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Original scientific paper

SUCCESS OF PROTOCOLS FOR SYNCHRONIZATION OF ESTRUS AND OVULATION IN DAIRY COWS IN SUMMER**

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Abstract: Cattle farming, as one of the most important branches of livestock production, has long been confronted with the chronic problem of the decline in reproductive performances. In 2005, the number of cattle around the world amounted to about 1,370,000,000 heads of cattle, while in 2012 this number was just over a billion, which indicates the importance of applying different reproductive protocols in order to increase production. Heat stress leads to disorders of the physiological and reproductive processes, as the rise in body temperature caused by heat stress has direct negative consequences on numerous cell functions. The study included a total of 54 Holstein-Friesian cows, 28 experimental and 26 control cows from PD Butmir farm with 5 lactations on average. Hormone protocols for estrus and ovulation were used in June and July 2013. Cows in the experimental group were subjected to a Presynch + 5dCoS2 protocol. Cows that did not conceive after this protocol were immediately subjected to resynchronization with Cosynch -72 protocol. The success rate of the Presynch + 5dCoS2 protocol was 19%, while in the case of Cosynch 72 it was 33%, which was statistically significant ($p <0,05$). The first presynchronization and synchronization protocol (5dCoS2) did not significantly help to improve the conception rate after the first insemination postpartum, but it is evident that they had a positive effect on another protocol (COS72) in the form of reduction in embryonic mortality in the summer months when embryonic loss is the greatest.
The COS72 protocol provides satisfactory results in the summer, but good fitness and health management, as well as heat stress reduction in accordance with the location and farm design are the preconditions for a successful estrus and ovulation synchronization program prior to artificial insemination.
Key words: dairy cows, heat stress, hormonal protocol, reproduction

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INTRODUCTION

Cattle farming, as one of the most important branches of livestock production, has long been confronted with the chronic problem of the decline in reproductive performance. In 2005, the number of cattle around the world amounted to be approximately 1,370,000,000, while in 2012 this number was just over a billion, which indicates the importance of applying different reproductive protocols in order to increase production. For successful reproduction, several prerequisites are needed such as the successful development of oocytes in the follicles, the expression of oestrous behavior and coordinated ovulation, the optimum uterine lining for the development of embryos and effective reproductive management.

The age of cattle is also very important for reproductive efficacy. At the age of 700-750 days, primiparous cows have 118-days service period, first artificial insemination after 66 days, conception rate after the first insemination of 36.4% and an artificial insemination index of 2.85 (Ettema et al., 2004). Fertility begins to decline with age, compared to older cows, primiparous cows have a higher conception rate after the first insemination postpartum, while cows with ≥5 lactations have the lowest conception rate (Pursley et al., 1993).

Heritability of positive reproductive parameters is ≤ 3% (Hansen, 2000). Holstein-Friesian breed has a lower success rate of the first insemination postpartum than other breeds of dairy cows and cross-breed cows, as well as a greater inclination to metabolic disorders (Pursley et al., 1993, Hansen, 2000). Reproduction is more efficient in cross-breed cows because they have a better conception rate after the first insemination postpartum (51-56% cross-breed cows, 40% Holstein breed) and less embryonic deaths (7.5-10.1% cross-breed cows, 12.8% Holstein breed) (Rabiee et al., 2005). A higher metabolic and nutritive load due to higher milk production can be responsible for the decline in reproductive performance, as Holstein Friesian cattle produce the highest amount of milk with the highest fat and protein content, but they also have the shortest productive lifetime compared to other dairy cattle, for example Norwegian Red cattle breed (with the longest lifetime production) (Walch et al., 2008). High milk production may have an antagonistic effect on estrus expression, but not on the reactivation of ovarian function (Harrison et al., 1990) High-producing cows have the longest interval to the first ovulation and estrus with low intensity of estrus expression, while other authors claim that high-producing cows, due to better general health status and multiple double ovulation, have greater chances of conception (Peters and Pursley, 2002, Santos et al., 2004). Due to increased catabolism higher milk yield is associated with reduced reproductive performance and delayed ovulation but effective management can solve this problem (Santschi et al., 2011).

At higher ambient temperatures and heat stress, follicle growth is threatened, and hormone concentrations associated with earlier cycle (Reist et al, 2003) are also reduced. Heat stress can disrupt physiological and reproductive processes (Hansen and Arechiga, 1999). The rise in body temperature caused by heat stress has direct negative consequences on cell functions. At higher temperatures and heat stress, follicle growth is endangered and the concentrations of progesterone, triiodothyronine and thyroxine (E2, T3, T4) are reduced (Reist et al., 2003, Sota et al., 1998). Heat stress in the summer reduces the length and intensity of estrus and therefore there is 76 - 82% of undetected estrus events and 44-65% of them in winter. (Pursley et al., 1998). Heat stress can also cause increased cortisol secretion (ACTH response) which blocks estradiol- induced sexual behavior (Pursley et al., 1998). Stress caused by elevated air temperature reduces libido, fertility, increases body temperature and embryonic mortality (Santos et al., 2009). Negative effects of heat stress are reflected 42 days before and 40 days after the insemination (Jordan, 2003). Heat stress occurs when the air temperatures are> 20 ° C, while the upper critical temperature at the start of the heat stress is between 25-26 ° C (Al-katanani et al, 1999). Increased temperature and humidity reduce appetite and dry matter intake, expression and duration of estrus, colostrum quality, endocrine status, conception rate, early embryonic development and fetal growth and the interval partus-conception is prolonged to extend the state of the negative energy balance (NEB) (Pursley et al., 1998, Jordan, 2003, Hansen and Arechiga, 1999). Heat stress affects oocyte maturation in the dominant follicles (Wolfenson et al., 1997), resulting in less viable embryos.

