Ovarian luteal category at the time of exogenous progesterone treatment alters pre-ovulatory follicle size and pregnancy outcome but not initial GnRH treatment in repeat-breeder crossbred dairy heifers submitted to the 7-day fixed-time AI protocol

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ABSTRACT
Repeat breeding is a substantial problem in heifer and cow breeding leading to greater infertility for female dairy herds. The aim of present study was to investigate the impact of corpus luteum (CL) presence and category and the first gonadotropin-releasing hormone (GnRH) administration concurrent with exogenous progesterone (P4) treatment on the largest follicle (LF) size and pregnancy rate (PR) in repeat-breeder crossbred dairy heifers submitted to the fixed-time artificial insemination (AI) protocol. Heifers (n = 243) were synchronised with (+GnRH) or without (–GnRH) first GnRH in the 7-day P4-GnRH-prostaglandin F2α-based programme. Each GnRH group was divided on presence of CL into two groups (+CL and –CL) in a 2 × 2 factorial arrangement. The PR was similar among –GnRH–CL (20.7%), –GnRH+CL (68.8%), +GnRH–CL (30.4%), and +GnRH+CL (68.3%) groups. However, presence of CL in heifers produced a 43.6% increase in PR compared to PR of heifers without CL (odds ratio = 6.550). Heifers bearing large-sized CL had greater large-sized LF on the day of fixed-time AI and PR. Plasma P4 concentration was positively related with CL diameter (r = 0.845; p < 0.001). The diameter of ovarian LF on the day of fixed-time AI was positively associated with P4 concentrations (r = 0.512; p < 0.001).

We highlight that ovarian CL presence and category at the time of exogenous P4 treatment alters pre-ovulatory follicle size and PR but not initial GnRH treatment in repeat-breeder crossbred dairy heifers submitted to service with the 7-day fixed-time AI programme.

1. Introduction

In dairy cattle, repeat breeding have been defined as normal oestrous cycling females with no clinically detectable reproductive disorders which failed to conceive after at least three successive inseminations (Puglisi et al., 2013). The high incidence of repeat breeders has been found in 24.4–45.4% of female dairy herds (lactating and non-lactating cows and heifers) (Deka et al., 2021). The phenomenon of the repeat breeding leads to infertility in heifers (Singh et al., 2005) and cows (Yusuf et al., 2010). This phenomenon challenges the normal reproductive output of the dairy farm (Nebel & Jobst, 1998) and repeat breeding heifers has considerable impact on the economy of dairy

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farmers (Båge et al., 2002). Reproductive performance of heifers is crucial for the economics of the heifer enterprise because decreasing time to conception decreases the management and opportunity costs of herd replacement (Masello et al., 2021). Therefore, effective breeding programmes to manage the reproduction of repeat-breeder dairy heifers may have a pronounced effect on the overall reproductive ability of dairy herds (Yaginuma et al., 2019). To manage reproduction in the replacement heifer herds, the 7-day progesterone (P4)-gonadotropin (GnRH)-prostaglandin F2α (PGF2α)-based protocol has been applied to induce the ovulation of dominant follicle (DF) and inseminate with fixed-time artificial insemination (AI) in heifers (Mel- lieon et al., 2012; Stevenson et al., 2008). This protocol assumes that the first GnRH treatment induces the growing DF to ovulate, which leads to emergence of an ovulatory wave (new ovarian follicular wave) with a new DF (Colazo & Mapletoft, 2014). To control follicular growth and prevent early ovulation before the second GnRH treatment, P4 device is added to this programme for 7 days. Seven days after first GnRH, administration with PGF2α results in degeneration of the corpus luteum (CL) (Colazo & Mapletoft, 2014). In turn, on day 9 of the programme, a new DF will be induced to ovulate by the second injection of GnRH (Colazo & Mapletoft, 2014). The appearance of a new ovarian follicular wave is induced only when first GnRH administration causes ovulation (Mellieon et al., 2012). If the initial GnRH does not induce the emergence of an ovarian follicular wave, ovulation following the second GnRH injection may be poorly synchronised (Colazo & Mapletoft, 2014; Martinez et al., 1999). Unfortunately, according to Mellieon et al. (2012), Stevenson et al. (2008), and Sahu et al. (2014), treatment of first GnRH irrespective of oestrous cycle stage resulted in an ovulation rate of only 31–49% and depends on the maturation stage of the follicles (Colazo & Mapletoft, 2014) and ovarian structure (Urzáiz González et al., 2017) at the time of programme initiation. Because of the importance of ovarian structure, dairy cows with a functional CL at the time of first GnRH treatment had more pregnancy outcome than cows lacking a functional CL at first GnRH injection regardless of their ovulatory response to the first GnRH treatment (Carvalho et al., 2018). This implies that, although ovulatory response to first GnRH treatment of the GnRH-based protocol can affect pregnancy to timed AI, a functional CL at the time of programme initiation has a greater effect on pregnancy than ovulatory response to first GnRH treatment (Carvalho et al., 2018). However, relatively little is known about the importance of ovarian luteal presence at the time of first GnRH treatment in repeat-breeder dairy heifers subjected to the fixed- time AI protocol. On the basis of these observations, there are still two questions to be resolved in the 7-day P4-GnRH-PGF2α-based programme when this protocol is applied irrespective of oestrous cycle stage in repeat-breeder dairy heifers: (1) the impact of CL presence at the time of the P4 treatment on follicle and fertility and its relationship with pre-ovulatory follicle size and pregnancy rate (PR); and (2) the necessity of the initial GnRH administration at the time of the P4 treatment of the fixed-time AI protocol. However, the impact of ovarian luteal category, blood P4 concentrations, and first GnRH treatment at the of exogenous P4 administration on pre-ovulatory follicle size and pregnancy outcome had not yet been investigated in repeat-breeder crossbred dairy heifers with a random stage of the ovarian cyclicity submitted to the 7-day P4-GnRH-PGF2α-based protocol. Moreover, enhanced knowledge of ovarian biology is essential to increasing the reproductive ability of normal and infertile female ruminants (Palmieri et al., 2011; d’Orey Branco et al., 2016; U-krit et al., 2022; Yama et al., 2022).

