# Improving the Reproductive Efficiency by Zoo-Technical Methods at a Dairy Farm

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# Contents

This trial was conducted on a Hungarian dairy farm between July 2001 and December 2004. The objective of this work was to improve the reproductive efficiency with relatively high milk production. At the beginning of this trial blood and fodder samples were taken for checking the metabolic status of the animals in order to determine their health condition. The nutritive value of the daily ration for all groups met with the requirements of the Hungarian National Standard and almost all serum metabolic parameters differed between the milking and pregnant animals. Early pregnancy detection (by ultrasound) and ovulation synchronizing methods were introduced to optimize reproductive performance. The oestrus cycle was also checked by ultrasound and open cows were treated by the appropriate method in order to inseminate them as soon as possible. Efficiency of artificial insemination (AI) followed by a single prostaglandin  $F_{2\alpha}$  (PGF<sub>2</sub>) and Ovsynch treatment was similar (30.8% and 29%) and less effective than AI after natural heat detection (37.1%). Provsynch (Pre-synch followed by Ovsynch) was the most effective ovulation synchronization method (conception rate = 42.6%; p < 0.01). Although milk production increased between 2002 and 2004 by approximately 600 kg per cow, the calving period decreased by 20 days and the number of AIs per pregnancy was also improved (0.8 AI per conception). These findings are really beneficial economically because the decrease in the calving interval returns profit for the dairy farms; one open day costs \euro2.5/cow. Economical analysis showed a high profit ratio from the reproductive 'investment' on this farm. Every invested \euro yielded approximately \euro10.

# Introduction

Optimizing reproductive performance by integrated reproductive management in current cattle production systems is considered essential. Different stress factors of high milk production (physiological, environmental), inadequate nutrient intake, low body condition, and intensive management systems impair aspects of reproductive performance in dairy cattle (Thatcher et al. 2001). In the last decades a continuous elevation of the average milk production was recorded for all dairy cattle populations kept in intensive production systems. On the other hand a simultaneous, dramatic decrease appeared in reproductive performance, mainly because of the increased incidence of metabolic malfunctions (Huszenicza et al. 2003a). Because of poor heat expression and/or detection in high producing dairy cows, herd pregnancy rates are reduced. Anoestrus, low conception rates, and increased embryo mortality are typical in these herds (Al-Katanani et al. 1999).

Cows enter negative energy balance (NEBAL) at parturition as milk production rapidly increases and, in

parallel, the nutritional requirements shift abruptly. The duration and intensity of NEBAL is mainly related to dry matter intake, which in turn, is related to body condition at calving (Butler 1998). Disadvantageous effects of NEBAL or insufficient energy intake in early lactation cause reduced fertility of lactating cows. NEBAL also influences the fertility via the hormonal system, because it has effects on blood progesterone concentrations as well (Butler 2000).

In large commercial dairy herds oestrus detection is the main limitation for the optimal reproductive performance. Oestrus synchronization is a successful tool to improve oestrus detection rate due to control of the lifespan of the corpus luteum (CL; Lucy et al. 1986; Chenault 1992). During the last 25 years, researchers have developed reproductive management protocols that synchronize the time of oestrus using  $PGF_{2\alpha}$  to regress the CL. Synchronizing follicle development with occurrence of CL regression, allowed for precise control of the time of ovulation. This new technology ensured the option of fixed timed insemination and it also improved embryo survival rate. These programmes are successful in high producing dairy cows that experience a reduction in oestrus intensity and this contributes to undetected heats, re-occurring luteal phases without oestrus expression, or re-occurring waves of follicles that fail to ovulate (Thatcher et al. 2000).

The main objective of this work was to improve the reproductive efficiency in a Hungarian herd with relatively high milk production. For optimizing reproductive performance, early pregnancy detection and methods based on synchronizing oestrus were introduced. The aims were to decrease (i) the calving interval and (ii) the number of AIs per pregnancy and (iii) to increase the fertility rate after the first service.

