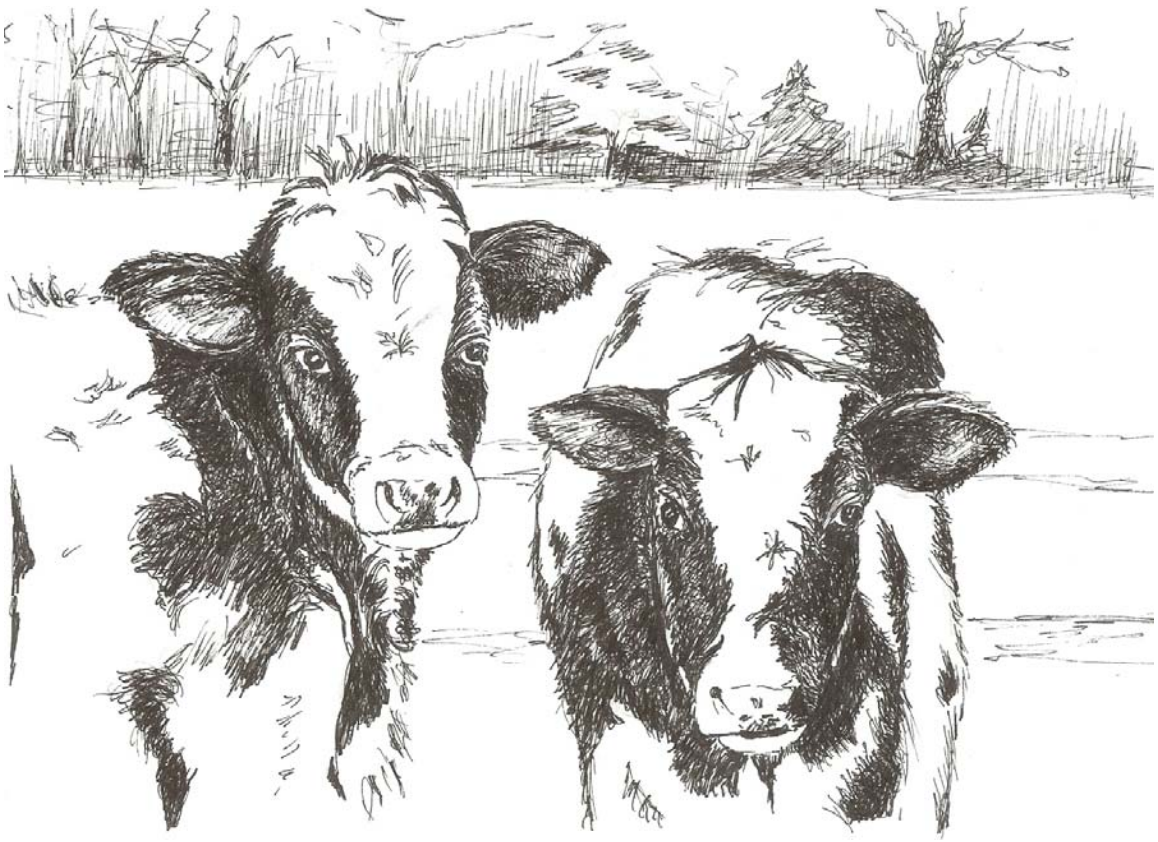
Strategies to improve ovulation and embryo development in timed insemination programs using GnRH, LH and hCG in post partum Holstein cows.



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Abstract

Fertility is a major problem in the dairy cow industry, the major cause of this problem is believed to be the rapid increase in milk yield of modern dairy cows. Due to poor fertility, calving intervals are prolonged, which resuls in economical losses due to reduced milk yield at the end of the lactation period and involuntary culling. As a result of these problems timed artificial insemination (TAI) procedures are being implemented on dairy farms. One of these procedures is 'Ovsynch' developed by Pursley et al in 1995. Ovsynch involves two injections of GnRH nine days apart and an injections with prostaglandin two days before the second GnRH injection. Artificial insemination follows one day after the second injection of GnRH. With this procedure pregnancy rates are around 40%. Conception rates are lower than when artificial insemination is based on observed oestrus and also early embryonic death is reported to be higher. In this study the GnRH injection is compared to LH and hCG injections. There were three treatment groups; treatment group 1 was given a normal Ovsynch protocol using GnRH, in treatment group 2 and 3 the GnRH injections replaced by respectively LH and hCG. The pregnancy rates of the treatment groups 1, 2 and 3 were 36%, 29% and 35% respectively, however no significant difference were found between the treatment groups.

Introduction

Reproduction performance in high yielding dairy cows is a major problem in the dairy industry worldwide. Fertility parameters, such as interval to first service, conception rates and oestrous detection, are poor. This leads to a prolonged calving interval, which results in economic losses. The causes for this problem are difficult to identify. High milk yield and poor oestrous detection are two major factors that play a role, but also early post partum diseases like mastitis, endometritis, retained placenta and cystic ovaries have been identified as causes for poor fertility (Lucy 2001). As there are so many contributing factors it is impossible to measure precisely the impact of one single parameter. However, several studies have calculated important relationships between farm and cow related factors involved, influencing poor reproductive performance.

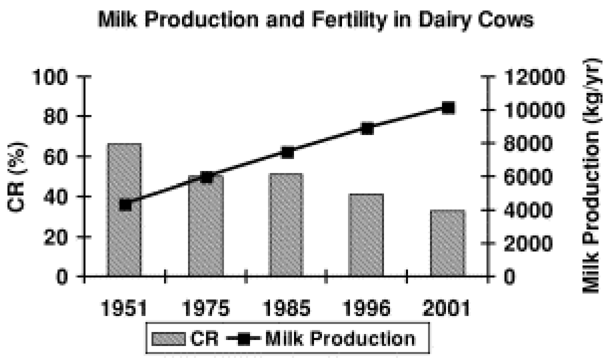

Fig 1 *The relationship between increased milk production and decreased conception rate (CR) over the last 50 years. (Butler 2003)*

Negative energy balance

In dairy industry there has been a selection for cows with high milk yield. In the last 50 years the average production of milking cows doubled, going from around 4000 kg/yr in the 1950's to almost 10000 kg/yr nowadays (figure 1) (Butler 2003). For this high milk yield a cow needs lots of energy and especially in the first weeks of the lactation this leads to problems. In order to fulfil to the energy requirements a cow has to ingest 4 to 6 times as much dry matter after parturition (Roche 2000). Because it is impossible to reach such an increase of feed intake the cow has a period of negative energy balance (NEB), which can last for 10-12 weeks post partum (Butler 2003). During NEB the cows fat and protein reserves are used, this means a high concentration of non-esterfied fatty acids (NEFA) in serum and triacylglycerol accumulation in the liver.

