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Silviu-Ionuț Borș *

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Article

Reproductive Outcomes in High-Yielding Holstein Cows Following Administration of GnRH on Day 5 after Artificial Insemination

Silviu-Ionuț Borş

Research and Development Station for Cattle Breeding Dancu, 707252 Iaşi, Romania;
Correspondence: bors.ionut@yahoo.com;

Simple Summary: In dairy cows, current methods for managing reproduction still need improvement. Future advancements will require new strategies to minimize additional interventions and maintain acceptance among veterinarians. As a result, the development of new therapies in dairy cows reproduction poses a significant challenge for improving reproductive performances. In recent years, there has been an increasing interest in inducing accessory corpus luteum in dairy cows, but the results have been controversial. It is still unclear for which parous cows this strategy is recommended. This study has clarified this aspect. Our work suggests that implementing this strategy on the farm is feasible only for repeat-breeder dairy cows with low genetic merit for fertility. It is not recommended as a herd management tool.

Abstract: The objectives of this study was to assess the impact of a reproductive approach in high yielding nulliparous and parous Holstein cows on the interestrus interval, estrus detection rates, conception rates, and pregnancy rates. The strategy involved giving GnRH on day 5 following artificial insemination (AI). For nulliparous cows, there appears to be no noticeable effect of GnRH therapy on the interestrus interval. In both groups, nearly all of the cows exhibited an 18–25-day cycle interval ($P < 0.05$). In the 18–25 day cycle period, the untreated multiparous cows exhibited the highest proportion ($P < 0.05$), while the treated group demonstrated the highest proportion ($P < 0.05$) in the 26–35 day cycle interval. The study found that the conception rate was higher in repeat breeder nulliparous and 3+ lactation treated Holstein cows compared with untreated ones. However, the cumulative heat detection rate, pregnancy rate and conception rates were similar in both groups ($P > 0.05$). Also, this study found that in the hottest months of the year, this therapeutic approach can't counteract the negative effect of heat stress in high-yielding dairy cows. Thus, it is not advised of using the hormonal method to create supplementary corpus luteum across the herd.

Keywords: high yielding cows, gonadorelin, accessory corpus luteum, reproductive parameters

1. Introduction

The effect of GnRH administration after artificial insemination for accessory corpus luteum (aCL) formation was confirmed in several studies **[1–6]**. The aCL is important in raising the level of progesterone (P4) in the bloodstream, which helps to address the deficiency in P4 that can lead to embryo mortality within the first 30 days after AI, especialy for high-yielding dairy cows **[7–9]**. Progesterone is crucial for maintaining the uterus during pregnancy, impacting the risk of embryo loss and pre-term labor **[10–12]**. It promotes an appropriate uterine environment for embryo elongation **[13–15]**. Compared to heifers, lactating dairy cows have lower plasma P4 levels due to increased liver blood flow and active P4 metabolism with rising milk production **[16]**. However, the data on the effectiveness of the treatment and the impact of aCL on reproductive performance are not consistent. In our previous study, we confirmed that administering GnRH between 7-14 days after artificial insemination (AI) can increase the number of aCL and improve reproductive performance in repeat-breeder dairy cows **[1]**. According to the results of Doležel et al., **[17]**, inducing aCL through gonadorelin administration was found to be more efficient in cows treated on day 5 following insemination, as compared to cows treated on days 6 or 7. A study conducted by Cunha et al. **[6]**

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found that administering 3,300 IU of hCG from day 5 to 7 of the estrous cycle increased the length of the cycle in non-inseminated lactating Holstein and Jersey cows. Furthemore, hCG treatments also prolonged luteolysis and interovulatory interval, and increased the incidence of cows with impaired luteolysis and delayed ovulation. These data indicate that forming aCL with the use of hCG disrupted natural luteolysis and ovulation, which are critical physiological events that regulate estrous cycle length **[18]**. Yan et al. **[19]** discovered that administering P4 between 3 and 7 days yielded benefits, while supplementation given earlier or later did not. Other studies have discovered that treatment with GnRH 10 days after AI is also conducive to pregnancy after AI **[5]**.

As indicated by the different studies, administration of GnRH or hCG at day 5–7 has the potential to improve fertility in dairy cows **[6,9,17]**. However, most of them failed to provide a clear consensus on the therapeutic benefits of such an approach **[20–23]**. To our knowledge, there have been no large-scale studies investigating the effects of GnRH treatment day 5 after AI on the fertility of nulliparous, primiparous, and multiparous cows. Thus, this study aimed to determine whether GnRH administration to nulliparous and parous cows on day 5 following AI could increase pregnancy and conception rates.

2. Materials and Methods

2.1. Ethical approval

All dairy cows were handled in accordance with the directives of the European Union on the protection of animals used for scientific purposes (Dir 2010/63/EU). The study protocol was approved by the Ethics Committee of Faculty of Veterinary Medicine, University of Life Sciences, 700489 Iasi, Romania. Efforts were made to minimize animal handling and stress.

2.2. Cattle herd management and study design

A Northeastern Romanian Holstein-Friesian dairy herd served as the subject of this study. The average number of lactating cows in the herd was 780 during the study period, and the average annual milk output per cow was 13.100 kg.

The cows were housed in free-stall barns with concrete floors, and straw beds and fed a Total Mixed Ration twice per day with ad libitum water access, according to the level of milk production and cow size. To keep the animals healthy, standard management practices, including a cooling system coupled with a weather station for hot months, were followed. During the study period, the farm milked about 780 cows three times a day at 0400, 1200, and 1900, for a daily average of 46 kg milk/cow/day.

