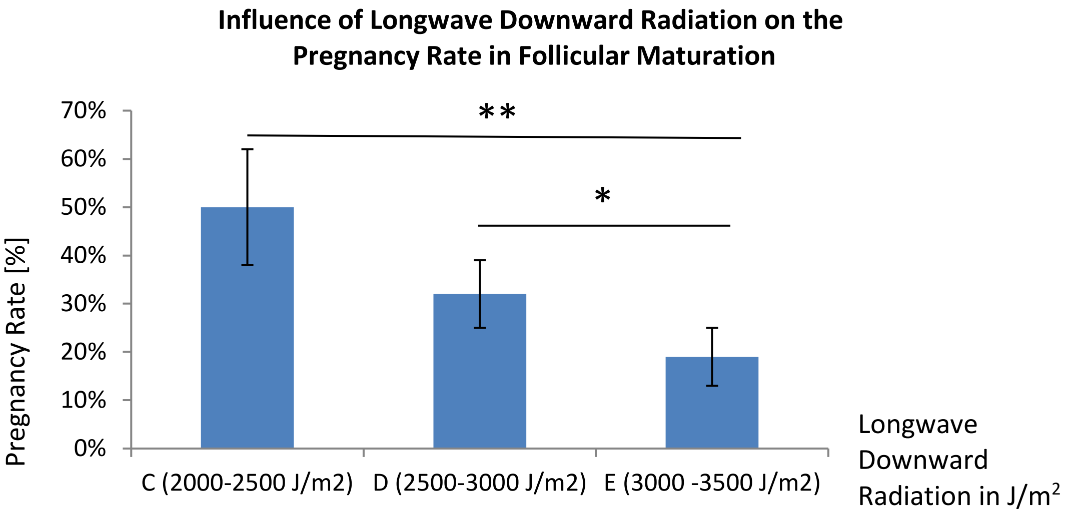
Air Pressure was divided into classes and taken as the independent variable and no significant effects (p>0.05) were seen.

9.2.3 Analysis: 4c + d: Minutes of Sunshine per Day

The minutes of sunshine per day were divided into classes (0 - 1000 minutes of sunshine per day and 0 - 250 minutes of sunshine per day) and taken as the independent variable but no significant effects (p>0.05) were seen.

9.2.4 Analysis 4e: Longwave Downward Radiation

In follicular maturation, longwave downward radiation had a significant effect (p=0.0146) on the pregnancy result. The higher the longwave downward radiation the less chance for a positive pregnancy result. Significant differences after correction for multiple comparisons were seen between the classes C and E (p=0.0051) and the classes D and E (p=0.043). The figure 9-3 represents the back-transformed least-square means and standard errors as well as the significant differences between the classes of longwave downward radiation in follicular maturation.



*   p ≤ 0.05: significant (error probability less than 5%)
** p ≤ 0.01: very significant (error probability less than 1%)

Figure 9-3: Influence of longwave downward radiation on the pregnancy rate [%] in follicular maturation (back-transformed least-square means and standard errors).

Furthermore in all phases the time point of AI had a significant effect (Follicular maturation: p=0.0094, ovulation: p=0.035, oviductal phase: p=0.0182, uterine phase: p=0.0121) on the pregnancy result.

9.2.5 Analysis 4f: Diffuse Solar Radiation

In the analysis were diffuse solar radiation was taken as the independent variable no significant effects (p>0.05) were seen.

9.2.6 Analysis 4g: Incoming Solar Radiation

In the analysis were incoming solar radiation was taken as the independent variable no significant effects (p>0.05) were seen.

9.2.7 Summary of the results of the Logistic Regression with Random Effects with Weather Parameters subdivided into Classes

The table 9-1 gives an overview:

Weather Parameter	Classes	Significance Level
a. THI	1. 30 – 40 2. 40 - 50 3. 50 - 60 4. 60 - 70 5. 70 - 80	$p>0.05$
b. Air Pressure (in hPa)	1. 1000 – 1005 2. 1005 -1010 3. 1010 - 1015 4. 1015 - 1020 5. 1020 - 1025	$p>0.05$
c. Sunshine (0 - 1000 Minutes per Day)	A = 0 - 250 Minutes B = 250 -500 Minutes C = 500 -750 Minutes D = 750 - 1000 Minutes	$p>0.05$
d. Sunshine (0 - 250 Minutes per Day)	A = 0 - 60 Minutes B = 60 - 120 Minutes C = 120 - 180 Minutes D = 180 - 250 Minutes	$p>0.05$
e. Longwave Downward Radiation [in J/m^2]	A = 0 - 1500 J/m^2 B = 1500 - 2000 J/m^2 C = 2000 - 2500 J/m^2 D = 2500 - 3000 J/m^2 E = 3000 - 3500 J/m^2	Follicular maturation: Significant effect ($p=0.0146$) Results visualized in tables in chapter 9.2.4.
f. Diffuse Solar Radiation [in J/m^2]	A = 0 - 500 J/m^2 B = 500 - 1000 J/m^2 C = 1000 - 1500 J/m^2	$p>0.05$
g. Solar Incoming Radiation [in J/m^2]	A = 0 - 1000 J/m^2 B = 1000 - 2000 J/m^2 C = 2000 - 3000 J/m^2 D = 3000 - 4000 J/m^2	$p>0.05$

Table 9-1: Summary of the results of the logistic regression with random effects with weather parameters subdivided into classes

9.3 Results of the Influence of the Light Program on Sunshine of Minutes per Day on the Pregnancy Result via the Logistic Regression with Random Effects with Weather Parameters subdivided into Classes (Glimmix in SAS 9.4) – Analysis 5

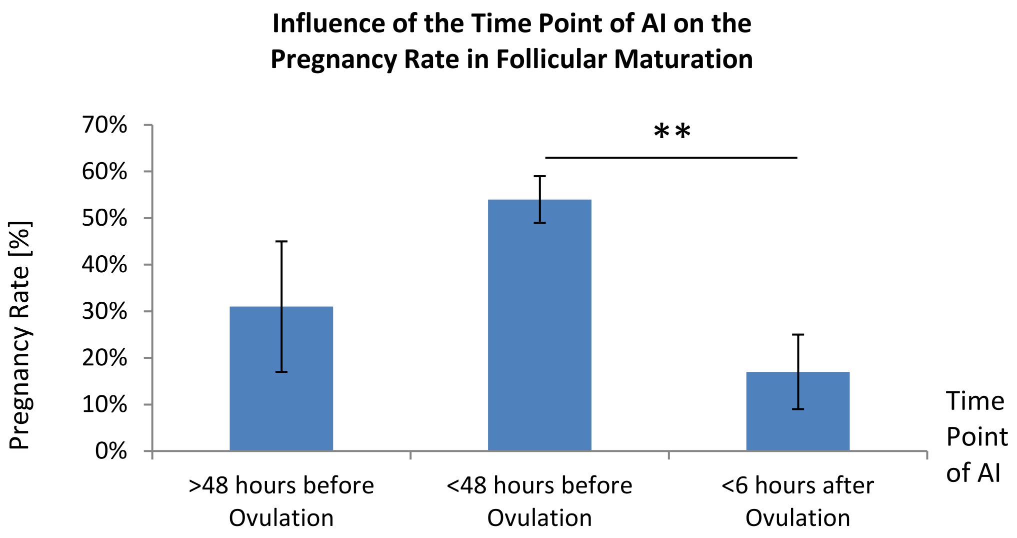
In order to prove the interaction between minutes of sunshine per day and the use of a light program the statistical analysis 5a to 5j showed the results.

9.3.1 Analysis 5a: Only data without the Light Program in all Biological Phases

No significant effects (p>0.05).

9.3.2 Analysis 5b: Only data without Light Program per Biological Phase

The time point of artificial insemination (AI) had a significant influence (Follicular maturation p=0.0091, ovulation p=0.0042, oviductal phase p=0.0068, uterine phase p=0.007) on the pregnancy result. Significant differences after correction for multiple comparisons were seen between the two time points of AI <48 hours before ovulation and <6 hours after ovulation (p<0.01). The figure 9-4 represents the back-transformed least-square means and standard error means as well as the significant differences between the time points of AI in follicular maturation.



** p ≤ 0.01: very significant (error probability less than 1%)

Figure 9-4: Influence of the time point of AI on the pregnancy rate [%] in Follicular Maturation: (back-transformed least-square means and standard errors)

In the ovulation the interaction of minutes of sunshine per day and light program had a significant effect (p=0.0467) on the pregnancy result. The odd ratio of 1.002 (estimate of 0.9987) showed that the interaction of minutes of sunshine per day and light program decreased the probability of a positive pregnancy result. However, there is no causality from the odds ratio as multiple others were analysed in this analysis.

9.3.3 Analysis 5c: Only data without the Light Program per Biological Phase in Classes in Minutes of Sunshine per Day (0 - 250 minutes)

No significant effects (p>0.05)

9.3.4 Analysis 5d: Only data without the Light Program per Biological Phase in Classes of Minutes of Sunshine per Day (250 - 1000 minutes)

No significant effects (p>0.05).

9.3.5 Analysis 5e: Only data without the Light Program per Biological Phase in Classes of Minutes of Sunshine per Day based on Frequency of Records (0 - 1000 minutes)

No significant effects (p>0.05).

9.3.6 Analysis 5f: Only data with the Light Program in all Biological Phases

No significant effects (p>0.05).

9.3.7 Analysis 5g: Only data with Light Program per Biological Phase

In the follicular maturation, the interaction of minutes of sunshine per day and light program had a significant effect (p= 0.0409) on the pregnancy result.

9.3.8 Analysis 5h: Only data with the Light Program per Biological Phase in Classes of Minutes of Sunshine per Day (0 - 250 minutes)

No significant effects (p>0.05).

9.3.9 Analysis 5i: Only data with the Light Program per Biological Phase in Classes of Minutes of Sunshine per Day (250 - 1000 minutes)

No significant effects (p>0.05).

9.3.10 Analysis 5j: Only data with the Light Program per Biological Phase in Classes of Minutes of Sunshine per Day based on Frequency of Data (0 - 1000 minutes)

No significant effects (p>0.05).

9.4 Results of the Influence of the Light Program on Longwave Downward Radiation on the Pregnancy Result via the Logistic Regression with Random Effects with Weather Parameters subdivided into Classes (Glimmix in SAS 9.4) – Analysis 6

In order to prove the interaction between longwave downward radiation and the use of a light program the statistical analysis 6a to 6f showed the results.

9.4.1 Analysis 6a: Only data without the light program in all biological phases

Longwave downward radiation had a significant influence (p=0.0071) on the pregnancy result. The higher the longwave downward radiation the lesser chance for a positive pregnancy result. The time point of artificial insemination (AI) had a significant influence (p=0.0063) on the pregnancy result as well. Significant differences after correction for multiple comparisons were seen between the two time points of AI <48 hours before ovulation and <6 hours after ovulation (p<0.01). The figure 9-5 represents the back-transformed least-square means and standard errors as well as the significant differences between the time points of AI.

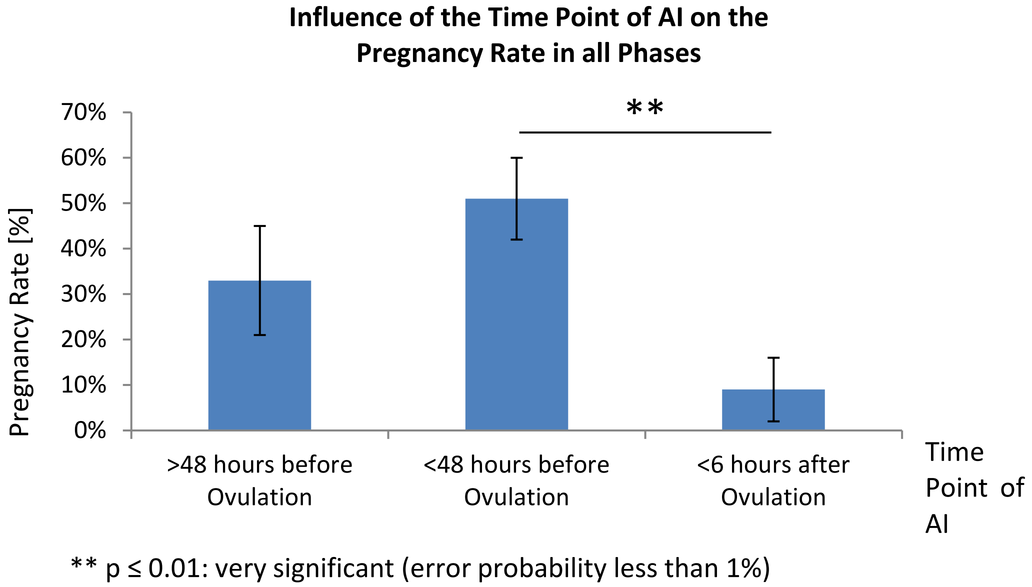
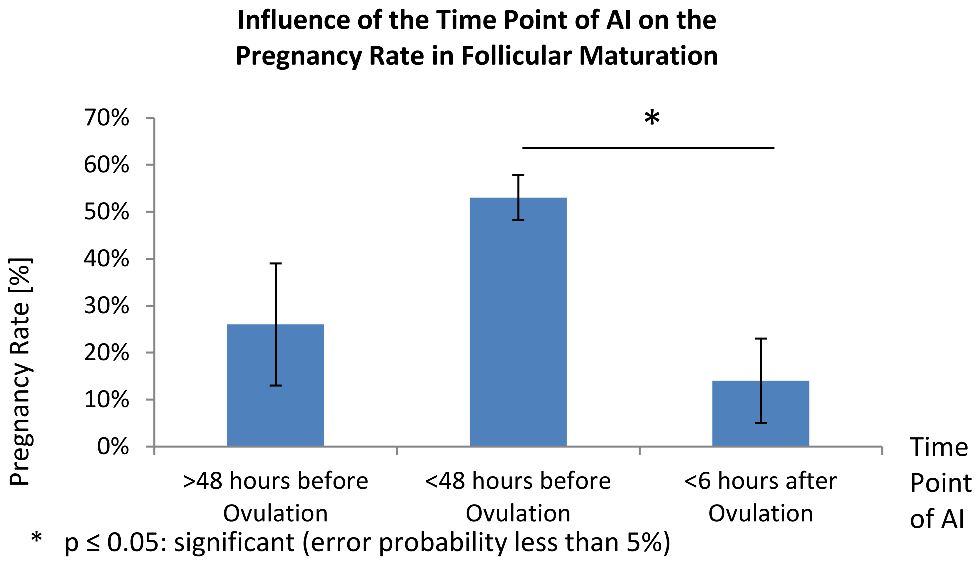


Figure 9-5: Influence of the time point of AI on the pregnancy rate [%] in all phases (back-transformed least-square means and standard errors)

9.4.2 Analysis 6b: Only data without light program per biological phase

In two phases the interaction of longwave downward radiation and light program had a significant effect (Follicular maturation p=0.0186, uterine phase, p=0.0122) on the pregnancy result.

