Efforts to improve reproductive efficiency in dairy heifers experiencing delayed puberty using heatsynch protocol

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Abstract. The aim of this study was to find out the application of Heatsynch protocol in overcome the delayed puberty of dairy heifers that suffered from infertility. This study was conducted at small dairy farms in Sub-district of Cendana, Enrekang Regency, Indonesia. The study was conducted in two stages. In the first stage, a total of 50 dairy heifers were used to determine the percentage of heifers that experienced delay in puberty. In the second stage, 20 dairy heifers with delayed puberty were divided into two groups. The first group, 10 dairy heifers were treated with Heatsynch and the other 10 dairy heifers were used as negative control (without Heatsynch). For control positive, 10 dairy heifers that did not suffered from delayed puberty were used. In Heatsynch treated dairy heifers, GnRH were injected on day-0, followed by PGF2α, estradiol injections on day-7 and day-8, respectively, and inseminated artificially on day-9. The results of this study showed that the percentage of dairy heifers that experienced in delayed puberty was 44%. The mean age at first pregnant after treated with Heatsynch was 545 days, shorter than those heifers in control negative (644 days). The percentage of dairy heifers become pregnant after treating with Heatsynch was 80%, higher than those untreated delayed puberty heifers (50%). It can be concluded that the incidence of delayed puberty in dairy heifers was still high. Heatsynch protocol can be one way that can be used to overcome the problem of delayed puberty in dairy heifers and to improve reproductive efficiency.

1. Introduction
Livestock breeding is strongly influenced by calving rates that have an impact on population growth, so that the occurrence of reproductive disorders resulting in low reproductive efficiency and prenatal mortality, which in turn leads to a decrease the livestock population [1]. Reproductive disorders resulting in infertility female animals, characterized by a low calving rate in cattle [2].

One of the reproductive barriers that usually occur in dairy heifers is delay of puberty. Generally, a heifer especially Holstein Friesian cattle gets puberty in the optimum months depending upon genetic and environment. In dairy heifer as a replacement of dairy cows, an essential reproductive phases included the time of puberty and first estrus, first mating, pregnant, first calving, until again first estrus after calving and become pregnant. The growth pattern of a heifer can affect the time of puberty as
well as first estrus. A heifer with slower in growth will experience the delay of puberty, first mating and first calving [3].

The onset of puberty based on their physiological status in heifer starting at 9-12 months after birth, but they are generally inseminated until the age of 15 months [4]. This suggest that the heifers would be able to get pregnant in the optimum time as well as first calving at 24 months or 33 month at the latest.

However, not all the dairy heifers get puberty and first estrus in an optimum time. Efforts to accomplish this problem in small holder farms has not been well performed. On the other hand, many efforts to overcome such this problem in dairy cows particularly delay estrus after calving such as induce the estrous using hormonal treatment or the use of a combination of hormones. One combinations that usually occupied to induce estrous and ovulation is Heatsynch protocol. Therefore, we hypothesized that some dairy heifers suffered from late puberty, and the incidence of delay puberty in dairy heifers especially in small holder dairy farms are quite high. The application of Heatsynch protocol [5] in dairy heifers are able to overcome the delay puberty or estrus. Therefore, the aim of this study was to find out the incidence of delay puberty in dairy heifers in the small holder dairy farms and application of Heatsynch protocol in overcome the delayed puberty of dairy heifers that suffered from infertility.

2. Materials and methods

2.1. Farms and reproductive examination
This study was conducted in the dairy small holder farms in Cendana District, Enrekang Regency, Indonesia. A total of 50 dairy heifers were used in the study among dairy small farms. All dairy heifers were recorded the age and body condition score (BCS). Clinically examination was performed for their general reproductive health, reproductive disorders through rectal palpation of the reproductive organs and assess the structure of the ovaries and uterus. The heifer that aged greater than 13 months and did not showing any palpable structure on both ovaries was considered delay puberty.

2.2. Study design and heatsynch protocol
The study was design and divided into three different groups. Group 1, normal dairy heifers (n=10) (Control positive group); heifers that onset of puberty within 13 months, group 2, dairy heifers (n=10) without any treatment (Control negative group) and group 3, dairy heifers (n=10) treated with Heatsynh (Heatsynch group). The dairy heifers that subjected to Heatsynch protocol were injected GnRH on day-0, followed by injections of PGF2α and estradiol on day-7 and day-8, respectively. Artificial insemination was conducted on day-9 in group 3, while in group 1 and 2 were conducted after estrous was detected. Heifers that become estrus after first AI were re-inseminated.