In all cows, Follicle Stimulating Hormone (FSH) waves start 5-7 days after parturition. FSH stimulates ovarian follicles to grow (Butler 2003). From this follicle growth wave a dominant follicle arises, which starts to produce oestradiol. Oestradial triggers a gonadotrophin surge which results in an ovulation. However in anoestrus cows the dominant follicle is not able to produce enough oestradiol to trigger a gonadotrophin surge and because of that there is no ovulation. This may result in two possibilities, either atresia of the dominant follicle or the dominant follicle becomes cystic. Both possible outcomes will delay the first ovulation with 20-50 days. (Butler 2003)

The reason of this decrease of oestradiol production is that NEB leads to lower glucose, insuline and IGF-I (Insuline Growth Factor) levels in blood, which are important for the development of follicles. Normally insuline up-regulates the LH receptors on the ovaries and IGF-I stimulates follicles and oestradiol production in follicle cells. (Roche 2000; Butler 2003)

Follicle maturation and ovulation depend on Luteinisation Hormone (LH) pulse frequency from the pituitary gland. During a state of NEB the LH production is depressed, probably by a decreased stimulation of Gonadotrophin Releasing Hormone (GnRH) produced by the hypothalamus. Because of this decrease in LH, there is less androgen production by the theca cells in ovaries. This decrease, plus the decrease in IGF-I leads to a decrease of oestradiol production in the dominant follicle by the granulosa cells. (Roche 2000)

Because of a NEB, the LH production is suppressed, dominant follicles are smaller and are less likely to ovulate. If ovulation does take place, the corpus lutuem (CL) formed is smaller and progesterone production may be impaired. This may leads to lower progesterone levels and eventually to lower pregnancy rates. (Roche 2000)

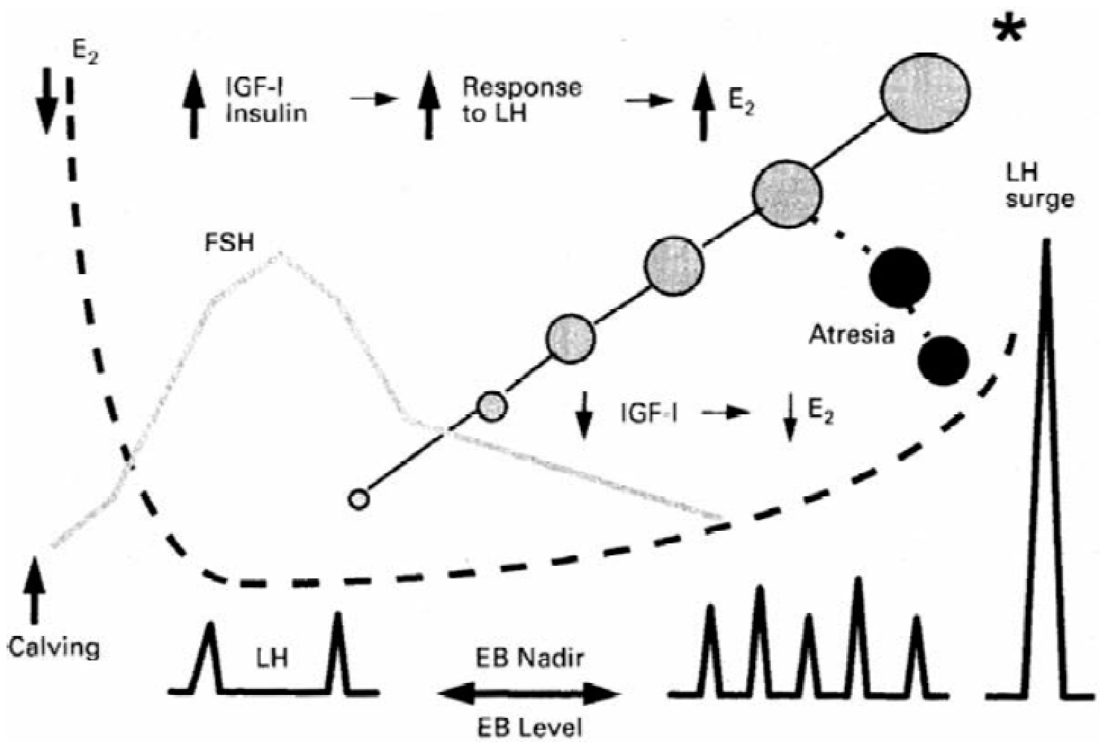

Fig 2: A schematic model describing dominant follicle development (circles and function in relation to changing metabolic and reproductive hormones, and energy balnce during the first follicular wave postpartum in dairy cows. The first follicle ovulates or undergoes atresia. (Butler 2003)

Management

The average farm size in Western countries has grown dramatically. In the Netherlands, farm size grew from 11 dairy cows per farm in the 1960's to more than sixty dairy cows per farm at the start of the 21st century (Bont de 2004). In the United Kingdom average herd size was 30 in 1970 and 71 in 1995. In the United States 30% of the farms have a herd size of over 500 cows (Lucy 2001). This means that management of a dairy farm changed as well, especially in farms with a herd size over 200 cows. The herdsman is not able to do all the work on his own and has to employ workers. This means the cows are handled by different persons and the herdspersons have less time per individual cow.

Oestrus detection traditionally was done by watching for oestrus signs, for example mounting another cow, licking, fighting and mowing (Vliet 1995). Not every cow shows when she is in oestrus clearly or in the same way, because of this it is important for a herdsman to know his cows individually. Often, only the cows that present standing or mounting oestrous are spotted and inseminated, but only 58% of the cows in heat show standing oestrous, and less when there is only one cow in oestrus (Roelofs 2005). This means that watching for oestrus in a large herd is time consuming and with several workers it is hard to get good results because the individual cow is less known.

