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EFFICIENCY OF USING ESTRUS SYNCHRONIZATION PROTOCOLS IN DAIRY COWS

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Abstract

As well as estrus synchronization programs have the role of increasing fecundity at the farm level, our experimental study was carried out during 2024-2025, on a herd of 111 dairy cows, with the aim of testing the efficiency of these protocols. Four groups were formed: group A consisting of 31 cows synchronized with OvSynch; group B consisting of 35 heads synchronized with OvSynch+PRID; group C consisting of 24 cows synchronized with d-cloprostenol and group D consisting of 21 cows inseminated on a spontaneous estrus cycle. The fecundity index, the services per conception rate, the calving to first services interval and the service period was monitored. Gestation control was performed with a portable ultrasound device, 30 days after insemination. The obtained results showed that the percentage of pregnancies was +8.61% in group A; +9.53% in group B; +16.67% in group C compared to group D. The analysis calving to first service interval shows that significant differences were recorded between groups of cows A - D ($p<0.05$; $p=0.03866$). Our findings support the implementation of hormone synchronization protocols to improve reproductive management in dairy herds.

Key words: *conception rate, dairy cows, OvSynch, reproductive performance.*

INTRODUCTION

The use of hormonal programs for estrus synchronization aims to improve reproductive performance in dairy cows (Strickland et al., 2024). Knowledge of how the endocrine system works has allowed the development of hormonal therapies for estrus synchronization. Currently, the use of these programs is a common practice (Macmillan, 2010). There are several estrus synchronization schemes, which are applied following a gynecological examination to establish the individual treatment/cow type.

The objectives of this study were to investigate the effectiveness of using hormonal synchronization programs in dairy cows in stimulating ovarian activity, their influence on twin pregnancies and abortions, in shortening the calving - first insemination interval, the service period and in improving herd fertility rates

Hormonal estrus synchronization protocols are developed to address ovarian pathology, stimulate ovarian hormonal activity, trigger ovulation, and enable timed artificial insemination, with the aim of improving fertility rates on farms (Simoes et al., 2024).

The use of hormonal therapy to synchronize the estrus phase may represent the solution to combat infertility in dairy cows in a context where more and more farms are faced with a decrease in the percentage of fecundity, an increase in the number of services/gestation, an increase in the open days period, and the calving interval in cows inseminated on a spontaneous heat cycle.

MATERIALS AND METHODS

This study was carried out during 2024-2025, in the Experimental Zootechnical Laboratory of the Research and Development Institute for Bovine Balotești, on a herd of 111 dairy cows from the Romanian Black Spotted breed owned by the Institute. The study followed the efficiency of estrus synchronization protocols in dairy cows.

The Experimental Zootechnical Laboratory is a medium-sized farm that had a herd of approximately 100 dairy cows during the experiment. The cows included in the research were selected based on several criteria: milk production in the first hundred postpartum days greater than 2500 kg, body condition score between 2.25 and 3.25 and the age of the animals to be no more than 3 lactations. The ovarian diagnosis was established by gynecological examination performed using an ultrasound device, by transrectal exploration. Based on the diagnosis, the group composition and hormonal treatment for estrus synchronization were determined.

Thus, the 111 cows were divided into four groups, namely: group A consisted of 31 cows diagnosed with follicular cysts and 'lazy' ovaries. These were synchronized through the OvSynch program. Group B, consisting of 35 cows diagnosed with ovarian hypotrophy and ovarian cysts, was synchronized through the OvSynch+PRID protocol. Group C, consisting of 24 cows diagnosed with persistent corpus luteum and luteal cysts, was synchronized with a synthetic analogue of prostaglandin Pg_2alpha called d-cloprostenol. Group D, consisting of 21 cows, was inseminated on a spontaneous heat cycle. The insemination of the cows was performed with conventional semen.

