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**Original Scientific Paper**

# NEGATIVE EFFECTS OF BEDDING AND LYING AREA ON SYNCHRONIZED ARTIFICIAL INSEMINATION IN DAIRY CAWS IN SARAJEVO KANTON

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## Summary

Dairy cattle breeding is one of the most important branches of livestock production, which has been facing, for several decades, the chronic problem of declining reproductive performance. In 2005, the number of cattle worldwide was about 1,370,000,000, while in 2015 that number dropped below one billion, and in 2021 it shows a slight recovery as it was 1,000,970. This indicates the importance of applying different reproductive protocols in order to increase the number of cows in production. The type of bedding on which the animals stay, as well as the characteristics of the lying area itself, shows a significant impact on numerous physiological functions such as food intake, chewing, milk yield, but also levels of sex hormones. The type of bedding and lying area, which causes chronic pain and stress, leads to disorders of physiological and reproductive processes, since stress has direct negative impact on numerous cellular functions. A total of 66 dairy cows, 50 Holstein-Friesian cows kept on PD Butmir and 16 Simmental cows kept on a private mini farm, were included in the study. At PD Butmir, cows were kept in tie-stall housing system, while on a mini-farm they were kept free. Hormonal protocols of estrus and ovulation synchronization were used in April, May and June 2019. Cows were subjected to two estrus synchronization and ovulation protocols, Ovsynch and Cosynch72. At PD Butmir, 25 cows were subjected to Ovsynch and Cosynch72 protocols, respectively. At the mini-farm only Ovsynch protocol was applied. The Ovsynch protocol applied on PD Butmir had success in conception rate of 12% (n = 3), while the Cosynch72 protocol gave a score of 36% (n = 9). On the mini-farm, Ovsynch resulted in a conception of 25% (n = 4). Based on our results, the Cosynch72 protocol, compared to the Ovsynch protocol, was a better choice in the case of Holstein-Friesian cows kept in the tie-stall housing system. In the Simmental cows kept in the free stall system, the Ovsynch protocol proved to be better choice in achieving conception, compared to the Holstein-Friesian cows. Therefore, it is necessary to test several different protocols of estrus and ovulation synchronization, in order to find the most optimal one for a certain breed, type of keeping and breeding.

**Keywords:** hormonal protocol, conception, lying area, dairy cattle

## INTRODUCTION

Nowadays, cattle are primarily oriented on production, meaning that healthy dairy cows should achieve the best possible reproductive performance and milk yield in the shortest possible time. Dairy cattle breeding is one of the most important branches of livestock production, which has been facing the chronic problem of declining reproductive performance for several decades. In 2005, the number of cattle worldwide was about 1,370,000,000, while in 2015 that number dropped below one billion, and in 2021 it showed a slight recovery as it was 1,000,970. This indicates the importance of applying adequate management in order to increase the number of cows in production. In intensive breeding of cows, where dairy cows are exposed to many unfavorable external factors (inadequate lying area and lack of adequate bedding), dietary mistakes (deficiency in energy, protein, vitamins, minerals), inadequate care of the hoof, increased body weight of animals with insufficient movement, lead to an increased risk of lameness (Kos, 2009). In intensive breeding of high-yielding dairy cows, 50% to 60% of cows have problems with limb diseases, and lameness of dairy cattle is an important problem worldwide because in addition to mastitis and reproductive disorders, it is one of the most common reasons for excluding cows from production (Weigele et al., 2018; Brkić, 2009). Hind limbs are most commonly affected (Blowey, 2005). Lack of comfort while walking and lying down is a significant risk for lameness (Olechnowicz and Jaskowski, 2011). Intense pain that occurs during inflammatory processes within the legs and impaired movement of animals that reduce daily food consumption, favors numerous health disorders, including worsening of reproductive parameters in the herd, which normalize much more slowly (Alawneh et al., 2011; Walker et al., 2008). The occurrence of chronic painful stress has a negative impact on reproductive hormones through the hypothalamic-pituitary-ovarian axis (Vasconelos et al., 1999) and lame cows have delayed ovarian cyclicity (Bicalho et al., 2007; Garbarino et al., 2004), as well as reduced dietary intake, which also contributes to the occurrence of a negative energy balance (Garbarino et al., 2004).

