EFFECTIVENESS OF DIFFERENT PROTOCOLS TO SYNCHRONIZE OESTRUS AND OVULATION IN NO CYCLING POSTPARTUM DAIRY COWS

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SUMMARY

The studies were performed in three farms for Holstein dairy cattle reared in indoor group boxes. Cows with no signs of oestrus between postpartum days 65 and 75 were examined by ultrasound. Experimental groups included animals, with no corpus luteum in ovaries, but possessing follicles with diameter ≥12 mm by the day of examination (day 0). They were randomly distributed as followed: Group A (n=*48*) were treated with gonadotropin-releasing hormone (GnRH, 100 μg Deferelin, Gonavet, Veyx, Germany) on day 0, and on day 7 – with prostaglandin (PGF2α, 500 μg, Veyx, Germany). The cows from the other 4 groups were treated with gonadotropin-releasing hormone on day 0 (GnRH, 100 μg Deferelin, Gonavet, Veyx, Germany), and on day 7 – with prostaglandin (PGF2α, 500 μg, Veyx, Germany), and prostaglandin on day 7 (PGF2α, 500 μg, Veyx, Germany). The cows from the other 4 groups were treated with gonadotropin-releasing hormone on day 0 (GnRH, 100 μg Deferelin, Gonavet, Veyx, Germany), and prostaglandin on day 7 (PGF2α, 500 μg, Veyx, Germany). The animals from group B were treated with GnRH (100 μg) 48 hours after PGF2α application and artificially inseminated 18 hours later (n=64). The cows from group C were treated with GnRH (100 μg) by the 48th hour and artificially inseminated 172 hours after receiving PGF2α (n=56). Cows from group D were treated with GnRH (100 μg) 66 hours after PGF2α application (n=62) and artificially inseminated at the same hour. Group E was treated with GnRH (100 μg) 72 hours after PGF2α and artificially inseminated at the same time (n=54). The application of the Select Synch protocol (group A) resulted in conception rate of 27.08%. The highest first service conception rate (32.81%) was achieved with the Ovsynch 66 protocol (group B). The use of the Ovsynch 72 protocol (group C) resulted in conception rate of 28.57%. The cows from group D (CoSynch 66) exhibited 24.19% conception rate, and the lowest one (20.37%) was registered in group E (CoSynch 72).

Key words*: dairy cows, anoestrus, oestrus and ovulation synchronisation*

Introduction

Over the last fifty years reproductive ability of lactating cows progressively decreases and mainly due to the steadily declining rate of establishment of estrus and fertilization (**Heershe and Nebel,** 1994; **Washburn et al.,** 2002). Numerous studies indicate that less than 50% of dairy cows were established oestrus (**Senger,** 1994; **Washburn et al.,** 2002) manifested in the long interval between insemination (**Stevenson et al.,** 1983). Fertility from approximately 70% (**Foote,** 1952; **Herman,** 1956) had reached 35% (**Lopez-Gaitius,** 2003; **Macfarlum and Pursley,** 2003) in the beginning of this century. Reasons of impaired reproductive productivity are multilateral. Most often they are genetics, breed, age and order of birth (**Fonceka et al.,** 1983), non-recovery of genital function of ovaries postpartum (**Thatcher and Wilcox,** 1973), nutrition (**Butler,** 2000), level of milk production (**Butler and Smith,** 1989; **Peters and Pursley, 2002**), state of the uterus (**LeBlanc et al., 2002**) etc. Those reasons have direct or indirect effect on the ovarian function expressed in varying degrees of follicular and luteal development (**Lucy,** 2000; **Sartory et al.,** 2002; **Sheldon and Dobson,** 2004).

