Analysis of Reproductive Indicators in Dairy Cows Inseminated with HeiferPlus Frozen Semen

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Abstract
More and more dairy cows are experiencing infertility problems caused by both the quality of the semen after thawing and the timing of artificial insemination. The present paper analyses reproductive indicators in a population of dairy cows that were artificially inseminated with HeifferPlus semen. This semen, according to those who sell it, increases the percentage of fecundity by 5-15% and the sex ratio is 65-85% in favour of the calf with the predetermined desired sex. The work was carried out at the Balotesti Bovine Development Research Institute, on a herd of 50 heads, Romanian Black Spotted cows, owned by the institute. The analysed data capture the farm's activity since 2020. The reproduction data were taken from the TAURINE.EXE farm program. The monitored reproductive indicators were: %fecundity, services per conception, service period interval, %fertility and sex ratio. From the analysed data the proportion of females obtained was 57.5%.

Keywords: artificial insemination, cow, reproduction, semen HeiferPlus.

1. Introduction

More and more dairy farms are facing infertility problems due to the thawing temperature of the frozen semen or the time chosen for insemination. Normally, the thawing of the semen is done in a water bath, at temperatures between 35ºC and 37ºC. The insemination of the semen is done in the oestrus phase of the ovarian cycle. The moment of insemination has broad limits and differs depending on the author. Thus, if we refer to the image below (Figure 1), the ideal time for insemination is considered to be the period between 4 and 16 after the onset of heat, approximately 16-28 hours before the onset of ovulation [1]. Other authors consider the optimal moment for artificial insemination to be between 6 and 24 hours after the onset of the oestrus phase.

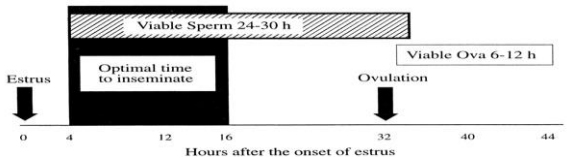

Figure 1. Diagram of the best moment for insemination in cows [1]

Other approved opinions claim that the optimal moment would be the period between 6 and 16 hours after the onset of oestrus and most consider that if a cow is diagnosed in the morning in the oestrus phase, it should be inseminated in the evening and if it is diagnosed in the evening, it should be inseminated in the morning [2]. The postpartum period in which the insemination takes place is also of major importance for the success of the insemination. From a reproductive point of view, insemination can be carried out starting with the second oestrous cycle, approximately 42 days after calving, the period that coincides with the end of the puerperal period and uterine involution.

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2. Materials and methods

The work was carried out at the Balotesti Bovine Development Research Institute, on a herd of 50 heads, Romanian Black Spotted cows, owned by the institute. They cows were artificially inseminated with HeiferPlus semen. The semen was thawed in a water bath at a temperature of 35ºC. The cows were divided into two groups. The first batch of 22 cows was inseminated 10-12 hours after the onset of heat and the second batch of 28 cows was inseminated 13-16 hours after the onset of the oestrus phase. The insemination was carried out in two periods. In the first period, cows were inseminated 42-84 days postpartum and in the second period, cows with 85-130 days of lactation were inseminated. The analysed data were taken from the TAURINE.EXE program. and capture the reproductive activity on the farm in 2020. The reproductive indicators monitored were: % fecundity, services per conception, service period interval, % fertility and sex ratio.
The statistical processing of the data was carried out with the help of the Microsoft Excel program.

3. Results and discussion

The thawing of the HeiferPlus semen used for the insemination of cows was carried out in a water bath at a temperature of 35ºC. The insemination was carried out 10-12 hours after the onset of the first heat, respectively 13-20 hours after the onset of the oestrus phase and 19-12 hours before the onset of ovulation.
Table 1 shows the reproductive performances recorded by cows inseminated 10-12 hours after the onset of the first heat.

Table 1. Insemination of cows 10-12 hours after the onset of heat

Statistical indicators	Reproduction indicators	%Fecundity	Services per conception (SPC)	Service period (SP)	%Fertility	Sex ratio
$\bar{X}$		72.24	1.9	97.38	66.6	58.5
Correl SPC - %Fecundity					-0.061859	
Correl SPC – Service period					-0.1801617	
Correl SPC - %Fertility					-0.2858843	
Chitest analysis %fecundity %fertility obtained ~ chitest %fertility %fertility desired					P =0.18243375; p>0.05	
CHITEST M/F Chitest analysis of the sex ratio obtained with HeiferPlus semen ~ the sex ratio obtained with conventional semen					P = 0.05611758; p≤0.05	