The OvSynch synchronization protocol consisted of administering 2.5 ml of gonadotropin-releasing hormone (Gn-RH) (Receptal®, MSD Animal Health, USA), injectable, intramuscular, on day 0. On day 7, 2 ml of d-cloprostenol (Sincrovall®, Mevet S.A.U., Spain), injectable, intramuscular, were administered, on day 9, 2.5 ml of Gn-RH, injectable, intramuscular, were administered and 16 hours after the last administration, insemination was performed.

The OvSynch+PRID protocol consisted of administering 2.5 ml of Gn-RH (Receptal®, MSD Animal Health, USA) intramuscularly on day 0 and inserting a progesterone (PRID Delta®, Ceva, France) device, containing 1.55 g into the vaginal cavity. On day 7, the PRID device was removed, and 2 ml of d-cloprostenol was administered intramuscularly. On day 9, 2.5 ml of Gn-RH was administered, and insemination was performed 16 hours after the last administration.

The synchronization protocol with the synthetic prostaglandin analogue $PgF_{2\alpha}$ (d-cloprostenol) consisted of administering 2 ml of, injectable, intramuscularly on day 0, and after 72 hours, the cows that responded to the treatment were inseminated.

Insemination on a spontaneous heat cycle was performed by monitoring the cows using the reproduction calendar, and those detected in estrus were inseminated 12 hours after the first signs of heat were triggered.

The efficiency of estrus synchronization protocols was interpreted using reproductive indicators. Thus, the fecundity index, number of services per conception (NSC), interval between calving and first insemination, service period, number of twin pregnancies, and abortion rate were analyzed. Gestation control was performed with a portable ultrasound device 30 days after insemination, and the re-control was performed 40 days after the diagnosis of gestation

The statistical analysis was performed using Microsoft Excel. The mean (X), standard deviation (SD) and coefficient of variability (CV%) were calculated for the 4 analyzed groups and significance tests of the F-test and CHITEST differences were performed. These were used to analyze and interpret the differences between the groups for each reproduction indicator. Thus, with the F-test, the significance differences were interpreted for the reproduction indicators the interval from calving to the first insemination, the service period and the number of services per conception, and with the CHITEST test, the differences between the results obtained and those expected for the reproduction indicators fecundity and number of services per conception were calculated. The differences were considered statistically significant at $p < 0.05$ and extremely significant at $p < 0.01$.

The sample size (n = 111 cows) was considered adequate for detecting biologically relevant differences in reproductive parameters between groups, based on similar studies reported in the literature

RESULTS AND DISCUSSIONS

Estrus synchronization programs in dairy cows aim to increase farm profitability by controlling ovarian pathology and improving reproductive

performance. They trigger the luteal phase (Gn-RH, progesterone), corpus luteum regression, progesterone reduction, follicular maturation ($PgF_{2\alpha}$), ovulation induction and artificial insemination at a fixed time (Gn-RH).

From Figure 1 it can be seen that group B was the most numerous. It was 12.9% larger than group A, 45.83% larger than group C and 66.66% larger than group D.

The analysis shows that group B recorded the best results among the three groups of cows diagnosed in the estrus phase and synchronized with different hormonal protocols. In this group, all cows were diagnosed with heat and inseminated. The percentage of cows inseminated in this group was +6.46% higher than that recorded in group A and +12.5% higher than the number of cows inseminated in group C. Similar results were recorded by other authors when detecting cows in the estrus phase following hormonal heat synchronization protocols (Mohammed et al., 2023)

The analysis of the fecundity index shows that the highest fecundity percentage, 50%, was obtained in group C. The result obtained is comparable to those presented in the specialized literature which reports a percentage of pregnancies between 40 and 65% in cows synchronized with a single injection of d-cloprostenol (Aregaw et al., 2024; Mohammed et al., 2021; Sharew et al., 2023). The second-best performance was recorded in group B, of 42.86%. The result obtained is comparable to that recorded by other authors, who reported a fecundity percentage in cows synchronized with OvSynch +PRID of up to 40% (Hölper et al., 2023). In group A, the fecundity percentage was 41.94%. The percentage of pregnancies obtained in this group is comparable to the results reported by other authors (Berean et al., 2025). The lowest fecundity percentage, 33.33%, was recorded in group D. This score is comparable to results reported in the literature for cows inseminated during a spontaneous heat cycle (Tippenhauer et al., 2023). This score is equivalent to poor fecundity.