Collectively, these observations led us to hypothesise that, during a random stage of the ovarian cyclicity, CL presence at the time of exogenous P4 treatment in the 7-day P4-GnRH-PGF2α-based protocol with or without first GnRH would result in enhanced diameter of the largest follicle (LF) on the day of fixed-time AI and PR after fixed-time AI with the 7-day P4-GnRH-PGF2α-based protocol.

2. Materials and methods

2.1. Ethics

The ethical approval for this experiment was obtained from the Animal Care and Use Committee of Maejo University (approval number: MACUC025A/2564).

2.2. Heifers and management

Repeat-breeder crossbred Holstein-Friesian (75.0–87.5%) dairy heifers (n = 243) were first selected based on their failure to conceive after three artificial inseminations (Arishtima et al., 2017) and no evidence of genital tract pathologies being diagnosed after ultrasonic evaluation (Puglisi et al., 2013). Heifers were raised indoors in a free-stall barn, and fed chopped maize silage and fresh Napier grass (Pennisetum purpureum) ad libitum supplemented with a commercial concentrate. Clean drinking water and mineralised salt bricks were provided ad libitum. Parameters of the dairy heifers, including age (mean ± standard deviation [SD]; 21.8 ± 5.3 mo), body weight (BW, 372.1 ± 63.0 kg), body condition score (BCS; [3.1 ± 0.5]), and insemination number (4.4 ± 0.9) were recorded prior to the initiation of the 7-day P4-GnRH-PGF2α-based protocol.

2.3. Experimental design, treatments, and fixed-time AI

A total of 243 repeat-breeder crossbred dairy heifers was distributed to a 2 × 2 (GnRH × CL) factorial arrangement. On a random stage of the ovarian cyclicity, heifers were synchronised with the 7-day P4-GnRH-PGF2α-based protocol. On the day of initiation of this protocol (day of the P4 treatment; day 0), heifers in the +GnRH group received a 10 µg dose (buserein) of first GnRH (Receptal, MSD Animal Health, New Zealand) while heifers in the −GnRH group did not receive the first GnRH. Luteal structures were evaluated using an HS-1600V ultrasound machine (Honda Electronics, Japan) with a 7.5 MHz linear-array probe to determine the CL presence. Heifers in both GnRH groups (−GnRH and +GnRH) were divided on presence of CL into two groups with [−CL] and without [−CL] in a 2 × 2 factorial arrangement: −GnRH−CL (n= 29), −GnRH+CL (n= 109), +GnRH−CL (n= 23), and +GnRH+CL (n= 82) groups.