Pregnancy diagnosis to the dairy heifers at each group was conducted at approximately 60 days or greater after AI by rectal palpation. Dairy heifers at each group was recorded for their pregnancy.

2.3. Parameter of the study
The parameters measured in this study were the percentage of heifer that had delayed puberty, body condition score (BCS), days to puberty, number of dairy heifers coming into estrus, service per conception (S/C), days to conception, and pregnancy rate.

2.4 Data analysis
The data collected in this study were tabulated using Microsoft Excel program, then analysed using SPSS software version 16.0. The percentage of heifer that had delayed puberty was presented in percentage. All percentages data parameters such as number of dairy heifers coming into estrus and pregnancy rate were compare using Chi-square test. Service per conception (S/C) and days to conception were analysed using ANOVA. Kaplan-Meier survival analysis was used to determine the rate of difference in the proportion of pregnant heifers in each treatment during the study.
3. Results and discussion

3.1 The incidence of delay puberty in dairy heifers

The incidence of delay puberty in dairy heifers in small holder farms is shown in Figure 1. Out of 50 dairy heifers examined in the present study, 22 of them (44%) suffered from delay puberty and the remaining 56% was considered normal, in which the onset of puberty within 13 months. Those delay puberty heifers had various ages even greater than two years.

![Figure 1. The proportion of normal and delayed puberty in dairy heifers](image)

Figure 1 shows various ages of the delay puberty heifers whereas 34% of them are between 14 and 19 months, 6% between 20 and 22 months, and the remaining 4% was greater than 22 months. This indicated that mostly delay puberty heifers within 20 months. Delayed mating in dairy heifers can be caused due to delayed puberty, generally puberty in dairy heifers starts at the age of 9-12 months after birth whereas a dairy heifer can be mated after 10-15 months [4]. Moreover, usually the score of body condition also determines whether the dairy heifers get puberty in time.

The BCS (Body Condition Score) of the dairy heifers in this study is presented in Figure 2. In the present study, it seems that the BCS of dairy heifers as shown in Figure 2 relatively similar between those heifers in normal puberty and those delayed puberty. Generally BCS of the dairy heifers both in normal delayed puberty were quite good, ranging from 2.75 to 4.0 (scale 1 – 5). This suggests that the BCS of the dairy heifers in small dairy farms in this region relatively in good condition. This indicated that feed management as well as feed additive or concentrate applied in the small dairy farms have been fulfilled the need of dairy heifers.
3.2 Effectiveness of Heatsynch protocol on dairy delayed puberty heifers

In order to know the effectiveness of Heatsynch protocol that treated to dairy delayed puberty heifers, the treatment was built in the present study to compare this treatment to normal dairy heifers and the dairy heifers that did not treated with Heatsynch protocol. The results of this treatments are shown in table 1.

Table 1. Effectiveness of Heatsynch protocol in normal and delayed puberty in dairy delayed puberty

| Item                              | Normal dairy heifers (Control positive) | Delayed dairy puberty heifers (Control negative) | Delayed dairy puberty heifers treated with Heatsynch protocol |
|-----------------------------------|----------------------------------------|-----------------------------------------------|------------------------------------------------------------|
| No. of dairy heifers             | 10                                      | 10                                            | 10                                                         |
| No. of dairy heifer coming into estrus (%) | 100                                     | 50                                            | 100                                                       |
| Service per conception (S/C)     | 2.1                                     | 5.6                                           | 2.7                                                       |

The table 1 shows that the percentage of dairy heifers coming into estrus during the study period in control positive, control negative, and in Heatsynch groups were 100%, 50%, and 100%, respectively. This indicated that only 50% dairy delayed puberty heifers without treated with Heatsynch protocol coming into estrus; significantly (P <0.01) lower than those in normal heifers and delayed puberty heifers treated with Heatsynch protocol. It means that the Heatsynch protocol improved the estrus rate of dairy delayed puberty heifers. This study is in line with the study of Yusuf et al [5] stated that, the purpose of the Heatsynch protocol is to synchronize estrus and facilitates artificial Insemination (AI).