From the mathematical analysis it is found that in groups synchronized by hormonal therapy the percentage of pregnancies obtained was higher than in cows inseminated on a spontaneous heat cycle as follows: in group C there were 16.67% more pregnancies recorded than in group D; in group B the percentage of fecundity was 9.53% higher than that recorded in group D and in group A the percentage of pregnancies obtained was 8.61% higher than that recorded in group D. Similar results were reported by other authors who found better fecundity in synchronous cows compared to those inseminated on a spontaneous heat cycle (Santos et al., 2017).

Figure 2 shows the relationship between the fecundity index, the percentage of twin pregnancies obtained and the percentage of abortions recorded in the 4 analyzed groups.

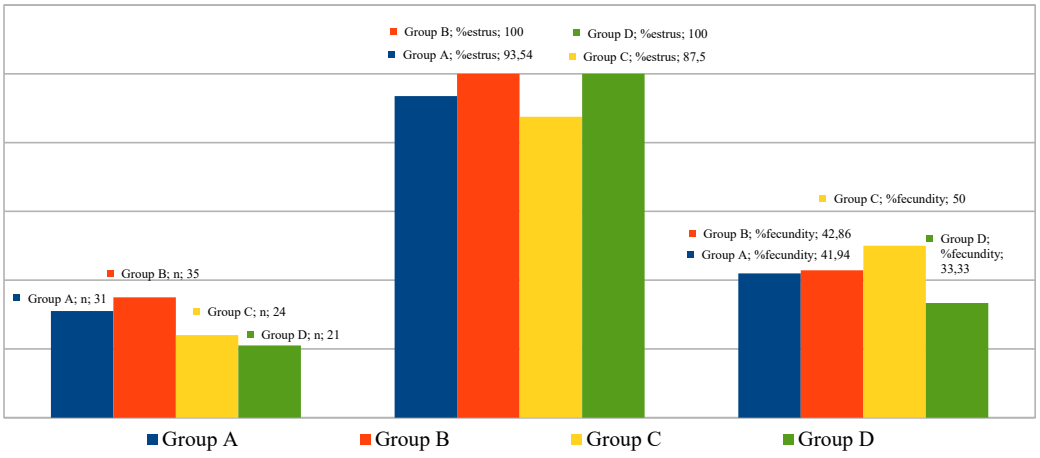

Figure 1. Distribution of cows from the 4 groups analyzed in relation to the fecundity index

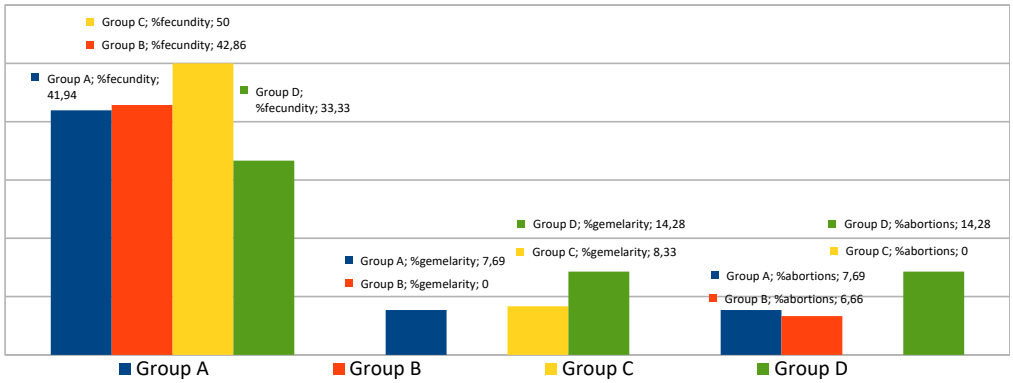

Figure 2. Analysis of the relationship between the fertility index, the percentage of twin pregnancies, and the number of abortions in cows from the 4 analyzed groups