On day 0, heifers in all groups were implanted with a P4-releasing device with 1.38 g of P4 (controlled internal drug release [CIDR], Eazi-Breed; Zoetis Inc., New Zealand) which were withdrawn on day 7. On day 7, all heifers received a 250 µg dose (cloprostenol) of PGF2α (Estrumate; MSD Animal Health, New Zealand). The day of the fixed-time AI was designated as day 9. On the day of the fixed-time AI, all heifers received a 10 µg dose (buserein) of GnRH. All heifers were inseminated with a dose of frozen-thawed bull semen at the same time as the last injection of GnRH.

2.4. Ovarian ultrasonography, assessment of luteal parameters, and ovarian LF

On day of the initiation of this protocol (day 0), ovarian follicular and luteal structures were evaluated using an HS-1600V ultrasound machine with a 7.5 MHz linear-array probe by a single examiner to evaluate the CL presence and diameter. To assess the ovarian luteal parameters, the location and diameter of CL were sketched on an ovarian map relative to each CL, and ultrasound images of each CL were
saved for further calculation. During ultrasound examination, the CLs were categorised according to size as class 1 (≤ 9.9 mm in diameter), class 2 (10.0–14.9 mm in diameter), class 3 (15.0–19.9 mm in diameter), or class 4 (≥ 20 mm in diameter) (Escalante et al., 2013). We followed the calculation of each CL cross-sectional area based on previously reported reports (Jitjumnong et al., 2020; Miura et al., 2015), using the following formulation (Eq. (1)):

$$CL_{cross-sectional area} = \pi/4 \times (meanDofCL)^2$$

where $\pi$ is 3.1416 and D is the diameter. If a CL cavity was appeared, each CL cross-sectional area was calculated according to the following formulation (Eq. (2)):

$$CL_{cross-sectional area} = \pi/4 \times (meanDofCL)^2 - \pi/4 \times (meanDofcavity)^2$$

where $\pi$ is 3.1416 and D is the diameter.

We followed the calculation of each CL volume based on previously reported (Tortorella et al., 2013), through the following formulation (Eq. (3)):

$$CL_{volume} = 4/3\pi r^3$$

where $\pi$ is 3.1416 and r is the radius.

On the day of the fixed-time AI (day 9), a sketch of the location and diameter of ovarian LF was recorded. During ultrasound examination, we followed the classification of the LFs based on previously reported (Escalante et al., 2013), according to diameter: small-sized (2.0–5.0 mm), medium-sized (6.0–9.0 mm), or large-sized (≥ 10.0 mm).

2.5. Blood sampling and hormonal assay

On day 0, all blood samples were taken from the coccygeal vein using venipuncture into plastic tubes containing anticoagulant. Plasma samples were centrifuged at 1200 × g for 15 min and stored at −80 °C until subsequent P4 determination. Plasma P4 concentration assays were performed using the competitive enzyme-linked immunosorbent assay. All plasma samples were pipetted in duplicate for each run, and the sensitivity of the assay was 0.02 ng/mL. The intraassay and interassay coefficients of variation were 9.6% and 11.7%.

2.6. Pregnancy diagnosis and calculations of PR and embryonic loss

On days 32 and 60 after fixed-time AI, all heifers were subjected to pregnancy diagnosis by transrectal ultrasonography. We followed the calculations of PR and embryonic loss based on previous reports (Urzúa Gonzalez et al., 2017) as follows. The PRs were defined as the proportion of heifers that were diagnosed as pregnant on days 32 or 60 after fixed-time AI, divided by the total number of bred heifers at fixed-time AI. The embryonic loss was presented as the proportion of heifers that lost their pregnancy between days 32 and 60 after fixed-time AI, divided by the total number of pregnant heifers on day 32 after fixed-time AI.

2.7. Statistical analysis

The first GnRH treatment and CL presence were included in the statistical model as class variables, and age, BW, BCS and insemination number were also included in the statistical model as covariates. Data were analysed using the generalized linear mixed model regarding the GnRH factor (with and without), the CL factor (with and without) and its interaction. In heifers with (+CL) and without (−CL) CL, the Duncan’s New Multiple Rang Test was used to compare the means of P4 concentrations and LF diameter among the CL categories. Data of P4 concentrations and LF diameter are expressed as the mean ± standard error of the mean (SEM). Linear regression was applied to evaluate the linear relationship between CL characteristics and ovarian LF sizes. The odds ratio (OR) and 95% confidence intervals (CI) were used to evaluate the association between the PR and GnRH treatments, CL presence, age, BCS, and BW. A probability of p-value ≤ 0.05 indicated a significant difference.