Differentiation of cows after birth of cycling and non-cycling is essential for their reproductive determination and their including in appropriate schemes for early breeding. The established share of non-cycling animals was in the frame 20 - 30% (**Silva et al.,** 2007; **Stevenson**

et al., 2006; **Lopez et al.,** 2005) with variation of 8% (**Watters et al.,** 2006) to 55.7% (**El-Zarkouny et al.,** 2004). The most commonly used methods for determining the status when it comes to the cow cycling for measurement of serum progestren concentrations (RIA) and ultrasound (U/S) test of ovarian. A comparative assessment of the two methods in different studies has been setting high correlation coefficient from 0.66 (**Silva et al.,** 2007) to 0.74 (**McDougall and Rhodes,** 1999). Sync stage of follicular development and regression of the corpus luteum using the combined treatment, first with GnRH and second with PgF2α after 7 days. This combination is known as GnRH-PgF2α (**Thatcher et al.,** 1989) or Select Synch protocol (**Downing et al.,** 1998). Treatment with GnRH induces ovulation or luteinization of the dominant follicle in cows which show oestrus at regular intervals and in cows who are anoestrus. Ovulation in anoestrus cows stimulates the development and function of yellowbody tissue manifested in the restoration of sexually cyclical activity. Treatment with prostaglandins leads to regression of the formed corpus luteum (Troxel et al., 1993). Later with aim to synchronize ovulation and doing of artificial insemination at a particular time have been developed two protocols subject to a further treatment with a GnRH after prostaglandin. First protocol is called Ovsynch (**Pursley et al.,**1995), and the other CoSynch (**Geary et al.,** 1998). In these protocols is performed synchronization stage of follicular development and regression of the corpus luteum in the same way like Select Synch protocol, but the second treatment with GnRH induces LH pic conducive to the development and ovulation of the ovulatory follicle. That additional treatment with GnRH synchronize ovulation in a much larger extent than the actual natural oestrus and allows artificial insemination to be carried out at a fixed time (artificial insemination at fixed time). The difference between the two protocols is expressed in different time of induction of artificial insemination and treatment with GnRH. In Ovsynch protocol artificial insemination is 16-18 hours after second treatment with GnRH while CoSynch protocol second treatment with GnRH and artificial insemination are carried out at the same time.

In cattle breeding farms in the country non-showing of the postpartum oestrus is spread widely. To increase the percentage of pregnant cows we aim to assess the effectiveness of the implementation of the protocols Select Synch, Ovsynch and Co-Synch in lacting cows non-showing oestrus to 65 days after birth.

Material and Methods

Experimental animals

The studies were performed in three lacting farms for Holstein Frisian cows reared in indoor group boxes. The average milk yield and herd size were respectively 7980 liters (148 cows, herd I), 8 440 liters (196 cows, herd II) and 7 560 liters (402 cows, herd III). Animals received rations twice daily complies with the requirements for lactating cows, water enough for their needs, double milking at first two farms and three times in the third.

Determination of anoestrus condition

Between 65 to 75-day postpartum was provided ultrasound testing of cows with non-registered oestrus. In experimental groups were included animals that had no visualized luteum in the ovaries, and were detected follicles with a diameter ≥ 12 mm in the day of experiment (day 0) and were randomly distributed in different groups (A, B, C, D, E) . An ultrasound test was performed with ultrasound apparatus Mindrey (China), transrectal linear transducer with a frequency of 5.0 MHz, and artificial insemination was provided by qualified staff working in each farm.

Group A (n=*48*) were treated with gonadotrophin-releasing hormone (GnRH, 100 µg Deferelin, Gonavet, Veyx, Germany) at day 0 and a prostaglandin preparation (PGF2α, 500 µg, Veyx, Germany) day 7. Cows were observed routinely for oestrus 3 times a day, from day 8 to day

12 and shown signs of oestrus has been inseminated under the rule am/pm. Cows of the next 4 groups were treated on day 0 with gonadotrophin-releasing hormone (GnRH, 100 μg Deferelin, Gonavet, Veyx, Germany),on day 7 with prostaglandin preparation (PGF2α, 500 μg, Veyx, Germany). Animals of group B were treated with GnRH (100 μg) on the 48-hour after implementation of PGF2α and artificial inseminated 18 hours later (n=64). Cows of group C were treated with GnRH (100 μg) on the 48-hour and artificial inseminated on 72th hour after treatment with PGF2α (n=56). Animals of group D were treated with GnRH (100 μg) on 66th hour after implementation of PGF2α (n=62)and artificial inseminated immediately. Group E treated with GnRH (100 μg) on 72th hour implementation of PGF2α and artificial inseminated in the same hour (n=54) (figure 1).