From Table 1, it can be seen that in the group of cows inseminated 10-12 hours after the onset of heat, a fecundity percentage of 72.24 was recorded. This score equates to a good fecundity but below the potential of dairy cows that are frequently expected to record 80% pregnancies. To obtain this fecundity percentage, 1.9 inseminations were performed. This score equates to good fecundity. Moreover, reproductive activity is considered to be good when the number of services per conception does not exceed 2 inseminations/gestation. In this group of cows, the duration of the service period was 97.38 days. If until the mid-80s this period was considered large and causing economic losses, today this period is considered acceptable [3]. The fertility obtained in this lot was 66.6%. This score equates to good fertility. For a cow to be efficient from a productive point of view, it must have a fecundity of at least 80% and a fertility of 60%. Thus, pregnancy losses of up to 20% are accepted. In the case of the analysed batch, pregnancy losses through embryonic death, abortion, dystocia births, morbidity or non-viable cattle were 8.46%. The ratio between the sexes shows that in this batch 58.5% females were obtained, 17% more than in the case of using conventional semen where the percentage of females is 50%. Moreover, the CHITEST test shows that there are significant differences between the females obtained in this group and the females obtained by insemination with conventional semen where the ratio between the sexes is 1/1 (p=0.05611758; p≤0.05). The analysis of the ratios between the number of services per conception and the % fecundity obtained shows that there is a positive correlation between these two indicators (0.18243375). This shows that in the group of cows inseminated 10-12 hours after the onset of heat, a higher fecundity can be obtained only by increasing the number of services per conception. The analysis of the ratios between the number of

services per conception and service period shows that a negative correlation was registered between these indicators (-0.1801617). This shows that the number of services per conception can decrease only by increasing the period of open days. A negative correlation was also registered in the ratios between the number of services per conception and the percentage of fertility obtained. Thus, it can be stated that a better fertility can be registered by decreasing the number of services per conception. In order to evaluate the fecundity and fertility performances obtained in this lot, the CHITEST test was performed. Thus, the data obtained for this batch were reported at the normal values of 80% fecundity and 60% fertility. The obtained result p =0.18243375; p>0.05 shows that there are no significant differences between the results recorded in this batch and the optimal results.

Table 2 shows the reproductive performances recorded by the cows that were inseminated 13-20 hours after the onset of heat.

Table 2. Insemination of cows 13-20 hours after the onset of heat

Statistical indicators	Reproduction indicators	%Fecundity	Services per conception (SPC)	Service period	%Fertility	Sex ratio
$\bar{X}$		83.21	1.7	82.3	73.2	56.5
Correl SPC - %Fecundity						-0.3474068
Correl SPC – Service period						-0.0470059
Correl SPC - %Fertility						-0.2688664
Chitest analysis %fecundity %fertility obtained ~ chitest %fertility %fertility desired						0.13527932
CHITEST M/F Chitest analysis of the sex ratio obtained with HeiferPlus semen ~ the sex ratio obtained with conventional semen						0.15329191

From Table 2, it can be seen that in the group of cows inseminated 13-20 hours after the onset of heat, a fecundity percentage of 83.21% was recorded. This score equates to a very good fecundity if we refer to the fact that from this species, we want to obtain an average percentage of pregnancies of 80%. To obtain this fecundity percentage, 1.7 services were performed per conception. This score equates to good fecundity [4]. Moreover, reproductive activity is considered to be good when the number of services per conception is 1.6-1.8 inseminations/gestation. In this group of cows, the duration of the service period was 82.3 days. This score is close to the ideal and thus allows the cows to achieve the desired yearling and calf [5]. The fertility obtained in this lot was 73.2%. This score equates to good fertility. If we refer to the fact that an average of 60% of viable cattle is obtained from one cow, it can be seen that in this lot 22% more viable cattle were obtained than usual. Pregnancy losses through embryonic death, abortion, dystocia births, morbidity or non-viable cattle were within acceptable limits, 13.67%. The ratio between the sexes shows that 56.5% females were obtained in this batch, 13% more than in the case of using conventional semen. The analysis of the CHITEST test shows that there are no significant differences between the females obtained in this batch and the females obtained by insemination with conventional semen where the ratio between the sexes is 1/1 (p=0.15329191; p>0.05). From the analysis of the ratios between the number of services per conception and the % fecundity obtained; it is found that there is a negative correlation between these two indicators (-0.3474068). This shows that in the batch of cows inseminated 13-20 hours after the onset of heat, a higher fecundity can be obtained only by decreasing the number of services per conception. There was also a negative correlation between the number of services per conception and the length of the service period (-0.0470059). This shows that the duration of the service period can decrease by increasing the number of services per conception. A negative correlation was also registered in the ratios between the number of services per conception and the percentage of fertility obtained (-0.2688664). Thus, it can be stated that a better fertility can be registered only by decreasing the number of services per conception. In order to evaluate the fecundity and fertility performances obtained in this lot, the CHITEST test was performed. Thus, the data obtained for this batch were reported at the normal values of 80% fertility and 60% fertility. The

obtained result p=0.13527932; p>0.05 shows that there are no significant differences between the results recorded in this batch and the optimal results.