In warm months (May-August) when air temperature and humidity is high, Bos taurus cows have lower oocyte quality (41 ± 9.5%) as well as lower embryo quality compared to the colder period of the year (75.9 ± 8%) (January-March) (Rocha et al., 1998). When it comes to reproduction hyperthermia is the most harmful on the day of estrus or the day after (Sota et al., 1998). Redirection of blood from the core to the periphery which results in reduced uterine and placental perfusion pressure is the adaptive response to heat stress (Hansen and Arechiga, 1999). Poor blood flow and tissue perfusion, disturbed endometrial and oviduct function and concentrations of steroid hormones enable secretion of prostaglandin F2α causing embryonic mortality (Pursley et al., 1998, Xu and Burton, 2000).

Dry matter intake and milk yield is often stimulated by increased protein intake, but this nutritional strategy also leads to reduced fertility, as excess of rumen degradable and rumen undegradable protein can contribute to reduced fertility in lactating cows (Butler, 1998). In order to stimulate milk yield, meals have> 17% of crude protein (SP) resulting in reduced reproductive performance. Excess protein in high-producing cows reduces reproductive efficiency and it doesn't increase milk yield. In NEB cases there is a lack of energy for detoxification of ammonia and there are changes in urea concentrations in blood and milk which are greater than 19 mg/ dl and can be harmful to conception and embryonic evelopment. Endometrial cells respond to this by intensifying the secretion of prostaglandis, decreasing progesterone concentrations and embryo survival rate (Butler, 1998).

Body condition score (BCS) reflects the body energy reserves available for metabolism, growth, lactation and ovarian activity (Montiel and Ahuja, 2005). This together with the presence of lameness speak about the welfare of animals on the farm, which is important for reproductive and productive performances. The occurrence and duration of NEB is associated with the interval to the first ovulation PP, and it is assumed that NEB in early lactation adversely affects the quality of the oocyte in 80 to 100 days period, reducing the conception in the first few inseminations (Patton et al., 2006). Adipose tissue constitutes the major β-carotene storage which affects the ovulation, luteal function, and progesterone levels (P4) and a loss in body condition results in decreased levels of β-carotene in plasma (Kawashima et al., 2009).

MATERIAL AND METHODS

The study included a total of 54 cows of Holstein-Friesian breed, 28 experimental and 26 control cows from PD Butmir farm (Canton Sarajevo). Average annual milk yield per cow on this farm is around 6,000 litres and 5 lactations. Estrus detection is carried out three times a day, visually on the basis of external signs and, if necessary using vaginal temperature measurements and transrectal examination. The voluntary waiting period for this farm is 60 days, followed by a clinical examination of the ovaries and uterus. After the examination it is decided whether to treat them or to introduce them into the artificial insemination program. The research was conducted from February 2013 to March 2014. The animals involved in the study stayed in the barn, in fenced spaces using tether system. Data about cow age, production, diet, health, reproductive abnormalities and excretions were obtained from the farm protocol or during animal experiments.

Hormonal treatment and artificial insemination

In order to stimulate ovarian function and accelerate the first ovulation On day15 , postpartum cows in the experimental group received an injection of 100 µg GnRH (Dephérélin Gonavet Veyx®, 1 ml = 0.05 mg or FertagylTM Intervet Schering-Plow Animal Health, 1 ml = 0.1 mg) Protocols for synchronization of estrus and ovulation were divided into presynchronization with two injections of prostaglandin F2α (Estrumate, Schering-Plow Animal Health, 1ml = 250µg Cloprostenol) in 14 days period and synchronization of ovulation 11 days after the last injection of prostaglandins (Presynch 11 + 5dCoS2).The presynchronization protocol was started at 53 ± 1 days postpartum, the synchronization protocol at 67 ± 1 days postpartum, and the first artificial insemination was performed at 77 ± 1 days postpartum. The next day artificial insemination was carried out between 8.00-13.00h. Pregnancy diagnosis was done by ultrasonography (Esaote MYLAB30VETGOLD, LV 513 Veterinary endorectal linear probe 10-4) 42 days after insemination, as earlier embryo can be covered with endometrial folds. Placenta development and the onset of fetal ossification in this period make the diagnosis easier. Non-pregnant cows were subjected to resynchronization with Cosynch 72 protocol, at the end of which they were immediately inseminated. Pregnancy diagnosis was repeated after 42 days.
These diagrams present a scheme of hormonal injection and insemination in performed protocols for synchronizing estrus and ovulation.