Pregnancy was diagnosed in the period 28th - 35th day after artificial insemination helped by the same ultrasound apparatus and a second test within two weeks.

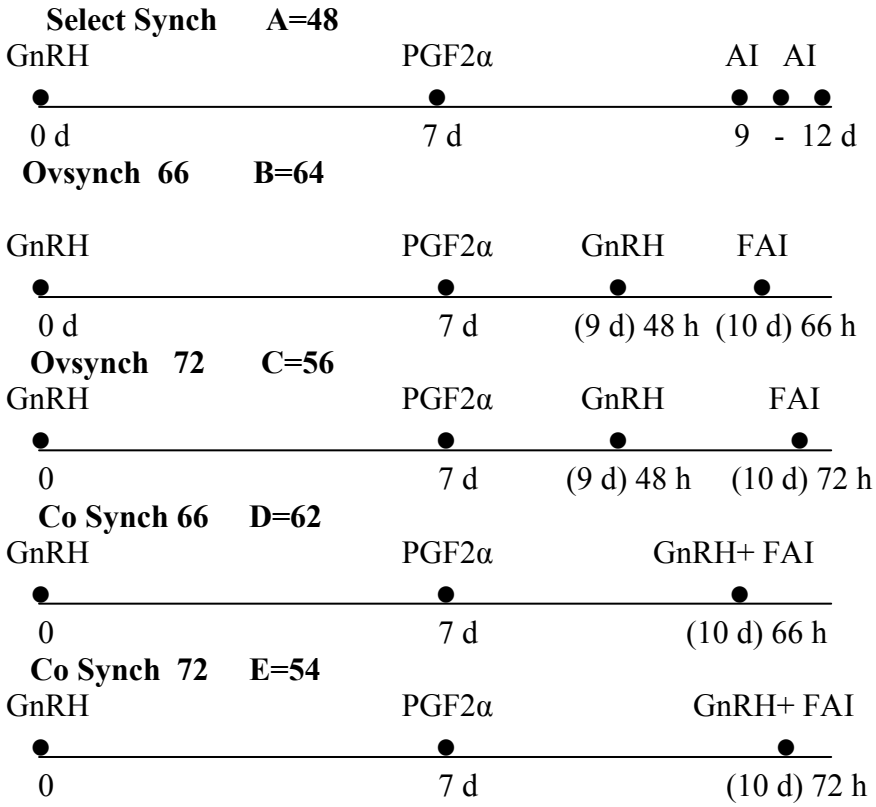

Fig. 1. Experimental design of applied synchronization programs

Results

The share of non-cycling cows in herds among which was performed study is 26.26%. The results obtained on pregnancy after first insemination, percent of gaved birth and late embryonic mortality in the application of different protocols are presented in Table 1.

Analysis of pregnancy on first artificial insemination after application of different protocols for synchronization of oestrus shows that between herds doesn't exist significant differences in this indicator. The percentage of pregnant animals from all protocols in individual herds is in the frames 24.62% -26.76%. Depending on the used protocol the established common pregnancy from first artificial insemination was in wide intervals (from 20.37% for group E to 32.81% for group B).Highest pregnancy from 32.81% after first artificial insemination has been reported in animals with applied Ovsynch protocol and artificial insemination on 66th hour after the second treatment

with gonadotrophin-releasing hormone (group B).In individual farms fertilization after application of this protocol is respectively 28.57% for herd1, 27.78% for herd2 and 37.54% for herd3. While using Ovsynch protocol and artificial insemination on 72th hour after the second treatment with gonadotrophin-releasing hormone, was established common fertility up to 28.57% (group C). Lowest fertility of 20.37% was recorded in Group E, in which animals has been treated with gonadotrophin-releasing hormone and artificial insemination on 72th hour with treatment with a prostaglandin preparation (CoSynch 72). Relatively low total fertility of 24.19% after first artificial insemination was obtained in animals of group D (CoSynch 66) treated with gonadotrophin-releasing hormone and artificial insemination on 66th hour with treatment with a prostaglandin preparation.