# **Materials and Methods**

This trial was conducted on a Hungarian dairy farm between July 2001 and December 2004. Primiparous and multiparous Holstein-Friesian × Hungarian Flekvieh crossbred (R2-R4) cows with an average yearly milk production of 7969 kg per cow in 2001, 8685 kg in 2002, 9300 kg in 2003 and 9250 kg per cow in 2004, respectively were studied. At the beginning of the trial blood and fodder samples were taken for checking the possible effect of NEBAL. Metabolic status of the animals (n = 56) was examined, in order to determine their health condition around the time of parturition. Cows were chosen during 2–5 days pre- (dry cows, n = 16) or post-partum (n = 20), and top production (40–60 days after parturition; n = 20). Routine laboratory examinations were carried out for feed (crude protein, fibre and fat) according to Hungarian National Standard methods (Hungarian Feed Codex, 2004). Clinical biochemical parameters were measured in blood sera (glucose, total protein, calcium, phosphorus, urea,  $\beta$ -carotene, AST, LDH, CK) using commercial kits (Diagnosticum Ltd., Budapest, Hungary; Randox Laboratories, Cork, Ireland). Small changes were made to improve the quality of the daily ration and for prevention of retained placenta. An intramuscular injection (i.m.; Esvex, Dunavet Rt, Dunaföldvár, Hungary) containing vitamin E and selenium 21 was given 21 to 7 days prior to parturition. An i.m. beta-carotene treatment (Carofertin, Werfft-Chemie, Austria) was also injected for the same purpose on 14-16 days prior to and after parturition. Fertility data were obtained during all of 2002. Year 2001 was used as the reference year of this trial.

#### Nutrition and milking

Cows were housed and fed in open-sided barns in several groups based on the quantity of their milk production. The daily ration consisted of forage (ground corn silage, alfalfa hay, grass hay, alfalfa roughage) and concentrate (corn, whole cottonseed, wet brewers grains, soybean meal, and wet sugar beet pulp). The animals were fed and milked two or three times a day depending on the level of milk production.

#### **Pregnancy detection**

The pregnancy detection was carried out 28–42 days post-insemination every other week by ultrasound equipment (Scanner 100, Vet LC, Pie Medical, Maastricht, The Netherlands). The ultrasound equipment was also used for monitoring the oestrus cycle (examination of different ovarian structures: 40–60 days post-partum) in order to decide the exact treatment for cows that were open after each AI (in the course of pregnancy detection).

### **Treatment groups**

# Prostaglandin F2a

Cycling cows (CL detected by ultrasound on ovary) were treated by a single 2 ml intramuscular injection of PGF<sub>2α</sub> (Estrumate, Schering-Plough, Kenilworth, N.J., USA). Expected ovulation occurred generally within 4 days after treatment and in the absence of oestrus symptoms. The treatment was repeated 11–14 days after the first injection followed by insemination 72–80 h afterwards.

# Ovsynch

Non-cycling cows (CL not detected by ultrasound) were put on an Ovsynch regimen. The Ovsynch program started with an i.m. injection of GnRH (150  $\mu$ g Fertagyl, Intervet, Boxmeer, The Netherlands). It was given 7 days before and again 48 h after an injection of  $PGF_{2\alpha}$ , (2 ml Estrumate, i.m.) and cows were inseminated 16–20 h after the second injection of GnRH. This system synchronized follicle maturation with regression of the CL before the GnRH-induced ovulation and timed insemination (Stevenson et al. 1999).

#### Provsynch

A programme defined as Presynch-Ovsynch was developed (Thatcher et al. 2001), and in which pre-synchronization was achieved with a standard oestrous synchronization protocol. The programme started at 35 days post-partum (PGF<sub>2α</sub>-2 ml Estrumate i.m. – was injected twice at a 14-day interval) and was followed by the Ovsynch programme, which was initiated 12 days after the second injection of PGF<sub>2α</sub>. An increase in pregnancy rates was attributed to manipulation of the oestrous cycle such that the timed insemination programme was initiated at the most favourable stage of the oestrous cycle.

#### No treatment

The animals were inspected for signs of oestrus at least three times a day. The voluntary waiting period from calving to first AI established for this dairy herd was 60 days.

#### **Economical analysis**

Fertility problems are associated with longer calving intervals, premature disposal and other costs (treatment costs and costs due to increase in number of inseminations). All accepted economic analyses are based on the costs of these losses. In the simplified static economic evaluation of this study the income implies a decrease in cost of reproductive failure (savings) by shortening the calving interval and diminishing the number of inseminations; the costs are the price of the products used up in the reproductive programme. The method of quantifying the losses was homologous with that written by Ozsvári and Kerényi (2004). The calculations are based on the reference year, 2001. The optimal number of AI per conception was assumed to be 2, and the cost of an AI was estimated to be \euro20 on average on the farm. The average cost of a dose of the different injection was: Estrumate \euro2.25; Fertagyl \euro1.28.

#### Statistical analysis

It was conducted using S-Plus 2000 (MathSoft Inc. 1999). Chi-square tests were conducted to determine effects of treatments. One-sample *t*-test analysis was used to determine the difference within the serum concentration of the metabolic parameters. The distribution of the calving to conception interval was not normal; therefore, ANOVA could not be used. An appropriate calving to conception interval (CCI) was selected (up to 120 days after parturition) and tested the proportions below and above CCI on different level of milk production by chi-square test.