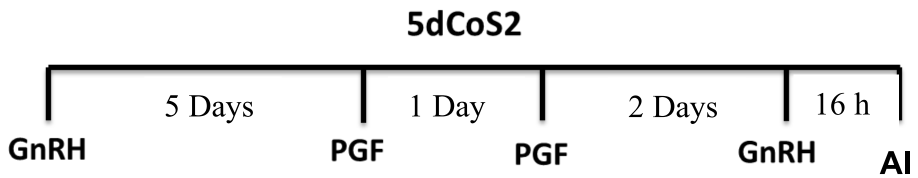


Diagram 1. 5dCoS2 protocol was applied after the process of presynchronization.

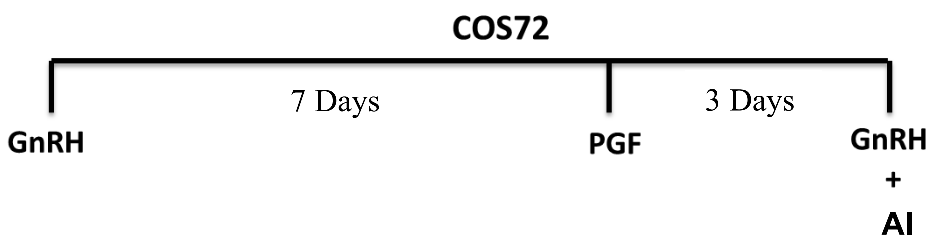
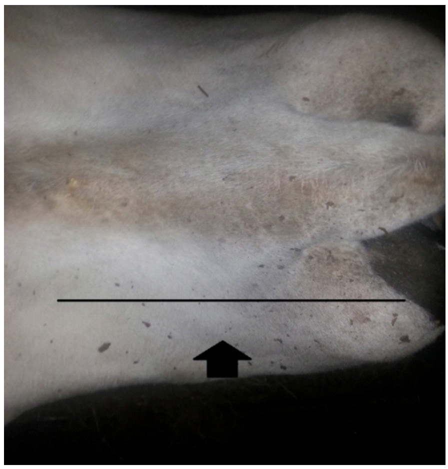


Diagram 2. COS72 protocol was applied after unsuccessful synchronization with protocol 5dCoS2.

The following reproductive parameters were analyzed: the interval to the first postpartum insemination, the success of the conception after the first and subsequent postpartum inseminations, insemination rate, the length of the service period, and the total number of pregnant and non-pregnant cows.

Ultrasound Measurements for Body Condition Score (BCS)

In addition to the first artificial postpartum insemination, body condition status in the experimental and control group was determined by sonography based on the thickness of the subcutaneous fat tissue. It is a fast, non-invasive method in which the ultrasonic probe is placed in the area of the sacrum and the root of the tail, because the largest deposits of adipose tissue are on the back. Hair doesn't need to be cut for the examination and a plant oil is used as a contact agent between the probe and the skin. The probe is positioned vertically and it must be held lightly because fat is compressed with pressure. The adipose tissue is seen as a dark (hypoechoic) narrower or thicker line, while the skin and profound fascia are light (hyperechoic). The thickness of the skin is always calculated, and the thickness of the subcutaneous adipose tissue is expressed in millimeters.



Figures 1 and 2. Location for placing an ultrasound probe during the measurement of adipose tissue.

Detection of lameness

Assessment of lameness was based on the observation of the standing cows, with an emphasis on the state of the limb and position of the back at the time of examination. Lamenes was assessed using Zinpro Locomotion Score method used for early detection of hoof disorders, monitoring prevalence of lameness, comparing the incidence and severity of lameness between herds and identifying individual cows for functional hoof trimming. The method is subjective. 353 cows, including the animals in the experiment, were examined prior to the start of the synchronization protocol and insemination in order to have an insight into the presence and expression of lameness in the breeding facility.

All obtained data were processed and displayed using the Microsoft Excel 2010 software package. All observed statistically significant differences were evaluated according to "p" values for $p < 0.05$, $p < 0.01$ and $p < 0.001$. The primary processing of results from examined cows was done using descriptive statistics method, and the secondary processing of the results was performed using a two-sample "t" test. The classification of observed statistical significances between the experimental and the control group is marked with stars *, ** and *** depending on the level of the "p" value.