3. Results

3.1. Main effect of the GnRH and CL factors and its interaction on plasma P4 concentrations, LF diameter, and PRs in repeat-breeder crossbred dairy heifers

Regardless of the stage of ovarian cyclicity, there was no interaction (GnRH × CL) effect on the plasma P4 concentrations on day 0 (p > 0.05), LF diameter on the day of the fixed-time AI (day 9) (p > 0.05), proportion of heifers with small-sized LF on day 9 (p > 0.05), PRs on days 32 and 60 after fixed-time AI (p > 0.05), and embryonic loss during 32–60 days after fixed-time AI (p > 0.05) (Table 1).

There was no significant effect of GnRH (−GnRH and +GnRH) on the plasma P4 concentrations on day 0 (p > 0.05), LF diameter on day 9 (p > 0.05), proportion of heifers with small-sized and large-sized LFs on day 9 (p > 0.05), PRs on days 32 and 60 after fixed-time AI (p > 0.05), and embryonic loss during 32–60 days after fixed-time AI (p > 0.05) (Table 1). There was no significant effect of CL (−CL and +CL) on the embryonic loss during 32–60 days after fixed-time AI (p > 0.05) (Table 1). However, a significant effect of CL (−CL and +CL) was observed on the plasma P4 concentrations on day 0 (p < 0.05), LF diameter on day 9 (p < 0.05), proportion of heifers with small-sized, medium-sized and large-sized LFs on day 9 (p < 0.05), and PRs on days 32 and 60 after fixed-time AI (p < 0.05) of repeat-breeder crossbred dairy heifers (Table 1). There was an GnRH × CL interaction effect on the proportion of heifers with medium-sized and large-sized LFs on day 9 (p < 0.05) (Table 1). The proportion of heifers having medium-sized LF on day 9 was greater (p < 0.05) in −GnRH–CL group than −GnRH+CL, +GnRH–CL, and +GnRH+CL groups (Table 1). On the other hand, the percentage of heifers with large-sized LF was less (p < 0.05) in −GnRH–CL group than −GnRH+CL, +GnRH–CL, and +GnRH+CL groups (Table 1).

3.2. Influence of different CL categories on plasma P4 concentrations, ovarian LF size, and PRs

In heifers with CL (+CL) on their ovaries, plasma P4 concentrations prior to start the exogenous P4 treatment on day 0 was greatest (p < 0.05) in heifers bearing CL class 4 compared with heifers bearing CL classes 3, 2, and 1 (8.6 ± 0.36 ng/mL vs. 6.1 ± 0.15 ng/mL, 3.3 ± 0.30 ng/mL, and 1.4 ± 0.14 ng/mL, respectively; Fig. 1a). In heifers with (+CL) and without (−CL) CL, ovarian LF size on the day of the fixed-time AI (day 9) was greatest (p < 0.05) in heifers bearing CL classes 2, 3, and 4 (12.6 ± 0.19 mm, 13.5 ± 0.17 mm, and 13.9 ± 0.18 mm, respectively) compared with heifers bearing CL class 1 and without CL (10.2 ± 0.22 mm and 11.1 ± 0.64 mm; Fig. 1b). On day 9, the percentage of heifers having small-sized LF was fewest (p < 0.05) in heifers bearing CL classes 1, 2, and 3 (3.0%, 2.0%, and 0.0%, respectively; Fig. 1c) compared with heifers without CL (11.5%); however, it was not different when compared with heifers bearing CL class 4 (3.7%) on their ovaries (Fig. 1c). The percentage of heifers having medium-sized LF was higher (p < 0.05) in CL class 1 (48.5%) and without CL (32.7%) than in CL classes 2, 3, and 4 (14.3%, 11.0%, and 3.7%, respectively; Fig. 1d). On the other hand, the percentage of heifers with large-sized LF was higher (p < 0.05) in CL classes 2 (83.7%), 3 (69.0%), and 4 (92.6%) than in CL class 1 (48.5%) and without CL (55.8%; Fig. 1e). The PRs on days 32 (Fig. 1f) and 60 (Fig. 1g) after fixed-time AI were greater (p < 0.05) for heifers bearing CL classes 3 and 4 than for heifers bearing CL classes 1 and 2 and without CL on day 0 (87.8% and 81.5% vs. 51.5% and 59.2% and 28.9% for PR on day 32; 82.9% and 77.8% vs. 48.5% and 53.1% and 25.0% for PR on day 60, respectively).
4. Discussion

The purpose of this study was to gain a better understanding of the impact of CL presence and category, and the first GnRH administration concurrent with exogenous P4 treatment on the ovarian LF size and PR in repeat-bred crossbred dairy heifers with a random stage of the ovarian cyclicity submitted to the fixed-time AI protocol. To the best of the researchers’ knowledge, this is the first investigation to provide data on the important factors (hormonal treatment and luteal stage) for the improvement of fertility in crossbred dairy heifers with repeat breeding syndrome.

