VETERINARY MEDICINE TEACHING AND RESEARCH CENTER

An Evaluation of the Heatime® System on a Dairy farm in California

A prescriptive study about the differences of the heat detection program Heatime® compared to the breeder and related to progesterone concentrations on a dairy farm with 3900 Holstein-Friesian cows in California, United States of America

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Abstract

The objective of this study is to detect possible differences between the breeder and the heat detection system Heatime® related to progesterone concentrations on a large dairy in central California. In total 132 cows have been sampled in a period of seven working days. Three groups were used: group 1 consisted of cows that were positively assigned as in heat by the breeder as well as by Heatime®. Group 2 consisted of cows only assigned as in heat by Heatime®. Group 3 consisted of cows only assigned as in heat by the breeder. The dairy also used OvSynch as a synchronization program on several cows. This research found a significant difference between the positive predictive value (PPV) of Heatime® and the breeder (p <0,001). When excluding the synchronized cows, this significance disappeared (p = 0,103). When checking the synchronized cows only, there was a significant difference between the PPV of Heatime® and the breeder (p <0,001). It is an added value that Heatime® is able to detect cows that are actually in estrus, where the breeder has difficulties to recognize them. Heatime® gave several false positives, but the system is said to be 'better safe than sorry'. Therefore the opinion of the breeder remains important. Because of specific settings of the He atime® systemon this dairy it was difficult to analyze it for scientific purposes. In the field it works very well.

As a final note there can be said that Heatime® is a good additional measuring device for heat detection, but it still is a matter of human judgment and it needs to be used with good care by a well-trained breeder.

Prefactory Note

Within the study of Veterinary Medicine at the University of Utrecht, all students are obliged to fulfill a research project within a subject of own choice. This paper is the final report of the project carried out by Sylvia Strik at the Veterinary Medicine Teaching and Research Centre, University of California, Davis.

The research was executed to evaluate the efficiency and accuracy of Heatime® to detect estrus on a dairy farm in California milking 3900 Holstein Friesian cows.

1 Introduction

In this introduction several aspects will be highlighted which are of importance in the dairy industry concerning reproduction. First the basic biology of the bovine cyclicity will be explained focusing on the follicular and the luteal phase. After that, a closer look will be given on the bovine pregnancy.Also the different ways to correctly determine pregnancy like rectal palpation and the use of specific proteins in blood samples will be discussed. As heat detection is crucial, the final paragraph will be about the signs of estrus, heat detection and the correct moment of insemination and the potentialof heat detection systems.

In the last decades, the Western dairy industry has become more and more efficient to produce as much milk as possible for the lowest costs. For optimal production a cow needs to have a calf every year. This involves artificial insemination and most dairy farmers in the Western world depend onthe use of this technology. The fewer 'open days' and smaller expenses on semen, the better. Cows can produce over forty liters of milk every day during their peak production. Unfortunately, there is a negative correlation between the characteristics of 'production' and 'fertility' (Yaniz et al., 2006). To obtain a bigger milk yield there has been a selection on bigger liver capacity. The metabolic clearance of steroid hormones is being processed by the liver. The reduction of behavioral manifestation of estrus could be caused by the fact that with a bigger liver more hormones are being cleared (Santoset al., 2006). High producing cows (more than 39.5 kg/day) have a lower serum estradiol concentration on the day of estrus, and compared with lower producers, the duration of estrus was greatly reduced.

Each kilogram increase in milk yield seems to be correlated with a 1.6% decrease in walking activity. Walking, however, is not alone related to estrus. (Roelofs et al., 2010). On the contrary, other articles reported that there is no correlation between estrus expression and milk yield (Arney et al., 1994; Valenza et al., 2012).

There are numerous factors that can influence estrus: breed, season, nutrition, milk yield, presence of a bull, lactation number, stress and especially the number of cows that are in estrus the same time (Noakes et al., 2009). Several studies have proved that the more cows there are in estrus, the better the estrus expression will be. Each additional cow in estrus at the same time can be associated with a 6.1% increase in walking activity. Also, the number of mounting activities can be multiplied by five if the number of cows in heat at the same time is multiplied by four or more (Roelofs et al., 2010).

Cows do not show their heat as good as they used to and farmers do not always allocate enough time to look closely at the cows for a long period of time (Saumande, 2002). Research shows that the estrus period of cows can be as short as eight hours and a lot of times a significant part of the heat occurs during the night. Therefore, it is important to use systems which can detect heat and the optimal time for insemination. (Noakes et al., 2009; Roelofs et al., 2010) It has seemed to become typical that heat detection efficiency is lower than 50% in most dairy herds (Saumande, 2002). Poor estrus detection has been related to annual cost greater than US$300 million by the USA dairy industry due to significant decreases in milk production, delays in days to first service, prolonged days open, and therefore extended calving intervals which are all contributing factors to this loss. (Roelofs et al., 2010; www.livestocktrail.uiuc.edu, n.d.)

Because of all the above reasons, it has become more and more difficult to successfully inseminate and impregnate dairy cows (Saumande, 2002; Gabor et al., 2007). Several products and systems are available on the market to support the farmer with heat detection to determine the optimal moment of insemination.

This report has focused on the efficiency and accuracy of one of these heat detection systems, called Heatime®.

1.1 Basic Biology of Cyclicity

Starting at the age of seven up to eighteen months, the female cow enters puberty which is the onset of cyclicity (Noakes et al., 2009). Numerous hormones are involved with the cyclicity, but in this report the most important ones will be discussed.

Starting at puberty, the plasma progesterone levels indicate ovarian activity even when no estrus behavior has been seen (Donaldson et al., 1970). From puberty onwards, the average length of the estrus cycle in the cow is twenty-one days and ranges between seventeen and twenty-four days (Noakes et al., 2009; Pope et al., 1969; Senger et al., 2003; Youngquist et al., 2007). Heifers show an average length of the estrus cycle of twenty days with normal ranges between eighteen and twenty-two days (Noakes et al., 2009). Estrus itself lasts about fifteen hours but can be as short as six hours (Senger et al., 2003). Especially in high producing dairy cattle, estrus can be relatively short with an average of eight hours (11.3 +/- 6.9 hours in heifers and 7.3 +/- 7.2 hours in pluriparous cows) (Noakes et al., 2009; Saumande, 2002).

Around twenty-four to thirty-two hours after the onset of estrus, the ovulation occurs which is on average twelve hours after the end of estrus (Noakes et al., 2009). Figure 1 shows the events happening in the estrus cycle of the cow. Not only does it illustrate the hormonal cyclicity, but it also shows the follicular waves and the differences between normal cyclicity and pregnancy. Ovulation takes place approximately eighteen hours after the LH surge (Senger et al., 2003; Roelofs, 2005a).

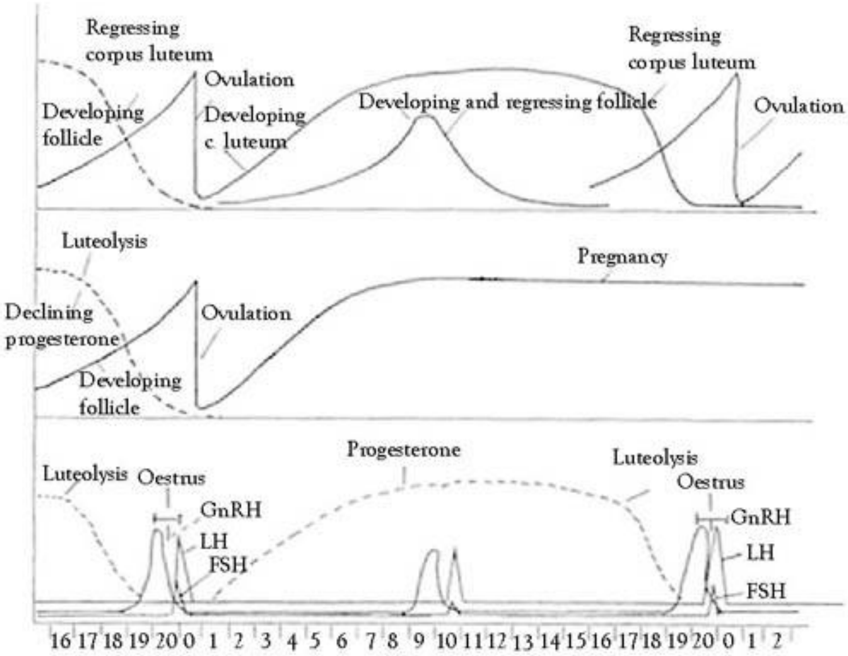

Figure 1: Events in the estrus cycle of the cow. From: Ibrahim et al., 2000.

1.1.1 Follicular phase (pro-estrus and estrus)

The follicular phase starts with the atresia of the corpus luteum which stops the negative feedback loop of the hormone progesterone on GNrH. Therefore GNrH isn't suppressed anymore and the GNrH release from the hypothalamic gland stimulates the pituitary gland to release the hormones FSH and LH. This causes the follicles to continue their growth and the production of estrogen in these follicles. (Noakes et al., 2009) Estrogen in the absence of progesterone gives a positive feedback loop to the hypothalamic gland which augments the release of LH (Senger et al., 2003; Roelofs, 2005a).

In cattle there can be two or three follicular waves. There is a difference between heifers and cows as heifers tend to have three waves more often than cows (33.3% against 14.3%). (Noakes et al., 2009; Senger et al., 2003).These waves occur around day two and eleven, or around day two, nine and sixteen (Noakes et al., 2009). Although these follicles tend to grow out to dominant follicles, no pre-ovulatory follicle will be able to develop because of the high progesterone level which prevents a LH surge. Only when the progesterone block falls away the dominant follicle will be able to develop to a pre-ovulatory follicle and will ovulate. The dominant follicle starts to produce the hormone inhibin which inhibits the release of FSH. This makes the other follicles go into atresia. The pre-ovulatory GNrH surge, and following this, the pre-ovulatory LH surge marks the ovulation of the pre-ovulatory follicle. (Noakes et al., 2009; Senger et al., 2003; Youngquist et al., 2007) Whenever there is an ovulation, progesterone concentrations have to be low. Progesterone levels near the time of ovulation are lower than 2 ug/mL, independent of the signs of estrus (Pope et al., 1969).

Even during periods as pregnancy, post-partum or anestrus, the follicular growth continues. Follicles can grow up to a dominant follicle although they seem to be smaller in diameter. Because no LH surge will occur, the follicles go into atresia (Noakes et al., 2009).

1.1.2 Luteal phase (metestrus and diestrus)

This is the phase in between ovulation up till luteolysis. After ovulation a corpus luteum is rapidly created primarily from the granulosa and theca cells. The stimulus for the formation and maintenance of the corpus luteum are the hormones prolactin and LH (Noakes et al., 2009). The corpus luteum starts to produce progesterone from day three to five and onwards. This inhibits the GNrH release and thus the release of LH and FSH so there will not be able to grow a pre-ovulatory follicle although there will be follicular waves in the ovaria. (Noakes et al., 2009; Senger et al., 2003; Youngquist et al., 2007)

The mean peak progesterone level in the peripheral plasma is 9.0 ng/mL and occurs on average thirteen days after ovulation (Pope et al., 1969). *Noakes et al.* states that peak values are being reached at day seven to eight (Noakes et al., 2009).

After seventeen to eighteen days after ovulation, the uterus will start to produce $PGF_2\alpha$ in episodes of six hours which will reach the ovaria via the countercurrent system between ovaria and uterus. The production of $PGF_2\alpha$ is being stimulated by the release of oxytocin that comes from the corpus luteum and thus is the corpus luteum itself responsible for its breakdown. The $PGF_2\alpha$ will also stimulate the ovary to produce oxytocin. (Noakes et al., 2009). This cascade will cause the corpus luteum to break down within one to three days and the most rapid drop in serum progesterone concentration will be around four days before ovulation (Noakes et al., 2009; Senger et al., 2003; Pope et al., 1969). Taking away the negative feedback on the hypothalamic gland this is the onset for the follicular phase which is described above in *1.1.1*.