Economics

Infertility leads to an extended calving interval (CI). Economical losses are mainly due to reduced milk production per year, decreased income from calf sales and an increase of replacements cows. (Noakes 2001) The exact losses are hard to determine and are variable between farms, but an accepted belief is that every extra open day before a new conception takes place costs around $3. (Arbel 2001) However, Arbel et al 2001 found that a 60 day voluntary waiting period had economic advantages and a CI of 12 to 13 month was favourable. However when the CI extended this 13 month substantial losses were calculated. A total cost of $24.46 per cow per year because of breeding problems is estimated. Every 1% drop in conception rate a $7,36 loss per cow per year is estimated. (Noakes 2001)

The current average first service conception rate after AI is approximately 40% in cows. Conception rates after AI based on observed oestrous are higher (45%) than when timed AI (TAI) is used, TAI is when hormone protocol are used to predict ovulation so no oestrous detection is needed (Lucy 2001). However when observed oestrous is used conception rate and pregnancy rate are not the same. An estimate of the pregnancy rate can given by the formula conception rate x oestrous detection (Vries de 2006).

De Vries et al (2006) used a modelling program to calculate the economic effects of variations on different reproduction parameters. Using this program they mimicked TAI and found that with a conception rate of 40%, net return per cow per year increased from $17.93 to $51.41. The control group in this study had an oestrous detection rate of 40%. Although this calculation used prices based on the United States, it showed that with a 40% conception rate a TAI protocol can be profitable (Vries de 2006).

Improving fertility

There are many different methods designed to improve fertility, including oestrous detection aids, hormone treatment to predict oestrous, preventing NEB with food regimes and improving housing in which signs of oestrous are better shown. (Roche 2000; Lucy 2001)

A lactating dairy cow may only be observed in oestrous for as little as 11 hours per cycle (Roelofs 2005). In this time frame the cow has to be detected and inseminated. Observation is the most common way to detect oestrous. With this technique an observer looks for signs of oestrous. The efficiency of observation depends largely on the competence of the observer and the time spend observing. It is advised by farmer manuals to observed at least 4 times each day for 20 minutes. (NRS BV 2006) If done correctly, observation may have an efficiency of more than 90% (Hulsen 2004). However studies have indicated that oestrus detection is time consuming and especially difficult in large herds. Using observation as a detection method could result in 80% detection rate, however, detection rates often do not exceed 60%. (Phillips 2001) An average oestrus detection rate of around 55% is reached during several studies. (Firk 2002)

In the United States and Canada, herd sizes often exceed 500 cows. With herds of this size it is understandable that the time spent on each individual cow is less than in smaller herds. Oestrous detection aids such as hormonal assays, pedometers and continuous video recording have been developed to support the farmer with oestrous detection. Using these methods the efficiency of oestrous detection is increased, although most detection aids are costly in either money or time. In table 1 some oestrus detection methods and aids are listed. (Fields 2002)

Detection Method	Advantage	Disadvantage	Efficiency (%)	Accuracy (%)	Cost
Observation	Actual observation of animals	Time commitment is required	51-94	50-98	Time
Painted/chalked	Simple and inexpensive	Repeated chalk application is required	66+		$1/cow
Continuous video recording	A continuous visually recorded record of activity is produced	Placement and maintenance of video equipment and video evaluation time	56-94	~50	$400-800 plus videotape
Chin ball marker on bulls and androgenized cows	Bulls and androgenized cows are very effective detectors	Animal and equipment must be maintained	79+		$150 plus ink
Hormonal assay – progesterone	It is the best able to confirm oestrus detection errors	Poor accuracy of detection	60-100	<50	$4-10/cow

Table 1. Summary of several detection methods and aids with a brief mention of their advantage, disadvantage, efficiency, accuracy and cost.

Hormone treatments

Hormones to improve fertility can be divided into two categories. First, hormones can be used to treat specific individual animals with abnormal oestrus cycles, for example anoestrus cows or cows which suffer from a follicular or luteal cyst on one of their ovaries. The second use of hormones is in oestrus synchronization and timed artificial insemination (TAI) protocols. Using TAI protocols, the moment of ovulation will be predicted so artificial insemination can be done at a fixed time. Four hormones are frequently used in TAI and synchronization protocols: those are, progesterone, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), GnRH, estradiol.