Furthermore, the time point of artificial insemination (AI) had a significant influence in every phase (Follicular maturation p= 0.0172, ovulation p=0.0385, oviductal phase p=0.0329, uterine phase p=0.0133) on the pregnancy result. Significant differences after correction for multiple comparisons were seen between the two time points of AI <48 hours before ovulation and <6 hours after ovulation (p<0.05).The figure 9-6 represents the back-transformed least-square means and standard errors as well as the significant differences between the time points of AI.



* p ≤ 0.05: significant (error probability less than 5%)

Figure 9-6: Influence of the time point of AI on the pregnancy rate [%] in Follicular Maturation (back-transformed least-square means and standard errors)

9.4.3 Analysis 6c: Only data without the Light Program in all Biological Phases in Classes of Longwave Downward Radiation (1500 - 3500 J/cm^2)

In every phase (Follicular maturation p= 0.054, ovulation p=0.024, oviductal phase p=0.0337, uterine phase p=0.0156) a significant influence was seen on the pregnancy result. Significant differences after correction for multiple comparisons were not seen.

9.4.4 Analysis 6d: Only data with the Light Program in all Biological Phases

No significant effects (p>0.05).

9.4.5 Analysis 6e: Only data with Light Program per Biological Phase

No significant effects (p>0.05).

9.4.6 Analysis 6f: Only data with the Light Program in all Biological Phases in Classes of Longwave Downward Radiation (1500 - 3500 J/cm^2)

No significant effects (p>0.05).

9.5 Results on Influence of Weather Parameter around Artificial Insemination via the Logistic Regression with Random Effects - Analysis 7

The average temperature had a positive and significant (p=0.0345) effect on the pregnancy result when inseminating <48 hours before ovulation. Furthermore, the time point of artificial insemination (AI) had a significant effect (p=0.0085) on the pregnancy result five days prior to AI. Significant differences after correction for multiple comparisons were seen between the two time points of AI <48 hours before ovulation and <6 hours after ovulation (p=0.0418). The figure 9-7 represents the back-transformed least-square means and standard error means as well as the significant differences between the time points of AI.

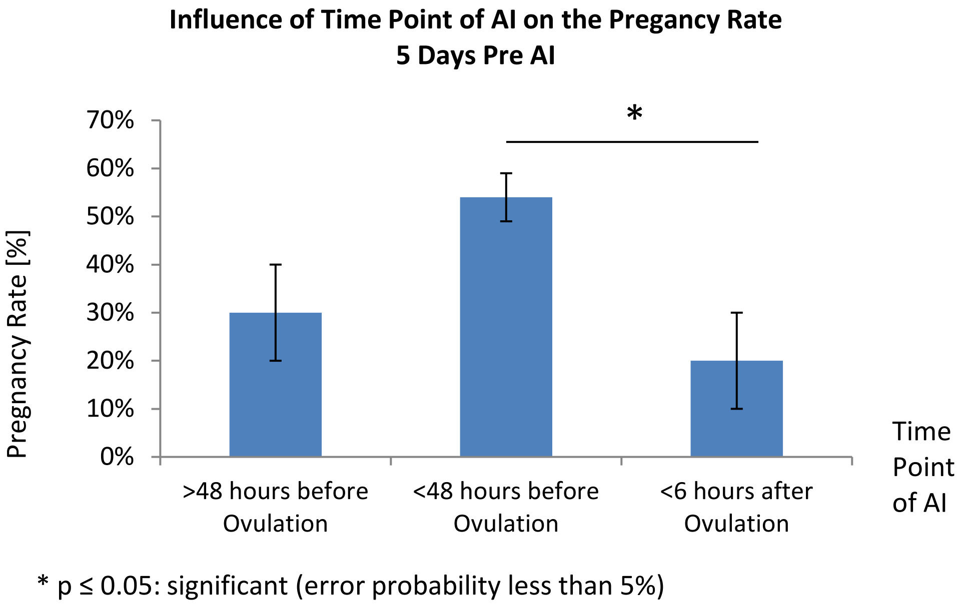
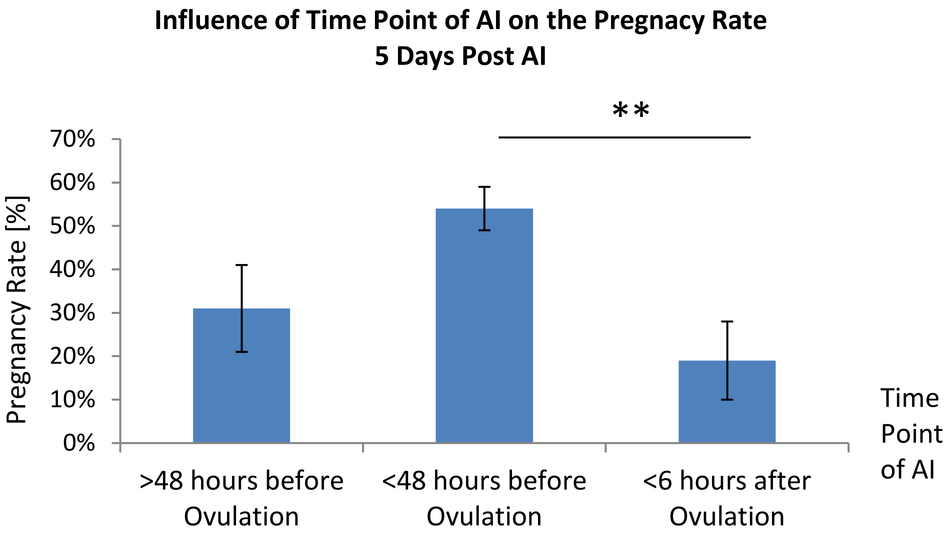


Figure 9-7: Influence of time points of AI in the 5 days pre AI (back-transformed least-square means and standard errors)

Significant differences after correction for multiple comparisons were seen between the two time points of AI <48 hours before ovulation and <6 hours after ovulation (p=0.0097). The figure 9-8 represents the means and standard error means as well as the significant differences between the time points of AI.



** p ≤ 0.01: very significant (error probability less than 1%)

Figure 9-8: Influence of time points of AI in the 5 days post AI (back-transformed least-square means and standard errors)

9.6 Results of Weather Parameter around Artificial Insemination via the Logistic Regression with Random Effects subdivided into Classes (Glimmix in SAS 9.4) – Analysis 8

No significant effects (p<0.05) could be seen in any analysis on classes of maximum temperature, average temperature and THI.

10 Discussion

With regard to the examined weather parameters, the present study showed only a moderate influence of weather on fertility and the probability of pregnancy in mares.

The timing of the insemination seemed more important. In particular, the timing of insemination within 48 hours before ovulation resulted in the highest pregnancy rates. This finding was previously published and considered a known factor (Sieme et al., 2003). In the present study, the time point of insemination was included and corrected in the model. Thus, when considering the results, there was no need to consider the time of insemination and the results were easier to interpret.

The present study showed that the Temperature-Humidity Index (THI) as occurred in the moderate climate of the present study, had no influence on fertility. There are no studies in equine on the effect of THI and fertility, so no direct comparison to published literature is possible. However, studies in other animal species show that the THI had a significant impact on fertility. In cattle in particular, it was shown that certain THI values (THI>25) had an influence on the expression of oestrus. Expression of oestrus was measured by examining sexually associated behaviour as indicated by incidence of chin pressing, licking and sniffing other cows (Rodtian et al., 1996). THI values higher than 60 led to a reduction in fertility; at a THI of 62, milk yield and quality was reduced (Brügemann et al., 2011). Comparing this with the present study, THI values averaged 56 but 44% of THI values were higher than 60. Therefore, the author had expected an impact on fertility in the mares. A possible explanation could be that horses can cope better with heat stress in general, likely due to the fact horses compared to cows and other livestock produce less metabolic heat due to lower (or no) milk production as well as due to the fact that horses are well able to control body temperature through extensive sweating. Nevertheless, certain combinations of temperature and humidity but also the two weather parameters by themselves have shown to influence the mare. Environmental temperature was reported to be a crucial variable influencing fertility in mares (Allen, 1987, Ju et al., 2002). The fact that more pregnancies can be achieved at moderate temperatures (in TNZ) was previously investigated in horses (Warriach et al., 2014). In the present study only 13% of days had an ambient temperature higher than 25 °C; ergo the moderate outside temperatures assisted the horses to remain in the TNZ (Morgan, 1998). In the present study, the results showed that there were very few cold days (7%) where mares were outside their TNZ. Mares were in paddocks for approx. 6 hours per day in winter and the remainder of time were stabled, where temperatures were probably generally higher due to emitted body heat. Therefore, the effect of reduced pregnancy rates in the present study during the cold winter months was unlikely due to a colder outside temperature, but rather due to other parameters, e.g. not enough daylight. No published equine data was identified on low temperatures and pregnancy rates. Reportedly, anoestrus lasts longer when outside temperatures were below the horse's TNZ (Allen, 1987). However, in these studies, it was unlikely only temperature that was responsible as the horses were not continuously exposed to the cold weather, but stabled and/or blanketed at night. Analysing the data from the present study on humidity, the measurements showed that humidity was 72.8% on average and the percentage of values with humidity above 75% was 21.1%. Previously reported studies on the influence of humidity on reproductive activities in horse mares showed an influence of humidity higher than 75% (Zeller, 2000, Amundson et al., 2006). Some studies showed a positive influence of higher air humidity on reproduction in horses (Meliani et al., 2011). In cattle, reportedly a THI of 60 effects fertility (Sanker et al., 2013). Observing the variables behind the THI, for example, air humidity of 70% with temperature of 24 °C leads to a THI of 70 and affected fertility in cattle (Brügemann et al., 2011).

As discussed before, studies on the influence of weather parameters such as temperature, humidity and THI are limited but the published studies outside of reproduction also show that horses cope better with heat stress and high humidity than cows. There are physiological reasons for this. When a horse is sweating, moisture wets the surface of the skin to cause cold evaporation and to cool the body down. Around 80% of the heat is released through evaporation (Guthrie et al., 1998). In addition, horses can dissipate about 20 to 30% of excess heat by respiration (Hodgson et al., 1993) especially with lower levels of humidity (<75% relative humidity) (Geor et al., 2000). However, studies also show that horses have considerable difficulty dissipating heat by evaporative cooling (Kohn et al., 1999). When interpreting these numbers and comparing them with cattle it has to should be noted that a horse sweats mainly on the neck and body and less on the legs. The sweat of the horse also shows a high concentration of the protein Latherin and, because of its molecular structure, it acts like a soap (Beeley et al., 1986). The watery sweat drops can be distributed evenly over the entire fur. Cows, on the other hand, can sweat a bit on the back and in the pelvic area, but compared to their intensive metabolism, the body surface of a cow is too small for efficient heat dissipation at higher ambient temperatures (Kolb, 1980). If the sweating is no longer sufficient for cooling, cows also begin to pant. In addition, when cows eat grass, the cellulose is fermented in the forestomach by microorganisms. The properties of ruminants, such as high metabolic activity and microbiological feed degradation in the rumen, mean that a lot of heat is generated in the cow's body, namely up to two degrees Celsius above the usually present rectal temperature (38.5 °C +/- 1 °C) (Owens et al., 1983, Renaudeau et al., 2012). This process produces waste heat, which heats up the cows from the inside. At temperatures around freezing, dairy cows have a total heat output of 2.200 watts and at an outside temperature of 35 °C, this power is still about 1.500 watts (Spiers, 2003). At high temperatures and humidity, the cow therefore has to release this energy into the environment almost exclusively by evaporating water, by way of sweating and increased breathing frequency to regulate the body temperature (Habeeb, 1992). In particular, dairy cows utilize considerable metabolic energy for milk production. It was shown that the TNZ is between -2 °C and +10 °C for dairy cows with a daily milk yield of 30 kg, and between -6 °C and +6 °C for cows with 40 kg milk (Berman, 2011). In addition, evaluations by Bavarian weather stations show that an average of 1.000 heat stress hours occur for dairy cows each year (Bayerische Landesanstalt für Landwirtschaft, 2020). It can thus be determined that the heat production of dairy cows (in MJ per year) is dependent on the annual output (kg ECM per cow and year) and the live weight of the cows (kg) (Jentsch et al., 2001, Kadzere et al., 2002). At the same time, the heat generation and distribution in the different tissues is not the same. With increasing milk production, for example, the heat build-up in the liver (increasing amount of gluconeogenesis or lipoprotein synthesis) and in the mammary gland increases significantly (Skibiel et al., 2018). In conclusion, it can be determined that the tolerance for heat and humidity is much higher in horses than in cattle. This leads to the question if the THI as it is defined for cattle is suitable as measurement for the influence of heat/humidity in horses, because the requirements of the animal species are completely different and the values and the weighting of heat and air humidity do not reflect the values that influence the horse.

Regarding the weather parameters temperature, humidity and THI, it can be determined that in the present study the THI as defined for cattle had no significant influence on fertility of horse mares. This would lead us to conclude that a different kind of THI is needed for horses. This would require establishing a THI range for horses that would consist of the temperature greater than 30 °C (Mortensen et al., 2009, Campbell, 2014) and humidity values greater than 75% (Zeller, 2000) so that an effect on the mare's body and thus on reproduction can be measured. In any case, a different THI was already used in a study, namely when measuring equine thermoregulatory responses during summertime road transport and stall confinement (THI = 0.7 * twb + 0.3 * tdb; where: twb = wet bulb temperature and tdb = dry bulb temperature (Green et al., 2007). However, this THI had no air humidity included, but put a high factor on the temperature, which in turn was questionable with average ambient temperature data measured from 28.5 °C to 35.6 °C including a maximum of 37.6 °C. For this reason, the author of the present study opted to utilize the THI as defined and established for cattle. However, a specific horse THI makes sense in terms of the history of previously published THIs. Since Thom (1959) described the THI for cattle, several equations were developed that can be used to calculate THI. The equations give more or less impact of either dry bulb or wet bulb temperature (ranging from the factor 0 to 0.35). Only one of them takes humidity into account: the "The Oklahoma Mesonet Cattle Heat Stress Index", which was designed to indicate the level of heat stress of outdoor cattle used by the authors cited in the present study (National Research Council, 1971). Overall, it we can assume that temperature in combination with humidity may have a greater impact on fertility in mares than previously known. Moreover, there seem to be large differences between animals and their sensitivity to temperature and humidity, again individually and in combination. These differences could make the data contradictory to studies where temperatures had little effect on horse fertility (Palmer et al., 1983, Guerin et al., 1994). Based on the three weather parameters (temperature, humidity and THI) it can be summarized that on the one hand the moderate climate here in Germany did not influence the mares to an extent that there would have been an effect on fertility and, on the other hand, that the THI as used in the present study is not appropriate for use in horses.