1.2 Pregnancy

This part will focus on the biology of pregnancy and on embryonic losses. Also the methods as rectal palpation and the use of specific proteins to detect early pregnancy will be discussed.

1.2.1 Basic biology of pregnancy

Progesterone is the leading hormone for maintaining pregnancy. This progesterone comes from the corpus luteum; the bovine placenta produces only slight amounts of progesterone (Pope et al., 1969). The concentrations of progesterone during the first fourteen days are similar to those of diestrus with concentrations around four to six ng/mL. (Pope et al., 1969). But, in a pregnant cow there is only a slight decline around day eighteen whereas the progesterone concentrations of non-pregnant cows decline sharply to induce the next ovulation. After this small decline the concentration increases slightly during pregnancy with a peak concentration of seven to eight ng/mL around 240 days of gestation until it starts to decline again around thirty days prepartum (Donaldson et al., 1970; Senger et al., 2003; Noakes et al., 2009).

The ovulated oöcyte makes its way through the oviduct and turns into an oöcyst. In the ampulla the sperm fertilizes the oöcyst which then becomes a zygote. When it reaches the uterus at three to four days, it rarely ever migrates to the contra-lateral uterine horn, and now it is called a conceptus. The conceptus measures the full length of the gravid horn but its diameter is minimal. The amniotic sac has a spherical form at twenty-eight days of pregnancy and is about two cm in diameter. It uses the free portion of the gravid horn. The embryo itself is 0.8 cm long. Starting at thirty-five days the allantochorionic sac starts to distend the gravid cornu and will become noticeable to the veterinarian as a small distension. (Noakes et al., 2009)

To retain pregnancy, the embryo produces a hormone called bovine IFN-Ƭ which has a maximal secretion between day sixteen and day nineteen of gestation and continues to be secreted until day thirty-eight. Bovine IFN-Ƭ modifies the oxytocin receptors so it will inhibit the release of $PGF_2\alpha$ and thus inhibits the onset of the cycle all over again as described earlier in this report. (Noakes et al., 2009)

Starting at forty days cotyledons will be formed where the allantochorion contacts the uterine caruncles. In total around 120 cotyledons can be found which are larger towards the centre of the allantochorion. (Noakes et al., 2009)

Estrogen concentrations are around 100 pg/mL through 250 days of pregnancy. After that, it rises up to 8,2 ng/mL two to five days prepartum and rapidly declines about eight hours prepartum to very low levels. FSH and LH concentrations remain low during the full pregnancy and do not show significant fluctuations (Noakes et al., 2009).

1.2.2 Early embryonic loss

Most embryo losses occur before fifteen days after insemination. The cow returns into estrus within the normal cyclicity and no extra time is lost except from the extra cycle and the extra insemination (Gabor et al., 2007). The critical period appears to be the moment that the morula enters the uterus and is developing into a blastocyst, six to seven days after service. The embryonic loss can be as high as 24-40%, as stated by *Lamming and Royal* in 2001, which highlights the importance of embryonic mortality to the dairy industry. *Santos and Thatcher* published in 2006 that up to 33% of fertilized oöcytes will not result in a live calf due to embryonic loss up to forty-two days of insemination.

1.2.3 Pregnancy check

Early and accurate detection of pregnant and open cows has become essential for controlling and monitoring the fertility status of dairy cattle (Scenzi et al., 1998a). The earlier a non-pregnant cow can enroll in a synchronization program, the less open days remain which results in a higher milk production per year. Diagnosing pregnancies can be performed in several ways. The classical way for pregnancy determination is rectal palpation. Also, pregnancy kits may be used based on progesterone or specific pregnancy proteins in milk or blood plasma.

The accuracy of progesterone tests for the determination of pregnancy is between 60% and 84.5%. The false positive diagnosis will partly be due to early embryonic deaths which may result in a prolonged cyclicity, and also because of natural variance in the lengths of cyclicity. (Nakao et al., 1983).

1.2.3.1 Rectal palpation

Rectal palpation is a skill which should be familiar to every veterinarian, and experienced veterinarians are able to accurately diagnose pregnancy status by thirty- five days after insemination (Roberts et al., 1986). An embryonic vesicle and a membrane slip can be felt in the pregnant horn at this stage. Also, there may be felt a slight asymmetry between the two horns and the pregnant horn may have less muscular tone (Pieterse, 2001). This method will not be discussed into detail in this article as it is assumed to be known.

1.2.3.2 Pregnancy kits

In 1982 two proteins specific for pregnancy called pregnancy associated proteins (PAG's) were discovered. These PAGs belong to a large family of aspartic peptidases and are being expressed by the placenta. This protein is secreted by binucleate throphoblast cells within the fetal placenta as early as fifteen to twenty-two days in copious amounts during gestation in the peripheral circulation. (Green et al., 2006; King, 1993; Scenzi, 1998b)

The pregnancy test used in this project is based on the presence of this specific protein. The production of PAG starts early in gestation and persists up to ninety days after parturition because of the long half life time of 7,3 days and the residual of placental tissue in the caruncles for a certain period post-partum (King, 1993). *Scenzi et al.* even states that it can be detected in the serum up to hundred days. Even so, IDEXX states that their test can be used from sixty days post-partum onwards. With ELISA PAG can be detected in blood serum accurately and reliably from twenty-eight to thirty days after insemination. (Gabor et al., 2007; Green et al., 1986; Scenzi 1998b). The detection of PAG with ELISA twenty-seven days after insemination can be as high as 95%. Because PAG will remain in the blood for a period of time after production by the throphoblast cells this elevates the chance on false positives because of the possibility of early fetal death. Also the test can give false positives in fresh cows up to ninety days in milk because of the persistence after parturition (Gabor et al., 2007; Scenzi 1998b).

1.3 Signs of estrus and moment of insemination

Estrus behavior is a very important marker for the detection of estrus and ovulation in the dairy industry. It is very important to know what kind of behavior is related to estrus and when it takes place. The behavior is mediated by the estrogens produced by the pre-ovulatory follicle. Estrus behavior may contain the following elements: standing to be mounted, which means that the cow is standing immobile when she is mounted by another cow. Although this is the clearest behavior for estrus, it is seen in less than 50% of the cows in heat. *Roelofs and Lopez Gatius* report that it is seen in about 60% of the cows. Attempts to mount other cows is seen in 80% of the cows in estrus, and 54% of the cows are being mounted but will not stand (Roelofs, 2005a). Most cows have a higher activity and use more vocalization during estrus. Also licking, rubbing and aggressive behavior like head butting may occur (Roelofs et al., 2010). Sniffing the vulva of other cows and resting with the chin on the back of another cow are additional signs. (Senger et al., 2003; Roelofs, 2005a). Because of mounting and chin-resting the hair of the tail-head is often ruffled and the skin sometimes becomes excoriated because of mounting by other cows. (Noakes et al., 2009) These specific signs are being used by almost every dairy herdsman for heat detection and are reinforced by spraying paint or applying chalk on the tail-heads and checking all the cows for evidence that the paint or chalk has been rubbed off due to estrous activity.

As *Roelofs and Lopez-Gatius* state, the number of lactation can have a large influence on the signs of estrus. Higher walking activities were registered in primiparous cows than in multiparous cows. Each additional lactation gave a decrease in walking activity during estrus by 21.4%. On the other hand, the times of standing-to-be-mounted increases with parity. Because in the USA most dairy cows are in their first or second lactation, this is believed not to be of great importance for this report. (Roelofs et al., 2010)

A non- behavioral sign that is evident for estrus is a vulval discharge of transparent mucus, the elasticity of which causes it to hang in strands from the vulva, sometimes all the way to the ground. The vulva may be slightly swollen and of a dark pink color. After two days, there is a bright red sanguineous discharge with the blood coming mainly from the uterine caruncles. (Noakes et al., 2009) All these signs are activated and influenced by the hormone estradiol from the pre-ovulatory follicle (Noakes et al., 2009; Roelofs et al., 2010)

The body temperature changes during estrus; it drops about 0.5°C the day before estrus, increases during estrus and drops again at ovulation with on average 0.38°C (Noakes et al., 2009).

1.3.1 Detection methods for estrus

Barr calculated in 1975 that USA dairies appeared to be losing twice as many days due to failure in detecting heat as due to conception failures. Nowadays within the large herds frequently seen in

Californian dairies and their management type, it is difficult to have regular periods of estrus observation; the manpower input per cow has decreased too much (Saumande, 2002). Therefore other detection methods are necessary. One of them is using a measurement device based on the activity of the individual cow. An increased activity during the estrus seems to be largely independent of animal characteristics and so can very well be used for heat detection (Arney, 1994).

As stated in *Noakes et al.*, *Lewis & Newman* found in 1984 that pedometer activity was doubled in estrus compared with the luteal phase of the cycle. They showed in their study that 75% of the cows showed peak activity on the day of onset of estrus whereas 25% peaked one day after estrus. *Kiddy* found in 1977 that cows in free stalls were about four times as active during estrus compared to cows not in estrus. Also there tends to be natural estrus synchronization in groups of cows. (Noakes et al., 2009)

There appears to be no difference concerning the true duration of estrus compared to the duration of estrus measured with a heat detection device as a pedometer (Valenza et al., 2012)

Early studies executed to determine the best time for insemination showed that maximum pregnancy rates were obtained from midestrus until a few hours after the end of standi ng behavior. The studies were based on a very frequent heat detection (four to twelve times a day). Nowadays this is not possible anymore. More recent studies with pedometer activity evaluations described the best time for insemination would be five to seventeen hours after the increase of activity (Roelofs et al., 2005, Roelofs et al., 2010) and an estimated optimum at 11,8 hours after the activity increase (Maatje et al., 1997). There is quite a big variability between cows though and on big dairies it is not possible to inseminate a cow at exactly the right moment (Valenza et al., 2012). It is important to optimize the systems we already have to get the best economic result possible what heat detection is concerned.

1.4 Research objectives

The developer of Heatime® claims that the system will give more pregnancies per month by increasing the heat detection in the herd. In chapter *2.1* the potential and advantages of the Heatime® system will be explained. As discussed above the reproduction of cows on a dairy is dependent of many factors. Therefore the hypothesis in this research is that there will be no significant differences between Heatime® and progesterone concentrations on one side, and the breeder and progesterone concentrations on the other in those animals scored in heat by either Heatime® and/ or breeder. This research project has tried to find an answer to the following question:

> "To what extent is there a difference between the observations of the breeder and the measurements of Heatime®?"

This question will be answered in chapter *3 Results* and discussed in chapter *4 Discussion*. Finally, a conclusion will be extracted in chapter *5 Conclusion*.

2 Material and Methods

In this chapter an overview of the dairy farm will be given together with the different heat detection systems and computer tools used on the farm. After that an explanation will be given about the materials and methods used in this research.

2.1 Dairy farm

The farm is located in the heart of the St. Joaquin Valley in Tulare, California, United States of America. It houses 7500 cows in total with about 3900 dairy cows. The dairy raises its own replacement heifers and sometimes it also houses some beef calves. From these 3900 cows, 1500 are in first lactation, 1030 are in second and 1400 are in third or more lactation. The dairy farm has both open feedlot systems for calves and springers and freestall housing with a small outside paddock for all productive h eifers and cows.

Because of the size of the farm it is inevitable to use complex computer- systems to have control. In this farm they use DairyComp305, a very frequently used database at dairy farms. DairyComp305 can generate almost anything the owner would like to know about the status of the farm and individual cows or groups of cows. All the information found below has been generated from the DairyComp305 database.

The voluntary waiting period is set on fifty days in milk (DIM) for cows and seventy DIM for heifers. The milk production is 12.147 kg/305 days. This includes the dry cows as well so although they don't produce at the moment their number has been taken into account. All year round 16% of the herd is in her dry period. The average days in milk of all cows is 178 days. The DIM goes up to 680 days but this represents only a few cows. Only cows with a DIM between 48 and 323 days have been used in this research, which represents 95% of the total herd. The services per conception up to 323 days in milk for all lactations are on average 3.0. The lowest amount of services of 2.6 is found at 71 DIM and the highest is 5.0 at 260 DIM. The insemination risk, also called heat detection risk (possibility of a cow being detected in heat in the eligible herd), is on average 76%. The exception is the group of cows between 50- 71 DIM which has an insemination risk of 39%. The pregnancy risk (the possibility of a pregnancy in the eligible herd) is on average 19% with again the exception at 50- 71 DIM which has a risk of 13%. The conception risk (possibility of a pregnancy in the group of cows inseminated) is on average 25%.