Oestrus cycle

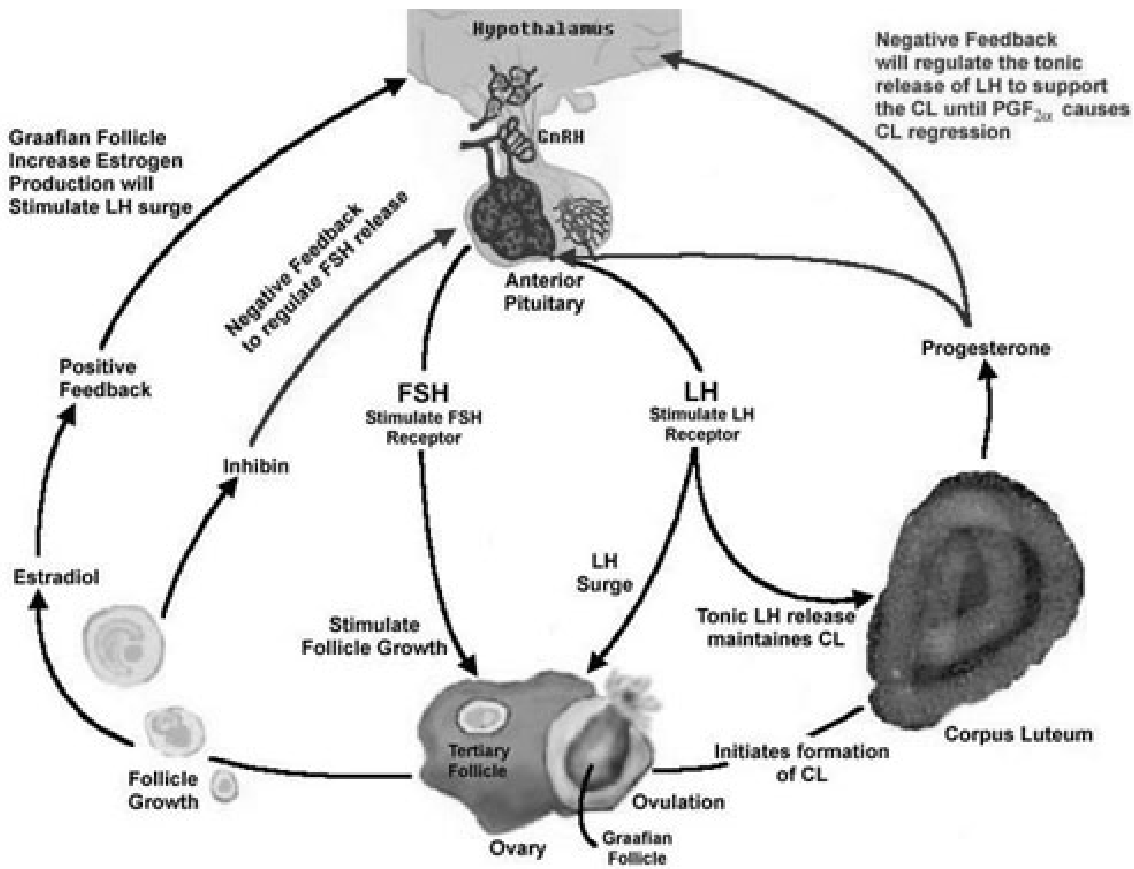

Fig 3: *Hormones secreted and the target areas of the hormones during oestrus in cows (Intervet 2008)*

The oestrus cycle of a cow has an average duration of 21 days, with a spread between 19 and 24 days. The oestrus cycle starts with an ovulation initiated by a LH surge 24 hours before. LH will initiate luteinization of the granulosa and thecal cells of the follicles. These cells will form the corpus luteum (CL), that produces progesterone. Progesterone prepares the uterus for a potential embryo, but it also has a negative feedback on the hypothalamus and the pituitary gland, inhibiting GnRH, FSH and LH release and

suppressing further maturation of follicles. Under the influence of oxytocin, produced by the CL, $PGF_{2\alpha}$ is produced in the endometrial cells in the uterus. $PGF_{2\alpha}$ is transported to the ovaries, where it causes luteolysis. The part of the oestrus cycle where a CL is present is called the luteal phase and last from day 1 till day 17. When the CL is no longer producing progesterone the follicular phase starts.
Since there is no suppressing of progesterone after lyteolysis, there is an increase in GnRH production and subsequently an increase in FSH and LH production. Under the influence of FSH, follicles in the ovaries will start to develop. One of the follicles selected will become dominant and will produce increasing levels of oestradiol. Oestradiol initiates LH surge by positive feedback on the hypothalamus inducing a GnRH release. This LH surge initiates the ovulation of the dominant follicle. Oestradiol has three major functions. It causes oestrus behaviour, which is important for oestrus detection. Oestradiol causes changes in the reproductive tract, allowing copulation, making sperm passage possible and facilitating the transport of the oocyte through the fallopian tubes. The follicular phase lasts from day 18 till day 21 (day 0). (Ball 2004)

Progesterone

To synchronize the oestrus cycle of a group of cows, progesterone treatment can be used. Progesterone suppresses the release of LH by negative feedback on the hypothalamus and pituitary gland, and thus suppressing follicular maturation and ovulation in the ovaries. When a group of cows is treated with progesterone and the treatment is stopped simultaneously the cows should come in oestrus synchronized. Exogenous progesterone does not have any influence on the length that the CL is present, so to ensure complete synchronization of a group the treatment should outlive the CL's present in the cows. To ensure this, the treatment should last for at least 16 days. However long-term progesterone treatment results in poor pregnancy rates. (Ball 2004) Shorter-term progesterone treatment (7-12 days) generally results in acceptable pregnancy rates, but has the disadvantage of not synchronizing all animals. (Ball 2004) To get acceptable pregnancy rates and ensure a successful synchronization, the progesterone treatment requires a combination with other hormones like prostaglandins (discussed further on).

Progesterone is usually administered intra-vaginal, by a device that gradually releases progesterone. Two intra-vaginal progesterone devices available and frequently used are CIDR (controlled intravaginal drug release, Pfizer Animal Health) and PRID (progesterone-releasing intravaginal device, Ceva Sante Animale). Both are inserted into the vagina by means of an applicator. After 7 days the devices are removed and the cows are observed for signs of oestrus for the next 4 days and inseminated if they show heat.

Prostaglandins

$PGF_{2\alpha}$ have a luteolytic effect, this means the life of a CL can be shortened with a $PGF_{2\alpha}$ treatment. A single injection of $PGF_{2\alpha}$ causes regression of the CL, and hence a drop of progesterone. Due to the drop of progesterone LH release from the pituitary gland is no longer suppressed and ovulation can take place in 2-5 days after the injection, depending on which stage of de follicle maturation the injection was given. When the injection is given in the early stage of a follicular wave it's takes longer for a follicle to ovulate. (Nebel 1998) A susceptible CL has to be present for a $PGF_{2\alpha}$ treatment to have an effect. A CL becomes susceptible for $PGF_{2\alpha}$ 5 days after ovulation. After day 17 the CL is no longer functional. This means that there is a 7 day window, from day 18 till day 4 of the next cycle, in the oestrus cycle in which $PGF_{2\alpha}$ treatment has no effect. (Ball 2004)

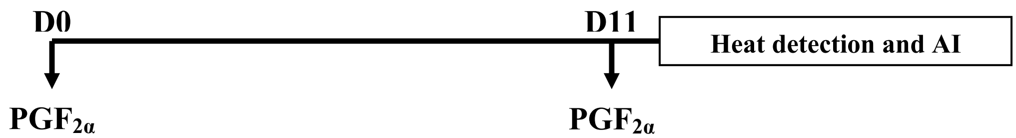

Fig 3: *Two plus two or Double injection system with $PGF_{2\alpha}$*

A method of two $PGF_{2\alpha}$ injection has been developed to ensure a high percentage of susceptible CL's in a group of cows on the moment the second injection is provided (Figure 3). This so called two plus two method or double injection system involves two injections of $PGF_{2\alpha}$ 11 days apart. The first injection causes luteolysis in cows between day 5 and 18 of the oestrus cycle, ovulation will take place and a new CL is formed. This CL is susceptible for $PGF_{2\alpha}$ at the time the second injection is given. In the cows that are between day 18 and day 4 the first $PGF_{2\alpha}$ injection has no effect on the oestrus cycle. Those cows will be between day 8 and day 15 of the oestrus cycle, with a susceptible CL on the moment of the second $PGF_{2\alpha}$ injection (Ball 2004). After the second injection the cows are checked for oestrus signs and are artificially inseminated on observed oestrus.