In the present study, the influence of sunlight on fertility was analysed. In most breeding regimes, standardized light programs (e.g. 8 hours dark, 16 hours light) are established. The analyses in the present study focused on influence and interaction between the sun and light program as horses were exposed to a light program in two years during the month September to April and in addition had access to paddock/pasture. With regard to sunshine, it was not only important to determine the number of hours of sunshine, but also consider radiation, as both are tied to one another. The results of the present study showed that sunshine during ovulation had a positive effect on fertility. This confirms the results of other studies that reported a positive influence of exposure to sunshine (Astudillo et al., 1960, Dini et al., 2019). The positive influence of sunshine and light is probably due to hormonal influences, which were extensively investigated in studies on lighting programs (Sharp et al., 1975, Palmer et al., 1983, Squires, 2008). There is evidence that light duration and intensity are critical for the level of melatonin secretion and therefore for its influence on the reproductive axis (Nagy et al., 2000). In mammalian species, melatonin secreted by the pineal gland is the neuromediator which then mediates the influence of light on the hypothalamo-pituitary-ovarian axis. During seasonal anoestrus, the activity of the gonadotrophin releasing hormone (GnRH) pulse initiator is inhibited and the amount of luteinizing hormone (LH) released from the pituitary is not high enough to stimulate reproductive ovarian functions. In all seasonal-breeding animals, photoperiod perception is crucial for timing of important physiological events. In the horse, photoperiod influences the onset of ovulation and cyclic shedding of the heavier winter coat, and the timing of parturition. Seasonal variation of different blood parameters/serum concentrations of prolactin, thyroxine and triodothyronine occurs relative to season and the estrous cycle (Johnson, 1987). If the reproductive rhythm of horses is examined, it is not surprising that the sun starts the cycle in motion, ensuring fertilization takes place at a time that influences birth of the foal under conditions favourable for survival.

Regarding radiation, results of the present study showed that a moderate diffuse solar radiation (<1000 J/cm^2) in the oviductal phase positively influenced fertility, whereas high longwave radiation (>2500 J/cm^2) in follicular maturation resulted in a negative effect on fertility. In addition, in the uterine phase, the interaction of longwave downward radiation and light program was reported to have a positive effect on the pregnancy result. Thus far, no studies on the influence of radiation in horses are published, which prevents a direct comparison. However, there appears to be a certain range of radiation that has negative effects. In addition, data from studies in cattle support the results from the present study. In particular, Roth et al. (2000) reported a negative influence of direct solar radiation on follicle growth and size. Cows were kept outside and only had an open shade structure for shelter and were therefore exposed to some amount of radiation. In chickens, the effect of high radiation together with green light was rated as negative (Baxter et al., 2014), and the combination of radiation with red light was rated as positive (Zhang et al., 2014). The results of the present study showed a significant influence in follicular maturation and ovulation with regard to the interaction of the sun and the light program. With additional hours of sunshine along with the light program, there were higher pregnancy rates than without a light program. The present data supports published data that a light program can significantly increase the likelihood of pregnancy and therefore including a light program in the winter months to get the mares into oestrus sooner may be beneficial. The present study, in addition, supports study results in cattle where very similar light programs were utilized not only to increase fertility, but also to ensure the overall cattle well-being for those not housed outside (Werner, 2019).

In other species it was reported that fertility and probability of pregnancy was affected by weather parameters during the days around insemination, mainly temperature (heat stress) (Lublin et al., 1984, Wettemann et al., 1985, Santolaria et al., 2014) and THI (Brügemann et al., 2013). The present study showed that the ambient temperature near the time of insemination had an impact on the success of pregnancy. The average outside temperature in the three days prior to insemination of the mare had an effect on the pregnancy result. This result is very important to consider when timing insemination in the 48 hours before ovulation as there can be a direct impact on fertility. These outcomes confirm the previous results of published data that reported that when mares were in the TNZ, the conditions for pregnancy were favourable. No significant effects were observed in any analysis on the THI, in contrast to published studies in cattle (García-Ispierto et al., 2007, Brügemann et al., 2013). The reason that no effect of THI was observed in the present study when only analysing the days around ovulation was probably the same as analysing the whole reproductive cycle of the individual mare (in the present study) as previously discussed.

Although the present study did not explicitly investigate hormonal activity, it can be assumed that the mares were in hormonal balance during the research period due to the moderate outside temperatures and lack of exposure to stress. This hormonally-balanced condition presumably created favourable conditions for fertility. Furthermore, analysing the month and considering the natural rhythm of equine reproduction, it can be concluded that pregnancy rates are generally lower in the summer months. While the highest fertility was achieved during the months of early spring that entail the most favourable conditions for the foals' survival under natural conditions. In addition, it can also be assumed that the mare's body can fully concentrate on becoming pregnant when mares are in the TNZ.

A potential point of criticism in the present research could be that neither stallions not their sperm were tested. It was assumed that stallions were in good health and in their ideal thermal comfort zones. There was also no differentiation between fresh sperm or frozen sperm as the information was not completely available. However, we must assume that the type and quality of the stallions and the sperm must play a significant part in the achievement of pregnancy in the respective mares. In the present study, 79% of all inseminations were performed with four stallions, including one stallion used in 43% of the cases. It would therefore have been interesting to look at the data of this stallion, if available, to obtain data on type and quality of the sperm. An analysis of the pregnancies achieved only with this stallion could display comparability. It would be reasonable to surmise that weather parameters can have an influence on sperm production and sperm quality as already described in other species (Myer et al., 2001). Meyer et al. (2001) showed that it was very clear that stallions undergoing heat stress had poorer sperm quality. Furthermore, in Holstein-Friesian and Jersey bulls it was shown that mass motility of semen in both breeds was significantly lower during wet summer (Fiaz et al., 2010).

Further research should focus on predicting the optimum time point of insemination. Moreover, additional evaluation research should analyse weather effects on the reproductive cycle of mares outside of their TNZ, with a focus on heat stress (>30 °C) and high humidity (>75%). If possible, a horse specific THI could be set up to include temperature with a higher factor compared to cattle to mimic the higher impact of temperature as well as humidity on the horse. Further research should be conducted long-term. Other parameters in addition to pregnancy result should be measured, including heart rate and cortisol levels, so as to build a broader picture of the impact on body condition and its effect on fertility. Sunshine, diffuse solar radiation, and longwave downward radiation are interesting parameters to observe, but do not seem to heavily influence the reproductive cycle as long as light programs are used or if horses are bred during the natural breeding season. In moderate climates such as Germany, increased exposure to sunshine should also support the overall wellbeing of mares, as they will likely be in the TNZ during winter months. Similarly, the effect of weather parameters on stallions outside of the TNZ should also be evaluated.

As no specific THI for horses exists currently, horse breeders do not have the possibility to utilize technical aids (e.g. as an app for the smartphone or a simple Excel calculation) as is done in cattle. However, according to the present study, horse breeders could benefit from allowing their horses outside as much as possible (pasture or paddock) in months with a moderate climate, while ensuring that horses have access to shade. Horses need a shelter (or trees) not only to escape the direct sun due to heat stress in the warm and humid summer months, but as protection from radiation, which can be high even at low temperatures. To determine heat and humidity, breeders can use the data of the German Weather Service (DWD, 2019). Here, breeders cannot only look at historical data to find out which months in the past generally had high temperatures, humidity and radiation, but can also access the forecast for the coming days. Depending on temperature, humidity and radiation values, the breeders can decide whether and when mares that are planned to be bred/inseminated are able to be outside in a paddock or at pasture or should be stabled. It is always important to observe the individual horse and to see how it reacts to the weather, and whether there are signs of heat stress such as excessive sweating. Furthermore, it is generally sensible to minimize heat stress and radiation for broodmares and to enable them to feel comfortable. The basic prerequisite for the success of all of the following measures is timely action before the occurrence of heat stress. Even simple measures such as providing good quality water in sufficient quantities from clean containers are beneficial. However, structural measures such as those that are already well-established in the cattle sector could also be implemented. Stables that allow air to move or reduce the radiant heat from the roof of the barn through light roofing or insulating sandwich elements should be considered. This implies that no light panels should be included and/or shading is possible. In the future it would be desirable to have a horse THI, which could then be deployed through similar tools used in cattle, e.g. as an app for the smartphone, websites (www.cool-cows.com) or a simple Excel calculation, to determine the THI on site.

11 Literature

Abdalla, E. B., Kotby, E. A. and Johnson, H. D. (1993). **"Physiological responses to heat-induced hyperthermia of pregnant and lactating ewes."** Small Ruminant Research 11(2): 125-134.

Aboul-Naga, A. and Aboul-Ela, M. (1987). **"Performance of subtropical Egyptian sheep breeds, European breeds and their crosses. I. Egyptian sheep breeds."** World Review of Animal Production.

Aguilar, I., Misztal, I. and Tsuruta, S. (2009). **"Genetic components of heat stress for dairy cattle with multiple lactations."** Journal of Dairy Science 92(11): 5702-5711.

Al-Katanani, Y., Paula-Lopes, F. and Hansen, P. (2002). **"Effect of season and exposure to heat stress on oocyte competence in Holstein cows."** Journal of Dairy Science 85(2): 390-396.

Al-Tamimi, H. J. (2007). **"Thermoregulatory response of goat kids subjected to heat stress."** Small Ruminant Research 71(1-3): 280-285.

Ali, A., Alamaary, M. and Al-Sobayil, F. (2014). **"Reproductive performance of Arab mares in the Kingdom of Saudi Arabia."** Tierärztliche Praxis G: Großtiere/Nutztiere 42(03): 145-149.

Allen, W. (1987). **"Endogenous hormonal control of the mare's oestrus cycle."** Proceedings of the Bain-Fallon Memorial 1987, Lectures: 2-13.

Allen, W. and Antczak, D. (2000). **"Reproduction and modern breeding technologies in the mare."** The Genetics of the Horse: 307-341.

Alves, B. G., Alves, K. A., Lúcio, A. C., Martins, M. C., Silva, T. H., Alves, B. G., Braga, L. S., Silva, T. V., Viu, M. A. O., Beletti, M. E., Jacomini, J. O., Santos, R. M. and Gambarini, M. L. (2014). **"Ovarian activity and oocyte quality associated with the biochemical profile of serum and follicular fluid from Girolando dairy cows postpartum."** Animal Reproduction Science 146(3): 117-125.

Amir, D. and Volcani, R. (1965). **"The sexual season of the Awassi fat–tailed ewe."** The Journal of Agricultural Science 64(1): 83-85.

Amundson, J., Mader, T. L., Rasby, R. J. and Hu, Q. (2006). **"Environmental effects on pregnancy rate in beef cattle."** Journal of animal science 84(12): 3415-3420.

Armstrong, D. (1994). **"Heat stress interaction with shade and cooling."** Journal of dairy science 77(7): 2044-2050.

Aroyo, A., Yavin, S., Arav, A. and Roth, Z. (2007). **"Maternal hyperthermia disrupts developmental competence of follicle-enclosed oocytes: In vivo and ex vivo studies in mice."** Theriogenology 67(5): 1013-1021.

Arrébola, F., Palacios, C., Gil, M.-J. and Abecia, J.-A. (2016). **"Management and meteorological factors affect fertility after artificial insemination in Murciano-Granadina goats."** Animal Production Science 56(11): 1906-1912.

Arrébola, F., Sánchez, M., López, M. D., Rodríguez, M., Pardo, B., Palacios, C. and Abecia, J.-A. (2016). **"Effects of weather and management factors on fertility after artificial insemination in Florida goats: A ten-year study."** Small Ruminant Research 137: 47-52.

Astudillo, C., Hajek, G.-A. and Diáz, O. (1960). **"The influence of some climatic factors on pregnancy duration in Thoroughbred mares. Preliminary account."** Zooiatria 2(35/38): 37-58.

Auchtung, T., Rius, A., Kendall, P., McFadden, T. and Dahl, G. (2005). **"Effects of photoperiod during the dry period on prolactin, prolactin receptor, and milk production of dairy cows."** Journal of Dairy Science 88(1): 121-127.

Aurich, C. (2009). **Manipulation des Sexualzyklus bei der Stute.** Georg Thieme Verlag.

Aurich, C. (2011). **Reproductive cycles of horses.** Georg Thieme Verlag.

Auvigne, V., Leneveu, P., Jehannin, C., Peltoniemi, O. and Sallé, E. (2010). **"Seasonal infertility in sows: A five year field study to analyze the relative roles of heat stress and photoperiod."** Theriogenology 74(1): 60-66.

Avdi, M., Vergos, V., Alifakiotis, T., Michailidis, I., Driancourt, M. and Chemineau, P. (1988). **"Seasonal variations of oestrus behaviour and ovulation rate in Chios and Serres ewes in Greece".** 3. Congres Mondial de Reproduction et Selection des Ovins et Bovins a Viande, Paris (France), 19-23 Jun 1988, INRA.

Ayyat, M. S. and Marai, I. F. M. (1998). **"Evaluation of application of the intensive rabbit production system under the sub-tropical conditions of Egypt."** World Rabbit Science 6: 213-217.

Bacci, M. L., Nannoni, E., Govoni, N., Scorrano, F., Zannoni, A., Forni, M., Martelli, G. and Sardi, L. (2014). **"Hair cortisol determination in sows in two consecutive reproductive cycles."** Reproductive Biology 14(3): 218-223.

Badinga, L., Thatcher, W. W., Diaz, T., Drost, M. and Wolfenson, D. (1993). **"Effect of environmental heat stress on follicular development and steroidogenesis in lactating Holstein cows."** Theriogenology 39(4): 797-810.