To increase the reproductive statistics the dairy uses several methods. Artificial insemination is the standard for most dairy farms. All heifers have a voluntary waiting period of seventy-five days and are being synchronised using DoubleOvsynch. For all cows that do not show heat within eigthy DIM they use a synchronisation program called OvSynch.

The heat interval gives an indication of the normal cyclicity of the cows eligible to be bred. The lowest services per conception can be found within the normal cyclicity of twenty-one days. The average days in milk of first breeding is seventy-two days.

In addition to these hormonal programs the use Heatime® as an extra aid for their reproductive program.

2.1.1 Cows

The cows included in this project are all eligible to breed or possibly pregnant but not yet checked by a veterinarian for the possible pregnancy. They are of any lactation possible. All included cows are wearing a Heatime® collar and have passed their voluntary waiting period. The eligible group is around 2000 cows. The cows that appeared not to be in heat as seen by the breeder, nor were they on the Heatime list or in a synchronization program were not included in this study.

There were no sampling size calculations done at the beginning of the project. The sampling size was given by convenience without interfering the breeder. Because of the short time span of this project blood sampling has been done within two weeks, sampling each cow only once. The total time span of the project contained three months, including analysing the data.

2.1.2 Artificial insemination

Artificial insemination is carried out by two experienced employees once a day during the morning hours for seven days a week. To do their job the best and most efficient way possible they used different systems: Heatime®, the OvSynch programs and their experiences with heat detection. Also they use spray paint and chalk on the tailheads of the cows to check if they are being rubbed of, which can be an indication of heat. They go through every pen on the dairy and therefore see every eligible cow every day. Once or twice a week they also check the pens where there should only be pregnant cows to see if there have been any abortions or possibly open cows. These cows are not wearing Heatime® collars and these cows have not been included in this research.

The breeders use different qualities of semen, depending on the amount of previous heats and inseminations.

2.1.3 Synchronization program

Most heifers and non-pregnant cows over eighty days in milk enroll in a synchronization program with OvSynch. This protocol consists of two injections of a GNrH analogue separated by a single administration of PGF2α (seven days- two days- one day). This is beyond the reach of this research and will not be discussed in detail in this report. Some authors claim that there could be a slight reduction in the duration of estrus (Roelofs et al., 2010). The synchronization should not have a big effect on the performances of Heatime® because it should still need to detect increased activity during estrus.

2.1.4 Heatime®

The estrus detection system Heatime® *(SCR Engineers Ltd, Electronic Industries Milking Systems POB 564, Netanya, 42104, Israel)*, delivered and maintained by Micro Dairy Logic™ *(Micro Dairy Logic Advanced Dairy Solutions, PO Box 9262, Amarillo,Texas 79105)* has been evaluated on the above described dairy farm. The dairy has been using this system since one-and-a-half years.

Heatime® is an electronic device designed for the detection of heat in the bovine species. Its principle is based on the detection of all movements of an individual cow. The movements are being registered by an infra-red light. When the cow moves a droplet of quicksilver interferes with this infra-red light beam. It registers the total activity and the two-hour activity of the cow.

Cows are given the collar between one to seven days postpartum or when they leave the hospital pen. The tag needs to be put just below the left ear. It will stay there until there is a pregnancy confirmation of the veterinary specialist. It will take the tag approximately six days to adjust the measured activity for the individual cow. After this first week it will use the last six days average of the cow as reference. The system is able to differentiate between normal behavioral movements or movements because of estrus.

At the entrance of the milking parlor there is an ID transceiver unit which scans all the devices when cows pass by. This immediately updates the database. A list can be withdrawn from the central computer which shows all cows with increased activity selected by pen-number. An example of this list can be seen in attachment A. There is also a graphic display, which shows the activity per cow. An example of these graphic displays can be found in the attachments B, C and D.

The guarantee made by MicroDairyLogic™ was an increase of 3% more pregnancies a month compared to not using the system together with a heat detection rate up to 95%.

In an interview with the dairy herdsman he has told that since the dairy has been using Heatime® the average DIM of the herd has dropped about fifteen days going from 190 DIM to 175 DIM. Also with the total amount of days that the cows are open on average they dropped fifteen days, going from 145 to 130 days. The pregnancy rate is around 22%-23% and has gone up with 2%- 3% since last year, there are on average fifteen to twenty more pregnancies a month.

The system in total has cost the dairy $180,000.-; the collars cost $95,- a piece and the installation has cost $20.000,-. The support fee for the maintenance of the collars and the system itself is $0,84 per collar per month. There is a warranty of seven years with the repayment amount going down by the years. The system should start to make profit after three to four years. The dairy needed to employ one extra fulltime employee to do all the work with the system.

Heatime® has its own computer program called DataFlow1. The DataFlow 1 (version 3.7.118) program is being used for the collection of “activity” data on this dairy. Because of management reasons they use DairyComp305 for all other data in- and output. There is a link between the two systems so data can get transferred from DataFlow to DairyComp and so DairyComp can give a complete overview. DairyComp is the most often used computer system in the United States of America on dairy farms.

The Heatime® system has been installed and is being maintained by Micro Dairy Logic™. The front page of the system called “herd overview” not only shows an overview of the actual herd and the high activity cows, it also signals cows with decreased activity (which may be a sign for sickness) and signals problems with the tags (like unassigned or broken collars). This page can be found in attachment E.

The system uses “STD’s” to quantify the cow’s movements. STD stands for the individual standard deviation of the last twenty-four hours for every cow that has been measured the previous six days of wearing the collar. All cows with an individual increased activity higher than 8,0 STD will show up in the font color red. The STD is related with the hours since peak heat activity and is user defined. On the dairy this has been set at thirty-eight hours to identify cows that they would potentially re-breed. This means that a cow will show up on the list when the heat can be as far away in time as thirty-eight hours. This construction works as a filter to weed out animals that are no longer eligible to breed. The main reason of the thirty-eight hours is that this will ensure that the breeder will see the cow at least once and so he will not miss any cows potentially eligible to breed. The cows enrolled in this report showed peaks up to sixty STD.

For the purpose of this research program there has been a classification of the different STD heights into four groups, see table 1.

Cows with an increased activity above 8,0 STD (group Heatime® H) show up on the activity list and will be highlighted in the font color red. These cows are said to be clearly in heat.

Cows with an increased activity between 4,2 STD and 8,0 STD (group Heatime® M) will show up on the list but will not be highlighted in red. These cows may receive some extra attention for heat detection by the breeder. It can be seen as an escape mechanism of Heatime® because it shows the doubtful cases.

Also there is a specific group below 4,2 STD (Heatime® L) which still come up on the attention list. This is because the cows are being recorded as ‘in heat’ for as long as thirty-eight hours on this particular dairy farm. The system has a set amount of twenty-four hours which can be seen on the high activity lists as “New max peak STD last 24 hours” (see attachment A). For registration purposes in this research it was not possible to use another peak STD than the system has given. Another reason that some of these cows are included in this report is the fact that the breeder has assigned these cows as in heat, thought Heatime® does not show a peak in activity.

The last group shown here is the group with an STD below 4,2 (Heatime® N) which are not on the attention list nor are they considered as 'in heat' by the breeder. These cows are not included in this report.

Heatime® N	STD < 4,2	Not used in this report
Heatime® L	STD < 4,2; peak within 38 hours	1. Probably no heat considering the amount of time that has gone past (> 24 hours), though doubtful AND/OR 2. Breeder considers cow as 'in heat' without increased activity
Heatime® M	STD 4,2- 8	Doubtful if in heat, peak of activity lies within twenty-four hours
Heatime® H	STD > 8	Positively in heat

Table 1: classification of the different Heatime® groups

In attachment A you will find a print of the high activity list. Please notice that, because of the primary use of DairyComp and not DataFlow 1, many columns may not always be relevant (namely column 2, 3, 4, 7, 8, 9, 10 and 11).

The program also shows individual graphs per cow with the STD on the Y-axis and the date and time on the X-axis. This way the cycle and the intensity of the heat can be shown. Also the activity of the cow in between two heats may be relevant. The graph for 'raw' activity shows the raw average activity of the cow in movements for every two hours.

Attachment C shows the raw activity graph for cow 27643 as an example. It measures the amount of activity for every two hours. Attachment B shows how the raw information gets transformed into a 'weight activity' line in the graph. Together they give a good impression of the activity of the individual cow. Over time you will get a graph like the one showed in attachment D which gives an overview of the cyclicity of the cow. As you can see for this particular cow there are two peaks which stands for two heats. In between there are smaller peaks without any significance.

2.2 Blood samples

Previous research has shown that the concentration of plasma progesterone increases to a maximum of six to twelve ng/mL about day fourteen of the estrus cycle. The progesterone concentration decreases rapidly during the last four days of the cycle reaching low levels on the day before estrus (below 1,5 ng/mL). During estrus there are no significant changes in the plasma concentration. (Donaldson et al., 1970; Pope et al., 1969; Senger et al., 2003; Youngquist et al., 2007).

The plasma progesterone concentration will be relatively low one day before the onset of estrus and will be elevating slowly after estrus. Therefore, if the estrus is indicated correctly, the progesterone concentration will be low and one blood sample will be enough.

Blood samples were collected using the coccygeal vein. Blood was taken from cows that showed up at the Heatime® activity list of that day and the breeder himself believed they were in heat to evaluate the level of progesterone in the blood during heat. Also blood was collected of cows that did not show up on the Heatime® list but of which the breeder thought were in heat, and of cows that were on the Heatime® list but the breeder did not think they were in heat. Also occasional cows that were in the synchronization program and were in the right stage of the synchronization cycle (considered as 'in heat') were included in the project. The breeder was fully aware of the synchronization program, the Heatime® list and of his own registration.

Blood samples were collected using the Vacutainer® system with a double-pointed needle, a plastic holder and 10mL vacuum tubes with red tops.

Blood samples were cooled on the spot. A DYNAC II Clay Adams Centrifuge has been used to centrifuge the blood samples for ten minutes at 2400 RPM. Disposable polyethylene transfer pipets were used to put the serum in 2.0mL polypropylene graduated microcentrifuge tubes. All the samples were stored in a freezer at -79°C until further processing. The samples were sent to the laboratory of the Minnesota Veterinary College for progesterone concentration.

2.3 Pregnancy check

2.3.1 Rectal palpation

Rectal palpation is a skill every veterinarian has to be experienced in. The palpations are done by the clinicians and interns at VMTRC; they are very capable of this and their opinion on the pregnancy is therefore stated as the golden standard concerning the detection of pregnancy from thirty-five days onwards. For this project the pregnancies on average were checked at thirty-five to forty-two days after insemination, so there was no pregnancy testing during the sampling period.

2.3.2 Pregnancy kit

The pregnancy kits used are from Idexx Laboratories Inc (Maine). They are ELISA kits based on a specific protein (PAG). Unfortunately Idexx Laboratories Inc would not want to give information about the protein used or other specifications about this test because it was not commercially available during this project. No further details can be given. The PAG ELISA test can be performed from twenty-seven days onwards.

2.4 Statistics

For the evaluation of the results χ^2 en Fisher's exact testing have been used to compare proportions. Proportions summarize observations of a binary (qualitative) variable. (Petrie et al., 1999). If $p < 0.05$ the differences between results were considered significant.

The χ^2 testing has been executed by the online statistical program *Quantspy*: http://www.quantpsy.org/chisq/chisq.htm. The fisher's exact testing has been calculated with the online program *In Silico*: http://in-silico.net/tools/statistics/fisher_exact_test. The confidence intervals have been calculated with the online available program of *McCallum*: http://www.mccallum-layton.co.uk/stats/ConfidenceIntervalCalcProportions.aspx

3 Results

This research focuses on the differences in observation between the breeder and Heatime® compared to the measurements of the progesterone concentration. To examine this several statistical analyses have been executed.