# Results

In generally the nutritive value of the daily ration for all groups met with the requirements of the Hungarian National Standard, including that for post-partum and high milk producing cows. In contrast with these findings, almost all serum metabolic parameters differed between 40–60 day lactating cows and the 2–5 day per and post-partum cows. No significant differences were found between the serum metabolic parameters of 2–5 day per and post-partum animals.

The serum concentration of glucose and the activity of liver enzymes were higher (p < 0.001; and out of the physiological range) in the group of 40–60 days post-partum cows than in the 2–5 day per and post-partum. Most of the milking cows had low serum concentration of beta-carotene; however, this concentration was significant higher in the pregnant cows. These differences were significant (p < 0.01). During this examination the serum concentration of the other measured parameters (protein, calcium, phosphorus and urea) were found to be close to the physiological values in all groups.

Production data are summarized in Table 1. From 2002 to 2004 milk production increased by 5.9%, while the calving interval decreased by 20 days and the pregnancy rate after the first AI was improved by 14.5%.

The pregnancy rate (Table 2) followed by a single  $PGF_{2\alpha}$  or Ovsynch treatment was similar and less effective than the AI of the no treatment group. The Provsynch was the most effective synchronization method (p < 0.01).

Distribution of the pregnancy rate after Provsynch treatment is shown in Fig. 1. The best results were observed if AI was carried out 67–75 days post-partum. There was no correlation between the post-partum day of AI and pregnancy rate (r = -0.51; p = 0.1).

No significant differences were found among the pregnancy rates of the different milking groups. Quantity of daily milk production was not related to pregnancy rate except in the highest milk production

Table 1. Production and reproduction data between 2002 and 2004

	2002	2003	2004
Milk production (kg)	8685	9300	9250
Calving interval (days)	425	410	405
Number of AI per pregnancy	3.01	3.09	2.3
Pregnancy rate after the first service (%)	29.8	40.1	44.3

Table 2. Efficiency of the different hormonal treatments on the pregnancy rate between 2002 and 2004

	$PGF_{2\alpha}$	Ovsynch	Provsynch	No treatment	Total
Number of AI	390	224	430	517	1561
Number of pregnant cows	120	65	183	192	560
Pregnancy rate (%)*	30.8 <sup>a</sup>	$29.0^{\rm a}$	42.6 <sup>b</sup>	37.1 <sup>a,b</sup>	35.9
Mean day conception after calving	155.3	155.5	71.7	152.5	133.7

Values within rows not sharing a common superscript differ significantly (p < 0.01).

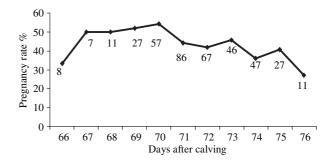


Fig. 1. Pregnancy rate of the Provsynch groups at different times of breeding after calving. Numbers at the data points are number of cows per group

group (over 45 kg), but this differences was not significant (p = 0.5).

# Discussion

High milk yield in cows is dependent on high levels of dietary protein as well as energy. As metabolism and utilization of dietary protein depends on energy availability, the effects of feeding high dietary protein superimposed on the preceding effects of NEBAL represents another important interaction of nutrition on reproductive performance in dairy cows (Butler 2000). In dairy cows the rapid increase in energy requirements at the onset of lactation results in NEBAL that begins a few days before calving. The detrimental effects of NEBAL in early lactation appear to be manifested as reduced fertility during the post-partum period. In normal dairy herd situations direct assessment of NEBAL in individual cows is not possible, but changes in body condition score (BCS) provide an indirect measure (Huszenicza et al. 2003b).

The metabolic examination showed that the serum concentration of most of the measured parameters were close to the physiological range. The serum concentration of glucose and the activity of liver enzymes in most of the milking cows (40-60 days post-partum) were elevated, as was serum concentration of beta-carotene in pregnant cows. Although in milking cows most of the examined parameters were found to be out of the physiological range, these are very typical for cows with high milk production within the first 90 days postpartum. That's why, based on our finding, these parameters are not really useful for the prediction of metabolic status for cows with high milk production. Keeping these parameters for the prediction of the health status of milking cows require re-defining the appropriate range for cows with high milk production.