Likewise, based on statistical analysis showed that there was a significant difference (P <0.05) on service per conception (S/C) among the treated dairy heifers groups. The S/C of normal dairy heifers group was significantly (P <0.01) lower than those heifers in both delayed dairy puberty heifers and delayed dairy puberty heifers treated with Heatsynch protocol groups (2.1 vs. 5.6 and 2.7 times). This results suggests that although the S/C of dairy delayed puberty heifers treated with Heatsynch protocol group showed significant difference (P <0.05) to the normal dairy heifers group but the point of difference is only 0.6. While the difference point of S/C between the group of delayed dairy puberty heifers and delayed dairy puberty heifers treated with Heatsynch protocol group was ultimately high (2.9 points). This suggests that there was a significant improvement in S/C to the dairy delayed puberty heifers after treating with Heatsynch protocol as well as improves pregnancy rate.
The pregnancy rate of dairy heifers at different groups are shown in Figure 3. The dairy delayed puberty heifers group shows significantly (P <0.05) lower pregnancy rate in comparison to those in normal dairy heifers group and dairy delayed puberty heifers treated with Heatsynch protocol group (50% vs. 100% and 80%). To achieve high pregnancy rate in dairy heifers, at the best that the dairy heifers should be detected the estrous frequently at least twice a day. Moreover, insemination twice at detected estrus are much more chances to the heifers become pregnant [6], although more semen straws are required.

In the present study, the percentage of heifers become pregnant in dairy delayed puberty heifers treated with Heatsynch protocol group was 80%. This result is significantly (P <0.05) higher than the pregnancy rate of those in dairy delayed puberty heifers group. Therefore, improvement in pregnancy rate after treating with Heatsynch protocol in dairy delayed puberty heifers could increase the reproductive efficiency in heifers. This result is in line with the study of Stevenson et al [7] using this protocol and improved the pregnancy rate of 89.21%.

In figure 4 shows the rate of dairy heifers to become pregnant at three different groups in small dairy farms. Normal dairy heifers group shows significantly (P <0.01) shorter days to become pregnant in comparison to dairy delayed puberty heifers group and dairy delayed puberty heifers treated with Heatsynch protocol group. Likewise, days to become pregnant in dairy delayed puberty heifers treated with Heatsynch protocol group had significantly (P <0.01) shorter days to become pregnant in comparison to dairy delayed puberty heifers group. This indicated that improvement the days to become pregnant in dairy heifers was achieved in the present study. Shorter days to become pregnant in dairy heifers could bring the producers of dairy farms to achieve high performance of the dairy cattle as well as reduce the possibility to cull the heifers due to infertility. Furthermore, increasing the conception rate of dairy heifers could reduce the incidence of repeat breeding.

Basically, conception rate of dairy heifers after estrous synchronization ranging from 25 to 75% [8,9] and to achieve high rate of the conception, it is necessary to have good body condition at the time of mating [10]. This condition brings the days of heifers to become pregnant as shorter as possible. Subsequently, increase the purpose of dairy heifers as replacement of the dairy cows.
As shown in Figure 4, the average days to conception after birth of the normal heifers group was 309 days, significantly (P < 0.01) shorter than those in heifers with delayed puberty groups. Heifers with delayed puberty group had significantly (P < 0.01) longer days to conception compared to the heifers with delayed puberty treated with Heatsynch protocol (644 vs. 545 days). Therefore, it can be supposed that Heatsynch protocol can be shorten the days to conception of the dairy heifers suffered from delayed puberty.

In fact, the days to conception and first calving of dairy heifers in this region is still longer. This condition allows the lack number of calves born in ratio with the number of heifers throughout of the year, and lower number of offspring per individual heifer during their lifetime resulting decreasing the reproduction performance. Essentially, the ability of dairy heifers as a candidate to substitute the dairy cows to reach puberty, normal cycles, conception in time, maintaining pregnancy, calveding normally, and first lactation are critical components of dairy farming [11].

4. Conclusion
It can be concluded that the incidence of delayed puberty in dairy heifers was still high. Heatsynch protocol can be one way that can be used to overcome the problem of delayed puberty in dairy heifers and to improve reproductive efficiency.

Acknowledgement
The authors are thanks to the Ministry of Research, Technology, and Higher Education of Indonesia through Directorate of Research and Community Services and The Centre of Research and Community Services, Hasanuddin University for funding this study through Excellent Research of Higher Education.

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