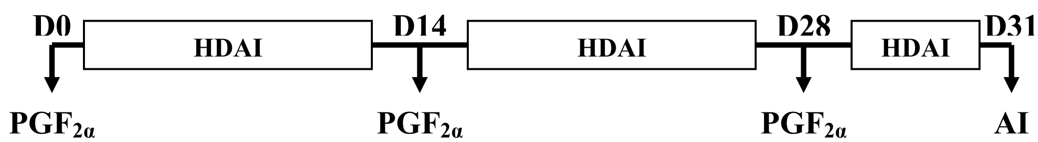

Fig 4: *Targeted Breeding protocol (HDAI: Heat detect and AI)*

Another method of multiple $PGF_{2\alpha}$ injections is the Targeted Breeding program (figure 4). All the cows are given an injections of $PGF_{2\alpha}$ and observed for oestrus. Those cows showing heat were inseminated. Fourteen days later the cows that did not come in heat are given second injection with $PGF_{2\alpha}$. After the second injection the cows are again observed for oestrus an inseminated when they are observed in oestrus. The cows that do not show signs of oestrus are given a third injection of $PGF_{2\alpha}$ after another 14 days and inseminated on observed oestrus, if they are not observed in oestrus within 72-80 hours after the last injection they are inseminated. The 14 day interval was designed so that cows are in mid cycle when the second injection of $PGF_{2\alpha}$ is administered. Pregnancy rates obtained with the Targeted Breeding program are around 39%. (Nebel 1998)

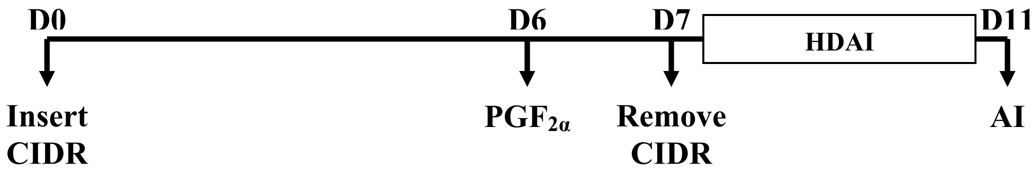

Fig 5: *CIDR Synch or Heat Detect protocol (HDAI: Heat detect and AI)*

In CIDR Synch or CIDR Heat Detect a progesterone treatment and a $PGF_{2\alpha}$ treatment are combined (figure 5). Cows are treated with progesterone for 7 days, and given a single injection of $PGF_{2\alpha}$ on day 6 of the treatment. After the removal of the progesterone device on day 7, the cows are observed for oestrus for the next 5 days and inseminated if they come into heat. After 5 days all the cows that that did not come into heat where artificially inseminated. (Graves 2003)

GnRH

In general, after a $PGF_{2\alpha}$ injection in a cow with a susceptible CL, ovulation takes place after 2 till 5 days, depending on the follicle maturation stage. Because of this spread in days it can not be used for timed insemination without oestrous detection. To be able to better predict the moment of ovulation, $PGF_{2\alpha}$ and progesterone treatments are combined with GnRH. With GnRH injections a follicular waves can be synchronized, resulting in a predicted timing of ovulation. With the use of this hormone, the observation for oestrus is no longer needed and the cows are inseminated on a defined time after the second GnRH injection, thus making oestrous detection rate 100%. (Nebel 1998)

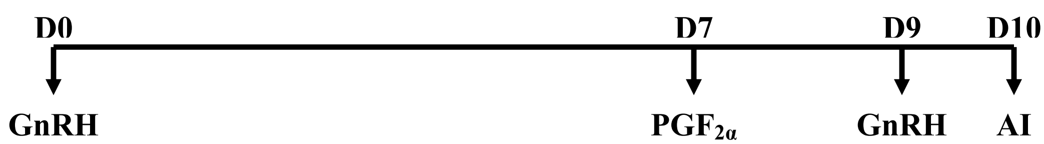

Fig 6: *Ovsynch protocol*

Pursley et al (1995) designed the Ovsynch protocol where the $PGF_{2\alpha}$ injection is preceded and followed by GnRH injections (figure 6). Using this technique, the hormone treatment can be used to breed cows without the need of oestrus detection. The first injection of GnRH is given 7 days prior to the $PGF_{2\alpha}$ injection. This starts a new growth wave of follicles which ensures a newly induced dominant follicle 7 days later. It may also cause ovulation or luteinization of a dominant follicle, if one is present on the moment of injection. This should ensure there is a responsive CL and a dominant follicle on the moment the $PGF_{2\alpha}$ is given. The $PGF_{2\alpha}$ causes luteolysis which causes a new ovulation as described in a previous paragraph. The second injection of GnRH is given 48 hours after the $PGF_{2\alpha}$ injection. With this injection, the LH surge and thus the ovulation will be more precisely determined and the cows are synchronised within a 8 hour time frame. The cows are bred 16 hours after the second GnRH injection. (Pursley 1997)

Pursley et al (1997) found pregnancy rates after Ovsynch treatment to be 43.4% in lactating cows after a voluntary waiting period (VWP) of 76 days. If the VWP was shorter (between 60 and 75 days) a pregnancy rate of 26.0% was found (Pursley 1997). The VWP is the number of days between the calving and the moment the cows are started to be be inseminated to get the optimal (farm depended) calving interval.