3.1 Overview

In this study 132 cows have been included. Group 1 are cows that were positively detected as 'in heat' by the breeder and by Heatime®. Group 2 included cows that were positively detected as 'in heat' by Heatime® alone, and group 3 included cows that were positively detected as 'in heat' by the breeder alone.

Groups	Number of cows considered as "in heat"	P_4 <2 ng/ml	P_4 >2 ng/ml	PPV ± CI95
1 (Heatime® & Breeder)	69	66	3	0,96 ± 0,033
2 (Heatime® only)	29	15	14	0,52 ± 0,085[a]
3 (Breeder only)	34	31	3	0,91 ± 0,016[b]
	132	**112**	**20**	

Table 2: An overview of the cows used in this report. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level. Outcomes with no superscripts (a,b) in common differ significantly

The results show a considerable difference of the positive predictive values (PPV) between group 2 (Heatime® = H) and group 3 (Breeder = B). The PPV gives the likelihood of a correct result when the outcome is positive. As group 1 has both breeder as Heatime® positive animals there is an overlap within group 1 compared to group 2 and 3. Therefore it is of no use of calculating significancies between group 1 and 2 and group 1 and 3.

A χ^2 test shows a significant difference between group 2 and group 3 (χ^2 = 12.364 (with 1 df) and p = 0.0004.

Because the double negative samples (all the cows that have not shown up on the Heatime® list nor were they detected by the breeder) are unknown, the sensitivities nor the specificities of the separate groups can be calculated.

As can be seen in group 1 (H&B); three cows were wrongly detected as in heat related to their high progesterone level. 'High' is stated here as a concentration above 2 ng/ml. One of these cows turned out to be already pregnant at the time of sampling which corresponds with the high progesterone concentration. The other two cows were not pregnant at that time, no explanation can be given for them to be considered as 'in heat'.

In group 2 (H only); fourteen cows had high progesterone levels. Eleven of these cows turned out to be pregnant at the time of sampling. These cows were in all ranges of lactation number and lactation stage.

In group 3 (B only); three cows had high progesterone levels. None of these were pregnant during the sampling. Of the three cows, two of them never showed positive on the Heatime list (at least not for the current lactation). Of the thirty-one cows with low progesterone levels fifteen cows did not show positive with Heatime at any other moment of the current lactation. These cows were in all levels of lactation ranging from the first lactation up to the sixth and from 49 DIM up to 264 DIM. Three of these cows had aborted the previous two months. None of the cows had been diagnosed with diseases or lameness of any kind.

3.1.1 Comparison Heatime® and P_4 and the breeder and P_4

Table 3 shows the comparison with the Heatime® system (group 1 and group 2 combined, for the original numbers see table 2) and the height of the progesterone concentration. Table 4 shows the same comparison but this time with the breeder's observations (group 1 and group 3 combined, for the original numbers see table 2). Tables 3 and 4 show that there is a difference in PPV between Heatime® and the breeder compared with the progesterone concentrations, whereby the breeder has a higher positive predictive value.

	Number of cows with $[P_4]$ < 2ng/ml	**Number of cows with $[P_4]$ > 2ng/ml**	**Total**	**PPV (CI95%)**
Heatime® +	81	17	**98**	0,83 ± 0,064
Heatime® -	31	3	**34**	
Total	**112**	**20**	**132**	

Table 3: Comparison with the observations of Heatime® and the progesterone concentration of the same cows. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level

	Number of cows with $[P_4]$ < 2ng/ml	**Number of cows with $[P_4]$ > 2ng/ml**	**Total**	**PPV (CI95%)**
Breeder +	97	6	**103**	0,94 ± 0,040
Breeder -	15	14	**29**	
Total	**112**	**20**	**132**	

Table 4: Comparison with the observations of the breeder and the progesterone concentration of the same cows. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level

3.1.3 Comparison Heatime® L, Heatime® M and Heatime® H with P_4

As specified in the chapter *2.1.4 Heatime®,* table 1, the system assigns cows into four different groups: Heatime® N, Heatime® L, Heatime® M and Heatime® H. Heatime® N has not been used in this report. Heatime® L and Heatime® M are the ones of most interest as these are the groups of which the system itself says they're doubtful.

Splitting out the results with the different Heatime® groups there can be seen that there are significant differences between the three alerts calculated with a Fisher's exact test: $p < 0{,}001$. Calculating the differences between the groups separately it gives the following results (correction with Bonferroni): Heatime® L with Heatime® M: $p = 0{,}0018$; Heatime® L with Heatime® H: $p = 0{,}0831$; Heatime® M with Heatime® H: $p < 0{,}001$. So in the end Heatime® L and Heatime® M are responsible for the significance.

	Number of cows considered as "in heat"	Number of cows with $P_4 < 2$ ng/ml	Number of cows with $P_4 > 2$ ng/ml	PPV (CI95%)
Heatime® L	40	35	5	0,88 ± 0,055 [a]
Heatime® M	26	12	14	0,46 ± 0,085 [b]
Heatime® H	66	65	1	0,99 ± 0,017 [a]
Total	**132**	**112**	**20**	

Table 5: Heatime® L: probably no heat as by Heatime®, though doubtful. Heatime® M: doubtful if in heat. Heatime® H: positive heat. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level. Outcomes with no superscripts (a,b) in common differ significantly

3.1.4 Comparison Heatime® L, M and H with the breeder

As can be seen in table 5 there are no considerable differences within Heatime® L and Heatime® H concerning the positive predictive value. The next Fisher's exact test shows that within the alerts (L, M and H) only the results of M are significant (L: $p = 0{,}2448$, M: $p = 0{,}0017$, H: $p = 0{,}197$) Table 6 and 8 show the results within Heatime L and H which are not significantly different. The differences therefore need to be within Heatime® M as can also be seen in table 7 concerning the differences in the PPV.

Groups within Heatime® L (n=40)	Number of cows considered as "in heat"	Number of cows with P_4 <2 ng/ml	Number of cows with P_4 >2 ng/ml	PPV (CI95%)
1 (Heatime® & Breeder)	22	21	1	0,95 ± 0,068 [a]
2 (Heatime® only)	4	3	1	0,75 ± 0,134 [a]
3 (Breeder only)	14	11	3	0,79 ± 0,126 [a]

Table 6: Groups within Heatime® L. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level

Groups within Heatime® M (n=26)	Number of cows considered as "in heat"	Number of cows with P_4 <2 ng/ml	Number of cows with P_4 >2 ng/ml	PPV (CI95%)
1 (Heatime® & Breeder)	9	7	2	0,78 ± 0,159[a]
2 (Heatime® only)	14	2	12	0,14 ± 0,133[b]
3 (Breeder only)	3	3	0	1,00[c]

Table 7: Groups within Heatime® M. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level. Outcomes with no superscripts (a,b,c) in common differ significantly

Groups within Heatime® H (n=66)	Number of cows considered as "in heat"	Number of cows with P_4 <2 ng/ml	Number of cows with P_4 >2 ng/ml	PPV (CI95%)
1 (Heatime® & Breeder)	53	53	0	1,00[a]
2 (Heatime® only)	11	10	1	0,90 ± 0,072[a]
3 (Breeder only)	2	2	0	1,00[a]

Table 8: Groups within Heatime® H. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level.

In Heatime® M eleven cows of the total of twenty-six cows were later diagnosed as pregnant during the time of sampling. Seventeen cows of this group had a divergent amount of days between this heat and the previous heat (as signaled by Heatime® and/or the breeder). They were of all kinds of lactation number and had DIM in between 74 DIM and 308 DIM. Some of these cows had already been inseminated for eight times.

3.2 Results when excluding the synchronized cows

To avoid the bias of the synchronization program the next data are excluded of these thirty-two cows. A remaining number of hundred cows will be analyzed. As can be seen in table 9 there is still a remaining difference concerning the PPV between group 2 and group 3. X^2 testing shows the differences are not significant though with $\chi^2 = 2{,}651$ (with 1 df) and p = 0,103.

Groups	Number of cows considered as "in heat"	Number of cows with P4 <2 ng/ml	Number of cows with P4 >2 ng/ml	PPV (CI95%)
1 (Heatime® & Breeder)	59	56	3	0,95 ± 0,043
2 (Heatime® only)	24	14	10	0,58 ± 0,097[a]
3 (Breeder only)	17	14	3	0,83 ± 0,074[a]
	100	**84**	**16**	

Table 9: An overview of the cows used in this report when excluding the synchronization program. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level. Outcomes with no superscripts (a,b) in common differ significantly

3.2.1 Comparison and Heatime® and P_4 breeder and P_4

Table 10 shows the comparison with the Heatime® system (group 1 and group 2 combined, for the original numbers see table 9) and the progesterone concentration. Table 11 shows the same comparison but this time with the breeder (group 1 and group 3 combined). These two tables again show that there is a remaining difference in PPV between the two of them compared with the progesterone concentrations, whereas the breeder has a higher positive predictive value.

	Number of cows with [P_4] < 2ng/ml	Number of cows with [P_4] > 2ng/ml	Total	PPV (CI95%)
Heatime® +	70	13	**83**	0,84 ± 0,072
Heatime® -	14	3	**17**	
Total	**84**	**16**	**100**	

Table 10: Comparison with the observations of Heatime and the progesterone concentration of cows excluding the synchronization program. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level

	Number of cows with [P_4] < 2ng/ml	Number of cows with [P_4] > 2ng/ml	Total	PPV (CI95%)
Breeder +	70	6	**76**	0,92 ± 0,053
Breeder -	14	10	**24**	
Total	**84**	**16**	**100**	

Table 11: Comparison with the observations of the breeder and the progesterone concentration of the cows excluding the synchronization program. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level

3.2.3 Comparison Heatime® L, Heatime® M and Heatime® H with P_4

Splitting out the results with the different Heatime® alerts there can be seen that there are significant differences between the three alerts calculated with a Fisher's exact test: $p < 0{,}001$.

Calculating the differences between the groups separately it gives the following results (correction with Bonferroni): Heatime® L with Heatime® M: p = 0,157; Heatime® L with Heatime® H: p = 0,0237; Heatime® M with Heatime® H: p < 0,001.

	Number of cows considered as "in heat"	Number of cows with P_4 < 2 ng/ml	Number of cows with P_4 > 2 ng/ml	PPV (CI95%)
Heatime® L	24	19	5	0,79 ± 0,080[a]
Heatime® M	19	9	10	0,47 ± 0,098[a]
Heatime® H	57	56	1	0,98 ± 0,027[b]
Total	**100**	**84**	**16**	

Table 12: Heatime® L: probably no heat as by Heatime®, though doubtful. Heatime® M: doubtful if in heat. Heatime® H: positive heat. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level. Outcomes with no superscripts (a,b) in common differ significantly

3.2.4 Comparison Heatime® L and Heatime® M with the breeder

The main differences still need to be within Heatime® M as can be seen in table 14. The Fisher's exact test within Heatime® M is significant (p = 0,0334).

Groups within Heatime® L (n=24)	Number of cows considered as "in heat"	Number of cows with P_4 <2 ng/ml	Number of cows with P_4 >2 ng/ml	PPV (CI95%)
1 (Heatime® & Breeder)	7	6	1	0,86 ± 0,010[a]
2 (Heatime® only)	4	3	1	0,75 ± 0,068[a]
3 (Breeder only)	13	10	3	0,77 ± 0,120[a]

Table 13: Groups within Heatime® L. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level. Outcomes with no superscripts (a,b) in common differ significantly

Groups within Heatime® M (n=19)	Number of cows considered as "in heat"	Number of cows with P_4 <2 ng/ml	Number of cows with P_4 >2 ng/ml	PPV (CI95%)
1 (Heatime® & Breeder)	7	5	2	0,71 ± 0,204[a]
2 (Heatime® only)	10	2	8	0,20 ± 0,180[b]
3 (Breeder only)	2	2	0	1,00[c]

Table 14: Heatime® M between groups when excluding the synchronization program. PPV: positive predictive value. Outcomes with no superscripts (a,b,c) in common differ significantly

Within Heatime® M seven of the nineteen cows turned out to be already pregnant, all of them were from group 2. The group existed of cows with a lactation period from 76 up till 308 days and were of all lactations. Only six cows of this group had a regular cyclicity (as signaled by Heatime® and/or the breeder).