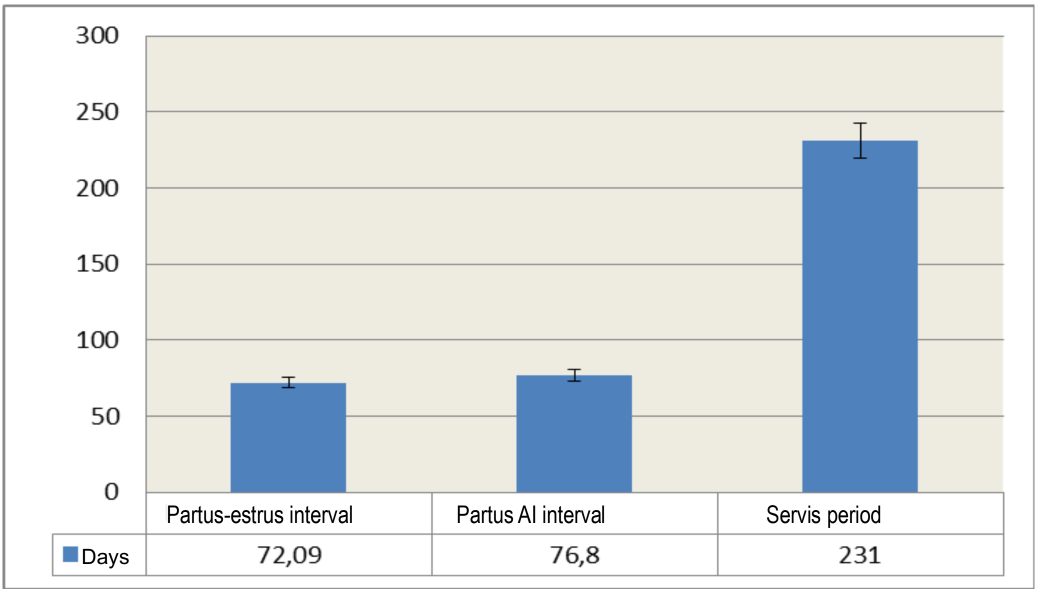
RESULTS

Table 1. Relation of cow categories in research

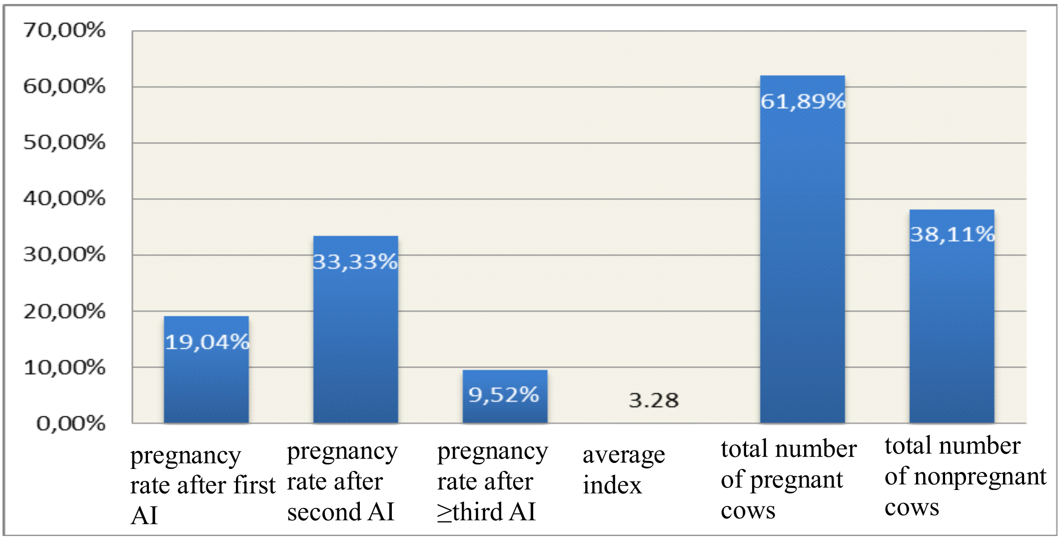
	Total
Primiparous cows	n=25
Multiparous cows	n=29
Normal puerperium	n=33
Abnormal puerperium	n=21

Reproductive parameters

Experiment:

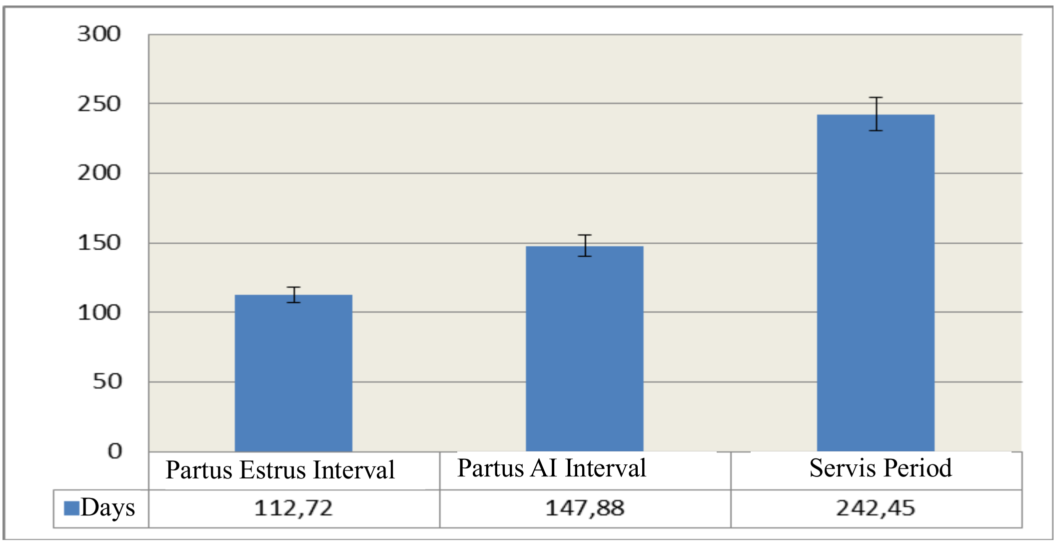


Graph 1. The time interval of days before the first detected estrus, artificial insemination and the length of the service period for all cows within the experimental group, as well as the mean value of the parameters presented with the subtracted and added standard deviation of the mean value (x ± Sx).