Badinga, L., Thatcher, W. W., Wilcox, C. J., Morris, G., Entwistle, K. and Wolfenson, D. (1994). **"Effect of season on follicular dynamics and plasma concentrations of estradiol-17β, progesterone and luteinizing hormone in lactating Holstein cows."** Theriogenology 42(8): 1263-1274.

Baker, J. (2004). **"Effective environmental temperature."** J. Swine Health Production 12(3): 140-143.

Barash, H., Silanikove, N., Shamay, A. and Ezra, E. (2001). **"Interrelationships among ambient temperature, day length, and milk yield in dairy cows under a Mediterranean climate."** Journal of Dairy Science 84(10): 2314-2320.

Barbari, M. and Conti, L. (2009). **"Use of different cooling systems by pregnant sows in experimental pen."** Biosystems engineering 103(2): 239-244.

Barrett, R. D. H. and Schluter, D. (2008). **"Adaptation from standing genetic variation."** Trends in Ecology & Evolution 23(1): 38-44.

Bassuny, S. (1999). **"Performance of doe rabbits and their weanlings as affected by heat stress and their alleviation by nutritional means, under Egyptian conditions."** Egyptian J. Rabbit Sci. 9: 61-72.

Baxter, M., Joseph, N., Osborne, V. and Bedecarrats, G. (2014). **"Red light is necessary to activate the reproductive axis in chickens independently of the retina of the eye."** Poultry Science 93(5): 1289-1297.

Bayerische Landesanstalt für Landwirtschaft, L. (2020). **Gesunde Kälber, gesunde Kühe**. Info-Tage Grub. Grub, Germany.

Beeley, J. G., Eason, R. and Snow, D. (1986). **"Isolation and characterization of latherin, a surface-active protein from horse sweat."** Biochemical journal 235(3): 645-650.

Benoit, J. and Assenmacher, I. (1966). **"Research on the photosensitivity of superficial and deep neural receptors in gonad stimulation by visible radiation in the immature Pekin duck."** Comptes rendus hebdomadaires des seances de l"Academie des sciences. Serie D: Sciences naturelles 262(26): 2750-2752.

Benthien, O., Braun, M., Riemann, J. C. and Stolter, C. (2018). **"Long-term effect of sheep and goat grazing on plant diversity in a semi-natural dry grassland habitat."** Heliyon 4(3): e00556.

Berman, A. (2011). **"Invited review: Are adaptations present to support dairy cattle productivity in warm climates?"** Journal of Dairy Science 94(5): 2147-2158.

Bernabucci, U., Biffani, S., Buggiotti, L., Vitali, A., Lacetera, N. and Nardone, A. (2014). **"The effects of heat stress in Italian Holstein dairy cattle."** Journal of dairy science 97(1): 471-486.

Bernabucci, U., Lacetera, N., Danieli, P. P., Bani, P., Nardone, A. and Ronchi, B. (2009). **"Influence of different periods of exposure to hot environment on rumen function and diet digestibility in sheep."** International Journal of Biometeorology 53(5): 387-395.

Black, J. L., Mullan, B. P., Lorschy, M. L. and Giles, L. R. (1993). **"Lactation in the sow during heat stress."** Livestock Production Science 35(1): 153-170.

Blackshaw, J. and Blackshaw, A. (1994). **"Heat stress in cattle and the effect of shade on production and behaviour: a review."** Australian Journal of Experimental Agriculture 34(2): 285-295.

Bloemhof, S., Van der Waaij, E., Merks, J. and Knol, E. (2008). **"Sow line differences in heat stress tolerance expressed in reproductive performance traits."** Journal of Animal Science 86(12): 3330-3337.

BMEL. (2016). **"Tierschutz-Nutztierhaltungsverordnung, TierSchNutzVO vom 22. April 2016."** Retrieved 01.06.2019, from https://www.bmel.de/SharedDocs/ExterneLinks/Rechtsgrundlagen/T/Tierschutz-Nutztierhaltungsverordnung.html.

Bohmanova, J., Misztal, I. and Cole, J. B. (2007). **"Temperature-humidity indices as indicators of milk production losses due to heat stress."** J Dairy Sci 90(4): 1947-1956.

Bouraoui, R., Lahmar, M., Majdoub, A., Djemali, M. and Belyea, R. (2002). **"The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate."** Animal Research 51(6): 479-491.

Boyazoglu, J. and Morand-Fehr, P. (2001). **"Mediterranean dairy sheep and goat products and their quality: A critical review."** Small Ruminant Research 40(1): 1-11.

Brown-Brandl, M. T., Beck, M., Schulte, D., Parkhurst, M. A. and DeShazer, A. J. (1997). **"Temperature Humidity Index for Growing Tom Turkeys."** Transactions of the ASAE 40(1): 203-209.

Brown-Brandl, T., Nienaber, J., Eigenberg, R., Hahn, G. and Freetly, H. (2003). **"Thermoregulatory responses of feeder cattle."** Journal of Thermal Biology 28(2): 149-157.

Brown-Brandl, T. M., Nienaber, J. A., Xin, H. and Gates, R. S. (2004). **"A literature review of swine heat production."** Transactions of the ASAE 47(1): 259.

Brügemann, K., Gernand, E., von Borstel, U. and König, S. (2011). **"Genetic analyses of protein yield in dairy cows applying random regression models with time-dependent and temperature x humidity-dependent covariates."** Journal of Dairy Science 94(8): 4129-4139.

Brügemann, K., Gernand, E., von Borstel, U. and König, S. (2013). **"Application of random regression models to infer the genetic background and phenotypic trajectory of binary conception rate by alterations of temperature× humidity indices."** Livestock Science 157(2): 389-396.

Burkhardt, J. (2009). **"Transition from anoestrus in the mare and the effects of artificial lighting."** The Journal of Agricultural Science 37(1): 64-68.

Cammack, K., Mesa, H. and Lamberson, W. (2006). **"Genetic variation in fertility of heat-stressed male mice."** Theriogenology 66(9): 2195-2201.

Campbell, M. (2014). **"Embryo transfer in competition horses: Managing mares and expectations."** Equine veterinary education 26(6): 322-327.

Campos, R., Rodas, Á., Cruz, C. and González, F. H. D. (2004). **"Thyroid hormones in native Colombian bovine breeds."** Revista Brasileira de Ciência Veterinária 11(3).

Carnevale, E. and Ginther, O. (1995). **"Defective oocytes as a cause of subfertility in old mares."** Biology of reproduction 52(monograph_series1): 209-214.

Carnevale, E. M. and Ginther, O. J. (1992). **"Relationships of age to uterine function and reproductive efficiency in mares."** Theriogenology 37(5): 1101-1115.

Carnevale, E. M., Hermenet, M. J. and Ginther, O. J. (1997). **"Age and pasture effects on vernal transition in mares."** Theriogenology 47(5): 1009-1018.

Cavestany, D., El-Wishy, A. and Foote, R. (1985). **"Effect of season and high environmental temperature on fertility of Holstein cattle."** Journal of Dairy Science 68(6): 1471-1478.

Charmantier, A., McCleery, R. H., Cole, L. R., Perrins, C., Kruuk, L. E. B. and Sheldon, B. C. (2008). **"Adaptive Phenotypic Plasticity in Response to Climate Change in a Wild Bird Population."** Science 320(5877): 800-803.

Chebel, R. C., Santos, J. E. P., Reynolds, J. P., Cerri, R. L. A., Juchem, S. O. and Overton, M. (2004). **"Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows."** Animal Reproduction Science 84(3): 239-255.

Chemineau, P. (1993). **"Medio ambiente y reproducción animal."** World Animal Review 77(1): 2-14.

Chemineau, P., Malpaux, B., Pelletier, J., Leboeuf, B., Delgadillo, J. and Pobel, T. (1996). **"Emploi des implants de mélatonine et des traitements photopériodiques pour maîtriser la reproduction saisonnière chez les ovins et les caprins."** Productions Animales 9(1): 45-60.

Christopherson, R. and Young, B. (1986). **"Effects of cold environments on domestic animals."** NATO advanced science institutes series: Series A: Life sciences (USA).

Cilek, S. (2009). **"The survey of reproductive success in Arabian horse breeding from 1976-2007 at Anadolu State farm in Turkey."** Journal of Animal and Veterinary Advances 8: 389-396.

Claus, R. and Weiler, U. (1985). **"Influence of light and photoperiodicity on pig prolificacy."** Journal of Reproduction and Fertility. Supplement 33: 185-197.

Close, W. H. and Mount, L. E. (2007). **"The effects of plane of nutrition and environmental temperature on the energy metabolism of the growing pig: 1. Heat loss and critical temperature."** British Journal of Nutrition 40(3): 413-421.

Coffey, R. D., Parker, G. and Laurent, K. (1995). **"Feeding growing-finishing pigs to maximize lean growth rate."** University of Kentucky.

Collier, R. J., Dahl, G. E. and VanBaale, M. J. (2006). **"Major Advances Associated with Environmental Effects on Dairy Cattle."** Journal of Dairy Science 89(4): 1244-1253.

Colston, J. M., Ahmed, T., Mahopo, C., Kang, G., Kosek, M., de Sousa Junior, F., Shrestha, P. S., Svensen, E., Turab, A. and Zaitchik, B. (2018). **"Evaluating meteorological data from weather stations, and from satellites and global models for a multi-site epidemiological study."** Environmental Research 165: 91-109.

Crawford, H., Morin, D., Wall, E., McFadden, T. and Dahl, G. (2015). **"Evidence for a role of prolactin in mediating effects of photoperiod during the dry period."** Animals 5(3): 803-820.

Curtis, S. E. (1983). **Environmental management in animal agriculture**, Iowa State University Press.

Cymbaluk, N. and Christison, G. (1990). **"Environmental effects on thermoregulation and nutrition of horses."** The Veterinary clinics of North America. Equine practice 6(2): 355-372.

Da Silva, R. G., Guilhermino, M. M. and De Morais, D. A. E. F. (2010). **"Thermal radiation absorbed by dairy cows in pasture."** International Journal of Biometeorology 54(1): 5-11.

Dahl, G. and Petitclerc, D. (2003). **"Management of photoperiod in the dairy herd for improved production and health."** Journal of Animal Science 81(15_suppl_3): 11-17.

Dankó, G. N. (2003). **"Some practical and biotechnological methods for improving reproduction traits in sheep."** Comunicado Técnico on line 11: 1-6.

Dash, S., Chakravarty, A., Singh, A., Upadhyay, A., Singh, M. and Yousuf, S. (2016). **"Effect of heat stress on reproductive performances of dairy cattle and buffaloes: A review."** Veterinary World 9(3): 235.

Dawson, R. D., Lawrie, C. C. and O'Brien, E. L. (2005). **"The importance of microclimate variation in determining size, growth and survival of avian offspring: experimental evidence from a cavity nesting passerine."** Oecologia 144(3): 499-507.

De Rensis, F. and Scaramuzzi, R. J. (2003). **"Heat stress and seasonal effects on reproduction in the dairy cow—a review."** Theriogenology 60(6): 1139-1151.

De Vries, A. (2006). **"Economic value of pregnancy in dairy cattle."** Journal of Dairy Science 89(10): 3876-3885.

Delgadillo, J. A., Fitz-Rodríguez, G., Duarte, G., Véliz, F. G., Carrillo, E., Flores, J. A., Vielma, J., Hernandez, H. and Malpaux, B. (2004). **"Management of photoperiod to control caprine reproduction in the subtropics."** Reproduction, Fertility and Development 16(4): 471-478.

Devendra, C. (1989). **Comparative aspects of digestive physiology and nutrition in goats and sheep.** "Satellite Symposium on Ruminant Physiology and Nutrition in Asia, VII International Symposium on Ruminant Physiology, 28 August to 1 September, 1989, Sendai, Japan.".

Dikmen, S. and Hansen, P. (2009). **"Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment?"** Journal of Dairy Science 92(1): 109-116.

Dillon, P., Berry, D. P., Evans, R. D., Buckley, F. and Horan, B. (2006). **"Consequences of genetic selection for increased milk production in European seasonal pasture based systems of milk production."** Livestock Science 99(2): 141-158.

Dini, P., Ducheyne, K., Lemahieu, I., Wambacq, W., Vandaele, H. and Daels, P. (2019). **"Effect of environmental factors and changes in the body condition score on the onset of the breeding season in mares."** Reproduction in Domestic Animals 54(7): 987-995.

Diskin, M. and Morris, D. (2008). **"Embryonic and Early Foetal Losses in Cattle and Other Ruminants."** Reproduction in Domestic Animals 43(s2): 260-267.

Donadeu, F. X. and Watson, E. D. (2007). **"Seasonal changes in ovarian activity: Lessons learnt from the horse."** Animal Reproduction Science 100(3): 225-242.

Dourmad, J., Etienne, M., Prunier, A. and Noblet, J. (1994). **"The effect of energy and protein intake of sows on their longevity: a review."** Livestock Production Science 40(2): 87-97.

Du Preez, J., Giesecke, W. and Hattingh, P. (1990). **"Heat stress in dairy cattle and other livestock under southern African conditions. I. Temperature-humidity index mean values during the four main seasons."** The Onderstepoort journal of veterinary research 57(1): 77.

DuBois, P. R. and Williams, D. J. (1980). **"Increased incidence of retained placenta associated with heat stress in dairy cows."** Theriogenology 13(2): 115-121.

DWD. (2019). **"Deutscher Wetterdienst."** Offenbach, Germany, from https://www.dwd.de/.

Ealy, A. D., Drost, M. and Hansen, P. J. (1993). **"Developmental Changes in Embryonic Resistance to Adverse Effects of Maternal Heat Stress in Cows."** Journal of Dairy Science 76(10): 2899-2905.

Egbunike, G. N. and Steinbach, J. (1980). **"Seasonal Changes in Sow Fertility in a Humid Tropical Environment."** Zentralblatt für Veterinärmedizin Reihe A 27(2): 109-117.

Eicker, S. and Fetrow, J. (2003). **"New tools for deciding when to replace used dairy cows".** Proc. Kentucky Dairy Conf., Cave City, KY. Univ. Kentucky, Lexington.

El-Tarabany, M. S. (2016). **"Effect of thermal stress on fertility and egg quality of Japanese quail."** Journal of thermal biology 61: 38-43.