The analysis of the number of cows that were diagnosed with twin pregnancies is justified, especially in groups of cows that were synchronized by hormonal therapy. Numerous authors have found that synchronization of cows with the help of hormonal treatments led to obtaining a higher number of twin pregnancies (Vazquez et al., 2012; Gatius et al., 2023; Arciniega et al., 2025;)

From Figure 2, it can be seen that the lowest percentages of twin pregnancies were recorded in groups of cows synchronized by hormonal treatments and the highest percentage of twin pregnancies was diagnosed in group D. The data obtained show that the use of synchronization programs in dairy farms does not influence the occurrence of twin calving, which is most often unwanted. The higher percentage of twin pregnancies recorded in group D is due to chance. Similar results were recorded by other authors who reported higher percentages of twin pregnancies obtained in cows inseminated on a spontaneous heat cycle compared to cows synchronized by hormonal treatments (Gatius et al., 2020).

The analysis of the incidence of abortions in early gestation (period 45-90 days of gestation) was performed because in the specialized literature, this interval is presented as a critical one with an incidence between 8% and 12% of the abortion rate (Pohler, 2020; Albaaj et al., 2022).

The analysis of embryonic mortality in the 4 groups shows that in groups A, B and C the percentage of abortions was between 0% and 7.69%. The highest percentage of abortions, 14.28%, was recorded in group D. The results obtained show that the incidence of abortions in groups A, B and C was lower compared to that reported by Pohler in 2020 and by Albaaj et al. in 2022 and higher in group D. This indicates that the use of hormonal therapy for estrus synchronization in dairy farms decreases the abortion rate by a percentage between 6.59% and 14.28%. The results recorded are similar to those presented by other authors who found that the use of hormonal therapy for estrus synchronization in dairy cows decreased the incidence of abortions (Madureira et al., 2019).

Fecundity is the ability of a cow to remain pregnant over a given period of time. It is interpreted using statistical and reproductive indicators and thus determines the economic efficiency of the herd. It is influenced by a number of factors such as milk production, energy balance and body condition score (Furnaris & Constantin, 2024).

The specialized literature estimates that fecundity in dairy farms is satisfactory when 50% of pregnant cows in the herd are diagnosed and a maximum of two services per conception are performed to obtain a pregnancy (Hutu, 2019).

Table 1. Analysis of cow fecundity in relation to the number of services per conception

Statistical indicators	Group A	Group B	Group C	Group D
n	31	35	24	21
Pregnant cows	13	15	12	7
% fecundity	41.94	42.86	50	33.33
Number of services per conception (NSC)	2.38	2.33	2	3
Comparative analysis of the significance of differences between group A vs. D				0.83282
Comparative analysis of the significance of differences between group B vs. D				0.81797
Comparative analysis of the significance of differences between group C vs. D				0.71751
Analysis of the significance of the differences between the obtained and expected fecundity percentage in group A (chitest)				0.20454
Analysis of the significance of the differences between the obtained and expected fecundity percentage in group B (chitest)				0.266210
Analysis of the significance of the differences between the obtained and expected fecundity percentage in group C (chitest)				1
Analysis of the significance of the differences between the obtained and expected fecundity percentage in group D (chitest)				0.003230