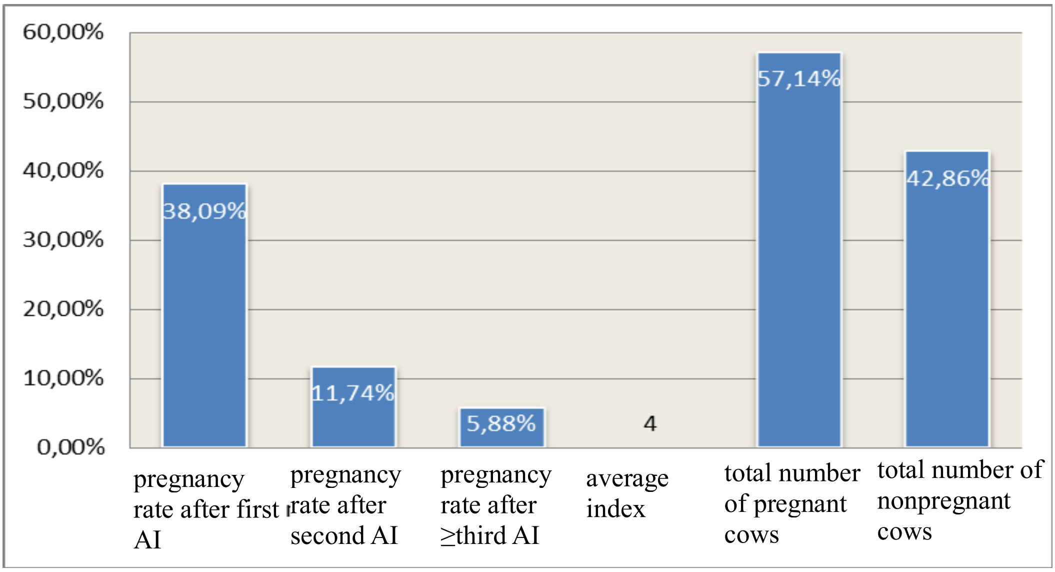


Graph 2. Recorded performance of 1, 2, ≥3. artificial insemination related to all cows from the experimental group, with an average insemination index and the percentage of pregnant and non-pregnant cows until the end of the research.