The goal is to achieve less than 30% of cows in a herd with more than 120 days of service period, and it can be obtained by correcting management and using hormonal protocols (Horan et al., 2005). There are no significant differences in response after the application of hormones to different breeds of cows (LeBlanc et al., 1998). The advantage of the hormonal protocol of estrus synchronization and ovulation, as well as insemination at a fixed time, is that it does not require estrus detection for insemination (Jordan et al., 2002). Ovulation is induced in 94-97% of cows after completion of the estrus synchronization and ovulation protocols (Gumen et al., 2003), and several studies showed the advantage of this protocol over insemination protocol in observed estrus with conception up to 41% (Lima et al., 2009; Vasconelos et al., 1999). In ovulation synchronization, the beginning of the protocol can be applied in a targeted or random phase of the estrous cycle, where the goal is to create a new follicular wave and the ovulation of the dominant follicle. Ovulation occurs 24-32h after the last hormonal treatment, and insemination is obtained 0-32h after the last hormonal dose (Jobst et al.,

2000; Pursley et al., 1998). This is called the Ovsynch protocol. There are different presynchronization and synchronization protocols, which also give different conceptions, as well as more or less embryonic deaths (Pursley et al., 1998). Some researchers have managed to get a conception of as much as 45.5 to 46.6% with modified hormonal protocols (Bisinotto et al., 2010), but of course this only applies to cows that were free from any health disorders. Other studies showed that the success of conception, 30 days after Ovsynch protocol, was up to 36.6%, but also that, due to embryonic deaths, the number of pregnant cows after 60 days was significantly reduced, even up to 19.8% (El-Zorkouny et al., 2004).

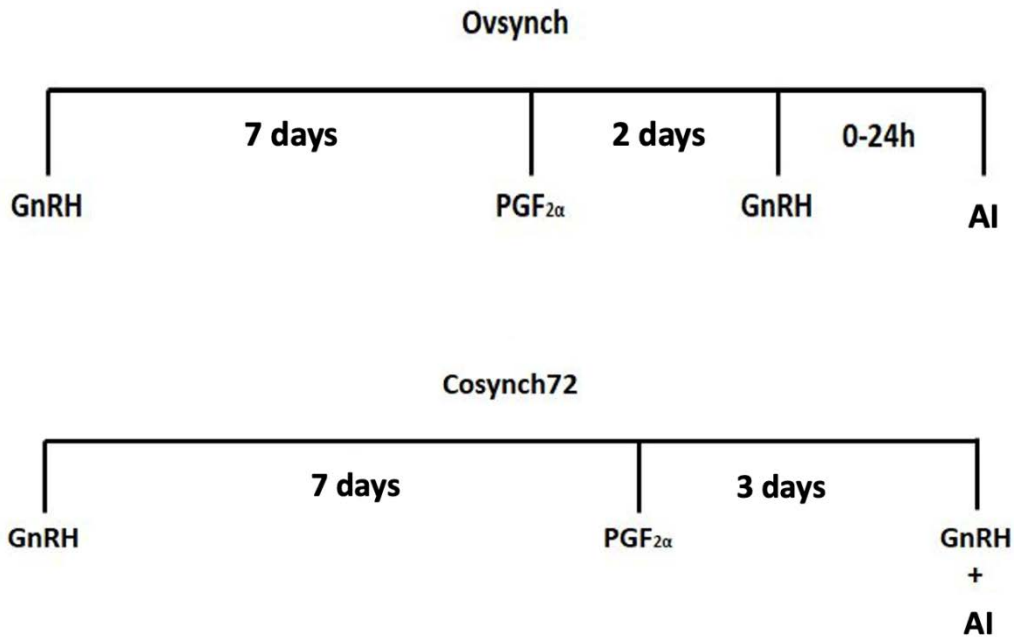
The aim of this study that included 66 dairy cows (50 Holstein-Friesian cows kept on PD Butmir farm and 16 Simmental cows kept on a private mini farm) was to improve conception after estrus and ovulation synchronization, as well as to assess the effects of existing bedding and laying area types on conception success.