Stage of lactation	Pregnancy rate		
	Control	Ovsynch	Total
	%		
60 – 75 d	39.4	26.0	31.3
≥ 75 d	38.8	43.4	41.0
P	0.88	0.04	0.12

Table 2 *Pregnancy rates of lactating cows after treatment with $PGF_{2\alpha}$ (control) and Ovsynch (Pursley 1997)*

A large variety of modifications were made on Ovsynch, either to improve the pregnancy rate, or to reduce the number of times the cows need to be handled. One of the modifications frequently used to improve the pregnancy rates of a Ovsynch treatment, is to synchronize the cows before they are treated. This is called a presynchronization (Presynch) protocol. Presynch protocols are implemented because the synchronization rate of an Ovsynch treatment is largest when the cows are in the middle of the oestrus cycle at the moment of the first injection of GnRH. Progesterone and $PGF_{2\alpha}$ treatment are frequently used in Presynch protocols. (Peters 2002)

Rabiee et al (2005) did a meta-analysis and compared pregnancy rates of Ovsynch to those of cows bred after observed oestrus, synchronization with $PGF_{2\alpha}$ treatment and a modified Ovsynch protocol with presynch. They found that Ovsynch produced better or the same pregnancy rates compared to cows bred on observed oestrus. This was largely because of the poor sensitivity of the heat detection with the natural bred cows. Pregnancy rates of Ovsynch were slightly better then after a $PGF_{2\alpha}$ treatment and they found no significant difference between Ovsynch and a Presynch-Ovsynch protocol. (Rabiee 2005)

Fig 7: *Heatsynch protocol*

Another modification of Ovsynch that has been made, is replacing one or both GnRH treatments with another hormone treatment. An example of this is Heatsynch (figure 7). In this protocol, the second GnRH treatment is replaced by an estradiol injection on day 8. Because estradiol has a positive feedback on LH, it has been used to synchronize oestrus. Pancarci et al found that Heatsynch performed better than Ovsynch in cycling cows (resp 34,3% and 28%), but had poor pregnancy rates in anoestrus cows. In this group Ovsynch performed better (resp 15,3% and 23,4%). (Pancarci 2002)

Objective

Although the pregnancy rates showed in some studies are acceptable there is still room for improvement of the TAI programs. Thus making it a better management tool that can be implemented on farm to improve fertility. With Ovsynch one of the major factors of poor reproductive performance, the management status on a dairy farm, is clearly addressed by making the heat detection 100%. However, the conception rate after Ovsynch is around 40%, which is less then when artificial insemination is used after observed oestrous.

The objective of this study was to compare the effects of exogenous GnRH with exogenous LH or hCG on ovarian response, conception rate and embryonic losses in lactating dairy cows subjected to a timed insemination protocol. The preovulatory LH surge induced by GnRH treatment lasts only for 4 to 6 h, which is substantially shorter than a spontaneous LH surge. The administration of exogenous LH may provide better progesterone production, CL function, and embryo development. As hCG has a LH like effect, this is hypothesised to induce a better ovulation and CL function. (Rajamahendran 2007)

Materials and methods

Animals

For this experiment 67 lactating Holstein Friesian cows were randomly assigned to 3 groups. The cows were between 2 and 8 years of age, uniparous or multiparous and at least 55 days in milk (DIM). The cows were group housed according to the guidelines of the Canadian Council of Animal Care (Olfert 1993). Cows were housed together with cows not involved in this experiment, and group sizes ranged from 12 till 74.

The average number of lactations, number of insemination in current lactation before the start of experiment (service), weight, days in milk on start of treatment and body condition score (BCS), of the three different groups are given in table 1.

Group	N	# lactations (±SD)	Service (±SD)	Weight (±SD)	DIM (±SD)	BCS (±SD)
1	25	2.40 (±1.58)	0.64 (±1.35)	647 (±71)	103.4 (±66.1)	3.12 (±0.37)
2	25	2.52 (±1.61)	0.44 (±0.71)	652 (±83)	96.6 (±39.9)	2.87 (±0.34)
3	17	2.53 (±1.55)	0.41 (±0.87)	646 (±82)	92.9 (±48.2)	3.00 (±0.35)

Table 3 *Group averages of number of lactions, service (number of inseminations in current lactation before the start of the experiment), weight, days in milk on the start of the treatment and bodyconditionscore.*

Experiment

The three groups each received a different synchronization treatment, group one (control group) was treated according to the Ovsynch protocol, with two injections of 2 ml GnRH (Fertiline®, Vétoquinol) on days -10 and -1, and a injection of 2 ml PGF (Estrumate®, Schering-Plough Animal Health) on day -3. Group 2 was treated with 5 cc LH (Lutropin-V, Bioniche Animal Health) on day -10 and -1, and 2 ml PGF on day -3. Group 3 was treated with 1 ml hCG (Chorulon®, Intervet) on day -10 and -1, and 2 ml PGF on day -3. All injections were given intramuscular in the hind limbs. In all groups the injections on day -3 and -1 were given between 4 and 5 o'clock pm. In figure 8 the treatments are given schematically.

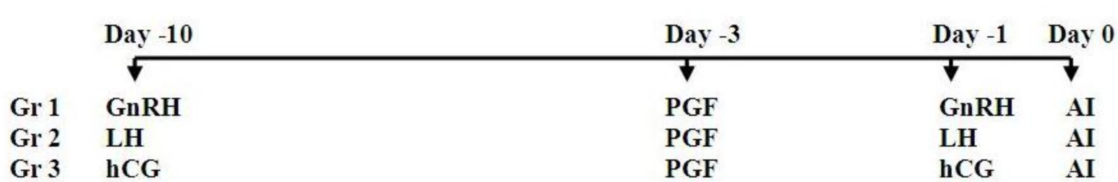

Fig 8: *Protocol of the three treatment groups.*

On day 0 the all the cows were artificially inseminated around 10 o'clock am. If a cow came into heat before day -5, she was not bred before day 0. If she came into heat between day -5 and day 0 the cow was bred and given their last injection of either GnRH, LH or hCG, the day she was observed in oestrus.

On day -10, -3 and -1 the cows were also palpated rectally and scanned with an ultrasound scanner (Aloka SSD-500, Aloka Co.) with a 7,5 MHZ transrectal linear array transducer. The size of the ovaries was assessed, and the follicles and CL's present on the ovaries were measured and recorded.

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On day -10 the size of the uterus was assessed and the BCS was determined. During the treatments the cows were weighed over 2 successive days , at approximately the same time of the day.