The present results confirm that eliminating first GnRH treatment during a random stage of the ovarian cyclicity concurrent with exogenous P4 treatment (day 0) in the 7-day P4-GnRH-PGF2α-based protocol for repeat-breeder crossbred dairy heifers did not reduce optimal size of ovarian LF and did not decrease the appearance of large-sized LF on the day of the fixed-time AI (day 9) as indicated by the success in synchronous emergence of preovulatory follicular wave, similar to a previous report on ovarian follicular dynamics in pasture-based dairy heifers (Sahu et al., 2014). Although no evaluation of ovulatory response to the treatment with first GnRH concurrent with exogenous P4 treatment in the 7-day P4-GnRH-PGF2α-based protocol was unnecessary for the recruitment of the new follicular wave (preovulatory follicular wave) and was not essential to achieving acceptable PR in crossbred dairy heifers with a random stage of the ovarian cyclicity.

Interestingly, the results strongly imply that mean diameter of LF and the proportion of repeat-bred crossbred dairy heifers having large-sized LF (>10.0 mm) on the day of the fixed-time AI, as indicated by the success in synchronous emergence of preovulatory follicular wave,
Fig. 1. Box plot showing the distribution and variability of plasma P4 concentrations (a) and LF diameter (b) on the day of the fixed-time AI (day 9) for repeat-breeder crossbred dairy heifers without CL (n = 52) and for repeat-breeder crossbred dairy heifers appearing different CL categories (n = 191) at the time of exogenous P4 treatment (day 0). The proportions of heifers bearing small-sized LF (c), medium-sized LF (d), and large-sized LF (e) on day 9 with different CL categories on day 0. PRs on days 32 (f) and 60 (g) after fixed-time AI of heifers appearing different CL categories on their ovaries. Values with different superscript letters indicate significant differences among animal groups at p-value < 0.05. CL, corpus luteum; AI, artificial insemination; LF, largest follicle; PR, pregnancy rate; P4, progesterone.
were greater in heifers with CL (luteal phase) at the time of exogenous P4 treatment than heifers without CL (follicular phase) on their ovaries. This implies that the appearance of a functional CL (optimal P4 production) on the ovary at the time of exogenous P4 insert leads to an increase in the percentage of dairy heifers with synchronous emergence of preovulatory follicular wave and an increase in fertility (Yusuf et al., 2016). As stated above, although the secretory patterns of LH were not evaluated in the present experiment, we speculate, based on previous data (Kinder et al., 1996), that low circulating P4 levels (sub-luteal concentrations) increased the pulse frequency of LH secretion, supporting the DF in retaining its dominance over other ovarian follicles, thus preventing a new follicular wave (preovulatory follicular wave) from emerging (Atanasov et al., 2015; Bridges & Fortune, 2003; Kinder et al., 1996).

Overall, in the present study, repeat-breeder heifers attained 62–66% of PR in the 7-day P4-GnRH-PGF2α-based programme. This increase in PR was probably due to the fact that a combination of GnRH and PGF2α in the P4-based fixed-time AI programme results in synchronous follicular wave emergence, timely follicular development, synchronous ovulation following the last injection of GnRH (Kim et al., 2007), and it can overcome the problem of delayed ovulation and anovulation in repeat-breeder dairy cattle (Bhattacharyya & Hafiz, 2009). As stated above, repeat breeder heifers have a smaller preovulatory LH surge than virgin heifers (Gustafsson et al., 1986) and, therefore, an increase in the spontaneous LH surge that results from the treatment of GnRH at onset of the final stage of DF development affects the fertility favourably (Ahmed et al., 2016; Kaim et al., 2003).