El-Wishy, A., El-Sayed, M., Seida, A., Ghoneim, I. and Serur, B. (1990). **"Some aspects of reproductive performance in Arabian mares in Egypt."** Reproduction in Domestic Animals 25(4): 227-234.

Erlich, S. S. and Apuzzo, M. L. (1985). **"The pineal gland: anatomy, physiology, and clinical significance."** Journal of Neurosurgery 63(3): 321-341.

Ferreira-Dias, G., Claudino, F., Carvalho, H., Agrícola, R., Alpoim-Moreira, J. and Silva, J. R. (2005). **"Seasonal reproduction in the mare: possible role of plasma leptin, body weight and immune status."** Domestic animal endocrinology 29(1): 203-213.

Fiaz, M., Usmani, R., Abdullah, M. and Ahmad, T. (2010). **"Evaluation of semen quality of Holstein Friesian and Jersey bulls maintained under subtropical environment."** Pak. Vet. J 30(2): 75-78.

Finocchiaro, R., Van Kaam, J., Portolano, B. and Misztal, I. (2005). **"Effect of heat stress on production of Mediterranean dairy sheep."** Journal of Dairy Science 88(5): 1855-1864.

Fisher, A., Stewart, M., Duganzich, D., Tacon, J. and Matthews, L. (2005). **"The effects of stationary periods and external temperature and humidity on thermal stress conditions within sheep transport vehicles."** New Zealand Veterinary Journal 53(1): 6-9.

Flowers, B., Cantley, T. C., Martin, M. J. and Day, B. N. (1989). **"Effect of elevated ambient temperatures on puberty in gilts."** Journal of Animal Science 67(3): 779-784.

Foreman, J. H. and Ferlazzo, A. (1996). **"Physiological responses to stress in the horse."** Pferdeheilkunde 12(4): 401-404.

Fradinho, M. J., Correia, M. J., Grácio, V., Bliebernicht, M., Farrim, A., Mateus, L., Martin-Rosset, W., Bessa, R. J. B., Caldeira, R. M. and Ferreira-Dias, G. (2014). **"Effects of body condition and leptin on the reproductive performance of Lusitano mares on extensive systems."** Theriogenology 81(9): 1214-1222.

Fraser, D. (1985). **"Selection of bedded and unbedded areas by pigs in relation to environmental temperature and behaviour."** Applied Animal Behaviour Science 14(2): 117-126.

Fuquay, J. (1981). **"Heat stress as it affects animal production."** Journal of animal science 52(1): 164-174.

Garcia-Hernandez, R., Newton, G., Horner, S. and Nuti, L. C. (2007). **"Effect of photoperiod on milk yield and quality, and reproduction in dairy goats."** Livestock Science 110(3): 214-220.

García-Ispierto, I., López-Gatius, F., Bech-Sabat, G., Santolaria, P., Yániz, J., Nogareda, C., De Rensis, F. and López-Béjar, M. (2007). **"Climate factors affecting conception rate of high producing dairy cows in northeastern Spain."** Theriogenology 67(8): 1379-1385.

Gaughan, J., A. Tait, L., Eigenberg, R. and Bryden, W. (2004). **"Effect of shade on respiration rate and rectal temperature of Angus heifers."** Animal Production in Australia 25(1): 69-72.

Gaughan, J., Goopy, J. and Spark, J. (2002). **Excessive heat load index for feedlot cattle**. Meat and livestock-Australia project report, FLOT. 316.

Gaughan, J., Mader, T., Holt, S., Sullivan, M. and Hahn, G. (2010). **"Assessing the heat tolerance of 17 beef cattle genotypes."** International Journal of Biometeorology 54(6): 617-627.

Gaughan, J., Mader, T. L., Holt, S. and Lisle, A. (2008). **"A new heat load index for feedlot cattle."** Journal of Animal Science 86(1): 226-234.

Geers, R., Dellaert, B., Goedseels, V., Hoogerbrugge, A., Vranken, E., Maes, F. and Berckmans, D. (1989). **"An assessment of optimal air temperatures in pig houses by the quantification of behavioural and health-related problems."** Animal Science 48(3): 571-578.

Geiser, S., Arlettaz, R. and Schaub, M. (2008). **"Impact of weather variation on feeding behaviour, nestling growth and brood survival in Wrynecks Jynx torquilla."** Journal of Ornithology 149(4): 597-606.

Geor, R. J., McCutcheon, L. J., Ecker, G. L. and Lindinger, M. I. (2000). **"Heat storage in horses during submaximal exercise before and after humid heat acclimation."** Journal of Applied Physiology 89(6): 2283-2293.

Ginther, O. J. (1974). **"Occurrence of anestrus, estrus, diestrus, and ovulation over a 12-month period in mares."** American Journal of Veterinary Research 35(9): 1173-1179.

Gizaw, S., Komen, H. and van Arendonk, J. A. (2009). **"Optimal village breeding schemes under smallholder sheep farming systems."** Livestock Science 124(1-3): 82-88.

Glimp, H. A. (1995). **"Meat goat production and marketing."** Journal of Animal Science 73(1): 291-295.

Gomes Da Silva, R. (2006). **"Weather and climate and animal production."** Guide to agricultural meteorological practices (GAMP) Retrieved 22.04.2019, from http://www.agrometeorology.org/files-folder/repository/gamp_chap_11.pdf.

Gourdine, J. L., Renaudeau, D., Noblet, J. and Bidanel, J. P. (2016). **"Effects of season and parity on performance of lactating sows in a tropical climate."** Animal Science 79(2): 273-282.

Green, A. R., Gates, R. S. and Lawrence, L. R. (2007). **"Equine thermoregulatory responses during summertime road transport and stall confinement."** Revista Brasileira de Engenharia de Biossistemas 1(1): 83-92.

Groenendyk, S., English, P. and ABETZ, I. (1988). **"External balance of water and electrolytes in the horse."** Equine veterinary journal 20(3): 189-193.

Grubaugh, W., Sharp, D., Berglund, L., McDowell, K., Kilmer, D., Peck, L. and Seamans, K. (1982). **"Effects of pinealectomy in pony mares."** Journal of Reproduction and Fertility. Supplement 32: 293-295.

Guerin, M. V. and Wang, X. J. (1994). **"Environmental temperature has an influence on timing of the first ovulation of seasonal estrus in the mare."** Theriogenology 42(6): 1053-1060.

Guerra-Galdo, E. H., Sanz, S. C., Barber, F. E. and López-Jiménez, P. A. (2015). **"CFD model for ventilation assessment in poultry houses with different distribution of windows."** International Journal of Energy and Environment 6(5): 411.

Guillen-Muñoz, J. M., Meza-Herrera, C. A., Rivas-Muñoz, R., Zuñiga-Garcia, Z., Calderon-Leyva, G., Mellado, M. and Veliz-Deras, F. G. (2018). **"The use of female estrogenized goats as sexual stimulator of crossbred dairy males subsequently exposed to acyclic goats during two phases of the anestrous season."** Theriogenology 119: 175-182.

Guitierrez, N. (1986). **"Economic constraints on sheep and goat production in developing countries."** Small ruminant production in the developing countries: 138-147.

Gupta, M., Kumar, S., Dangi, S. and Jangir, B. L. (2013). **"Physiological, biochemical and molecular responses to thermal stress in goats."** Int J Livest Res 3(2): 27-38.

Guthrie, A. J. and Lund, R. J. (1998). **"Thermoregulation: base mechanisms and hyperthermia."** Veterinary Clinics of North America: Equine Practice 14(1): 45-59.

Guzeloglu, A., Ambrose, J. D., Kassa, T., Diaz, T., Thatcher, M. J. and Thatcher, W. W. (2001). **"Long-term follicular dynamics and biochemical characteristics of dominant follicles in dairy cows subjected to acute heat stress."** Animal Reproduction Science 66(1-2): 15-34.

Gwazdauskas, F. C., Wilcox, C. J. and Thatcher, W. W. (1975). **"Environmental and Management Factors Affecting Conception Rate in a Subtropical Climate1."** Journal of Dairy Science 58(1): 88-92.

Habeeb, A. (1992). **"Heat stress."** Farm animals and the environment: 27-47.

Habeeb, A., Aboul-Naga, A. and Khadr, A. (1999). **"Deterioration effect of summer hot climate on bunnies of acclimatized rabbits during suckling period".** 1st International Conference on Indigenous versus Acclimatized Rabbits, El-Arish, North Sinai, Egypt.

Hafez, E. S. E. (1964). **"Effects of high temperature on reproduction."** International Journal of Biometeorology 7(3): 223-230.

Hafez, E. S. E. and Hafez, B. (2013). **Reproduction in farm animals**, John Wiley & Sons.

Hahn, G., Nienaber, J. and DeShazer, J. (1987). **"Air temperature influences on swine performance and behavior."** Applied Engineering in Agriculture 3(2): 295-302.

Hammami, H., Rekik, B., Soyeurt, H., Bastin, C., Stoll, J. and Gengler, N. (2008). **"Genotype x environment interaction for milk yield in Holsteins using Luxembourg and Tunisian populations."** Journal of Dairy Science 91(9): 3661-3671.

Hansen, P. (1997). **"Strategies for enhancing reproduction of lactating dairy cows exposed to heat stress".** Proceedings of the 16th Annual Convention American Embryo Transfer Association, Madison.

Hansen, P. and Areéchiga, C. (1999). **"Strategies for managing reproduction in the heat-stressed dairy cow."** Journal of Animal Science 77(suppl_2): 36-50.

Hansen, P. J. (2007). **"Exploitation of genetic and physiological determinants of embryonic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress."** Theriogenology 68 Suppl 1: S242-249.

Hansen, P. J. (2007). **"To be or not to be - Determinants of embryonic survival following heat shock."** Theriogenology 68: S40-S48.

Hansen, P. J., Drost, M., Rivera, R. M., Paula-Lopes, F. F., Al-Katanani, Y. M., Krininger, C. E. and Chase, C. C. (2001). **"Adverse impact of heat stress on embryo production: causes and strategies for mitigation."** Theriogenology 55(1): 91-103.

Hartmann, E., Hopkins, R. J., Blomgren, E., Ventorp, M., von Brömssen, C. and Dahlborn, K. (2015). **"Daytime shelter use of individually kept horses during Swedish summer."** Journal of Animal Science 93(2): 802-810.

Hauser, E. R., Kamwanja, L. A. and Hansen, P. J. (1983). **"Photoperiod Influences Age at Puberty of Heifers."** Journal of Animal Science 57(4): 985-992.

Henneke, D., Potter, G., Kreider, J. and Yeates, B. (1983). **"Relationship between condition score, physical measurements and body fat percentage in mares."** Equine veterinary journal 15(4): 371-372.

Her, E., Wolfenson, D., Flamenbaum, I., Folman, Y., Kaim, M. and Berman, A. (1988). **"Thermal, Productive, and Reproductive Responses of High Yielding Cows Exposed to Short-Term Cooling in Summer."** Journal of Dairy Science 71(4): 1085-1092.

Hinrichs, K. (1998). **"Production of embryos by assisted reproduction in the horse."** Theriogenology 49(1): 13-21.

Hintz, H., Williams, J., Hillman, R., Houssay, H. and Reyes, R. F. (1992). **"Effect of month of breeding on duration of gestation period of thoroughbreds in Argentina."** Equine practice 14: 16-20(12).

Hjalmarsson, F., Olsson, I., Ferneborg, S., Agenäs, S. and Ternman, E. (2014). **"Effect of low light intensity at night on cow traffic in automatic milking systems."** Animal Production Science 54(10): 1784-1786.

Hodgson, D., McCutcheon, L. J., Byrd, S. K., Brown, W. S., Bayly, W., Brengelmann, G. and Gollnick, P. (1993). **"Dissipation of metabolic heat in the horse during exercise."** Journal of Applied Physiology 74(3): 1161-1170.

Holcomb, K., Tucker, C. and Stull, C. (2013). **"Physiological, behavioral, and serological responses of horses to shaded or unshaded pens in a hot, sunny environment."** Journal of Animal Science 91(12): 5926-5936.

Holcomb, K. E., Tucker, C. B. and Stull, C. L. (2014). **"Preference of domestic horses for shade in a hot, sunny environment."** Journal of Animal Science 92(4): 1708-1717.

Howell, C. and Rollins, W. (1951). **"Environmental sources of variation in the gestation length of the horse."** Journal of Animal Science 10(4): 789-796.

Hsia, L., Fuller, M. and Koh, F. (1974). **"The effect of water sprinkling on the performance of growing and finishing pigs during hot weather."** Tropical Animal Health and Production 6(3): 183-187.

Hurtgen, J. P. and Leman, A. D. (1980). **"Seasonal influence on the fertility of sows and gilts."** Journal of the American Veterinary Medical Association 177(7): 631-635.

Huynh, T., Aarnink, A., Verstegen, M., Gerrits, W., Kemp, B. and Heetkamp, M. (2004). **"Pigs' physiological responses at different relative humidities and increasing temperatures".** 2004 ASAE Annual Meeting, American Society of Agricultural and Biological Engineers.

Igono, M. O., Bjotvedt, G. and Sanford-Crane, H. T. (1992). **"Environmental profile and critical temperature effects on milk production of Holstein cows in desert climate."** International Journal of Biometeorology 36(2): 77-87.

Ingraham, R., Gillette, D. and Wagner, W. (1974). **"Relationship of temperature and humidity to conception rate of Holstein cows in subtropical climate."** Journal of Dairy Science 57(4): 476-481.

Irvine, C. H. G. (1967). **"Thyroxine secretion rate in the horse in various physiological states."** Journal of Endocrinology 39(3): 313-320.

Istituto Nazionale di Geofisica e Vulcanologia, I. (2007). **"CIRCE - climate change and impact research: the mediterranean environment."** Retrieved 08.01.2020, 2020, from https://climate-adapt.eea.europa.eu/metadata/projects/climate-change-and-impact-research-the-mediterranean-environment.

Jackson, P., Guy, J. H., Sturm, B., Bull, S. and Edwards, S. A. (2018). **"An innovative concept building design incorporating passive technology to improve resource efficiency and welfare of finishing pigs."** Biosystems Engineering 174: 190-203.

Jeelani, R., Konwar, D., Khan, A., Kumar, D., Chakraborty, D. and Brahma, B. (2019). **"Reassessment of temperature-humidity index for measuring heat stress in crossbred dairy cattle of a sub-tropical region."** Journal of Thermal Biology 82: 99-106.