Fertility, late embryonic mortality and births in dairy cows included in different protocols for synchronization of oestrus and ovulation (in %)

Table 1.

Herd	procent pregnant	procent gave birth	procent c.e.mortality
GnRH и PGF			
1	20.00% (10)	20.00% (10)	00.00% (2)
2	23.53% (17)	17.65% (16)	25.00% (4)
3	33.33% (21)	33.33% (21)	00.00% (7)
Total	27.08% (48)	25.00% (47)	07.69% (13)
GnRH 48 +TAI 66			
1	28.57% (14)	28.57% (14)	00.00% (4)
2	27.78% (18)	27.78% (18)	00.00% (5)
3	37.54% (32)	35.48% (31)	08.33% (12)
Total	32.81% (64)	31.75% (63)	04.76% (21)
GnRH 48 +TAI 72			
1	26.67% (15)	26.67% (15)	00.00% (4)
2	29.41% (17)	25.00% (16)	20.00% (5)
3	29.16% (24)	29.16% (24)	00.00% (7)
Total	28.57% (56)	27.27% (55)	06.25% (16)
GnRH 66 +TAI 66			
1	21.43% (14)	15.38% (13)	33.33% (3)
2	21.05% (19)	21.05% (19)	00.00% (4)
3	25.00% (29)	25.00% (29)	00.00% (8)
Total	24.19% (62)	22.58% (61)	06.67% (15)
GnRH 72 +TAI 72			
1	25.00% (12)	25.00% (12)	00.00% (3)
2	18.75% (16)	18.75% (16)	00.00% (3)
3	19.23% (26)	12.50% (24)	40.00% (5)
Total	20.37% (54)	17.31% (52)	18.18% (11)

When were provided twice ultrasound was identified percent late fetal mortality. Depending on the applied protocol highest share of fetal mortality was registered in groups D and E. The

highest proportion of late fetal mortality were registrated in herd 3 with applied protocol CoSynch 72, respectively 40.00%.

Discution

During performance of economic analyzes was founded that animals with aoestrus cause serious losses to the dairy farm by increasing the cost of mating, fruitless days and scrapping of animals (**De Vries et al.,** 2006). In recent years, the most common treatment in non-cycling animals is Ovsynch protocol and some of his types. By using this biotechnological method is inducing ovulation in the majority of anestral animals (**Gumen et al.,** 2003). Comparing the results of studies in cycling and non-cycling cows, become clear that at the first induction of synchronous oestrus and fertility are higher (**Moreira et al.,** 2001).

Select Synch program is intended for breeding dairy cows in farms with good registration system of oestrus and preferably from owners cows are inseminated only by found signs of oestrus. There are two opinions on the implementation of artificial insemination. In the first cows were observed for oestrus from 3 to 5 days after treatment with prostaglandin and when is established oestrus are inseminated (**Geary et al.,** 2000). Within the second, all animals are inseminated at 72th hour after treatment with prostaglandin (**DeJarnette,** 2001b;2003).In this protocol price is lower only because cows with no signs of oestrus are treated for the second time with gonadotrophin preparation. In this study, cows were inseminated with established signs of oestrus. Middle fertility was 27.08%, and the highest percentage was achieved in herd3, respectively 33.33%. Better results in this farm we believe are due to the triple made observations for detection of oestrus cows, and also the data about the activity of the animals detected by computer program.

