The effect of heatsynch protocol on repeat breeding dairy cows

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Abstract. The aim of this study was to know the incidence of repeat breeding in dairy cows and to know the effectiveness of Heatsynch protocol on dairy repeat breeding cows. The study was conducted in two stages. The first stage, all dairy cows were recorded and clinically examined through palpation per rectum and for their reproductive statuses. Parameter measured in the study was the incidence of repeat breeding. The second stage of the study was that the repeat breeder cows subjected to Heatsynch protocol for estrus induction. The protocol was GnRH injection on day-0, followed by PGF2α, estradiol injections on day-7 and day-8, respectively, and inseminated artificially on day-9. The results of the study indicated that the incidence of repeat breeding was 41.6%. The Heatsynch protocol was successfully increase the number of pregnant repeat breeder cows; 75%, higher compared to untreated repeat breeder cows; 20%. It can be concluded that high incidence of repeat breeding in dairy cows. Heatsynch protocol can be used to treat repeat breeder cows and improve the reproductive efficiency.

1. Introduction
Dairy cattle are one of the most widespread cattle populations in Indonesia, especially in areas whose agricultural production can support dairy cattle development. Enrekang Regency, especially Cendana District, is one of dairy cattle breeding center in South Sulawesi. Although the potential and prospects of dairy cattle development in Enrekang Regency, particularly in Cendana District is quite large, but this development is still facing various obstacles that result in low productivity. There are many factors that affect; one of them is the number of cases of reproductive disorders.

One of the most common reproductive disorders in dairy cattle is repeat breeding. Repeat breeding is a condition of a dairy cows that is failing to become pregnant after being mated three or more times in the absence of observed abnormality [1]. Repeat breeding cows are generally characterized by long calving interval (18-24 months), low conception rate (<40%), and high service per conception (>3) [2]. The causes of repeat breeding cows are basically due to failure of fertilization and early
embryonic death. Failure of fertilization and early embryonic death are generally caused due to hormonal disorders, infection factors, environment, nutrition, and management [3].

The Heatsynch protocol is a synchronization method using a combination of GnRH, PGF2α and estrogen that is expected to have simultaneous estrus and ovulation and can be used for AI [4] application without the need to detect any signs of estrus and AI can be performed with a fixed time. The purpose of the Heatsynch protocol is to induce estrous and ovulation to perform AI. This protocol showed 89.21% success in improving the efficiency of dairy cattle reproduction [5], therefore, it is expected that Heatsynch protocol can also be one effective method in solving repeat breeders cases. Therefore, this study was conducted to determine the incidence of repeat breeding dairy cows and the effectiveness of Heatsynch protocol for repeat breeders.

2. Materials and methods

2.1. Dairy farms and treatment
This study was conducted during a period of January 2017 to April 2017 in Panette Village, Cendana District, Enrekang Regency, South Sulawesi. A total of 12 dairy small farms were used in the study. The farms size ranged from two to 15 dairy cows with housing system was tie-stall. All cows in the farms were subjected to reproductive examination. The study was conducted in two stages. The first stage, all dairy cows (n=77) were recorded and clinically examined through palpation per rectum and for their reproductive statuses. History of repeat breeding in dairy cows was noted to expect the incidence. The second stage of the study was the use of Heatsynch protocol to the repeat breeders. During the Heatsynch protocol, 18 dairy cows with repeated breeding were divided into two groups. The first group consisted of 8 cows that subject to Heatsynch protocol, while the other 10 were used as negative control (without Heatsynch). For positive control, 10 dairy cows that did not experience repeat breeding were included in the protocol. The protocol was GnRH injection on day-0, followed by PGF2α, estradiol injections on day-7 and day-8, respectively, and inseminated artificially on day-9. Pregnancy diagnosis was conducted two months or more after artificial insemination using rectal palpation and/or ultrasonography.

2.2. Parameters of the study
Parameters measured in this study were (1) the incidence repeat breeding and (2) Effectiveness of Heatsynch protocol in repeat breeding cows. The data was also recorded percentage of estrus; relative number of cows showed sign of estrus after treatment, interval between calving and pregnancy, service per conception (S/C), conception rate; percentage of cows conceived after artificial insemination.

2.3. Study design and data analysis
This study was designed to determine the incidence of repeat breeding of dairy cows in Panette Village, Cendana District, Enrekang Regency, and effectiveness of Heatsynch protocol to repeat breeders. The data was tabulated using Microsoft Excel program and then analyzed using SPSS version 16.0. The interval between calving and pregnancy and service per conception was analyzed using Kaplan-Meier survival to determine the rate of difference in proportion of pregnant cows and the number of Artificial Insemination (AI) in each treatment during the study. The pregnancy rate at each treatment was compared using chi-square test.

3. Results and discussion

3.1. The incidence of repeat breeding
The incidence of repeat breeding cows in the present study was 41.6% as shown in Figure 1. Likewise, normal cows was 58.4%. The results obtained in this study is higher than reported by Prihatno et al. [3] that the incidence of repeat breeding at the farmers level of in Yogyakarta was 29.4%, however the results of this study is lower than reported by Yusuf et al. [6] that incidence of repeat breeding in the
tropics can reach 62%. This suggests that the incidence of repeat breeding cows vary depending on regional, environmental and management factors.