3.3 Results when only using the synchronized group

To see the effect of the synchronization of the cows and the outcome of the heat detection by the breeder and by Heatime® the data of the synchronized cows were analyzed separately. Using a χ^2 test it shows a significant difference between Heatime® and the breeder, but because of the low numbers caution needs to be taken in the interpretation. $\chi^2 = 16{,}622$ and $p < 0{,}001$ (with 1 df).

Groups	Number of cows considered as "in heat"	Number of cows with P_4<2 ng/ml	Number of cows with P_4 >2 ng/ml	PPV (CI95%)
1 (Heatime® & Breeder)	10	10	0	1,00
2 (Heatime® only)	5	1	4	0,20 ± 0,138[a]
3 (Breeder only)	17	17	0	1,00[b]
	32	**28**	**4**	

Table 15: An overview of the cows used in this report when only analyzing the synchronized cows. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level. Outcomes with no superscripts (a,b) in common differ significantly

3.3.2 Comparison Heatime® and P_4 and Breeder and P_4

Table 16 shows the comparison with Heatime (group 1 and group 2 combined) and the progesterone concentration. Table 17 shows the comparison with the breeder (group 1 and 3 combined) and the progesterone concentration. Table 17 shows there is no significant difference between the measurements of the progesterone concentration and the opinion of the breeder. In this situation it is a logical conclusion as this is the group with the synchronized cows so the breeder has a large bias.

	Number of cows with [P_4] < 2ng/ml	Number of cows with [P_4] > 2ng/ml	Total	PPV (CI95%)
Heatime® +	11	4	**15**	0,73 ± 0,154
Heatime® -	17	0	**17**	
Total	**28**	**4**	**32**	

Table 16: Comparison with the observations of Heatime® and the progesterone concentration of the synchronized cows. PPV: positive predictive value. CI95%: confidence interval with 95% desired confidence level

	Number of cows with [P_4] < 2ng/ml	Number of cows with [P_4] > 2ng/ml	Total	PPV
Breeder +	27	0	**27**	1,00
Breeder -	1	4	**15**	
Total	**28**	**4**	**32**	

Table 17: Comparison with the observations of the breeder and the progesterone concentration of the synchronized cows. PPV: positive predictive value.

4 Discussion

As shown in the results there is a difference between Heatime® and the breeder on this dairy which is facilitated by the use of a synchronization program. The significance disappears when taking out the synchronized group. This may partly be because of the low numbers of cows in the dataset, but it is a clear statement that the breeder under the current dairy conditions has an advantage above Heatime®. This is supported by evaluating solely the synchronized cows. Although not significant, there is a slight remaining difference in the positive predictive value of the different groups. As can especially be seen within the Heatime® M group, Heatime® appears to give several false positives. This is partly because Heatime® tends to be 'better safe than sorry'. Finally, Heatime® points out cows that do not show any visible heat sign. Hence, it is of considerable added value on the dairy.

The research has not been executed as a double blind research, nor was it randomized. It is important that thorough research meets certain requirements. Unfortunately, under the circumstances of this small study it was not possible to create an ideal research environment.

4.1 Breeder

In general, the human component of a breeder's work is very important. Even though devices like Heatime® can improve heat detection, the human touch remains essential.

As agreed with both the owner and the veterinarian of the dairy one can enact that their breeder is one of the best in the neighborhood. Not only does he detect the heats very well, but he also takes the rectal exam into account before insemination. A possible bias in this report is this same talented breeder. The breeder has many years of experience and is used to work with his own expertise. One-and-a-half years ago the dairy invested in Heatime® without consulting the breeder on forehand. Therefore, on the one hand, it could very well be that he is trying to beat the system and paying more attention to heat signs. On the other hand, the dairy has been using Heatime® for one-and-a-half year. Hence, it would take great lengths for the breeder trying to beat the system. Considering this, Heatime® could be used as a tool to keep employees sharp and focused on their job.

4.2 Synchronization

An important bias in this research is the fact that the breeder was fully aware of the schedules of the synchronization programs. Thus, he had an advantage to use for his heat detection and insemination work compared to Heatime®. The results are therefore not independent from each other. As can be seen in the results, there are no significant differences between Heatime® and the breeder when the synchronised cows are excluded from analyses. Simultaneously, only analysing the synchronised cows showed a significant difference between the breeder and Heatime®. Hence, the breeder indeed has an advantage compared to Heatime®.

As stated in the introduction of this report, synchronization should not have an effect on a cow to display heat signals. The results however show otherwise. Synchronization, apparently, seems to make it more difficult for a heat detection system like Heatime®. Research of Lu Meng Chao substantiates this. His research showed both a smaller intensity of the walking activity and a shorter duration of estrus than a natural-estrus control group. The pedometer, though, used in his research still detected the heat. (Lu Meng Chao et al., 2010). Future research should include similar tests with Heatime® to investigate if there is indeed a difference.

4.3 Heatime®

Another important bias is the fact that there has been a selection for cows that show heat signs. The cows that did not show any heat signs nor were on the Heatime® list are not included in this study. This gives a distorted view of the real situation. Heat detection is stated inefficient when the percentages of unobserved or missed heats increase and say therefore something about the false negatives. Therefore, at this moment, nothing can be said about the efficiency of Heatime®.

Including more cows in the research over a longer period of time, and also including the cows that do not show any heat nor included on the Heatime® list would provide a more realistic overview of the situation and would possibly reveal differences between Heatime® and breeder techniques.

False negative influences on Heatime® can be lameness of the particular cow, bad pasture and bedding, and conditions on the physical fitness of the cow. Aungier et al. state that lameness did not have a significant effect on the Heatime® score, but as they already acknowledged themselves, this was not trustworthy because of the low number of lame cows in their research (Aungier et al., 2012). Thus, Heatime® could still be an indicator for this type of problems as it shows the total activity of the cow. Activity declines will consequently be visible as well. This would be interesting to investigate in future research.

The Heatime® L group contains a lot of cows only indicated as in heat by the breeder. This is an important group to signal, as this particular group could have other problems, like being lame. Besides that, this could contain cows with a silent ovulation (ovulation without clear heat signals) as described by Ranasinghe et al. in 2010. An experienced breeder might be able to discover this.

Finally, in this Heatime® L group, there were animals that showed a peak in activity more than twenty-four hours away. It is important for the breeder to check these animals if necessary. Because of the fixed settings of the system (with the maximum amount of hours since peak activity of thirty-eight), this is a difficult group to analyze. Future research could include this particulate group.

Especially in the Heatime® M group the amount of false positives within the system was significantly higher compared to the breeder. Heat detection is stated inaccurate when cows are inseminated, but they are not really in estrus, which says something about the false positives. The breeder should identify these false positive cows when using the Heatime® list and not inseminate them. Apparently the Heatime® system detects a change in behavior of these cows and puts these animals on the list. As said earlier, this way the system is 'better safe than sorry' and in about half of the cases (fifteen cows) the cows turned out to be in heat. This is an important positive aspect of the system to acknowledge, since those specific cows can be seen as an 'extra' on top of the ones that the breeder would have found.

Valenza et al. investigated the Heatime® system in 2012, but they did not include the possible effects of the breeder in their results and they used only synchronized cows. Valenza concluded that Heatime® had a PPV of 0,95. This is a lot higher than this research found (0,73), which is comparable to the research of Aungier et al., who had a PPV of 0,72 (Aungier et al., 2012). The reason for this discrepancy might be differences in the experimental design and herd. Aungier et al. also found 32% of false positives by Heatime®. They made a difference in the height of the peak activity level as scored by Heatime®. They related this to the different cyclic phases (luteal, follicular, pregnancy) diagnosed by ultrasound (Aungier et al., 2012). This is not comparable with this report. In the report of Valenza et al., 13% of the cows that were actually in heat were missed by Heatime®. No difference was made between the height of the weighed activity. As the breeder has an important role in the process, he should be incorporated in the results, as well as the differences between natural estrus and induced estrus.

Holman et al. executed a research with different heat detection systems, including Heatime®, comparing it to a breeder. They did not find any differences between the heat detection systems and the breeder in sixty-seven cows, since all of them had around 60% heat detection. Combining the Heatime® system with a breeder's observations gave the best results with a PPV of 0,92 (Holman et al., 2011). These results correspond with the current research.

4.4 Moment of insemination

Because the cows are inseminated only once a day in the morning, not all cows will be inseminated at the ideal moment. As the oocyte only has a short life span, the time from artificial insemination to ovulation is critical. Early studies about the best time for insemination showed that maximum pregnancy rates were obtained from midestrus until a few hours after the end of standing behavior. More recent studies with pedometer activity evaluations described the best time for insemination as five to seventeen hours after the increase of activity (Maatje et al., 1997; Roelofs et al., 2010; Valenza et al., 2012). This discrepancy between the ideal moment and the true moment will likely have its influence on pregnancy rates.

Valenza et al. performed artificial insemination twice a day when using the Heatime® system (Valenza et al, 2012). This might be better than the situation on the dairy in this report. But because of the size of this dairy, it might not be time and cost efficient.

People need to be cautious of the fact that Heatime® will signal cows up to thirty-eight hours after peak activity. On the one hand, this will ensure that all cows will be seen by the breeder at least once. On the other hand, there will not be any need to breed a cow after that period of time, because the chance of pregnancy will be very small.

4.5 Hormonal factors

In this research the progesterone concentration was used as a reference for whether a cow was in heat. As told in the introduction the progesterone already drops to low concentrations a few days before ovulation and climbs back up to several ng/ml in a couple of days. A low concentration of progesterone could mean several things. Firstly, when a cow is cyclic a low progesterone, it does not mean she is actually fertile. It only gives an indication whereabouts the cow is in the follicular phase. Secondly, a low progesterone concentration however could also mean the cow is not cyclic at all. Thirdly, it might be an option that she has a follicular cyst. These last two options have not been taken into account in this report. Future research should include a rectal examination of every cow to know whether the cow is cyclic or has fertility problems.

Because high producing cows have bigger livers, they tend to break down steroid hormones easier than lower producers. The duration of estrus therefore is reduced, which could make it harder for the breeder to detect heat (Santos et al., 2006). In this case, a heat detection system might be of help. Because no difference has been made between the high and low producing cows in this report, the clearance effect of the liver cannot be evaluated.

4.6 Pregnancy

Where some literature states that 6% of pregnant cows display signs of heat, a recent study claims this for 19% of the cows (Sturman et al., 2000, cited by Roelofs et al., 2010). This report also included twelve cows that were indeed pregnant, but either showed up on the Heatime® list (eleven cows) or the breeder believed they were in heat (one cow). The breeder therefore needs to put a lot of attention to heat detection, not only to the signs visible on the outside, but also to typical aspects discovered with rectal palpation. Inseminating pregnant cows is useless. It might even be dangerous for the developing embryo and cause embryonic mortality or abortion. Manipulating the reproductive tract while the embryo is nesting in the uterus might endanger that delicate process (Roelofs et al., 2010). In this research the pregnancy testing was not done at the moment of sampling, but afterwards. Immediate pregnancy testing could be useful, but because the breeder is not a trained veterinarian, it is not possible.

The developer of Heatime® claims that the system will give 3% more pregnancies. Because Heatime® by itself has detected fifteen cows (from group 2, Heatime® only) that were actually in heat and could have been inseminated at that very moment, Heatime® does increase the amount of pregnancies in a herd. With the current pregnancy rate of 25% there could have been an extra three to four pregnancies in this sample group of in total 132 cows. Because there are no accurate numbers of pregnancies in this report, no precise number can be given on the percentage of pregnancies. More cows including their pregnancies should be included over a longer period of time in the results to give a better comparison.