Figure 2 shows the comparative analysis of the results obtained by the two groups for the monitored reproductive indicators.

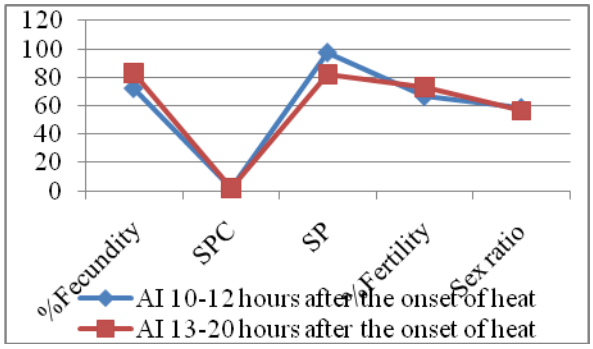

Figure 2. Comparative analysis of the monitored reproduction indicators

Legend:
%fecundity = percent fecundity; SPC = services per conception; SP = service period; %fertility = percent fertility; AI = artificial insemination

Figure 2 shows that in the group of cows inseminated 13-20 hours after the onset of oestrus and 19-12 hours before ovulation, the results recorded are better. Thus, it can be observed that regarding the %fecundity indicator, 15.18% more pregnancies were obtained compared to the inseminated group 10-12 hours after the onset of heat. When analysing the number of services per conception, it was found that 11.76% less HeiferPlus semen was used in the insemination group at 13-20 hours. The analysis of the service period indicator shows that in the group of cows inseminated at 13-20 hours, the period of open days was 18.32% shorter. The analysis of the %fertility indicator shows that in the group of cows inseminated at 13-20 hours, 9.9% more viable calves were obtained. The analysis of the ratio between the sexes shows that in the group inseminated at 10-12 hours, 3.53% more females were obtained.

Table 3 shows the reproductive performances recorded by the cows that were inseminated 42-84 days after the onset of lactation.

Table 3. Analysis of insemination of cows 42-84 days postpartum

Statistical indicators	Reproduction indicators	%Fecundity	Services per conception (SPC)	Service period	%Fertility	Sex ratio
$\bar{X}$		75.07	1.9218107	77.485	70.370370	55
Correl SPC - %Fecundity						0.05976143
Correl SPC – Service period						-0.0502836
Correl SPC - %Fertility						0.28676967
Chitest analysis %fecundity %fertility obtained ~ chitest %fertility %fertility desired						0.147676139
CHITEST M/F Chitest analysis of the sex ratio obtained with HeiferPlus semen ~ the sex ratio obtained with conventional semen						0.317310813

Table 3 shows that in the group of cows inseminated 42-84 days after calving, a fecundity percentage of 75.07% was recorded. This score equates to good fecundity if we mean that from this species, we want to obtain an average percentage of pregnancies of 80%. To achieve 75.07% pregnancies, 1.92 services were performed per conception. This score represents an acceptable result from the point of view of farm management. In this group of cows, the duration of the service period was 77.48 days, which is equivalent to the potential of the species. The fertility obtained in this batch was 70.37%. If we refer to the fact that an average of 60% viable calves are obtained from a cow, it can be seen that in this batch 17.28% more viable calves were obtained than usual. Pregnancy losses due to embryonic death, abortion, dystocia births, morbidity or non-viable calves were 6.67%, well below acceptable limits. The gender ratio shows that in this batch 55% were women, 22% more than men.

The analysis of the CHITEST test shows that there are no significant differences between the females obtained in this batch and the females obtained by insemination with conventional semen where the ratio between the sexes is 1/1 (p=0.317310813; p>0.05). From the analysis of the ratios between the number of services per conception and % fecundity, it is found that between these two indicators there is a positive correlation (0.05976143). This shows that improving the %fecundity indicator can only be achieved by increasing the number of services

per conception. The analysis of the ratios between the number of services per conception and the period of open days shows that between these two indicators there is a negative correlation (-0.0502836). This shows that as the service period increases, the number of services per conception will decrease. It can be said that with the decrease in milk production, the number of services per conception will also decrease. The analysis of the ratios between the number of services per conception and the recorded %fertility shows that there is a positive correlation between these two indicators (0.28676967). Thus, it can be stated that more inseminations increase the chances of obtaining better fertility. In order to evaluate the fecundity and fertility performances obtained in this lot, the CHITEST test was performed. Thus, the data obtained for this batch were reported at the normal values of 80% fecundity and 60% fertility. The obtained result p=0.1476761392; p>0.05 shows that there are no significant differences between the results recorded in this batch and the optimal results.