In the base year of the trial (2001) the number of AIs per conception and the parturition interval were high (3.95 and 439 days respectively). Although the milk production increased between 2002 and 2004 by approximately 600 kg per cow, the calving interval decreased 20 days and the number of AIs per pregnancy also improved (0.8 AI per conception). These findings are really beneficial economically because decreasing the calving period returns profit for the dairy farms; one open day costs \euro2.5/cow (Table 4).

From 2002 different treatments (PGF<sub>2 $\alpha$ </sub>, Provsynch and Ovsynch) were used in parallel on the farm and became a complex reproduction management system, therefore their costs and their savings were grouped together in the economical calculation.

The ultrasonic examination of oestrus cycle was used to decide the exact treatment routes for the cows. As the first step the healthy cows were inseminated followed by Provsynch treatment. Cows that returned to oestrus were natural breeding (without treatment) approximately 8–12 h after the first signs of oestrus were observed. Cows that were open after Provsynch were either treated by a single injection of PGF<sub>2α</sub> or were put on an Ovsynch regimen based on their ultrasonic examination. The cycling cows (CL detected by ultrasound on ovary) were treated by PGF<sub>2α</sub> and re-inseminated generally within 4 days, generally. Non-cycling cows (CL not detected by ultrasound on ovary) were put on an Ovsynch regimen and re-inseminated 16–20 h after the second injection of GnRH.

### Prostaglandin $F_{2\alpha}$ treatment

Prostaglandin  $F_{2\alpha}$  has been successfully used in the treatment of anoestrous in cycling cows which are then inseminated at the detected oestrus (Lucy et al. 1986; Archbald et al. 1992). However the use of PGF<sub>2\alpha</sub> results in ovulations scattered over a 5 days period, and as a result the conception rates after PGF<sub>2\alpha</sub> treatment are higher for inseminations at the observed oestrus than for fixed-timed inseminations (Gaines et al. 1989; Archbald et al. 1992).

Synchronization with  $PGF_{2\alpha}$  was successful when cows were bred to a detected oestrus, because oestrus detection rates increased and management of AI was more efficient than daily detection of oestrus (Stevenson and Pursley 1994). Nevertheless, this management tool still did not control the time of AI, because oestrus detection continued to be necessary.

#### Ovsynch

Success of the Ovsynch programme is dependent on whether lactating dairy cows are anoestrus or cycling (Moreira et al. 2001). In this trial Ovsynch was only used in non-cycling cows; that is why the conception rate (29%) was more than acceptable although a significantly higher pregnancy rate was found by the treatment with Provsynch. One of the more subtle advantages from using this regimen is that there was an induction of ovulation and luteinization of a follicle in anovulatory cows (Pursley et al. 2001). The authors found that cows respond by approximately 90% synchronized ovulation for this treatment.

#### Provsynch

Hormonal control of the ovarian activity for further optimization of fertility (30–75 days post-partum) resulted in a higher conception rate, shorter calving interval and decreased number of AIs per conception (Table 1). Pregnancy rate after the first service was improved by almost 50% following the Provsynch treatment. An-

Level of milk production (kg)	< 20	20–25	25–30	30–35	35–40	40–45	>45
Number of AI Calving to conception	122 184 <sup>a</sup>	207 192 <sup>a</sup>	343 138 <sup>a,b</sup>	379 116 <sup>b,c</sup>	318 103 <sup>b,c</sup>	185 87°	91 73°
interval (day) Pregnancy rate (%)	35.2	37.7	36.2	34.8	36.8	37.8	33

Values within rows not sharing a common superscript differ significantly (p < 0.01).

Table 4. The cost-benefit analysis (profitableness) of improved fertility results in 2002–2004

	Savings* (€) (reduced cost)	Cost** (€)	Profit*** (€)
2002	13002	1627	11375
2003	25392	2136	23255
2004	31863	2575	29289
Total (\euro)	70257	6338	63919

\*Savings by reducing calving interval and AI costs.

\*\*Cost of products for treatment.

\*\*\*Herd level.

other beneficial 'side effect' of this protocol is very similar to that of Ovsynch: 80% of the open cows remained in cycle. That ensured the option for a single PGF<sub>2α</sub> treatment after the early pregnancy check. While animals treated by Provsynch showed 42.6% pregnancy rate (Table 2), the remaining open cows treated by PGF<sub>2α</sub> after the early pregnancy detection (mean 35 day post-insemination) had a 34.5% conception rate. This resulted in keeping the calving period within 390 days for these cows.

Another benefit of this protocol is that conception did not relate to milk production while (as Table 3 shows) increased milk production generally has a slightly moderate negative effect on pregnancy rate. The conception rates differed significantly depending on the time (post-partum day) of AI. The best conception rate was found when AI was performed between 67 and 70 days post-partum (Fig. 1).