Jentsch, W., Derno, M. and Weiher, O. (2001). **"Wärmeabgabe der Milchkühe in Abhängigkeit von der Leistung–eine Studie."** Archives Animal Breeding 44(6): 599-610.

Johnson, A. L. (1986). **"Serum Concentrations of Prolactin, Thyroxine and Triiodothyronine Relative to Season and the Estrous Cycle in the Mare1,2."** Journal of Animal Science 62: 1012-1020.

Johnson, H. and Ragsdale, A. (1962). **Effect of various temperature-humidity combinations on milk production of Holstein cattle**.

Johnson, H. D. (1987). **Bioclimatology and the adaptation of livestock**, Elsevier Amsterdam.

Johnston, L. J., Ellis, M., Libal, G. W., Mayrose, V. B., Weldon, W. C., Arthur, R. D., Brumm, M. C., Christenson, R. K., Crenshaw, J. D., Hines, R. H., Jesse, G. W., Jones, D. D., Leibbrandt, V. D., Rozeboom, D. W. and Zimmerman, D. R. (1999). **"Effect of room temperature and dietary amino acid concentration on performance of lactating sows."** Journal of Animal Science 77(7): 1638-1644.

Ju, J.-C., Peh, H.-C., Hsu, J.-C., Cheng, S.-P., Chiu, S.-C. and Fan, Y.-K. (2002). **"The reproductive characteristics of the mare in subtropical Taiwan."** Asian Australasian Journal of Animal Sciences 15(4): 494-499.

Kadzere, C. T., Murphy, M. R., Silanikove, N. and Maltz, E. (2002). **"Heat stress in lactating dairy cows: a review."** Livestock Production Science 77(1): 59-91.

Kahi, A. K. and Nitter, G. (2004). **"Developing breeding schemes for pasture based dairy production systems in Kenya: I. Derivation of economic values using profit functions."** Livestock Production Science 88(1): 161-177.

Kendall, P. E., Verkerk, G. A., Webster, J. R. and Tucker, C. B. (2007). **"Sprinklers and Shade Cool Cows and Reduce Insect-Avoidance Behavior in Pasture-Based Dairy Systems."** Journal of Dairy Science 90(8): 3671-3680.

Kermabon, A.-Y., Prunier, A., Djiane, J. and Salesse, R. (1995). **"Gonadotropins in lactating sows exposed to long or short days during pregnancy and lactation: serum concentrations and ovarian receptors."** Biology of reproduction 53(5): 1095-1102.

King, R. (1987). **"Nutritional anoestrus in young sows."** Pig News and Information 8(1): 15-22.

King, S. R. B. and Gurnell, J. (2010). **"Effects of fly disturbance on the behaviour of a population of reintroduced Przewalski horses (Equus ferus przewalskii) in Mongolia."** Applied Animal Behaviour Science 125(1): 22-29.

Kinne, O. (1964). **"The effects of temperature and salinity on marine and brackish water animals : 2. Salinity and temperature-salinity combinations."** Oceanography and Marine Biology : an Annual Review 2: 281-339.

Klinedinst, P. L., Wilhite, D. A., Hahn, G. L. and Hubbard, K. G. (1993). **"The potential effects of climate change on summer season dairy cattle milk production and reproduction."** Climatic Change 23(1): 21-36.

Kohn, C. W., Hinchcliff, K. W. and McKeever, K. H. (1999). **"Effect of ambient temperature and humidity on pulmonary artery temperature of exercising horses."** Equine veterinary journal. Supplement 30: 404-411.

Kojima, T., Udagawa, K., Onishi, A., Iwahashi, H. and Komatsu, Y. (1996). **"Effect of heat stress on development in vitro and in vivo and on synthesis of heat shock proteins in porcine embryos."** Molecular Reproduction and Development 43(4): 452-457.

Kolb, E. (1980). Die Regulation der Körpertemperatur. **Lehrbuch der Physiologie der Haustiere**, VEB Gustav Fischer Verlag, Jena: 594-612.

Kondrashov, F. A. (2012). **"Gene duplication as a mechanism of genomic adaptation to a changing environment."** Proceedings of the Royal Society B: Biological Sciences 279(1749): 5048-5057.

Kooistra, L. H. and Ginther, O. J. (1975). **"Effect of photoperiod on reproductive activity and hair in mares."** American journal of veterinary research 36(10): 1413-1419.

Kosgey, I. S., van Arendonk, J. A. M. and Baker, R. L. (2003). **"Economic values for traits of meat sheep in medium to high production potential areas of the tropics."** Small Ruminant Research 50(1): 187-202.

Kosgey, I. S., Van Arendonk, J. A. M. and Baker, R. L. (2004). **"Economic values for traits in breeding objectives for sheep in the tropics: impact of tangible and intangible benefits."** Livestock Production Science 88(1): 143-160.

Krijgsveld, K. L., Visser, G. H. and Daan, S. (2003). **"Foraging behavior and physiological changes in precocial quail chicks in response to low temperatures."** Physiology & Behavior 79(2): 311-319.

Kunavongkrit, A. and Heard, T. (2000). **"Pig reproduction in south east Asia."** Animal Reproduction Science 60: 527-533.

Landaeta-Hernández, A. J., Yelich, J. V., Lemaster, J. W., Fields, M. J., Tran, T., Chase, C. C., Rae, D. O. and Chenoweth, P. J. (2002). **"Environmental, genetic and social factors affecting the expression of estrus in beef cows."** Theriogenology 57(4): 1357-1370.

Lassoued, N., Rekik, M., Mattoufi, F. and Salem, I. B. (2008). **"Summer solar radiation and reproductive performances in Barbarine sheep raised in semi-arid conditions."** Livestock and Global Climate Change: 201.

Lewis, P. D. and Morris, T. R. (2000). **"Poultry and colored light."** World's Poult. Sci. J. 56: 189-207.

Lu, C. D. (1989). **"Effects of heat stress on goat production."** Small Ruminant Research 2(2): 151-162.

Lublin, A., Wolfebson, D. and Berman, A. (1984). **Circulatory adaptations to heat stress and their relationship to fertility.** Proceedings of 5th Annual Meeting of the EAAP. The Hague, Netherlands.

Lucas, E. M., Randall, J. M. and Meneses, J. F. (2000). **"Potential for Evaporative Cooling during Heat Stress Periods in Pig Production in Portugal (Alentejo)."** Journal of Agricultural Engineering Research 76(4): 363-371.

Lucy, M. (2001). **"Reproductive loss in high-producing dairy cattle: where will it end?"** Journal of dairy science 84(6): 1277-1293.

Mahrose, K. (2000). **Environmental studies on growth and reproduction traits in rabbits**, M. Sc. Thesis, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Malik, S., Rani, S. and Kumar, V. (2004). **"Wavelength dependency of light-induced effects on photoperiodic clock in the migratory blackheaded bunting (Emberiza melanocephala)."** Chronobiology international 21(3): 367-384.

Malpaux, B., Maurice-Mandon, F., Daveau, A. and Chemineau, P. (1995). **"Utilisation de la lumière et de la mélatonine pour la maîtrise de la reproduction des ovins et des caprins.".** 2e Rencontres autour des Recherches sur les Ruminants, 13-14 décembre 1995, Paris.

Malpaux, B., Robinson, J., Brown, M. and Karsch, F. (1988). **"Importance of changing photoperiod and melatonin secretory pattern in determining the length of the breeding season in the Suffolk ewe."** Journal of Reproduction and Fertility 83(1): 461-470.

Marai, I., Ayyat, M. and El-Monem, U. A. (2001). **"Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions."** Tropical Animal Health and Production 33(6): 451-462.

Marai, I., El-Darawany, A., Fadiel, A. and Abdel-Hafez, M. (2008). **"Reproductive performance traits as affected by heat stress and its alleviation in sheep."** Tropical and Subtropical Agroecosystems 8(3).

Marai, I. F. M., El-Darawany, A. A., Fadiel, A. and Abdel-Hafez, M. A. M. (2007). **"Physiological traits as affected by heat stress in sheep - A review."** Small Ruminant Research 71(1): 1-12.

Marai, I. F. M., Habeeb, A. A. M. and Gad, A. E. (2002). **"Rabbits´ productive, reproductive and physiological performance traits as affected by heat stress: a review."** Livestock Production Science 78(2): 71-90.

Mari, G., Morganti, M., Merlo, B., Castagnetti, C., Parmeggiani, F., Govoni, N., Galeati, G. and Tamanini, C. (2009). **"Administration of sulpiride or domperidone for advancing the first ovulation in deep anestrous mares."** Theriogenology 71(6): 959-965.

Marlin, D., Scott, C. M., Schroter, R., Harris, R., Harris, P. A., Roberts, C. and Mills, P. (1999). **"Physiological responses of horses to a treadmill simulated speed and endurance test in high heat and humidity before and after humid heat acclimation."** Equine veterinary journal 31(1): 31-42.

Mauro, L. J., Youngren, O. M., Proudman, J. A., Phillips, R. E. and El Halawani, M. E. (1992). **"Effects of reproductive status, ovariectomy, and photoperiod on vasoactive intestinal peptide in the female turkey hypothalamus."** General and comparative endocrinology 87(3): 481-493.

McBride, G., Christopherson, R. and Sauer, W. (1985). **"Metabolic rate and plasma thyroid hormone concentrations of mature horses in response to changes in ambient temperature."** Canadian Journal of Animal Science 65(2): 375-382.

McClintock, J. B. and Watts, S. A. (1990). **"The effects of photoperiod on gametogenesis in the tropical sea urchin Eucidaris tribuloides (Lamarck) (Echinodermata: Echinoidea)."** Journal of Experimental Marine Biology and Ecology 139(3): 175-184.

McDonnell, S. M. (2000). **"Reproductive behavior of stallions and mares: comparison of free-running and domestic in-hand breeding."** Animal Reproduction Science 60-61: 211-219.

McDowell, R. E., Hooven, N. W. and Camoens, J. K. (1976). **"Effect of Climate on Performance of Holsteins in First Lactation."** Journal of Dairy Science 59(5): 965-971.

McGlone, J. J., Stansbury, W. F. and Tribble, L. F. (1988). **"Management of lactating sows during heat stress: effects of water drip, snout coolers, floor type and a high energy-density diet."** Journal of Animal Science 66(4): 885-891.

McKechnie, A. E. and Wolf, B. O. (2010). **"Climate change increases the likelihood of catastrophic avian mortality events during extreme heat waves."** Biology Letters 6(2): 253-256.

McManus, C., Paludo, G. R., Louvandini, H., Gugel, R., Sasaki, L. C. B. and Paiva, S. R. (2009). **"Heat tolerance in Brazilian sheep: physiological and blood parameters."** Tropical Animal Health and Production 41(1): 95-101.

Mee, J., Fahey, J. and Crilly, J. (2002). **"Breeding the Dairy Cow of the Future Today's Challenges".** National Dairy Conference 1999, Adare, Ireland.

Meliani, S., Benallou, B., Abdelhadi, S. A., Halbouche, M. and Naceri, A. (2011). **"Environmental factors affecting gestation duration and time of foaling of pure bred Arabian mares in Algeria."** Asian J Anim Vet Adv 6: 599-608.

Meyer, H. and Klug, E. (2001). **"Einfluss der Ernährung auf die Fruchtbarkeit der Stuten und die Vitalität neugeborener Fohlen."** Pferdeheilkunde 1: 47-62.

Mihm, M., Baguisi, A., Boland, M. and Roche, J. (1994). **"Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers."** Reproduction 102(1): 123-130.

Miller, A., Erdman, R., Douglass, L. and Dahl, G. (2000). **"Effects of photoperiodic manipulation during the dry period of dairy cows."** Journal of Dairy Science 83(5): 962-967.

Mills, P. C., Smith, N. C., Casas, I., Harris, P., Harris, R. C. and Marlin, D. J. (1996). **"Effects of exercise intensity and environmental stress on indices of oxidative stress and iron homeostasis during exercise in the horse."** European Journal of Applied Physiology and Occupational Physiology 74(1-2): 60-66.

Mobarkey, N., Avital, N., Heiblum, R. and Rozenboim, I. (2010). **"The role of retinal and extra-retinal photostimulation in reproductive activity in broiler breeder hens."** Domestic Animal Endocrinology 38(4): 235-243.

Morand-Fehr, P. and Boyazoglu, J. (1999). **"Present state and future outlook of the small ruminant sector."** Small Ruminant Research 34(3): 175-188.

Morel, M. C. D. (2015). **Equine reproductive physiology, breeding and stud management**, CABI.

Morgan, K. (1997). **"Effects of short-term changes in ambient air temperature or altered insulation in horses."** Journal of Thermal Biology 3(22): 187-194.

Morgan, K. (1998). **"Thermoneutral zone and critical temperatures of horses."** Journal of Thermal Biology 23(1): 59-61.

Morrison, S., Heitman, H. and Bond, T. (1969). **"Effect of humidity on swine at temperatures above optimum."** International journal of biometeorology 13(2): 135-139.

Morrison, S., Heitman, H., Bond, T. and Finn-Kelcey, P. (1966). **"The influence of humidity on growth rate and feed utilization of swine."** International journal of biometeorology 10(2): 163-168.

Morsing, S. and Jacobsen, D. (1997). **"A decision support tool for designing animal ventilation systems"**. Proc. 5th Int. Livestock Environment Asae, USA. Vol. II**:** 843-850.

Mortensen, C., Choi, Y., Hinrichs, K., Ing, N., Kraemer, D., Vogelsang, S. and Vogelsang, M. (2009). **"Embryo recovery from exercised mares."** Animal Reproduction Science 110(3-4): 237-244.

Morton, J. M., Tranter, W. P., Mayer, D. G. and Jonsson, N. N. (2007). **"Effects of Environmental Heat on Conception Rates in Lactating Dairy Cows: Critical Periods of Exposure."** Journal of Dairy Science 90(5): 2271-2278.

Mosheim, R. and Lovell, C. A. K. (2009). **"Scale Economies and Inefficiency of U.S. Dairy Farms."** American Journal of Agricultural Economics 91(3): 777-794.

Mount, L. (1974). **"The concept of thermal neutrality."** Heat loss from animals and man: 425-439.

Murphy, B. A. (2019). **"Circadian and Circannual Regulation in the Horse: Internal Timing in an Elite Athlete."** Journal of Equine Veterinary Science 76: 14-24.