Table 4 shows the reproductive performances recorded by the cows that were inseminated 85-130 days after the onset of lactation.

Table 4. Analysis of insemination of cows 85-130 days postpartum

Statistical indicators	Reproduction indicators	%Fecundity	Services per conception (SPC)	Service period	%Fertility	Sex ratio
X^{-}		79.88	1.76086957	86.8399209	73.9130435	60
Correl SPC - %Fecundity						-0.0358339
Correl SPC – Service period						0.158935
Correl SPC - %Fertility						0.09309916
Chitest analysis %fecundity %fertility obtained ~ chitest %fertility %fertility desired						0.07252262
CHITEST M/F Chitest analysis of the sex ratio obtained with HeiferPlus semen ~ the sex ratio obtained with conventional semen						0.04550027

Table 4 shows that in the group of cows inseminated at 85-130 days postpartum, a fecundity percentage of 79.88% was recorded [6]. To achieve this fecundity percentage, 1.76 services were performed per conception, which equates to a good score [7].

In this group of cows, the duration of the service period was 86.83 days this score shows that the service period is within limits that favour economic growth [8]. The fertility obtained in this batch was 73.91%. The sex ratio shows that 60% females were obtained in this batch, 20% more than when using conventional semen. The analysis of the CHITEST test shows that in this batch there are significant differences (p=0.04550027; p<0.05) between the percentage of females obtained with HeiferPlus semen and the percentage of females, 50%, [9] obtained with conventional semen.

From the analysis of the ratios between the number of services per conception and the percentage of fertility obtained, it is found that there is a negative correlation between these two indicators (-0.0358339). This shows that in the lot of cows inseminated 85-130 days after the onset of heat, higher fecundity can be achieved just by decreasing the number of services per conception. The ratios between the number of services per conception and the period of open days show that there is a positive correlation between them (0.158935). There was also a positive correlation between the number of services per conception and the recorded %fertility (0.09309916). To evaluate the fecundity and fertility performances obtained in this batch, the CHITEST test was performed. Thus, the data obtained for this batch were reported to the normal values of 80% fecundity and 60% fertility. The obtained result p=0.07252262; p>0.05 shows that there are no significant differences between the results recorded in this batch and the optimal results.

Figure 3 presents a comparative analysis between the results obtained by the two batches for the monitored reproduction indicators.

Figure 3 shows that in the group of cows inseminated 85-130 days after the onset of lactation, 6.36% more pregnancies were obtained compared to the group inseminated 42-84 days postpartum. Also, the percentage of viable calves obtained in the 85–130-day insemination group was 5.03% higher. To obtain these calves, 9.9% less semen was used compared to the batch

inseminated at 42-84 days. Also in this batch, 9.09% more females were obtained compared to the batch inseminated at 42-84 days.

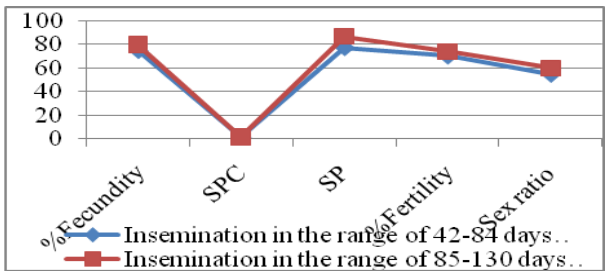

Figure 3. Comparative analysis of the reproductive performances of the two analysed groups of cows

Legend
%fecundity = percent fecundity; SPC = services per conception; SP = service period, %fertility = percent fertility

The analysis of the service period indicator shows that in the group of cows inseminated at 42-84 days, the period of open days was 13.53% shorter.

Conclusions

Insemination of cows 13-20 hours after the onset of heat offers more chances for obtaining a pregnancy.
The insemination of cows 85-130 days after the initiation of lactation offers a greater opportunity for obtaining a pregnancy compared to their insemination shortly after the end of the puerperal period.

Even if this increased fecundity comes with a longer period of open days, this increase is within acceptable limits.

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