Economical analysis showed a high profit ratio from the reproductive 'investment'. Every invested single \euro made approximately \euro10 profit. This profit based on the decreasing of the parturition interval (less open days: no nutrition fee for this time) and the lower number of AIs (Table 4).

In summary, during a 3-year period the reproductive efficiency was improved in a Hungarian herd with relatively high milk production. The reproductive performance was increased by early pregnancy detection and different synchronized of oestrus and ovulation methods. Decreasing the calving period by 20 days and by 0.8 the number of AI per pregnancy also helped to increase the fertility rate up to 44.3% after the first service. All these changes result in a higher profitability and better competitiveness.

### References

Al-Katanani YM, Webb DW, Hansen PJ, 1999: Factors affecting seasonal variation in 90-day nonreturn rate to first

service in lactating Holstein cows in a hot climate. J Dairy Sci **82**, 2611–2616.

- Archbald LF, Tran T, Massey R, Klapstein E, 1992: Conception rate in dairy cattle after timed insemination and simultaneous treatment with gonadotrophin-realising hormone and/or prostaglandin. Theriogenology **37**, 723–731.
- Butler WR, 1998: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J Dairy Sci 81, 2533–2539.
- Butler WR, 2000: Nutritional interactions with reproductive performance in dairy cattle. Anim Reprod Sci **60–61**, 449–457.
- Chenault JR, 1992: Pharmaceutical control of estrous cycles. In: Horn VH, Wilcox CJ (ed.), Large Dairy Herd Management. Am. Dairy Sci. Assoc., Savoy, IL, pp. 153.
- Gaines JD, Eicker S, Larson P, 1989: Effectiveness of prostaglandin treatment of anoestrus in dairy cows. Bov Pract 24, 98–105.
- Hungarian Feed Codex Committee: Hungarian Feed Codex 2004, National Institute for Agricultural Quality Control. ISBN: 963 8609753.
- Huszenicza GY, Kulcsár M, Kátai L, Balogh O, 2003a: The interrelation of nutrition, milk production and reproduction in high-yielding dairy cows. Part 2: The onset of cyclic ovarian function in the postpartum period. A review of literature. Magy Állatorv Lapja **125**, 75–82.
- Huszenicza GY, Kulcsár M, Dankó G, Balogh O, Gaál T, 2003b: The interrelation of nutrition, milk production and reproduction in high-yielding dairy cows. Part 4: Form and consequences of increased ketone production. A review of literature. Magy Állatorv Lapja **125**, 203–208.
- Lucy MC, Stevenson JS, Call EP, 1986: Controlling first service and calving interval by prostaglandin F2a, gonadotropin-releasing hormone, and timed insemination. J Dairy Sci **69**, 2186.
- MathSoft Inc, 1999: S-Plus 2000 User's Guide. Seattle, WA, USA.

- Moreira F, Orlandi C, Risco CA, Mattos R, Lopes F, Thatcher WW, 2001: Effects of pre-synchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J Dairy Sci 84, 1646–1659.
- Özsvári L, Kerényi J, 2004: Quantification of losses due to reproductive disorders on a large-scale Holstein-Friesian dairy farm (in Hungarian). Magy Állatorv Lapja **126**, 523–531.
- Pursley JR, Fricke PM, Garverick HA, Kesler DJ, Ottobre JS, Stevenson JS, Wiltbank MC, 2001: NC-113 Regional Research Project. Improved Fertility in Noncycling Lactating Dairy Cows Treated with Exogenous Progesterone During Ovsynch. Midwest Branch ADSA. Meeting, Des Moines, IA, pp. 63.
- Stevenson JS, Pursley JR, 1994: Use of milk progesterone and prostaglandin  $F2\alpha$  in a scheduled artificial insemination program. J Dairy Sci 77, 1755.
- Stevenson JS, Kobayashi Y, Thompson KE, 1999: Reproductive performance of dairy cows in various programmed breeding systems including ovsynch and combinations of gonadotropin-releasing hormone and prostaglandin F2α. J Dairy Sci 82, 506–515.
- Thatcher WW, Mattos R, Moreira F, Binelli M, Ambrose JD, 2000: Experimental manipulation of follicular growth. Reprod Domest Anim 6 (Suppl.), 27–33.
- Thatcher WW, Moreira F, Santos JEP, Mattos RC, Lopes FL, Pancarciland SM, Risco CA, 2001: Effects of hormonal treatments on reproductive performance and embryo production. Theriogenology 55, 75–89.

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