Rectal palpation and PAG testing are both good aids in the detection of pregnancy. As mentioned earlier, rectal palpation can be done accurately from thirty-five days onward. Pregnancy diagnosing should be done carefully. A false positive results in failure to re-inseminate the cow and therefore in longer days open. A false negative will cause the cow to abort after administration of PGF-2α, which is common in the United States.

4.7 Environmental circumstances

The average herd pregnancy in this dairy is 49%, which means on average 49% of the animals are pregnant at any stage. For the month October 2010 it was 54%. The mild weather of the summer of 2010 may have had something to do with it, as it is known that seasonal and acute heat stress can have a dramatic negative influence on steroid production by dominant follicles (Roelofs et al., 2010). The climate of the area has also to be taken into account. California can get very warm in summertime which can cause problems with the fertility. Because the blood sampling of this project was executed in seven working days (one-and-a-half week), the weather will not have an effect in between individual samples. High temperatures can also cause a false negative effect on Heatime®, as cows will tend to move less when temperatures increase. (Peralta et al., 2004)

Because the measurements have all been done within such sort amount of time, it also means that other influences than the weather are not taken into account. Future research could be done over a longer period of time, and with more animals, to see what effects there are relevant from outside.

4.8 Economics

An important factor in the dairy industry is the calving interval which indicates the two successive parturitions. The impact of the voluntary waiting period on the calving interval can be high, because a reduction of one day decreases the calving interval by 0.86 days. (Roelofs et al., 2010). Literature has pointed out that a one day increase in calving interval can give a reduction in profit between US$0,39 up till US$3,59, dependent on differences in production and pricing differences, considered calving intervals, method of calculation, assumptions, criteria and the considered time period (Plaizier et al., 1997). Senger estimated in 1994 that the losses due to erroneous detection of estrus is about $300 million annually for the North American dairy industry, and Maatje stated that failure in detection of estrus is the second largest cause of economic losses in the dairy industry, following mastitis (Senger, 1994; Maatje et al., 1997). Since these calculations are almost twenty years old, the current amount will probably be a lot higher.

As said before, heat detection is stated inefficient when the percentage of unobserved or missed heats increases. Efficiency, therefore, says something about the false negatives (Roelofsetal., 2010; Vries et al., 2006). The same applies for pregnancy diagnoses. Both errors of pregnancy diagnosis are costly: a false positive diagnosis results in failure to re-inseminate the open cow, whereas a false negative diagnosis might lead to abortion, if after the diagnosis prostaglandin is administered to regress the corpus luteum. Giving prostaglandin to cows trying to shorten the insemination intervalis a common method in America (Vries et al., 2006).

If we try to compare the Heatime® system as used at this dairy with a dairy that relies on synchronization completely, we can get the results as shown below. Please note that this is a rough calculation based on American prices in 2010 and that the actual calculations are dependent on a lot of variances. Also be aware of the fact that this is a calculation based on the American situation. In Europe it is not common to use hormones on such a big scale as in America.

Based on the amount of cows and the calving interval there will be 3472 parturitions a year. On average three inseminations per parturition are used. A GNrH injection will cost approximately $1.85, a PGF2a injection will be around $1,-. The total of treatments for Double OvSynch and reSynch will then be around $16.95. Heatime has a guarantee for seven years and says to give 3% more pregnancies a year, which means hundred-and-four pregnancies a year. A comparable dairythatwould use synchronization methods on all cows would pay approximately $411,950.- for seven years ofshots for all cows. The evaluated dairy in this report would have the following costs. $293,400,- for seven years of Heatime, $158,441.- for seven years of DoubleOvsynch heifers, $280,000.- for seven years of the extra employee and +$327,600.- as a profit for the extra pregnancies gained by the use of Heatime. This ultimately would give a total of $423,755.- for seven years. Compared to the full-synchronized dairy there is a negative balance of $11,805.-. So part of the profits might be in the form of the so called job satisfaction. And of course a lot is dependent on the circumstances and opportunitiesonthe farm itself.

Because Heatime® also detects a decrease in activity it can also be of use identifyinglamecows and sick cows. This way it can be of economic importance as it can be an early detection system for these cows.

5 Conclusion

There is a difference between Heatime® and the breeder on this dairy which is facilitated by the use of a synchronization program. The significance disappears when taking out the synchronized group. On one hand the system can detect cows in estrus that do not to show all heat signs and a lot of the heats occur overnight. Hence, Heatime® increases the amount of cows to be inseminated. On the other hand Heatime® shows more cows on the list than actually should be inseminated. Therefore it is necessary for the fine tuning that the cows need to be checked by the breeder as well. Heatime® gives the breeder an extra tool for heat detection, which ensures that the breeder can be more precise in his decision making.

As there seem to be no big financial differences, the personal preference of the dairyman also has to be taken into account. Furthermore, Heatime® can be of use identifying lame and sick cows, as it can also detect a decrease in activity.

Further research is needed on the importance of advanced heat detection devices as Heatime® within the dairy industry, and to what extent their claim to effectuate more pregnancies is feasible.

6 Acknowledgments

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7 References

- Arney DR, Kitwood SE, Phillips CJC. *The increase in activity during oestrus in dairy cows.* Applied Animal Behaviour Science 40 211-218, 1994
- Aungier SPM, Roche JF, Sheehy M, Crowe MA. *Effects of management and health on the use of activity monitoring for estrus detection in dairy cows.* Journal of Dairy Science, Vol 95 May, p. 2452-2466, 2012
- Barr HL. *Influence of estrus detection on days in dairy herds.* Journal of Dairy Sciences, 58:246, 1975
- Donaldson LE, Bassett JM, Thorburn GD. *Peripheral plasma progesterone concentration of cows during puberty, oestrous cyclus, pregnancy and lactation, and the effects of under-nutrition or exogenous oxytocin on progesterone concentration.* Journal of Endocrinology 48, 599-614, 1970
- Fricke PM. *Scanning the future- ultrasonography as a reproductive management tool for dairy cattle.* Journal of Dairy Sciences, 85: 1918-1926, 2002
- Gabor G, Toth F, Zsvari LO, Abonyi- Toth ZS, Sasser RG. *Early detection of pregnancy and embryonic loss in dairy cattle by ELISA tests.* Reproduction in Domestic Animals 42, 633–636, 2007
- Green JA, Roberts RM. *Ch 1. 9. Establishment of an ELISA for the detection of native bovine pregnancy-associated glycoproteinssecreted by trophoblast binucleate cells.* In *Placenta and Trophoblast*. Methods in Molecular Medicine™, Volume 122, IV, 2006
- Holman A, Thompson J, Routly JE, Cameron J, Jones DN, Grove-White D, Smith RF, Dobson H. *Comparison of oestrus detection methods in dairy cattle.* Veterinary Record 169, 47, 2011
- Ibrahim H. and Olaloku E. 2000. *Improving cattle for milk, meat and traction.* ILRI Manual 4. ILRI (International Livestock Research Institute), Nairobi, Kenya. 135 pp, n.d.
- Kiddy CA. Variation in physical activity as an indication of estrus in dairy cows. Journal of Dairy Science. Volume 60, Issue 2, February, 235-243, 1977
- King GJ. *Ch 3. Endocrinology of Pregnancy.* In *Reproduction in Domestic Animals.* Elsevier Sciences Publishers BV, 1993.
- Lamming GE, Royal MD. *Ovarian hormone patterns and subfertility in dairy cows.* Diskin MG (ed), Fertility in the high producing dairy cows. Vol 1. BSAS Occasional pub. 26, pp 105-118, 2001

- Lu Meng Chao, Sato S, Yoshida K, Kojima T, Kubota C. *Comparison of oestrus intensity between natural oestrus and oestrus induced with Ovsynch based treatments in Japanese black cows.* Reproduction of Domestic Animals 45, 168-170, 2010
- Maatje K, Loeffler SH, Engel B. *Predicting optimal time of insemination in cows that show visual signs of estrus by estimating onset of oestrus with pedometers.* Journal of Dairy Science, June; 80(6): 1098-105, 1997
- Nakao T, Sugihashi A, Kawata K, Saga N, Tsunoda N. *Milk progesterone levels in cows with normal or prolonged estrous cycles, referenced to an early pregnancy diagnosis.* Japanese Journal of Veterinary Science. 45 (4), 495-499, 1983
- Nation DP, Malmo J, Davis GM, et al.. *Accuracy of bovine pregnancy detection using transrectal ultrasonography at 28 to 35 days after insemination.* Australian Veterinary Journal; 81: 63-65, 2003
- Noakes DE. *Ch 1. Endogenous en exogenous control of ovarian cyclicity.* In Noakes DE, Parkinson TJ, England GCW. *Veterinary Reproduction and Obstetrics.* 9th ed. Saunders Elsevier, 2009.
- Noakes DE. *Ch 2. Development of the conceptus.* In Noakes DE, Parkinson TJ, EnglandGCW. *Veterinary Reproduction and Obstetrics.* 9th ed. Saunders Elsevier, 2009.
- Petrie A, Watson P. *Ch 9. Hypothesis tests 3- the Chi-squared test: comparing proportions.* In *Statistics for veterinary and animal science.* Blackwell Science ltd, 1999
- Peralta OA, Pearson RE, Nebel RL. *Comparison of three estrus detection systems during summer in a large commercial dairy herd.* Animal Reproduction Science 87, 59-72, 2005
- Pieterse MC. *Praktische tips fertiliteit rund.* International Veterinary Information Service, Ithaca New York, 2001
- Plaizier JCB, King GJ, Dekkers JCM, Lissermore K. *Estimation of economic values of indices for reproductive performance in dairy herds using computer simulation.* Journal of Dairy Sciences, 80: 2775-2783, 1997
- Pope GS, Gupta SK, Munro IB. *Progesterone levels in the systemic plasma of pregnant, cycling and ovariectomized cows.* Journal of Reproduction and Fertility. December; 20(3): 369-381, 1969
- Ranasinghe RMSBK, Nakao T, Yamada K, Koike K. *Silent ovulation, based on walking activity and milk progesterone concentrations, in Holstein cows housed in a free-stall barn.* Theriogenology 73, 942-949, 2010
- Roberts SJ. *Ch 2. Examination for pregnancy.* In *Veterinary obstetrics and genital diseases (theriogenology).* 3rd ed. Woodstock, Vt: David & Charles, 1986

- Roelofs J, Lopez-Gatius F, Hunter RHF, Van Eerdenburg FJCM, Hanzen Ch. *When is a cow in estrus? Clinical and practical aspects.* Theriogenology 74, 327-344, 2010
- Roelofs JB, Van Eerdenburg FJCM, Soede NM, Kemp B. *Pedometer readings for estrous detection and a predictor for time of ovulation in dairy cattle.* Theriogenology 64, 1690-1703), 2005
- Roelofs JB. *When to inseminate a cow? Insemination, ovulation and fertilization in dairy cattle.* PhD thesis, Wageningen University, the Netherlands, 2005
- Romano JE, Thompson JA, Forrest DW, et al. *Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle.* Theriogenology, 66: 1034-1041, 2006
- Santos, JEP, Thatcher WW. *Characterization of early embryonic death and prevention of pregnancy wastage.* January 9th, 2006. http://www.milkproduction.com/Library/Articles/Characterization_of_early_embryonic_death_and_prevention_pregnancy_wastage.htm
- Saumande J. *Electronic detection of oestrus in postpartum dairy cows: efficiency and accuracy of the DEC ® (showheat) system.* Livestock Production Science 77, 265-271, 2002
- Scenzi O, Taverne MAM, Beckers JF, Sulon J, Varga J, Borzsonyi L, Hanzen Ch, Schekk Gy. *Evaluation of false ultrasonographic diagnosis in cows by measuring plasma levels of bovine pregnancy associated glycoprotein 1*. Veterinary Record, 142, 304-306, 1998
- Senger PL. *The estrus detection problem: new concepts, technologies and possibilities.* Journal of Dairy Science 77, p2745-2753, 1994
- Senger PL. *Ch 6. Puberty.* In *Pathways to Pregnancy and Parturition.* 2nd ed. Current Conceptions Inc., 2003.
- Senger PL. *Ch 8. Reproductive Cyclicity- the follicular phase.* In *Pathways to Pregnancy and Parturition.* 2nd ed. Current Conceptions Inc., 2003.
- Senger PL. *Ch 9. Reproductive Cyclicity- the luteal phase.* In *Pathways to Pregnancy and Parturition.* 2nd ed. Current Conceptions Inc., 2003.
- Senger PL. *Ch 11. Reproductive behavior.* In *Pathways to Pregnancy and Parturition.* 2nd ed. Current Conceptions Inc., 2003.
- Senger PL. *Ch 13. Early embryogenesis and maternal recognition of pregnancy.* In *Pathways to Pregnancy and Parturition.* 2nd ed. Current Conceptions Inc., 2003.
- Szenci O, Beckers JF, Humblot P, Sulon J, Sasser G, Taverne MA, Varga J, Baltusen R, Schekk G. *Comparison of ultrasonography, bovine pregnancy-specific protein B, and bovine pregnancy-associated glycoprotein 1 tests for pregnancy detection in dairy cows.* Theriogenology. July 1; 50(1):77-88, 1998.