Murphy, B. A., Walsh, C. M., Woodward, E. M., Prendergast, R. L., Ryle, J. P., Fallon, L. H. and Troedsson, M. H. T. (2014). **"Blue light from individual light masks directed at a single eye advances the breeding season in mares."** Equine Veterinary Journal 46(5): 601-605.

Myer, R. and Bucklin, R. (2001). **"Influence of hot-humid environment on growth performance and reproduction of swine."** Respiration 38(40.6f): 39.30e.

Nabenishi, H., Ohta, H., Nishimoto, T., Morita, T., Ashizawa, K. and Tsuzuki, Y. (2011). **"Effect of the temperature-humidity index on body temperature and conception rate of lactating dairy cows in southwestern Japan."** Journal of Reproduction and Development: 1104050364-1104050364.

Nagy, P., Guillaume, D. and Daels, P. (2000). **"Seasonality in mares."** Animal Reproduction Science 60-61: 245-262.

Nardone, A., Ronchi, B., Lacetera, N. and Bernabucci, U. (2006). **"Climatic effects on productive traits in livestock."** Veterinary Research Communications 30(1): 75-81.

Nardone, A., Ronchi, B. and Valentini, A. (1991). **"Effects of solar radiation on water and food intake and weight gain in Sarda and Comisana female lambs."** Animal husbandry in warm climates 55: 149-150.

Nasr, A. (1994). **"Milk yield and some associated traits affected by season of kindling, parity, and kindling intervals in New Zealand White doe rabbits under Egyptian conditions."** Egyptian J. Rabbit Sci. 4(2): 149-159.

NASS. (2016). **"National Agricultural Statistics Service, Quick stats (2016)."** Retrieved 29.05.2019, from https://quickstats.nass.usda.gov/results/99223A11-A246-341D-9238-72B6FD0E25E9.

National Research Council (1971). **A guide to environmental research on animals.** Washington, National Academy of Sciences.

Nguyen, T. T., Bowman, P. J., Haile-Mariam, M., Nieuwhof, G. J., Hayes, B. J. and Pryce, J. E. (2017). **"Implementation of a breeding value for heat tolerance in Australian dairy cattle."** Journal of Dairy Science 100(9): 7362-7367.

Nikitchenko, I., Plyaschenko, S. and Zenkov, A. (1988). **Stresses and productivity of farm animals**, Urajai Publishing House, Minsk.

Notter, D. (2012). **"Genetic improvement of reproductive efficiency of sheep and goats."** Animal reproduction science 130(3-4): 147-151.

Olczak, K., Nowicki, J. and Klocek, C. (2015). **"Pig behaviour in relation to weather conditions–a review."** Annals of Animal Science 15(3): 601-610.

Olive, P. J. W. (1995). **"Annual breeding cycles in marine invertebrates and environmental temperature: Probing the proximate and ultimate causes of reproductive synchrony."** Journal of Thermal Biology 20(1): 79-90.

Oliveira, J. P., Jacob, J. C. F., de Jesus, V. L. T. and de Almeida Silva, P. C. (2015). **"Influence of temperature and humidity in a equine embryo transfer program, in the Baixada Fluminense, Rio de Janeiro."** Brazilian Journal of Veterinary Medicine 37(2): 158-162.

Oliveira, M., Guido, S. and Lima, P. (2001). **"Comparison of different protocols used to induce and synchronize estrus cycle of Saanen goats."** Small Ruminant Research 40(2): 149-153.

Olsson, K., Josäter-Hermelin, M., Hossaini-Hilali, J., Hydbring, E. and Dahlborn, K. (1995). **"Heat stress causes excessive drinking in fed and food deprived pregnant goats."** Comparative Biochemistry and Physiology Part A: Physiology 110(4): 309-317.

Orton, J. H. (2009). **"Sea-Temperature, Breeding and Distribution in Marine Animals."** Journal of the Marine Biological Association of the United Kingdom 12(2): 339-366.

Owens, F. and Bergen, W. (1983). **"Nitrogen metabolism of ruminant animals: historical perspective, current understanding and future implications."** Journal of animal science 57(suppl_2): 498-518.

Ozawa, M., Hirabayashi, M. and Kanai, Y. (2002). **"Developmental competence and oxidative state of mouse zygotes heat-stressed maternally or in vitro."** Reproduction 124(5): 683-689.

Ozawa, M., Tabayashi, D., Latief, T., Shimizu, T., Oshima, I. and Kanai, Y. (2005). **"Alterations in follicular dynamics and steroidogenic abilities induced by heat stress during follicular recruitment in goats."** Reproduction 129(5): 621-630.

Palacios, C. and Abecia, J. A. (2015). **"Meteorological variables affect fertility rate after intrauterine artificial insemination in sheep in a seasonal-dependent manner: a 7-year study."** International Journal of Biometeorology 59(5): 585-592.

Palmer, E. and Driancourt, M. A. (1983). **"Some interactions of season of foaling, photoperiod and ovarian activity in the equine."** Livestock Production Science 10(2): 197-210.

Palmer, E., Driancourt, M. A. and Ortavant, R. (1982). **"Photoperiodic stimulation of the mare during winter anoestrus."** Journal of Reproduction and Fertility. Supplement 32: 275-282.

Palmer, E. and Guillaume, D. (1992). **"Photoperiodism in the equine species—what is a long night?"** Animal Reproduction Science 28(1-4): 21-30.

Panzani, D., Zicchino, I., Taras, A., Marmorini, P., Crisci, A., Rota, A. and Camillo, F. (2011). **"Clinical use of dopamine antagonist sulpiride to advance first ovulation in transitional mares."** Theriogenology 75(1): 138-143.

Pearse, J. and Eernisse, D. (1982). **"Photoperiodic Regulation of Gametogenesis and Gonadal Growth in the Sea Star Pisaster Ochraceus."** Marine Biology 67(2): 121-125.

Pearse, J. S., Eernisse, D. J., Pearse, V. B. and Beauchamp, K. A. (1986). **"Photoperiodic Regulation of Gametogenesis in Sea Stars, with Evidence for an Annual Calendar Independent of Fixed Daylength."** American Zoologist 26(2): 417-431.

Pegorer, M. F., Vasconcelos, J. L., Trinca, L. A., Hansen, P. J. and Barros, C. M. (2007). **"Influence of sire and sire breed (Gyr versus Holstein) on establishment of pregnancy and embryonic loss in lactating Holstein cows during summer heat stress."** Theriogenology 67(4): 692-697.

Penev, T., Radev, V., Slavov, T., Kirov, V., Dimov, D., Atanassov, A. and Marinov, I. (2014). **"Effect of lighting on the growth, development, behaviour, production and reproduction traits in dairy cows."** Int. J. Curr. Microbiol. App. Sci 3(11): 798-810.

Peters, R., Chapin, L., Emery, R. and Tucker, H. (1981). **"Milk Yield, Feed Intake, Prolactin, Growth Hormone, and Glucocorticoid Response of Cows to Supplemented Light."** Journal of Dairy Science 64(8): 1671-1678.

Pinto, M. R., Miragaya, M. H., Burns, P., Douglas, R. and Neild, D. M. (2017). **"Strategies for increasing reproductive efficiency in a commercial embryo transfer program with high performance donor mares under training."** Journal of Equine Veterinary Science 54: 93-97.

Prunier, A., Dourmad, J. Y. and Etienne, M. (1994). **"Effect of light regimen under various ambient temperatures on sow and litter performance."** Journal of Animal Science 72(6): 1461-1466.

Prunier, A., Quesnel, H., de Bragança, M. M. and Kermabon, A. Y. (1996). **"Environmental and seasonal influences on the return-to-oestrus after weaning in primiparous sows: a review."** Livestock Production Science 45(2): 103-110.

Purswell, J. L., Dozier III, W. A., Olanrewaju, H. A., Davis, J. D., Xin, H. and Gates, R. S. (2012). **"Effect of temperature-humidity index on live performance in broiler chickens grown from 49 to 63 days of age".** 2012 IX International Livestock Environment Symposium (ILES IX), American Society of Agricultural and Biological Engineers.

Putney, D., Drost, M. and Thatcher, W. (1988). **"Embryonic development in superovulated dairy cattle exposed to elevated ambient temperatures between days 1 to 7 post insemination."** Theriogenology 30(2): 195-209.

Pycock, J. F. (2000). **"Breeding management of the problem mare."** Equine breeding management and artificial insemination 1: 195-228.

Randall, J. M. (1983). **"Humidity and water vapour transfer in finishing piggeries."** Journal of Agricultural Engineering Research 28(5): 451-461.

Ravagnolo, O. and Misztal, I. (2002). **"Studies on Genetics of Heat Tolerance in Dairy Cattle with Reduced Weather Information via Cluster Analysis."** Journal of Dairy Science 85(6): 1586-1589.

Ravagnolo, O., Misztal, I. and Hoogenboom, G. (2000). **"Genetic Component of Heat Stress in Dairy Cattle, Development of Heat Index Function."** Journal of Dairy Science 83(9): 2120-2125.

Ray, D. E., Halbach, T. J. and Armstrong, D. V. (1992). **"Season and Lactation Number Effects on Milk Production and Reproduction of Dairy Cattle in Arizona1."** Journal of Dairy Science 75(11): 2976-2983.

Regnault, T., Orbus, R., Battaglia, F., Wilkening, R. and Anthony, R. (1999). **"Altered arterial concentrations of placental hormones during maximal placental growth in a model of placental insufficiency."** Journal of Endocrinology 162(3): 433-442.

Renaudeau, D., Anaïs, C. and Noblet, J. (2003). **"Effects of dietary fiber on performance of multiparouslactating sows in a tropical climate."** Journal of Animal Science 81(3): 717-725.

Renaudeau, D., Collin, A., Yahav, S., de Basilio, V., Gourdine, J. L. and Collier, R. J. (2012). **"Adaptation to hot climate and strategies to alleviate heat stress in livestock production."** Animal 6(5): 707-728.

Renaudeau, D., Huc, E. and Noblet, J. (2007). **"Acclimation to high ambient temperature in Large White and Caribbean Creole growing pigs."** Journal of Animal Science 85(3): 779-790.

Rich, T. D. and Alliston, C. W. (1970). **"Influence of Programmed Circadian Temperature Changes on the Reproductive Performance of Ewes."** Journal of Animal Science 30(6): 966-969.

Rodtian, P., King, G., Subrod, S. and Pongpiachan, P. (1996). **"Oestrous behaviour of Holstein cows during cooler and hotter tropical seasons."** Animal Reproduction Science 45(1): 47-58.

Roman-Ponce, H., Thatcher, W., Buffington, D., Wilcox, C. and Van Horn, H. (1977). **"Physiological and production responses of dairy cattle to a shade structure in a subtropical Environment."** Journal of Dairy Science 60(3): 424-430.

Roth, Z. (2008). **"Heat Stress, the Follicle, and Its Enclosed Oocyte: Mechanisms and Potential Strategies to Improve Fertility in Dairy Cows."** Reproduction in Domestic Animals 43(s2): 238-244.

Roth, Z., Arav, A., Bor, A., Zeron, Y., Braw-Tal, R. and Wolfenson, D. (2001). **"Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows."** Reproduction Cambridge 122(5): 737-744.

Roth, Z., Meidan, R., Braw-Tal, R. and Wolfenson, D. (2000). **"Immediate and delayed effects of heat stress on follicular development and its association with plasma FSH and inhibin concentration in cows."** Journal of Reproduction and Fertility 120(1): 83-90.

Roth, Z., Meidan, R., Shaham-Albalancy, A., Braw-Tal, R. and Wolfenson, D. (2001). **"Delayed effect of heat stress on steroid production in medium-sized and preovulatory bovine follicles."** Reproduction Cambridge 121(5): 745-751.

Rua, M. A. S., Quirino, C. R., Rodrigues, A. C. C., Christo, M. M. and Barreto, M. A. P. (2018). **"Evaluation of environmental effects on reproductive characteristics of Mangalarga Marchador mares in a commercial embryo transfer program."** Animal Reproduction Science 195: 131-138.

Rutledge, J., Monson, R., Northey, D. and Leibfried-Rutledge, M. (1999). **"Seasonality of cattle embryo production in a temperate region."** Theriogenology 51(1): 330.

Ryan, D., Prichard, J., Kopel, E. and Godke, R. (1993). **"Comparing early embryo mortality in dairy cows during hot and cool seasons of the year."** Theriogenology 39(3): 719-737.

Sakatani, M. (2017). **"Effects of heat stress on bovine preimplantation embryos produced in vitro."** Journal of Reproduction and Development 63(4): 347-352.

Saldanha, C. J., Leak, R. K. and Silver, R. (1994). **"Detection and transduction of daylength in birds."** Psychoneuroendocrinology 19(5): 641-656.

Sanker, C., Lambertz, C. and Gauly, M. (2013). **"Climatic effects in Central Europe on the frequency of medical treatments of dairy cows."** Animal: an International Journal of Animal Bioscience 7(2): 316-321.

Santolaria, P., Yániz, J., Fantova, E., Vicente-Fiel, S. and Palacín, I. (2014). **"Climate factors affecting fertility after cervical insemination during the first months of the breeding season in Rasa Aragonesa ewes."** International journal of biometeorology 58(7): 1651-1655.

Scalco, R., Canisso, I., Silva, G., Almeida, T., Pazinato, F., Borba, L., Feijó, L., Nogueira, C. and Curcio, B. (2018). **"Temperature-humidity Index (THI) Is Associated with Gestation Length in Thoroughbred Mares."** Journal of Equine Veterinary Science 66: 254.

Schillo, K., Hansen, P., Kamwanja, L., Dierschke, D. and Hauser, E. (1983). **"Influence of season on sexual development in heifers: age at puberty as related to growth and serum concentrations of gonadotropins, prolactin, thyroxine and progesterone."** Biology of Reproduction 28(2): 329-341.

Schüller, L., Burfeind, O. and Heuwieser, W. (2013). **"Short communication: Comparison of ambient temperature, relative humidity, and temperaturehumidity index between on-farm measurements and official meteorological data."** J Dairy Sci 96(12): 7731-7738.

Schüller, L. K., Burfeind, O. and Heuwieser, W. (2014). **"Impact of heat stress on conception rate of dairy cows in the moderate climate considering different temperature–humidity index thresholds, periods relative to breeding, and heat load indices."** Theriogenology 81(8): 1050-1057.

Segnalini, M., Bernabucci, U., Vitali, A., Nardone, A. and Lacetera, N. (2013). **"Temperature humidity index scenarios in the Mediterranean basin."** International Journal of Biometeorology 57(3): 451-458.