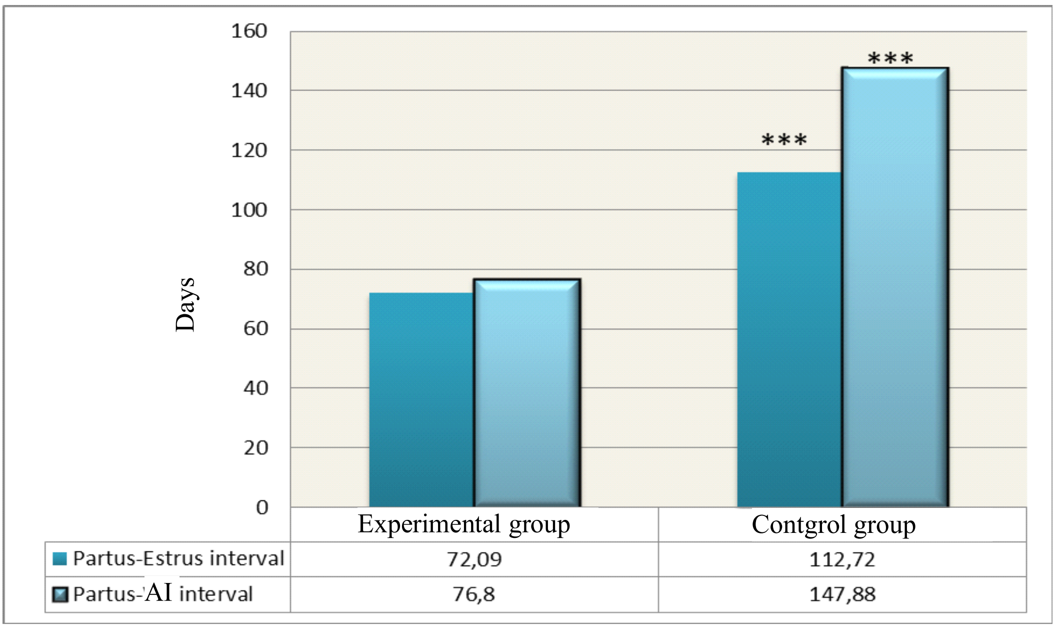
Control



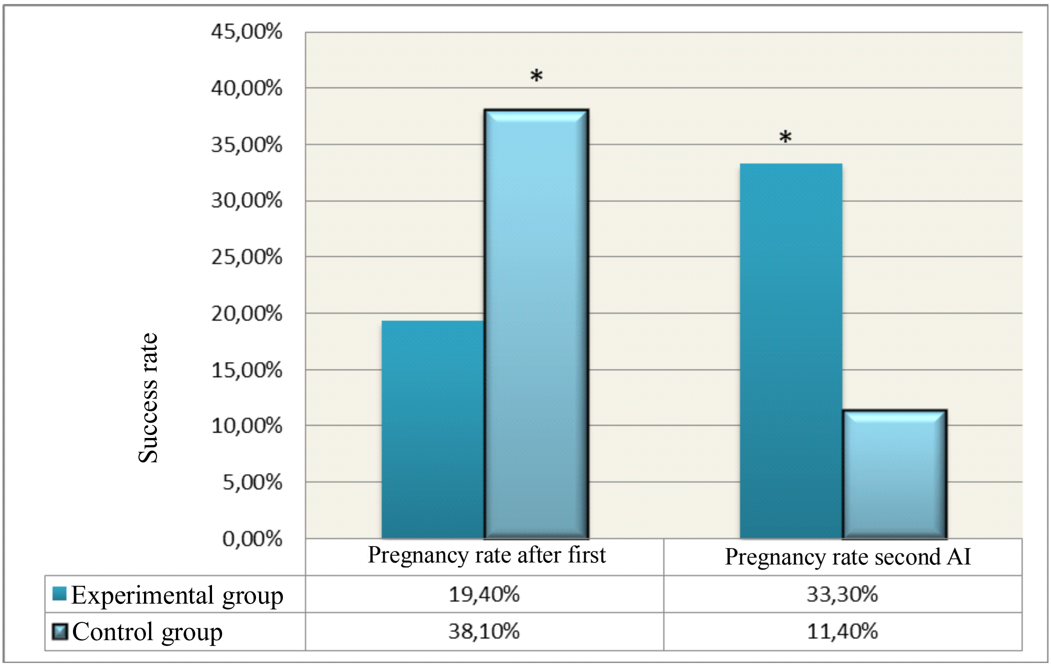
Graph 3. The time interval of days before the first detected estrus, artificial insemination and the length of the service period for all cows within the control group, as well as the mean value of the parameters presented with the subtracted and added standard deviation of the mean value (x ± Sx).



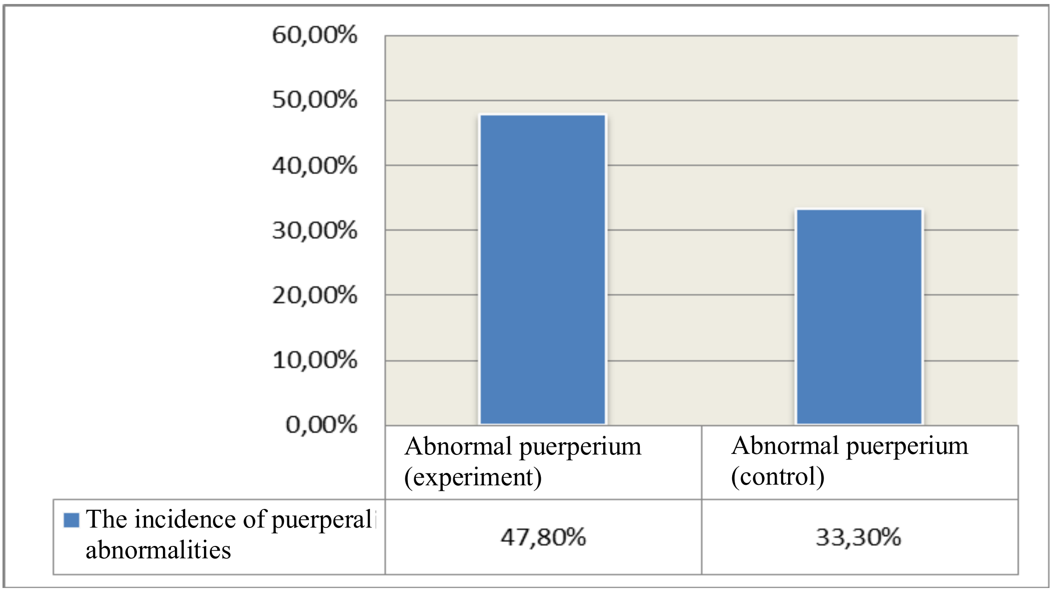
Graph 4. Recorded performance of 1, 2, ≥3. artificial insemination related to all cows from the control group, with an average insemination index and the percentage of pregnant and non-pregnant cows until the end of the research.



Graph 5. The ratio of the length of the first observed partus-estrus interval and the length of partus - artificial insemination interval between the experimental and the control group. Columns with *** (p <0.001) are statistically extremely significant.



Graph 6. The success ratio of the 1st and 2nd artificial insemination with a significant difference between them. Columns with * ($p < 0.05$) are statistically significant



Graph 7. The graph represents interrelations in the appearance of abnormalities of puerperium, the different etiologies between the investigated groups of cows.