Milk samples were taken on days -10, -3, 0, 7, 11, 14, 21, 25 and 28; all samples were treated with preservative and frozen by -10 degrees celcius. The samples were defrosted one day before they were analysed. Progesterone levels in milk samples were analysed with solidphase ^{125}I radioimmunoassay (Count to Count Progesterone, Diagnostic Products Corporation, Los Angeles). The progesterone levels were used to determine whether a CL was present on the day of sampling. If the level of progesterone was more than 1 ng/mL it was assumed there was a functional CL present. This procedure defined whether there was an ovulation on day 0 and whether there was a CL present after day 21, which could indicate pregnancy. On day 40 all cows were checked for pregnancy, by means of palpation and ultrasound scanning. By analyzing the progesterone levels embryonic death from day 21 till day 40 could be determined.

Statistical analysis

The results were analysed using a statistical programme, SPSS 15.0. The significant difference (P≤0.05) between the three different treatment groups was analysed using a One-way ANOVA model. The effects of the different characteristics on pregnancy rate and synchronisation rate was analysed using a logistic regression model.

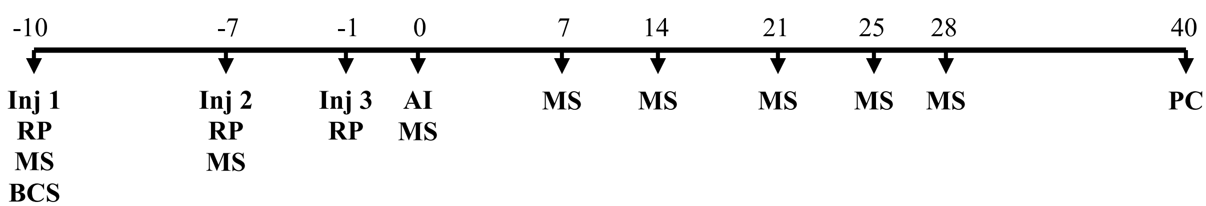

Fig 8: *Experimental design: Inj 1&3: GnRH, LH or hCG injection; Inj2: $PGF_{2α}$ injection; RP: rectal palpation; MS: milk sample; BCS: body condition score; AI: artificial insemination: PC: pregnancy check*

Results

The pregnancy rates from the three different treatments are displayed in table 4. In group 1, 9 out of 25 cows where diagnosed as being pregnant on day 40. In group 2 this was 6 of the 25, and in group 3, 6 of the 17. The pregnancy rates of the GnRH, LH and hCG treatments where 36%, 24% and 35% respectively. However LH and hCG treatments were not significantly different from GnRH (P = 0.76 and P = 0.927) .

		Pregnancy test on day 40		
		non-preg	pregnant	Total
Treatment	GnRH	16	9	25
	(control)	64,0%	36.0%	
	LH	19	6	25
		76,0%	24,0%	
	hCG	11	6	17
		64,7%	35,3%	
	Total	46	21	67
		68,7%	31,3%	100,0%

Table 4 *Treatment versus pregnancy between the three treatment groups.*

Cows were determined as synchronised when they had low progesterone on day 0, as is as shown in fig 9. The synchronised cows showed a clear drop of progesterone on day 0. None of the non-synchronised cows were pregnant on day 40.

In table 5 the synchronisation rate is listed. They were 87,5%, 93,8% and 72,7% respectively for groups 1, 2 and 3. There are only progesterone results from cows in the start of the experiment, because the these sample analyzed on a later date and the writer did not obtain the results. Because of this, the group sizes are smaller in this part of the experiment.

		Synchronization on day 0		
		non-sync	synchronized	Total
Treatment	GnRH	2	14	16
		12,5%	87,5%	
	LH	1	15	16
		6,3%	93,8%	
	hCG	3	8	11
		27,3%	72,7%	
	Total	6	37	43
		14,0%	86,0%	100,0%

Table 5 *Treatment versus synchronization rate, between the three treatment groups.*

Only five cases of possible early embryonic death between day 21 and day 40 where observed, three of these cases where in the LH treatment group and both of the other groups had one case each.

The effects of different characteristics (days in milk, body condition score, lactation and service number) on the pregnancy rate and synchronisation rate were analysed. Only the body condition score had a tendency to be of influence. If the BSC was < 3 then the pregnancy rate was 26%. If the BSC was ≥ 3 the pregnancy rate was 35%. However this result is not significant ($P = 0.111$). None of the other characteristics had a significant effect on the pregnancy or synchronisation rate.

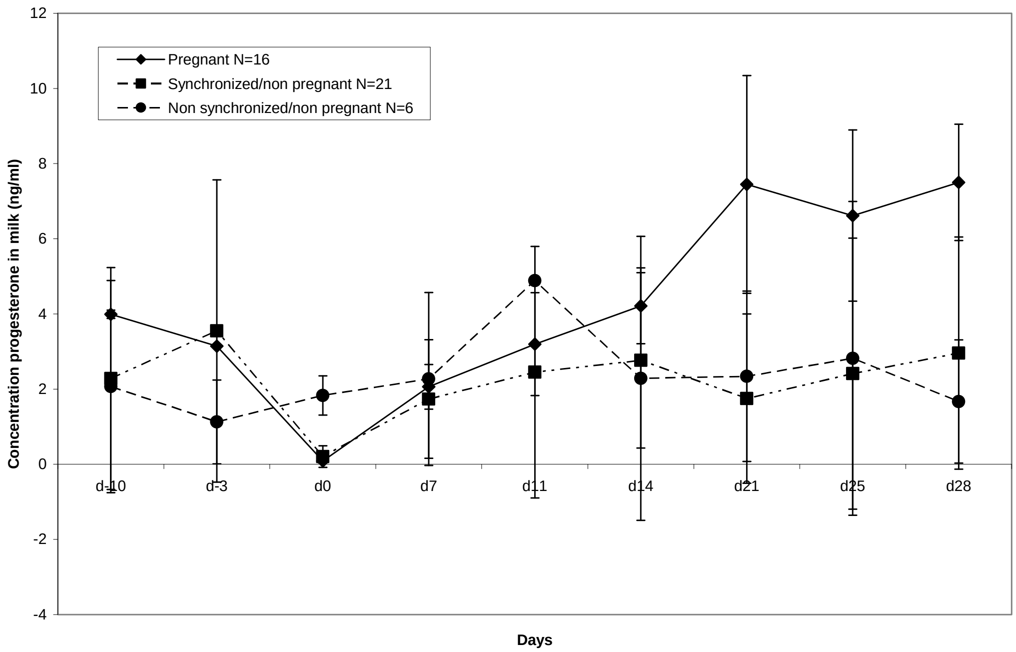

Fig 9: *Average progesterone levels of synchronized and pregnant, synchronized but not pregnant and not pregnant cows in the studie.*