5. Conclusion

Taken together, we conclude that initial administration of GnRH concurrent with exogenous P4 treatment in the 7-day P4-GnRH-PGF2α-based protocol is not essential to achieving acceptable fertility in repeat-breeder crossbred dairy heifers at a random stage of the ovarian
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Table 2
The OR for the risk factors contributing to the PR of repeat-breeder crossbred dairy heifers with (+CL) or without (-CL) CL on their ovaries that received (+1GnRH) or did not receive (-GnRH) first GnRH (GnRH) in the 7-day P4
GnRH-PGF2α-based protocol (n = 243).

| Variable | Pregnant heifers (n) | Non-pregnant heifers (n) | PR % | OR 3 | 95% CI 4 | p-value |
|----------|----------------------|--------------------------|------|------|----------|--------|
| 1GnRH administration (Ref = +1GnRH) 5 | | | | | | |
| +GnRH | 63 | 42 | 60.0 | (63/105) | | |
| -GnRH | 81 | 57 | 58.7 | (81/138) | 0.947 | 0.564-1.590 | 0.838 |
| Luteal presence (Ref = -CL) 6 | | | | | | |
| -CL | 13 | 39 | 25.0 | (13/52) | | |
| +CL | 131 | 60 | 68.6 | (131/191) | 6.550 | 3.416-12.558 | 0.001 |
| Age (mo) (Ref = <21 mo) 7 | | | | | | |
| <21 | 71 | 52 | 57.7 | (71/123) | | |
| 21-23 | 56 | 26 | 68.3 | (56/82) | 1.577 | 0.877-2.836 | 0.128 |
| >23 | 17 | 21 | 44.7 | (17/38) | 0.593 | 0.285-1.232 | 0.161 |
| BCS (Ref = <2.5) 7 | | | | | | |
| <2.5 | 7 | 10 | 41.2 | (7/17) | | |
| 2.5-3.5 | 116 | 74 | 61.1 | (116/190) | 2.239 | 0.832-6.031 | 0.111 |
| >3.5 | 21 | 15 | 58.3 | (21/36) | 2.000 | 0.618-6.472 | 0.247 |
| BW (kg) (Ref = <350 kg) 7 | | | | | | |
| <350 | 62 | 50 | 55.4 | (62/112) | | |
| 350-400 | 38 | 29 | 56.7 | (38/67) | 1.057 | 0.573-1.949 | 0.860 |
| >400 | 44 | 20 | 68.8 | (44/64) | 1.774 | 0.931-3.383 | 0.082 |
| Insemination number (Ref = 4-5) 7 | | | | | | |
| 4-5 | 130 | 82 | 61.3 | (130/212) | | |
| 6-7 | 12 | 14 | 46.2 | (12/26) | 0.541 | 0.240-1.218 | 0.138 |
| >7 | 2 | 3 | 40.0 | (2/5) | 0.421 | 0.072-2.451 | 0.335 |

1 GnRH, gonadotropin releasing hormone; CL, corpus luteum; BCS, body condition score; BW, body weight. 2 PR, pregnancy rate. 3 OR, Odds ratio. 4 CI, confidence interval. 5 First GnRH treatment concurrent with exogenous P4 treatment (day 0). 6 Luteal presence at the time of exogenous P4 treatment (day 0). 7 Age, BCS, BW and AI service number before the start of the synchronisation programme.

cyclicity. Repeat-breeder crossbred dairy heifers with the luteal stage when subjected to the 7-day P4-GnRH-PGF2α-based programme show improved fertility.

Ethical statement

The study was conducted according to the ethical principles and guidelines for the use of animals of the National Research Council of Thailand and approved by the Animal Care and Use Committee of Maejo University (approval number: MACUC025A/2564).

CRediT authorship contribution statement

Warunya Chaikol: Conceptualization, Methodology, Writing – original draft. Chayanon Yadmak: Conceptualization, Funding acquisition. Punnawut Yama: Methodology, Writing – original draft. Jakree Jitjumnong: Methodology, Writing – original draft. Molarat Sangkate: Methodology, Writing – original draft. Waritha U-krity: Methodology, Writing – original draft. Nalinthip Pramsoa: Methodology, Investigation. Assawadet Suriad: Methodology, Data curation. Raktam Methkrita: Methodology, Writing – review & editing. Julakorn Panatk: Methodology, Conceptualization. Hien Van Doan: Methodology, Writing – review & editing. Chien-Kai Wang: Methodology, Writing – review & editing. Pin-Chi Tang: Methodology, Writing – review & editing. Tossapop Moonmannee: Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare no conflict of interest.

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