Body condition assesment

Table 2. Presentation of the body condition status in the experimental and control group in the period of the first postpartum insemination.

Group	Days	Thickness (mm)	BCS
Experimental	77	19,9	3
Control	148	23,8	3,5

Expression of lameness on the farm

Table 3. Percentage of the lame cows on the farm, calculated at the beginning of the research, using the "Zinpro Locomotion Score" method. The most number of cases were in category 2 and 3, as well as in the experimental groups.

The presence of lameness according to "ZLS" rating	1	2	3	4	5
%	1,13	59,2	36,26	3,11	0,28
Numerically	4	210	128	11	1

Table 4. Percentage ratio of pregnancy between the groups at the end of the study period.

	Experimental group	Control group
Pregnancy rate in primiparous cows	63,6 %	71,4 %
Pregnancy rate in multiparous cows	60 %	50 %

DISCUSSION

The Holstein-Friesian breed generally has a lower success rate of the first artificial insemination compared to cross-breed cows and other breeds of dairy cows (Inchaisry et al., 2010). The conception rate after the first AI after 60-67 days postpartum period is two times higher in cold months than in warm months (45% in the winter and 22% in the summer) (Benmrad and Stevenson, 1986)

Cows that have been inseminated in estrus after 75 days postpartum have a better conception rate than those inseminated after 60-75 days or earlier (Jordan et al., 2002), which is probably the result of the establishment of regular cycles, the elimination of contaminants and infection of the uterus, the end of lactation peak and preferred BCS. Synchronization for insemination in postpartum can increase the number of pregnant animals in the following order: up to 81 days 26.1%, up to 102 days 39.8%, up to 123 days 50.1% and up to 144 days 59.2% (Jordan, 2002). The facts that experimental cows were inseminated with an average of 77 days postpartum and with a success rate of 19%, the second time with 128 ± 1 days with a success rate of 33%, and that the success of the first insemination at 148 days postpartum in the control group was 38%, speak of a similar growth pattern of the conception rate.

It is better for cows in detected estrus to be inseminated much later in lactation for a better conception rate and cost-effectiveness of insemination. If this is to be started earlier, it is better to apply protocols for synchronizing estrus and ovulation (Jobst et al., 2000, Tenhagen et al., 2004).

With good management reflected through the appearance of lameness and body condition status, it is possible to achieve up to 69% success rate in primiparous cows inseminated within 100 days postpartum and 63% success rate in multiparous cows. For those inseminated within 180 days success rate in primiparous cows can go up to 88% and 92% in multiparous cows (Sakaguchy et al., 2004). This is confirmed by some other authors (Stevenson et al., 1996).

In our case primiparous cows in both groups had a higher conception rate than multiparous cows and it was significantly more pronounced in the control group which is consitent with Sakaguchy's claim. Some studies have shown that the conception rate is best when cows are inseminated within 90-110 days postparum (Waters et al., 2009) which is the period when they are not at peak lactation and have satisfactory BCS. It is often assumed that NEB in early lactation has a negative effect on oocyte quality even up to the first 80-100 days of lactation, thereby reducing the conception rate to the first few inseminations(Patton et al., 2006). Artificial insemination at estrus gives a better conception rate than fixed -time insemination but it also causes more embryonic deaths (Stevenson et al., 1999).

In the period up to 120 days of lactation, the conception rate is up to 40% and it stays at the same level (Thatcher et al., 2000). The results of Thatcher coincide with our results in the case of a control group, where the conception rate after the first insemination after 120 days was 38%. Cows that have BCS ≥2.75 at the moment of insemination gets pregnant in a higher percentage (Lima et al., 2009), which is observed in the control group that had BCS 3.5 during the first insemination and the success rate of the conception of 38%, indicating the need to establish a BCS assessment protocol before an insemination program.

The conception rate after the first insemination is expected to be in the range of 43-45% for cows with normal puerperium and 15-24% for cows with puerperal disorders (Stevenson et al., 1989). Bearing in mind that monitoring of the health condition found that puerperial disorders were frequent in both groups, a lower conception was understandable, especially after the first insemination in the experimental group. Holstein-Friesian cows have a lower success rate after the first insemination (Inchaisry et al., 2010), especially in the months of June and July, but also primiparous cows have a greater chance of getting pregnant. It should be emphasized that during June and July in Bos taurus cows only 6 ± 14.8% of embryos develop into morulae, while 46.6 ± 12.5% in cold months (Rocha et al., 1998). Other researchers agree with this (Stevenson et al., 1996), and despite the fact that after the first insemination, the conception rate was better in multiparous cows, the biggest number of pregnant cows at the end of the study was among primiparous cows, which coincides with his claims (Stevenson et al. al., 2012). Better results of presynchronization and synchronization of estrus and ovulation and less embryonic deaths are observed if there is corpus luteum in the ovaries at the beginning of hormonal treatment (Sterry et al., 2006), which was not the case in our study. Anestrous cows subjected to hormonal protocols still have increased embryonal mortality (15%) (Gumen et al., 2003, McDougal, 2010, Santos at al., 2009). These facts indicate the importance to establish an ovarian status prior to the beginning of the synchronization protocols, which is why COS72 protocol was used, since insemination immediately after the last GnRH application has the lowest embryonic mortality (Pursley et al., 1998).