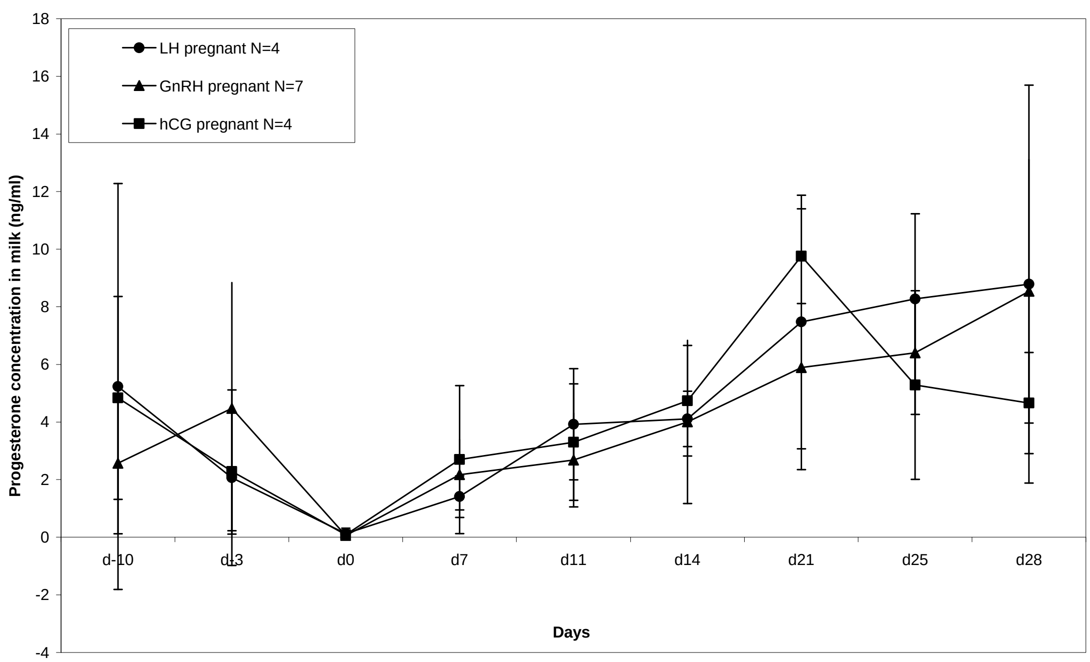

Fig 10: *Average progesterone levels of pregnant cows in the different treatment groups*

No difference in hormone levels was found between the different treatments, as seen in figure 10. However the progesterone concentration of the cows that where diagnosed pregnant on day 40 are higher on day -10, then the cows that were not pregnant.(figure 8) This difference is not significant (P = 0.114).

Discussion

The objective of the study was to compare different variance of the Ovsynch protocol with GnRH, LH and hCG, measured by ovarian response, pregnancy rates and embryonic losses. From the results obtained in this experiment, no conclusion can be drawn other than all three hormones can be used in ovulation synchronization. To make any conclusion about differences in pregnancy rates and synchronization rates, a larger experiment has to be conducted. So far there is no evidence to suggest that LH or hCG injections will improve the results of an Ovsynch treatment. Data found in this study suggests an Ovsynch protocol based on GnRH will result in better pregnancy rates, than based on LH or hCG.. Treatment with LH did result in the best ovulation synchronization rates (93,8%), but had the lowest pregnancy rates (24%). This might indicate that although LH is capable of synchronization, oocyte quality, CL quality or both are poor, resulting in low conception rates, early embryonic death, or both. This theory is further supported with the fact that most diagnosed embryonic death occurred in the LH treatment group. Again as none of the results appear to be significant a larger study would be beneficial in order to obtain more conclusive results.

Table 3 shows that 14% of the cows were not synchronized on day 0. Why these cows did not respond to the treatment is not known, at least half of the cows appeared to have a CL present on day -3, determined either by progesterone levels or rectal palpation. A possible explanation can be that the cows had an ovulation on day -10 or -9 and that the CL was not yet responsive to $PGF_{2\alpha}$.

Figure 11 shows that on day 21 the progesterone levels is of in the different groups was <1 ng/L as expected in a group in cycling cows that ovulated on day 0. This can be explained by embryonic death between day 21 and 40. These cows are diagnosed not pregnant on day 40 but have a functional CL on day 21.

So far, the costs of the Ovsynch protocol has not been mentioned. This is because it largely depends on the country and management on the farm. The cost of hormone treatment itself is reasonable, but because of the elimination of oestrus detection it is less time consuming. The hormone injections could be done in the milking pit, this way costs little time as long as the preparations are sufficient. Because there is a 40% conception rate, there is a need for a regularly pregnancy control by a veterinarian or other qualified person or for oestrus detection, to quickly identify the 60% of cows that will come into oestrus again. The decision whether to use Ovsynch or another synchronization protocol largely depends on the farm characteristics and management.

Timed artificial insemination in the Netherlands

In the Netherlands there also is a problem with fertility, however a major difference between the Netherlands and countries in which TAI is frequently used, for example Canada and the United States, is the farm size and management. In the Netherlands average farm size is around 70 dairy cows, and there only a few farms with more then one hundred dairy cows. On most farms the cows are only handled by one or two workers, which means that checking cows on oestrus can be done more effectively and takes less time. Because of this difference in farm size and management, oestrus detection is higher in the Netherlands, around 70% against 40% or less in the United States.(Hulsen 2004) However in the Netherlands there is tendency of an increase in farm size, indicating that in the near future a demand for TAI programas will increase, especially by the group of entrepreneurship farmers.

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