## MATERIALS AND METHODS

The study was conducted on 66 dairy cows, Holstein-Friesian and Simmental breed, during the months of April, May and June 2019. All examined cows were between the first and the fifth lactation. Holstein-Friesian cows on PD Butmir (n = 50) were kept in a tie-stall housing system, and had no possibility to leave the concrete laying area, but only to get up and lay down. Badding type was fine sawdust or straw and the lying areas were mostly dry. On a private mini-farm, Simmental cows (n = 16) were kept in a free stall system, but with relatively limited space for movement, on a concrete floor, with and without individual beddings and lying area, and the lying area was mostly covered with feces. The milking average at PD Butmir was 20 liters, and at the mini-farm was 22 liters per cow and in both cases, the cows were fed a mono meal (silage, hay, beer trope, protein and vitamin/mineral supplement). At both facilities, artificial insemination (AI) was performed by the competent veterinary service, after the signs of estrus were noticed by the farm employees.

Cows were subjected to two estrus synchronization and ovulation protocols, Ovsynch and Cosynch72, without prior determination of ovarian status. Figures 1 and 2 show the order of hormone application and the time period between them for different protocols, as well as the time of artificial insemination. At PD Butmir, 25 cows were subjected to the Ovsynch protocol and the Cosynch72 protocol, respectively. At the mini-farm, 16 cows were subjected to the Ovsynch protocol. Artificial insemination was performed by the competent veterinary service, and this protocol was performed 8-16 hours after the last hormonal application in cows that were subjected to the Ovsynch protocol. In cows subjected to the Cosynch72 protocol, artificial insemination was performed immediately after the last administration of the hormone. Diagnosis of pregnancy was performed in the period of 40-50 days after artificial insemination, using ultrasonography.

All data related to animals, which were assessed as diagnostically relevant, were used from the documentation of the farm or were obtained by on-site inspection.




**Figures 1 and 2** Ovsynch and Cosynch72 estrus and ovulation synchronization protocols

The outcome variable was the proportion of pregnancies achieved (number of animals with confirmed pregnancy 40-50 days after AI in relation to the total number of animals), post stratified according to the used estrus and ovulation synchronization protocol (Ovsynch and Cosynch72) and breed, as well as bedding type (PD Butmir - Holstein Friesian cattle and mini farm - Simmental cattle). Before comparing possible differences in the percentage of conception between the defined groups, the uniformity of the presence of associated characteristics in the groups (average lactation, condition, milk yield, general health) was confirmed.

## RESULTS

An overview of the keeping systems (beddings and lying areas) on the farms included in the study, is given in Figures 3 and 4. A large number of cows with mild to severe pathological changes in the locomotor system were observed at both types of farms (Figures 4, 5 and 6). The changes ranged from mild swelling of the joints, interdigital

dermatitis and ulceration to the formation of smaller and larger deformities of the joints. All cows in the study had some pathological alterations in the legs, and the most common location of pathological alterations was in the hind limbs with 70% (n = 46) cases, and the most often affected joints were tarsal and metatarsal, as well as the hoof (Figures 5 and 6). The remaining 30% of pathological alterations were located in the forelimbs, and the most often affected joint was the carpal and there was the presence of overgrown hooves (Figure 4). When it comes to the number of affected limbs, in 55% (n = 36) cases only one limb was affected, and in 45% (n = 30) two legs were affected.

**Figure 3** An overview of an object in which animals are kept free on a concrete lying space, without clearly marked beddings and lying areas.

**Figure 4** A facility where animals are kept in a tied system on a concrete lying area with sawdust bedding. It may be noticed that the hoof on the front limb is deformed and overgrown.

**Figure 5 and 6** Deformity of tarsal and metatarsal joints, as well as the hoof itself in the facility in which the animals are kept in a tied system, as well as on a concrete lying area with a sawdust bedding.

The success of hormonal protocols for estrus and ovulation synchronization is shown in Table 1.

**Table 1** The success of the conception according to the type of cow facility, the protocol used, and the breed.

| Cow facility | Breed | Hormonal protocol used | Conception success |
|---|---|---|---|
| PD Butmir | Holstein-Frisian (HF) | Ovsynch (n=25) | 12% (n=3) |
| | | Cosynch72 (n=25) | 36% (n=9) |
| Mini farm | Simmental (SM) | Ovsynch (n=16) | 25% (n=4) |

## DISCUSSION

Decreased reproductive performance is significantly affected by lameness, as a chronic, painful and stressful condition (Walker et al., 2008). This is probably one of the leading reasons why estrus detection and conception at both types of cow facilities is not at a satisfactory level. Cows that suffer from chronic painful stress and develop more or less pronounced lameness over time move and stand less, which results in a prolonged lying period and a reduced chance of manifesting estrous behavior. The marked presence of pathological alterations in the legs in the facilities where the study was conducted resulted in unsatisfactory estrus detection, a lower number of inseminations per cow and poorer conception results. This was the reason for applying two estrus synchronization and ovulation protocols in our study.