We probably have to agree with the above-mentioned claims because the most cows got pregnant in COS72 protocol, which was used for second fixed-time insemination. The reason for this could be a reduction in embryonic mortality, but it should be have in mind that the insemination was then done by resynchronization in the period 128 ± 1 day postpartum. This protocol was carried out in July, when high embryonic mortality should be expected due to high temperatures, but the concept rate was satisfactory. When the rectal temperature is 40 ° C as a result of exposure to higher daily temperatures of ≥ 32 ° C, the conception rate falls to 0% compared to 48% conception when the daily temperature is up to 21 ° C and rectal 38.5 ° C (Jordan, 2003). From May to September in spite of hormonal applications other researchers (Pursley et al., 1998) also had a low conception rate ranging from 4.5% to 20% or an average of 13.9%, while the control groups had much weaker results of 4.8%, where within 120 days postpartum about 27% cows got pregnant and 16.7% of cows in the controls. It seems that such decline in the conception rate at elevated temperatures influenced the lower conception rate in cows inseminated in June and July. Heat stress has a negative effect on fertility in days before insemination, and later through embryogenesis, most notably in the first 7 days, but also later (6-14), while the adverse effects of heat stress decrease as the embryo further develops (Hansen and Arechiga, 1999). Cows in the control group had significantly higher conception rate after the first postpartum insemination, but it happened significantly later in slightly colder months, so the percentage of pregnant and non-pregnant cows was similar in both groups.

Presinchronization of estrus with two prostaglandin applications at interval of 14 days and the beginning of the fixed-timed insemination 12-14 days later was developed to increase the number of pregnant cows after inseminatio (Galvao et al., 2007). At the end of the synchronization protocols cows should ovulate up to 92% if they respond to first GnRH and 79% if they don't respond (Vasconelos et al., 1999). By reducing presynchronization time of prostaglandins from 14 to 11 days and the period from the first GnRH injection to the first application of prostaglandins from 7 to 5 days and its repetition on the 6th day of the protocol, the luteolytic effect is intensified (Ricarda et al., 2009). Stronger luteolytic effect was also evident in our work, because signs of estrus were observed in all experimental animals on the day of fixed-time insemination. With the synchronization for estrus and ovulation, the results show a higher conception rate in cows that showed signs of estrus sign (45.8%) than in cows without signs of estrus (35.4%) (Jordan et al., 2002).

Using presynchronization with prostaglandins and synchronization of ovulation for the first postpartum insemination, it is possible to have estrus cycle of 80-90% (Rocha et al., 1998) and 43.3-45.1% pregnant cows (Galvao et al., 2007, Navanukraw et al., 2004, Ribeiro et al., 2011) as it was demonstrated in COS72 protocol that resulted in more pregnant cows. The use of presynch + Ovsynch protocols is probably the cheapest and most efficient way to prepare a large number of cows for the first postpartum insemination (Stevenson et al., 2001), but based on insights into cows' health status of cows in our results, we think that many prerequisites for reproductive and health management should be met.

Synchronization of estrus and ovulation should have noticeable positive effects on the reproductive performance of dairy cows (Stevenson, 2001), but in our case the presynchronization and synchronization protocol (5dCOS2) did not significantly improve the conception rate after the first postpartum insemination. But there was positive effect after the second fixed-time insemination (COS72) as embryonic mortality was decreased in the summer months, when it is usually more pronounced. In the performance analysis, it should be taken into account that COS72 protocol was carried out after more than 4 months postpartum, that it was resynchronization and that the previous protocol was likely to have an effect on P4 elevation that had a positive effect on the dominant follicles, and consequently to a better conception and less embryonic deaths. There are no significant differences in the total number of pregnant cows between the two investigated groups, which indicates the negative impact of all other management factors on the production of all cows regardless of the number and stage of lactation.

CONCLUSIONS

In problematic herds, insemination programs shouldn't start before the end of 90 days lactation period in order to eliminate most of the negative factors which reduce their effectiveness. Presynchronization ahead of synchronization is desirable when introducing a large number of cows into the program for first postpartum insemination. In cows that do not get pregnant after the first synchronized artificial insemination, resynchronization with the selected protocol is sufficient and the use of ovsynch and modified ovsynch protocols results in equalizing the ratio of pregnant primiparous and multiparous cows.

More primiparous cows get pregnant after artificial insemination, but they also have a longer service period due to frequent puerperal disorder. Protocol for synchronization protocol and ovulation such as COS72 gives satisfactory results in the summer period for cows with ≥120 days postpartum.

Prerequisites for a successful artificial insemination program are good BCS, general health status, reduced lameness, the elimination of heat stress in accordance with the location and farm design, since the negative environmental impacts and management failures considerably lower the success of the first but also of subsequent artificial insemination and extend service period.

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