From the data analysis, it is found that in the groups of cows synchronized, by hormonal therapy the fecundity obtained was higher and the NSC was lower compared to the group inseminated by spontaneous heat cycle. This is justified by the fact that by using hormonal programs, the optimal moment of insemination of cows is identified by monitoring progesterone (P4) in milk and blood plasma. In dairy cows, the onset of the luteal phase is represented by the increase in the concentration of progesterone in milk from <5 ng/mL to values > 5 ng/mL, and the luteal phase lasts as long as the concentration of P4 is >5 ng/m. The determination of the moment of ovulation is deduced both by the decrease in the progesterone value and by transrectal ultrasound examination (Constantin et al., 2024). Thus in group C 1 (one) less insemination was performed to obtain a pregnancy than in group D (50%, 2 NSC vs. 33.33%, 3 NSC), in group A 0.62 fewer services per conception were performed compared to group D (41.94%, 2.38 NSC vs. 33.33%, 3 NSC) and in group B 0.67 fewer services per conception were performed compared to group D (42.86%, 2.33 NSC vs. 33.33%, 3 NSC).

The comparative analysis of significance of the FTEST differences showed that there were no significant differences between the hormonally synchronized groups and the one inseminated on a spontaneous heat cycle. Thus, between groups A vs D p=0.83285; p>0.05; B vs D p=0.81797, p>0.05; C vs D p=0.71751, p>0.05. Similar results have been recorded by other authors (Valenza & Bach, 2025).

The analysis of the CHITEST test shows that in the groups of cows A, B and C there were no significant differences between the results obtained and the expected ones. The performance threshold was chosen to achieved a fecundity rate of 50% with a maximum consumption of 2 services per conception. This threshold was chosen because it coincides with a good fecundity. Thus in group A p=0.20454, p>0.05; in group B p= 0.266210, p>0.05; in group C p=1, p>0.05. Distinctly significant differences were recorded in group D, with p=0.003230 (p<0.01), indicating that the results obtained in this group differ substantially from the expected ones, leading to economic losses.

Table 2 presents the analysis of the calving-first insemination indicator. This indicator is crucial because it influences farm management. In cows, the puerperal period lasts an average of 42 days. Once uterine involution has ended, the cow can be inseminated in an attempt to obtain a new pregnancy. Most studies indicate that during this heat cycle, the chances of becoming pregnant are low. The optimal insemination period is between 60 and 90 days from calving (Stangafero et al., 2018). The longer the calving-first insemination interval, the greater the economic losses.

Table 2. Index analysis, calving - first insemination in cows from the four analyzed groups

Statistical Indicators	Group A	Group B	Group C	Group D
n	31	35	24	21
X ± SD	85.6±18.03	91.48±19.47	90.04±17.62	104.1±27.72
CV%	21.05	21.28	19.57	26.63
Comparative analysis of significance of differences between group A vs. D				0.03227
Comparative analysis of significance of differences between group B vs. D				0.06771
Comparative analysis of significance of differences between group C vs. D				0.03868

From Table 2 it is found that the longest anestrus period was recorded in group D, 104.1 days. In this group, the calving-first insemination period was + 18.5 days longer than in group A, + 12.62 days longer than in group B, and + 14.06 days longer than in group C. The data show that, on dairy farms, the use of hormonal therapy for heat synchronization is indicated because these methods shorten the interval between first insemination and calving. Similar results showing that the use of estrus phase synchronization protocols shortens the calving-first insemination interval were reported by other authors (Fricke & Wiltbank, 2022). Recent research shows that monitoring hormones involved in ante and postpartum reproductive activity can influence the reduction of time intervals and improve pregnancy rates (Constantin et al., 2023)

This is to the farmer's advantage because it reduces the open days period.

The standard deviation analysis indicates that the greatest dispersion was observed in group D. In this group, the deviation from the mean was 27.72 days, indicating that some cows experienced a very long anestrus period due to various factors, such as high milk production, ovarian pathology, absent heats, and farmer's inattention. In groups A, B and C, the deviation from the mean was lower (between 17.62 and 19.47 days), which shows a greater homogeneity of the groups due to the use of hormonal therapy to synchronize the estrous phase. The result obtained is comparable to those reported by other authors, who found a smaller variation in cows synchronized with hormone therapy (Mekonnen et al., 2023).