![Figure 1](image-url)  
Figure 1. The proportion of normal dairy cows and dairy cows with repeat breeding

### 3.2. Effectiveness of Heatsynch protocol on repeat breeding cows

The cause of repeat breeding is basically a failure of fertilization and the consequences of early embryonic death [7]. Failure of fertilization and early embryonic death are generally due to complex and interrelated factors, for example due to infection, hormonal, environmental, nutrition, and management [8]. Other factors that cause repeat breeding are ovarian disorders, infections, and reproductive disorders such as distocya, placental retention, and uterine prolapse, as well as ovarian, nutritional, AI technician, infection and hormonal activity [9].

Heatsynch protocol is one of the modification of hormone induction technology using a combination of GnRH, PGF2α and estrogen. This protocol is expected to overcome the hormonal disturbance occurring in dairy cows, induce simultaneously estrus and ovulation as well as conduct artificial insemination at fixed-time without estrous detection. The effectiveness of the Heatsynch protocol in repeat breeding cows in comparison to control groups is presented in Table 1.

| Table 1. Effectiveness of Heatsynch protocol in repeat breeding cows |
|---------------------------------------------------------------|
| Repeat breeding cows | Normal cows |
| Heatsynch | Kontrol (-) | Kontrol (+) |
| No. of cows | 8 | 10 | 10 |
| No. of cows showing estrus (%) | 100 | 100 | 100 |
| Interval between calving and pregnancy (day) | 488.3<sup>a</sup> | 553.1<sup>b</sup> | 94.9<sup>c</sup> |
| Service per conception (S/C) before Heatsynch treatment | 13.4<sup>a</sup> | 8.3<sup>b</sup> | 1.2<sup>c</sup> |
| Service per conception (S/C) after Heatsynch treatment | 2 | - | - |
| No. of cows pregnant (%) | 6/8 (75)<sup>a</sup> | 2/10(20)<sup>b</sup> | 10/10(100)<sup>c</sup> |

<sup>a, b, c</sup> different significantly (P<0,01)
The results showed that all treated dairy cows with Heatsynch protocol showed estrus, this mean that 100% have a response after hormonal induction. This may due to that estrogen work well in cows during treatment. Yusuf et al. [4] stated that, the purpose of the Heatsynch protocol is to synchronize estrus and ovulation after EB (Estrogen Benzoat) injection to allow for Artificial Insemination (AI).

Based on the survival analysis, the interval from calving to conception showed a significant difference (log rank test P <0.01), where the interval between calving and pregnancy in repeat breeding cows after treating with Heatsynch was 488.3 days, lower than those repeat breeders that did not treated with Heatsynch (control -); 553.1 days. However, in comparison to control (+), the normal cows had much lower interval; that was only 94.9 days. The results of this study had lower than the study reported by Yusuf et al. [10] that normal cows requires an average of 114 ± 3 days after calving to become pregnant. However, the results of this study on repeat breeding cows were much higher (488.3 days) compared with the report of Yusuf et al. [10] that cows with repeat breeding requires 211 ± 10 days after calving to become pregnant.

The survival analysis also showed a significant difference (log rank test P <0.001) at service per conception, where service per conception in normal cows was only 1.2 insemination per pregnancy while in repeated breeding cows, the average service per conception was much high; 13.4 inseminations per pregnancy. The repeat breeders in this study showed higher than those repeat breeders reported by Yusuf et al. [10] the average service per conception in normal cows was 1.7 ± 0.1 inseminations per pregnancy and in repeat breeding cows was 4.7 ± 0.2 inseminations per pregnancy. Rustamadji et al. [2] stated that repeat breeders is generally characterized by calving interval (18-24 months), low conception rate (<40%) and high service per conception (> 3).

Service per conception of dairy cows after the Heatsynch treatment showed an average of 2 inseminations per pregnancy, lower than before being treated. The effect of treatment on pregnancy rate showed a significant difference (chi-square test P <0.01). The percentage of repeat breeding cows that were successfully pregnant after the Heatsynch treatment was 75%, higher than repeat breeding cows that did not treated (control -); it was only 20%. This suggests that the Heatsynch protocol may increase pregnancy rate in repeated breeding cows. This is in line with the study of Stevenson et al. [5] that using the Heatsynch protocol showed 89.21% success in improving female dairy cattle reproductive efficiency.

Based on the results and discussion can be concluded that most dairy cows with repeat breeding can be cured by Heatsynch method so Heatsynch method can be one way that can be used to overcome the problem of repeat breeding that many occur in dairy cattle so it can decrease High recurring rate of recurring events and improved reproductive efficiency.

4. Conclusion
It can be concluded that high incidence of repeat breeding in dairy cows. Heatsynch protocol can be used to treat repeat breeder cows and improve the reproductive efficiency.

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