Sevi, A., Annicchiarico, G., Albenzio, M., Taibi, L., Muscio, A. and Dell'Aquila, S. (2001). **"Effects of solar radiation and feeding time on behavior, immune response and production of lactating ewes under high ambient temperature."** Journal of Dairy Science 84(3): 629-640.

Shafie, M., Murad, H., El-Bedawy, T. and Salem, S. (1994). **"Effect of heat stress on feed intake, rumen fermentation and water turnover in relation to heat tolerance response by sheep."** Egyptian Journal of Animal Production 31(2): 317-327.

Sharma, S., Morel, M. D. and Dhaliwal, G. (2010). **"Factors affecting the incidence of postpartum oestrus, ovarian activity and reproductive performance in Thoroughbred mares bred at foal heat under Indian subtropical conditions."** Theriogenology 74(1): 90-99.

Sharp, D. C. and Ginther, O. J. (1975). **"Stimulation of Follicular Activity and Estrous Behavior in Anestrous Mares with Light and Temperature."** Journal of Animal Science 41(5): 1368-1372.

Shelford, V. (1954). **"The antelope population and solar radiation."** Journal of Mammalogy 35(4): 533-538.

Shimizu, T., Ohshima, I. and Kanai, Y. (2000). **"Effects of heat stress on follicular development in PMSG-treated immature rats."** Nihon Chikusan Gakkaiho 71(1): 32-37.

Shkolnik, A., Borut, A. and Choshniak, J. (1972). **Water economy of the Bedouin goat.** Symp. Zool. Soc. London.

Shkolnik, A. and Silanikove, N. (1981). **Water economy energy metabolism and productivity in desert ruminants [goat (Bedouin goat), gazelles (Gazella dorcas)].** Nutrition et systemes d'alimentation de la chevre.-Nutrition and systems of goat feeding, 37-Tours (France), 12-15 May 1981, ITOVIC.

Sieme, H., Schäfer, T., Stout, T., Klug, E. and Waberski, D. (2003). **"The effects of different insemination regimes on fertility in mares."** Theriogenology 60(6): 1153-1164.

Silanikove, N. (2000). **"Effects of heat stress on the welfare of extensively managed domestic ruminants."** Livestock production science 67(1-2): 1-18.

Singh, J. N., A. Adams, G. (2000). **"The reproductive pattern and efficiency of female buffaloes."** Animal Reproduction Science 60-61: 593-604.

Skibiel, A. L., Zachut, M., do Amaral, B. C., Levin, Y. and Dahl, G. E. (2018). **"Liver proteomic analysis of postpartum Holstein cows exposed to heat stress or cooling conditions during the dry period."** Journal of Dairy Science 101(1): 705-716.

Snoeks, M. G., Moons, C. P. H., Ödberg, F. O., Aviron, M. and Geers, R. (2015). **"Behavior of horses on pasture in relation to weather and shelter—A field study in a temperate climate."** Journal of Veterinary Behavior: Clinical Applications and Research 10(6): 561-568.

Sölkner, J., Nakimbugwe, H. and Valle Zarate, A. (1998). **"Analysis of determinants for success and failure of village breeding programmes".** Proceedings of the 6th world congress on genetics applied to livestock production.

Sönmez, M., Demirci, E., Türk, G. and Gür, S. (2005). **"Effect of season on some fertility parameters of dairy and beef cows in Elazığ province."** Turkish Journal of Veterinary and Animal Sciences 29(3): 821-828.

Soto, A., Draghi, G., Boyezuk, D., Soni, C., Nigro, H., Cetra, B. and De-La-Sota, R. (1998). **"Use of shade during the breeding season to reduce heart stress and to increase the overall flock pregnancy rate in a subtropical environment."** Rev. Brasileira Reprod. Anim 22(2): 97-101.

Souza-Cácares, M. B., Fialho, A. L. L., Silva, W. A. L., Cardoso, C. J. T., Pöhland, R., Martins, M. I. M. and Melo-Sterza, F. A. (2019). **"Oocyte quality and heat shock proteins in oocytes from bovine breeds adapted to the tropics under different conditions of environmental thermal stress."** Theriogenology 130: 103-110.

Spiers, D. E. (2003). **How cows dissipate heat.** Dairy Management Conference June 21 & 22.

Squires, E. L. (2008). **"Hormonal Manipulation of the Mare: A Review."** Journal of Equine Veterinary Science 28(11): 627-634.

Srikandakumar, A., Johnson, E. and Mahgoub, O. (2003). **"Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep."** Small Ruminant Research 49(2): 193-198.

Srivastava, R. and Chaturvedi, C. M. (2012). **"Age, photoperiod and estrogen dependent variations in the shell gland and the expression of AVT in the ovary of Japanese quail."** Steroids 77(6): 578-588.

St-Pierre, N. R., Cobanov, B. and Schnitkey, G. (2003). **"Economic Losses from Heat Stress by US Livestock Industries."** Journal of Dairy Science 86: E52-E77.

Stevenson, J. S. (2001). **"Reproductive management of dairy cows in high milk-producing herds."** Journal of Dairy Science 84: E128-E143.

Stork, M. (1979). **"Seasonal reproductive inefficiency in large pig breeding units in Britain."** The Veterinary Record 104(3): 49-52.

Suriyasomboon, A., Lundeheim, N., Kunavongkrit, A. and Einarsson, S. (2006). **"Effect of temperature and humidity on reproductive performance of crossbred sows in Thailand."** Theriogenology 65(3): 606-628.

Tao, X. and Xin, H. (2003). **Temperature-humidity-velocity index for market-size broilers.** 2003 ASAE Annual Meeting, American Society of Agricultural and Biological Engineers.

Teague, H., Roller, W. and Grifo Jr, A. (1968). **"Influence of high temperature and humidity on the reproductive performance of swine."** Journal of Animal Science 27(2): 408-411.

Thom, E. C. (1959). **"The discomfort index."** Weatherwise 12(2): 57-61.

Thompson, J. A., Magee, D. D., Tomaszewski, M. A., Wilks, D. L. and Fourdraine, R. H. (1996). **"Management of summer infertility in Texas Holstein dairy cattle."** Theriogenology 46(3): 547-558.

Thurmond, M., Picanso, J. and Jameson, C. (1990). **"Considerations for use of descriptive epidemiology to investigate fetal loss in dairy cows."** Journal of the American Veterinary Medical Association 197(10): 1305-1312.

Torres-Júnior, J. d. S., De FA Pires, M., De Sa, W., Ferreira, A. d. M., Viana, J. H. M., Camargo, L. S. d. A., Ramos, A., Folhadella, I., Polisseni, J. and De Freitas, C. (2008). **"Effect of maternal heat-stress on follicular growth and oocyte competence in Bos indicus cattle."** Theriogenology 69(2): 155-166.

Trout, J. P., McDOWELL, L. R. and Hansen, P. J. (1998). **"Characteristics of the estrous cycle and antioxidant status of lactating Holstein cows exposed to heat stress."** Journal of Dairy Science 81(5): 1244-1250.

Tucker, C. B., Rogers, A. R. and Schütz, K. E. (2008). **"Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system."** Applied Animal Behaviour Science 109(2-4): 141-154.

Tucker, H. and Oxender, W. (1980). **"Seasonal aspects of reproduction, growth and hormones in cattle and horses."** Progress in reproductive biology: 155-180.

Vale, W. (2007). **"Effects of environment on buffalo reproduction."** Italian Journal of Animal Science 6(sup2): 130-142.

van Arendonk, J. A. and Liinamo, A.-E. (2003). **"Dairy cattle production in Europe."** Theriogenology 59(2): 563-569.

Van Dierendonck, M. C., Sigurjónsdóttir, H., Colenbrander, B. and Thorhallsdóttir, A. G. (2004). **"Differences in social behaviour between late pregnant, post-partum and barren mares in a herd of Icelandic horses."** Applied Animal Behaviour Science 89(3): 283-297.

Velasco, J., Reid, E., Fried, K., Gressley, T., Wallace, R. and Dahl, G. (2008). **"Short-day photoperiod increases milk yield in cows with a reduced dry period length."** Journal of Dairy Science 91(9): 3467-3473.

Verde, Quintero, B., Sifontes, L., Manzo, M., Benacchio, N. and Diaz, T. (2018). **"Seasonal Changes in Ovarian Activity and Estrous Behavior of Thoroughbred Mares in a Tropical Environment1."** Biology of Reproduction 52(monograph_series1): 469-474.

Villa-Mancera, A., Méndez-Mendoza, M., Huerta-Crispín, R., Vázquez-Flores, F. and Córdova-Izquierdo, A. (2011). **"Effect of climate factors on conception rate of lactating dairy cows in Mexico."** Tropical Animal Health and Production 43(3): 597-601.

Walsberg, G. E. (1981). **"Nest-Site Selection and the Radiative Environment of the Warbling Vireo."** The Condor 83(1): 86-88.

Walsh, S. W., Williams, E. J. and Evans, A. C. O. (2011). **"A review of the causes of poor fertility in high milk producing dairy cows."** Animal Reproduction Science 123(3): 127-138.

Warriach, H. M., Memon, M. A., Ahmad, N., Norman, S. T., Ghafar, A. and Arif, M. (2014). **"Reproductive Performance of Arabian and Thoroughbred Mares under Subtropical Conditions of Pakistan."** Asian-Australasian Journal of Animal Sciences 27(7): 932-936.

Werner, D. (2019). **"Bedarfsorientierte Beleuchtung für Mensch und Rind in der landwirtschaftlichen Milchviehhaltung."**

Wettemann, R. P. and Bazer, F. W. (1985). **"Influence of environmental temperature on prolificacy of pigs."** Journal of Reproduction and Fertility. Supplement 33: 199-208.

Wight, H. M. and Conaway, C. H. (1961). **"Weather influences on the onset of breeding in Missouri cottontails."** The Journal of Wildlife Management 25(1): 87-89.

Wilks, D. S. and Wilby, R. L. (1999). **"The weather generation game: a review of stochastic weather models."** Progress in Physical Geography 23(3): 329-357.

Wilson, S., Marion, R., Spain, J., Spiers, D., Keisler, D. and Lucy, M. (1998). **"Effects of controlled heat stress on ovarian function of dairy cattle. 1. Lactating cows."** Journal of Dairy Science 81(8): 2124-2131.

Wilson, S. J., Marion, R. S., Spain, J. N., Spiers, D. E., Keisler, D. H. and Lucy, M. C. (1998). **"Effects of Controlled Heat Stress on Ovarian Function of Dairy Cattle. 1. Lactating Cows."** Journal of Dairy Science 81(8): 2124-2131.

Wingfield, J. C. (1984). **"Influence of weather on reproduction."** Journal of Experimental Zoology 232(3): 589-594.

Wise, M. E., Rodriguez, R. E., Armstrong, D. V., Huber, J. T., Wiersma, F. and Hunter, R. (1988). **"Fertility and hormonal responses to temporary relief of heat stress in lactating dairy cows."** Theriogenology 29(5): 1027-1035.

Wolf, B. O. and Walsberg, G. E. (1996). **"Thermal Effects of Radiation and Wind on a Small Bird and Implications for Microsite Selection."** Ecology 77(7): 2228-2236.

Wolfenson, D., Lew, B. J., Thatcher, W. W., Graber, Y. and Meidan, R. (1997). **"Seasonal and acute heat stress effects on steroid production by dominant follicles in cows."** Animal Reproduction Science 47(1): 9-19.

Wolfenson, D., Roth, Z. and Meidan, R. (2000). **"Impaired reproduction in heat-stressed cattle: basic and applied aspects."** Animal Reproduction Science 60: 535-547.

Wolfenson, D., Thatcher, W., Badinga, L., Savio, J., Meidan, R., Lew, B., Braw-Tal, R. and Berman, A. (1995). **"Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle."** Biology of Reproduction 52(5): 1106-1113.

Woodfill, C. J. I., Karsch, F. J., Robinson, J. E. and Brown, M. B. (1989). **"Circannual Cycles of Luteinizing Hormone and Prolactin Secretion in Ewes during Prolonged Exposure to a Fixed Photoperiod: Evidence for an Endogenous Reproductive Rhythm."** Biology of Reproduction 41(6): 1034-1046.

Xue, J., Dial, G., Marsh, W. and Davies, P. (1994). **"Multiple manifestations of season on reproductive performance of commercial swine."** Journal of the American Veterinary Medical Association 204(9): 1486-1489.

Younas, M., Fuquay, J., Smith, A. and Moore, A. (1993). **"Estrous and endocrine responses of lactating Holsteins to forced ventilation during summer."** Journal of dairy science 76(2): 430-436.

Young, B. and Coote, J. (1973). **"Some effects of cold on horses."** Feeders' Day Dep Anim Sci Univ Alberta.

Yurkov, V. (1980). **"Effect of lighting on animal productivity."** Rosselhozizdat, Moscow.

Zeller, D. (2000). **"Effect of the environmental temperature and air moisture on some reproductive parameters in foaling mares."** Czech Journal of Animal Science 45(9): 385-388.

Zeron, Y., Ocheretny, A., Kedar, O., Borochov, A., Sklan, D. and Arav, A. (2001). **"Seasonal changes in bovine fertility: relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles."** Reproduction 121(3): 447-454.

Zhang, Z., Cao, J., Wang, Z., Dong, Y. and Chen, Y. (2014). **"Effect of a combination of green and blue monochromatic light on broiler immune response."** Journal of Photochemistry and Photobiology B: Biology 138: 118-123.

Zimbelman, R., Rhoads, R., Rhoads, M., Duff, G., Baumgard, L. and Collier, R. (2009). **A Re-evaluation of the Impact of Temperature Humidity Index (THI) and Black Globe Humidity Index (BGHI) on Milk Production in High Producing Dairy Cows**. Western Dairy Management Conference. March 9-11 Reno, Nevada, USA**:** 113-125.

Zulovich, J. and DeShazer, J. (1990). **"Estimating egg production declines at high environmental temperatures and humidities."** Paper-American Society of Agricultural Engineers(90-4021).

Zumbach, B., Misztal, I., Tsuruta, S., Sanchez, J. P., Azain, M., Herring, W., Holl, J., Long, T. and Culbertson, M. (2008). **"Genetic components of heat stress in finishing pigs: Development of a heat load function."** Journal of Animal Science 86(9): 2082-2088.