- Taverne M. *Ch 3. Pregnancy and its diagnosis.* In Noakes DE, Parkinson TJ, England GCW. *Veterinary Reproduction and Obstetrics.* 9th ed. Saunders Elsevier, 2009.
- Valenza A, Giordano JO, Lopes G jr, Vincenti L, Amundson MC, Fricke PM. *Assessment of an accelerometer system for detection of estrus and treatment with gonadotropin-releasing hormone at the time of insemination in lactating dairy cows.* Journal of Dairy Science 95:7115-7127, 2012
- Vries A de, Broaddus B, Bartolome J. *What is early pregnancy diagnosis worth?* May 17th 2006 http://www.milkproduction.com/Library/Articles/What_is_Early_Pregnancy_Diagnosis_Worth.htm
- Yaniz JL, Santolaria P, Giribet A, Lopez-Gatius F. *Factors affecting walking activity at estrus during postpartum period and subsequent fertility in dairy cows.* Theriogenology 66, 1943-1950, 2006
- Youngquist RS, Threlfall WR. *Current Therapy in Large Animal Theriogenology 2.* Saunders Elsevier, 2007
- University of Illinois. Comparison of two aids for detection of estrus, n.d. http://www.livestocktrail.uiuc.edu.uploads.dairynet.papers.Elect_det.pdf

8 Attachments

Please note that the cows and dates presented in the following tables and graphs are chosen randomly. They are only for clarifying the collected data.

Attachment A: High activity list October 18th 2010 generated by the DataFlow program

Attachment B: Raw graph from cow 27643 derived from the DataFlow program

Attachment C: Zoomed in at the high activity graph of cow 27643 derived from the DataFlow program

Attachment D: Overview of high activity of cow 27643 derived from the DataFlow program

Attachment E: Frontpage herd overview, dated on October 18th 2010 derived from the DataFlow program

Attachment A

Cow ID	Pen	Current Lactation Status	Days in Milk	New Max Peak STD Last 24 Hours	Hours from Peak Activity	Hours from Last ID	Last Heat Date	Last Breeding Date	Days since Breeding	Times Bred
26383	14	No Heat	639	4,6	10	3	15-9-2010		0	0
31126	5	Heifer Ready	0	4,6	14	5	2-10-2010		0	0
16395	12	No Heat	603	5	18	2	17-10-2010		0	0
30820	5	Heifer Ready	0	5,2	30	6	16-10-2010		0	0
29902	5	Heifer Ready	0	5,2	6	1	25-10-2009		0	0
16441	17	No Heat	764	5,7	12	1	17-10-2010		0	0
29757	5	Heifer Ready	0	6,4	12	4	4-10-2010		0	0
16274	8	No Heat	652	6,6	8	5	20-9-2010		0	0
28113	5	No Heat	837	6,9	8	2	22-12-2009		0	0
27694	3	No Heat	521	7,2	28	5	17-10-2010		0	0
30090	5	Heifer Ready	0	7,4	14	2	7-12-2009		0	0
16861	5	Heifer Ready	0	8	8	2	13-12-2009		0	0
28008	14	No Heat	538	8,2	18	2	17-10-2010		0	0
31816	5	Heifer Ready	0	8,2	6	5	27-9-2010		0	0
29637	5	Heifer Ready	0	9,4	26	3	17-10-2010		0	0
31620	5	Heifer Ready	0	10,4	20	6	17-10-2010		0	0
23149	7	No Heat	651	10,9	2	2	19-7-2010		0	0
28559	5	No Heat	734	10,9	28	5	17-10-2010		0	0
24895	19	No Heat	773	12	18	1	17-10-2010		0	0
28389	19	No Heat	749	12,2	4	3	26-9-2010		0	0
16700	19	No Heat	861	12,5	18	3	17-10-2010		0	0
26557	20	No Heat	795	13,8	4	2	29-12-2009		0	0
31407	5	Heifer Ready	0	15	16	4	5-9-2010		0	0
31720	5	Heifer Ready	0	15,7	16	4			0	0
26631	18	No Heat	760	16,2	10	10	22-9-2010		0	0
30251	5	Heifer Ready	0	16,8	20	5	17-10-2010		0	0
28381	19	No Heat	782	17,1	14	6	25-9-2010		0	0
25471	10	No Heat	573	17,5	8	0	24-8-2010		0	0
30173	5	Heifer Ready	0	17,9	10	2	7-12-2009		0	0
28846	14	No Heat	513	18,2	12	10	26-9-2010		0	0
26993	19	No Heat	784	18,9	18	5	17-10-2010		0	0
28839	12	No Heat	595	20,3	6	4	10-9-2010		0	0
31690	5	Heifer Ready	0	20,7	26	4			0	0
31198	5	Heifer Ready	0	21,6	20	4	17-10-2010		0	0
29181	16	Breeding	582	22,3	14	1	17-10-2010	10-8-2009	434	1
30248	5	Heifer Ready	0	24,7	8	2	18-11-2009		0	0

24111	8	No Heat	617	24,7	14	1	15-9-2010		0	0
16885	11	No Heat	520	24,8	6	5	22-9-2010		0	0
31684	5	Heifer Ready	0	25,1	22	5	17-10-2010		0	0
27643	3	No Heat	519	25,2	8	5	24-9-2010		0	0
29956	5	Heifer Ready	0	26,8	28	4	17-10-2010		0	0
16620	16	No Heat	754	27,7	10	10	27-9-2010		0	0
30966	5	Heifer Ready	0	27,8	24	5	17-10-2010		0	0
30698	5	Heifer Ready	0	28	6	5	11-9-2010		0	0
31548	5	Heifer Ready	0	28,8	24	4	17-10-2010		0	0
31136	5	Heifer Ready	0	30,8	24	4	17-10-2010		0	0
29164	12	No Heat	562	31,1	20	4	4-10-2010		0	0
31437	5	Heifer Ready	0	31,9	8	5	27-9-2010		0	0
29088	15	No Heat	578	32,2	16	3	28-9-2010		0	0
29814	2	No Heat	453	32,3	8	2	24-12-2009		0	0
28186	20	No Heat	724	34,7	6	2			0	0
30614	5	Heifer Ready	0	34,9	14	6	15-9-2010		0	0
24953	3	No Heat	518	35,9	16	3	4-10-2010		0	0
24841	8	No Heat	519	36	8	4			0	0
31738	5	Heifer Ready	0	36,5	8	6			0	0
29824	5	Heifer Ready	0	36,5	10	5	1-10-2010		0	0
31658	5	Heifer Ready	0	38,3	18	6			0	0
28409	19	No Heat	742	38,7	14	1	17-10-2010		0	0
22634	6	No Heat	744	39,7	6	4	27-9-2010		0	0
30034	5	Heifer Ready	0	42,2	8	4	31-10-2009		0	0
31744	5	Heifer Ready	0	44,3	16	6	26-9-2010		0	0
30062	5	Heifer Ready	0	44,5	4	2	28-1-2010		0	0
31343	5	Heifer Ready	0	50,5	26	4	17-10-2010		0	0
63										

Table 1: High activity list October 18th 2010 generated by the DataFlow program

Attachment B

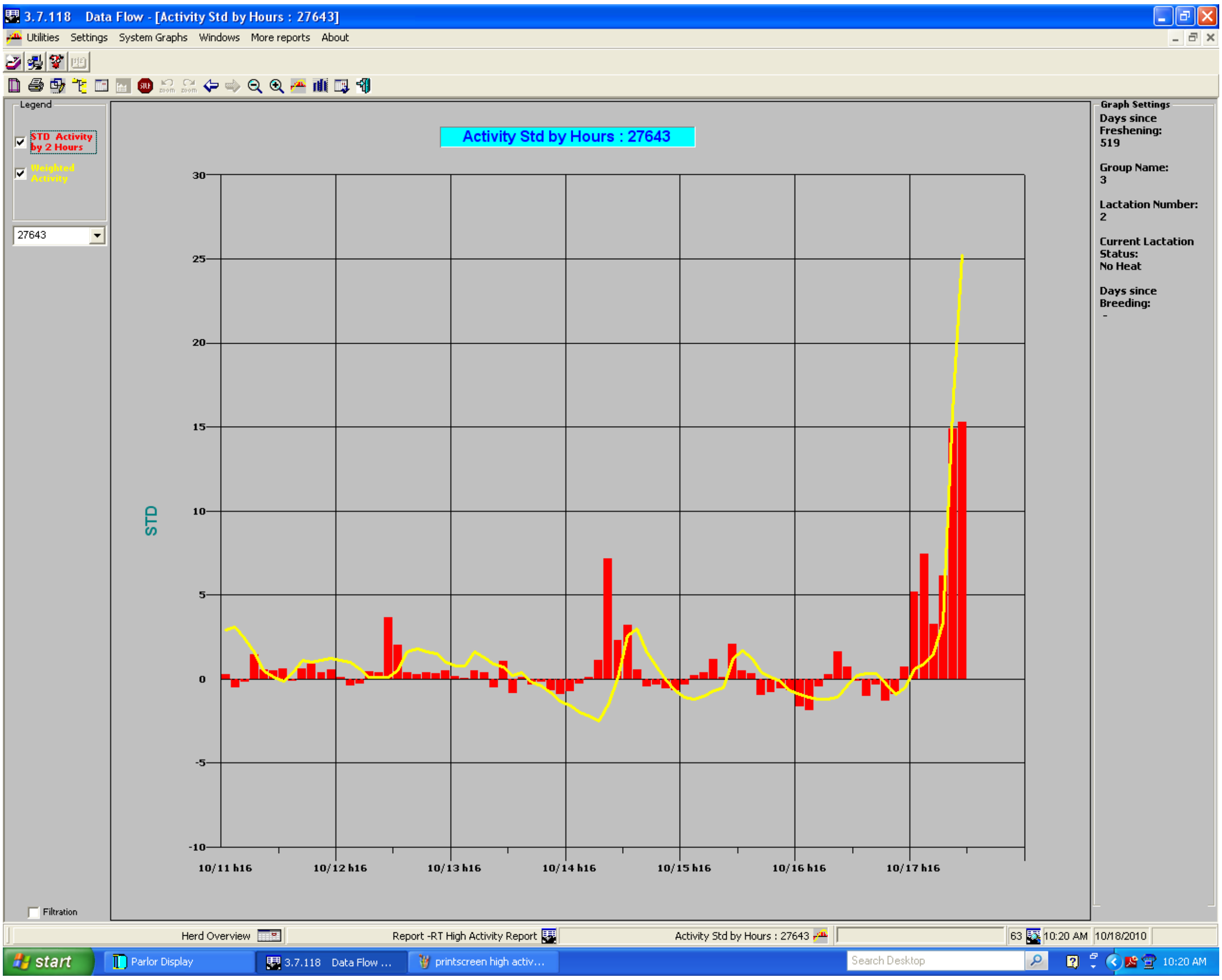

Figure 1: Zoomed in at the high activity graph of cow 27643 derived from the DataFlow program. On the Y-axis you will find the height of the STD = the activity of the cow. On the X-axis you will find the days. Red: raw acitivity. Yellow: weighted activity.