Ovsynch synchronization protocol with fixed time for artificial insemination has been developed, tested and intensively used in dairy cows (**Pursley et al.,** 1997; 1998**)**. When implementing the program in large dairy farms achieved pregnancy rate was between 30% and 40% without separating dairy cows in cycling and non cycling. Applying the Ovsynch protocol in dairy cows **Moreira et al.,** (2001) is registered fertility from 41.7% in cycling animals, compared to 22.4% in anestral. The results obtained in our study are higher than reported, respectively 32.81 for group B and 28.57 for group C. Similar to the established by our results are these registred by **Chebel et al.,** (2006), **Stevenson et al.,** (2006) и **Keskin et al.,** (2010) in terms of fertility of anestral animals after the application of the program Ovsynch, respectively 20.9%, 30.2% and 31.9%. For unsatisfactory results in non-cycling cows after application of Ovsynch protocol report **Gumen et al.,** (2003)and **Maiero et al.,** (2006), respectively 9% and 7% fertility. Resulting in low fertility in anestral cows after application of Ovsynch is explained by the short luteal phase (**Gumen et al.,** 2003). To remove this adverse effect is added exogenous progesterone between the first GnRH treatment and injection of prostaglandin preparation. While applying of this protocol fertility in increased up to 10% compared to untreated (**Stevenson et al.,** 2006). In studies conducted on various variants of the program Ovsynch without prior synchronization was reported fertility by 19.1% in cows treated with gonadotrophin-releasing hormone and artificial inseminated on 72th hour, 24.3% in cows treated with gonadotrophin-releasing hormone and artificial inseminated on 48th hour and 27.1%, in animals treated with gonadotrophin-releasing hormone and artificial inseminated on 48th hour and artificial inseminated on 72th hour (**Portaluppi and Stevenson,** 2005). Treatment with GnRH in the late afternoon on 56-60th hour after prostaglandin leads to act of artificial insemination in the hours before noon the next day with gonadotropic hormone at 48th hour, followed by artificial insemination in the afternoon and evening hours (**Overton and Heins,** 2012).

For the removal of some of the discomforts related to the application of Ovsynch protocol, as artificial insemination is carried out in the morning hours has been developed CoSynch protocol, but studies have shown fertility was lower than that of Ovsynch. Hypothesis testing the variety

CoSynch 72 was that will lead to a greater proportion of pregnant animals, to the applying to CoSynch 48 (Brusveen et al., 2008; Portaluppi and Stevenson, 2005). In the studies we registered higher fertility in the program Ovsynch (28.57% and 32.81%), to CoSynch (24.19% and 20.37%). To achieve better results about fertility reported **Brusveen et al.,** (2008), respectively in CoSynch 48 (29.2%), CoSynch 72 (25.4%) and for the group with Ovsynch protocol 38.6%. Lower performance when using CoSynch protocol are registered with research of **DeJarnette and Marshal,** (2003). Comparing these results with those obtained in our study were found that variant that was applied to the animals of group E has a low efficiency. Achieved low fertility in CoSynch 72 protocol is confirmed by research of **Cartmill et al.,** (2001); **Portaluppi and Stevenson,** (2005). CoSynch program is an alternative to the Ovsynch and more often used in meat-cattle (**Geary et al.,** 2001). **Small et al.,** (2001) haven't identified differences in fertility between CoSynch and Ovsynch protocols used in meat-cows. **Geary and Whitter,** (1998) registered weak but statistically significant advantage in terms of the benefit of fertility of Ovsynch program to CoSynch.

Many researchers report for loss of pregnancies in the range 10% -23%. They found that embryonic losses occur during early pregnancy periods before 28th day and with increasing of gestational age, the same reduced (**Fricke et al.,**1998; **Pursley et al.,**1998; **Moreira et al.,**2001; **Fricke et al.,**2003). In our study proportion of late fetal mortality is within the limits. The lowest proportion of late fetal losses are reported in applying Ovsynch protocol (4.76%), and highest in CoSynch 72 (18.18%). A high percentage (40.0%) fetal losses are reported by Alnimer et al., (2009)within the performed in synchronization of estrus and ovulation with Ovsynch and CoSynch 72 protocols in dairy cows during the summer season. **Cartmill et al.,** (2001) reported of 7.0% до 56.0% fetal losses.

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