From the analysis of the coefficient of variability (CV %) it is found that the highest dispersion was recorded in group D, 26.63%. In groups A, B and C the coefficient of variability was between 19.57% and 21.28%. This shows that the use of hormonal treatments for synchronizing heat in dairy cows leads to combating endogenous and exogenous factors and helps to improve reproduction indicators. Other authors (Carvalho et al., 2014) also found reduced variability in groups of cows synchronized by hormonal therapy.

The analysis of the F-test of significance of the differences found that there were insignificant differences between groups B vs D where $p=0.06771$, $p>0.05$. Other authors have also reported no significant differences in the calving-first insemination interval between cows synchronized with hormonal therapy and those inseminated during spontaneous heat cycles (Belandria et al., 2023). Significant differences were recorded between groups A vs D $p=0.03227$, $p<0.05$; respectively, C vs D where $p= 0.03866$, $p<0.05$. Significant differences in the first postpartum insemination between cows synchronized with hormonal therapy and those inseminated during a spontaneous heat cycle have also been reported by other authors (Valenza & Bach, 2026). The differences recorded between synchronized groups A and C vs D show the efficiency of these therapies in shortening the calving period, first insemination, and in improving farm management.

Table 3 presents the analysis of the open days period in cows from the 4 analyzed groups. Open days represent the mobile component of the calving interval (Gheorghe-Irimia et al., 2023). Ideally, this should last approximately 80-90 days (Vidu et al., 2025). The longer the interval, the lower the farm's managerial efficiency.

Table 3. Analysis of the open days period in the four groups

Statistical indicators	Group A	Group B	Group C	Group D
n	13	15	12	7
X ± SD (zile)	90.79±9.15	98.13±15.16	96.54±10.32	113.25±20.32
CV%	10.08	15.45	10.7	17.95
Comparative analysis of significance of differences between group A vs. D				0.013023
Comparative analysis of significance of differences between group B vs. D				0.322367
Comparative analysis of significance of differences between group C vs. D				0.038918

Table 3 reveals that group D recorded the longest period of open days, with 113.25 days. In this group the duration of the service period was higher by 22.46 days than in group A, by 15.12 days than in group B, and by 16.71 days than in group C. The analyzed data show that the use of heat synchronization programs in dairy cows shortens the duration of open days and promotes economic growth on the farm. The results obtained are comparable to those reported by other authors who found that the use of estrus synchronization hormone therapy shortens the service period in dairy cows (Santos et al., 2016).

The standard deviation analysis indicates that the greatest dispersion from the mean was observed in group D, with a standard deviation of ±20.32 days. In addition, these open days to the average service period of 113.25 days indicate that some cows in this group had open days approximately 53 days longer than the optimal 80-day period. This increase leads to a decrease in their performance and can decisively influence the farmer's decision to reform the cows. The results obtained are similar to those reported by other authors, who found, in the open days analysis, that dispersion is higher in cows inseminated during a spontaneous heat cycle than in cows synchronized through hormonal protocols (Santos et al., 2016).

The analysis of variance shows that group D had the highest variability, 17.95%, of the 4 groups analyzed. The high variability within this group indicates that inseminating cows during spontaneous heat cycles increases the open days period.

This variability in the service period is consistent with findings in the specialized literature, which indicates that it differs depending on the insemination strategy used (Sitko et al., 2023)

CONCLUSIONS

The use of estrus synchronization programs in dairy cows led to an increase in fecundity by 8.61% -16.67%

The use of estrus synchronization programs in dairy cows does not favor an increase in the percentage of twin pregnancies.

The use of heat synchronization programs tends to reduce the incidence of abortions in early pregnancy by up to 6.59% -14.28%.

The use of heat synchronization programs can reduce the number of services per conception in dairy cows by 0.62 - 1 services per conception.

The use of estrus synchronization programs can help shorten the interval between calving and first insemination by 12.62 days - 18.5 days

The use of estrus synchronization programs can shorten the service period by 15.12 days - 22.45 days.

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