Attachment C

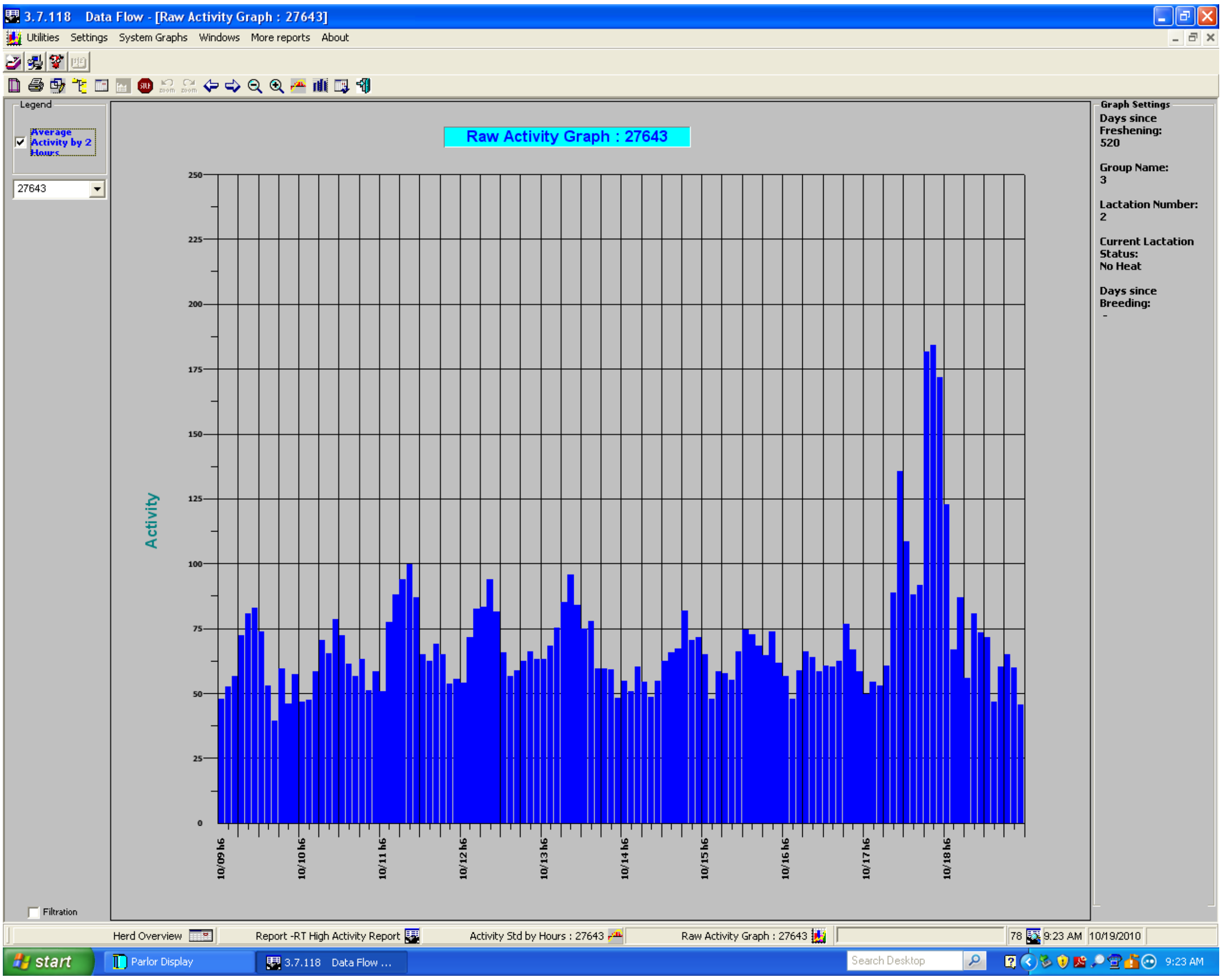

Figure 2: Raw graph from cow 27643 derived from the DataFlow program, a little more zoomed out than figure 1. On the Y-axis you will find the height of the STD = the activity of the cow. On the X-axis you will find the days. Blue: raw acitivity.

Attachment D

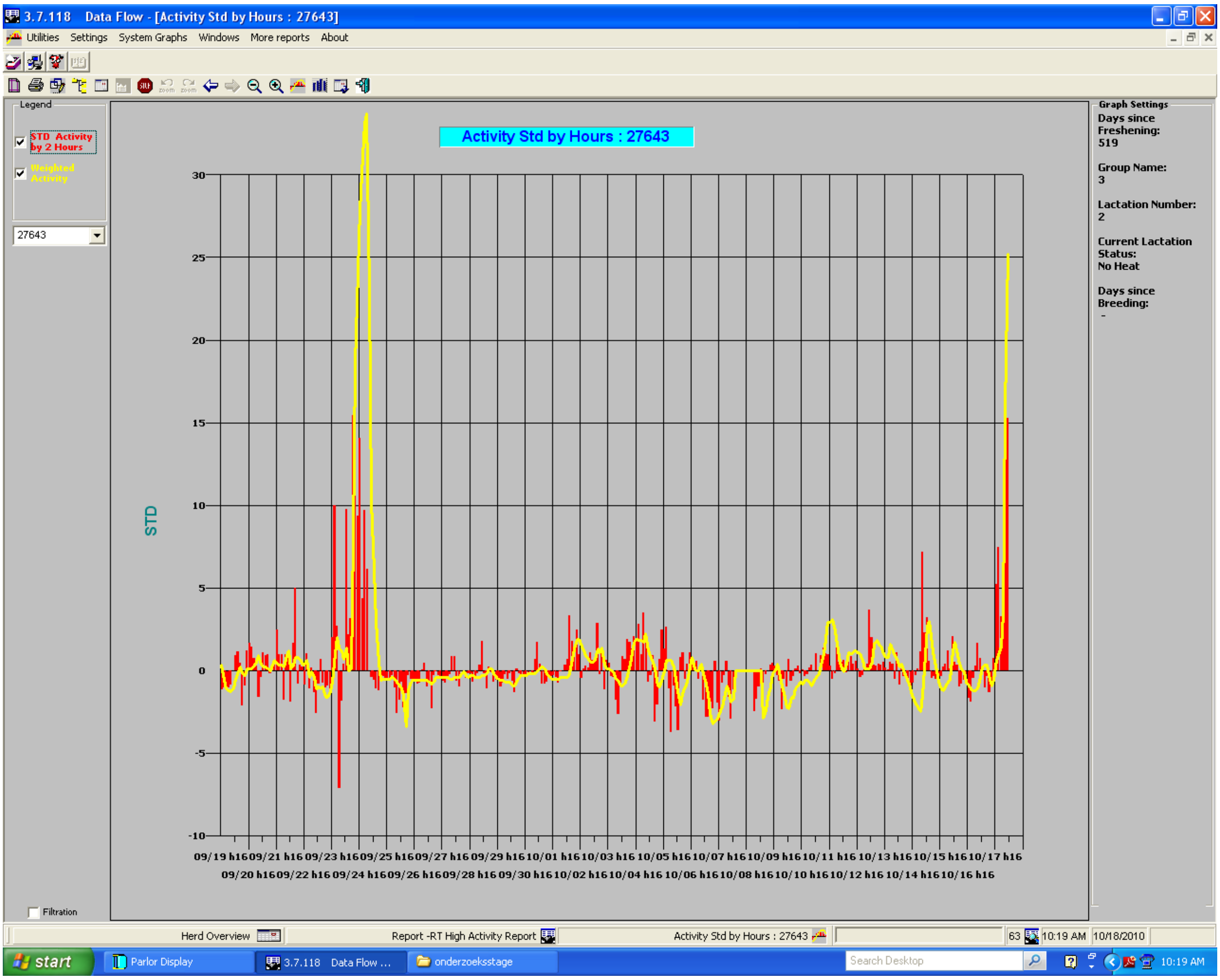

Figure 3: Overview of high activity of cow 27643 derived from the DataFlow program, you can see two heats. On the Y-axis you will find the height of the STD = the activity of the cow. On the X-axis you will find the days. Red: raw acitivity. Yellow: weighted activity.

Attachment E

As can be seen on this print screen the overview shows increased and decreased activity, as well as the total herd numbers, cystic cows and unassigned collars/ missing cows.

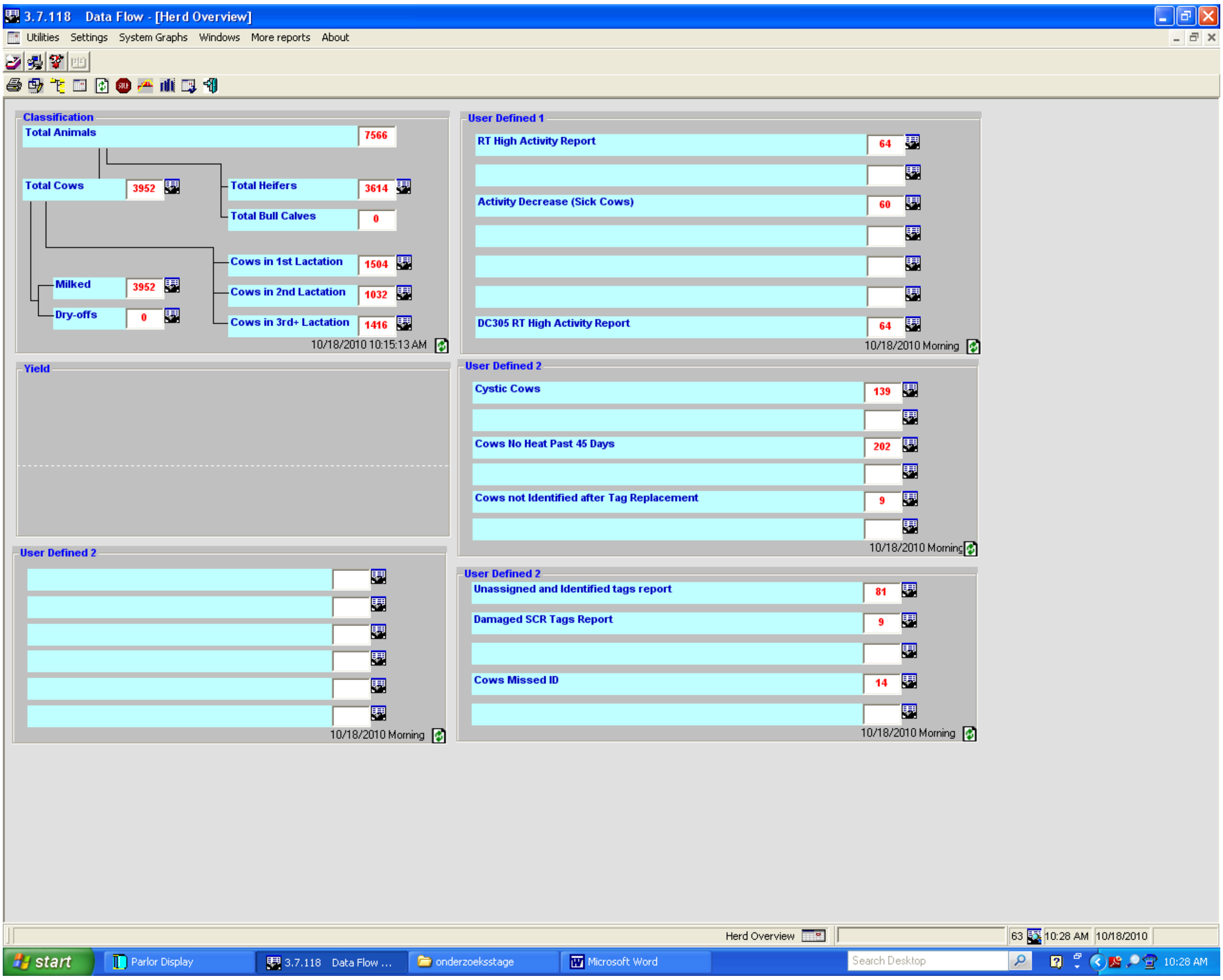

Figure 4: Frontpage herd overview, dated on October 18th 2010 derived from the DataFlow program