The significant presence of various pathological alterations in the legs is probably the result of the tie-stall housing system in which cows are kept in contact, then the type of surface on which cows move and lie down, and the effectiveness of routine methods of treating hoofs, as well as treatment of diseased joints. The lameness of Holstein-Friesian cows increases the risk of delaying ovarian cyclicity (Garbarino et al., 2004), which probably had an impact on poorer estrus detection and conception. In all cows involved in the study, the percentage of lameness was present to a greater or lesser extent. Lack of comfort while walking and lying down, as well as pain that occurs during inflammatory processes in the legs and difficulty in moving, reduces daily food consumption, which favors the occurrence of a range of health disorders, including deterioration of reproductive parameters in the herd (Olechnowicz and Jaskowski, 2011; Alawneh et al., 2011; Walker et al., 2008). This was evident through the poor results of the Ovsynch protocol on Holstein-Friesian cows, but also unsatisfactory results in Simmental cows.

Lameness has become one of the three most common reasons for weaning and economic losses in the dairy industry (Walker et al., 2008), making it the most representative indicator of animal welfare concern in the dairy industry. According to researchers (Weigele et al., 2018; Brkić, 2009) 50% to 60% of cows have chronic problems with limb diseases and lameness in intensive breeding of high-yield dairy cows. Hind limbs are most often affected (Blowey, 2005), as was also shown in our study.

The use of both hormonal protocols led to greater detection of estrus, but the results of conception differed considerably, both between protocols and between cow breeds. The success of the Ovsynch protocol shows dependence on the stage of the estrus cycle in which it begins, as if it is applied from 5 to 12 days of the estrus cycle, the possibility of conception could be from 40 to 41% (Vasconelos et al., 1999). In our study, both hormonal protocols started without prior detection of the estrus cycle phase. This could be one of the reasons for the lower performance of the standard Ovsynch protocol. Negative effects of the hard bedding could be observed through the low performance results of the Ovsynch protocol in both breeds of cows, although in the Holstein-Friesian breed the results were twice as bad as in the Simmental (12:25%).

Conception results obtained in the Holstein-Friesian breed became noticeably better with the use of the Cosynch72 protocol, which managed to overcome much worse results

using the Ovsynch protocol. The use of this protocol showed an advantage over the standard Ovsynch protocol, which in both breeds of cows did not produce the desired result that could be expected by results of other researchers (Bisinotto et al., 2010; Lima et al., 2009; Vasconelos et al., 1999). The good efficiency of the Cosynch72 protocol is supported by the fact that it was applied without prior establishment of the estrus cycle phase, and it is possible that its application at 5-12 days of the estrus cycle could increase efficiency even more. The low success rate of the Ovsynch protocol in the Holstein-Friesian and Simmental breeds is supported by other researchers, which report more embryonic deaths using this hormonal treatment (El-Zorkouny et al., 2004). In contrast to the Ovsynch protocol, which would probably have better results if the negative effects of hard surfaces and moisture were eliminated, the Cosynch72 protocol shows the potential to be a better choice of hormonal treatment, especially when accommodation conditions are far from optimal and the estrus cycle phase is unknown.

## CONCLUSION

For successful breeding of dairy cows with desirable reproductive performance and milk production, it is necessary to eliminate the negative effects caused by hard lying area, moisture, inadequate amount of bedding material or its absence. Management's failure to address these factors, which cause chronic pain in breeding animals, leads to a number of health disorders, including deterioration of reproductive parameters in the herd, which return to normal much more slowly. The application of different hormonal protocols shows a clear difference in performance in different breeds of cows. The use of the Cosynch72 protocol appears to be a significantly better choice in the Holstein-Friesian breed of cows compared to the standard Ovsynch protocol, which in turn is more effective in Simmental breed cows. Prerequisites for a successful artificial insemination program are good management and selection of an appropriate hormonal protocol. Therefore, it is necessary to test several different protocols of estrus and ovulation synchronization, in order to find the most optimal for a certain breed, type of keeping and breeding.

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