During the study period from January 2022 to December 2023, cows were grouped by days in milk (DIM) and parity. Calving dates, breeding dates, and DIM were obtained from the AfiMilk management software (AfiMilk, Kibbutz Afikim, Israel). From January 2022 to December 2022, approximately all the cows were included in the G0 group, which received no treatment. For the period from January 2023 to December 2023, each cow in the herd received a single dose of GnRH (dephereline: gonadorelin acetate [6-DPhe]; Gonavet Veyx, Veyx-Pharma GmbH, Schwarzenborn, Germany) on day 5 after AI to induce ovulation and aCL formation.

Estrous cows were identified using the AfiMilk (AfiMilk, Kibbutz Afikim, Israel) daily estrus report, and an experienced veterinarian team examined each one. Signs such as attempting to mount other cows, chasing herds mates, restlessness, chin resting, sniffing herd mates' vaginas, bellowing, congestion, relaxation, and mucus discharge from the vulva were considered suggestive of estrus. Additionally, the visualization of a cow standing while being mounted was regarded as a definitive sign of estrus.

The veterinary team performed the ultrasound examinations and hormone injections. Each animal in the G5 group received one dose of GnRH after each AI, regardless of the number of AIs. A 5–7.5 MHz rectal convex probe (BCF EasyScan, BCF Ultrasound Australasia, Mitcham, Victoria, Australia) was used to scan the uterus and diagnose pregnancy. Pregnancy confirmation was based on visualizing an anechoic fluid-filled uterine horn and the presence of an embryo, along with a

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corpus luteum (CL) on the same side of the uterus. The first pregnancy diagnosis was performed using transrectal ultrasonography on day 30 after AI and confirmed on day 60.

2.3. Statistical analysis

By using binary logistic regression (logistic procedure of PASW Statistics for Windows Version 21, SPSS Inc., Chicago, IL, USA) and adjusting for lactation, days in milk, milk yield, type of estrus, repeat breeder, sire, and technician, the effects of treatment on rates of pregnancy, twin pregnancy, and pregnancy loss were examined. Significance was set at $P < 0.05$.

3. Results

In nulliparous cows, treatment with GnRH on the fifth day following AI did not alter the distribution of the cycle. In both groups, the majority of the cows ($P < 0.05$) presented the 18–25 day cycle interval. The other three periods showed low proportions and were not comparable ($P > 0.05$) with the exception that in the G5 group the 5-17 days cycle interval presented the lowest proportion ($P < 0.05$) (Table 1).

When compared to the G0 group, the primiparous cows in the G5 group showed a low proportion ($P < 0.05$) of the 18–25 day cycle interval and a high proportion ($P < 0.05$) of the 36–60 day cycle interval. The majority of primiparous cows in both groups ($P < 0.05$) showed an interval of 18 to 25 days between estrus and a lowest proportion of the 5–17 days cycle intervals (Table 1).

In the study, it was observed that multiparous cows from the G5 group had a lower proportion ($P < 0.05$) of the 18–25 days cycle interval and a higher proportion ($P < 0.05$) of the 26–35 day cycle interval compared to the G0 group. The analysis of the entire farm's cycle distribution showed that the 18–25-day interval had the highest proportion of cows ($P < 0.05$) compared to the other three intervals. However, there were differences between the treated and untreated groups. The untreated group had the highest proportion ($P < 0.05$) of the 18–25 day cycle interval, whereas the treated group had the highest proportion ($P < 0.05$) of the 26–35 day cycle interval (Table 1).

Table 1. Distribution of cycles in G0 vs G5 group.

Distribution of cycles	Nulliparous %		1st lact. %		2nd lact. %		3+lact.%		All cows %		Total %	
	G0	G5	G0	G5	G0	G5	G0	G5	G0	G5	G0	G5
5 - 17 days	5.1^{b} (19/ 374)	1.5^{c} (6/ 400)	5^{c} (18/ 357)	0.9^{d} (2/ 234)	4.7^{c} (13/ 277)	2.7^{c} (11/ 403)	3.5^{d} (12/ 347)	2.9^{b} (11/ 382)	4.4^{d} (43/ 981)	2.4^{c} (24/ 1019)	4.6^{c} (62/ 1355)	2.1^{c} (30/ 1419)
18 - 25 days	79.7^{a} (298/ 374)	77.8^{a} (311/ 400)	58.8^{ax} (210/ 357)	46.2^{ay} (108/ 234)	55.2^{ax} (153/ 277)	47.9^{ay} (193/ 403)	49.3^{ax} (171/ 347)	35.1^{ay} (134/ 382)	54.4^{ax} (534/ 981)	42.7^{ay} (435/ 1019)	61.4^{ax} (832/ 1355)	52.6^{ay} (746/ 1419)
26 - 35 days	5.9^{b} (22/ 374)	9.5^{b} (38/ 400)	14.6^{b} (52/ 357)	21.8^{c} (51/ 234)	17^{by} (47/ 277)	25.8^{bx} (104/ 403)	19.3^{cy} (67/ 347)	29.8^{ax} (114/ 382)	16.9^{cy} (166/ 981)	26.4^{bx} (269/ 1019)	13.9^{by} (188/ 1355)	21.6^{bx} (307/ 1419)
36 - 60 days	9.4^{b} (35/ 374)	11.3^{b} (45/ 400)	21.6^{by} (77/ 357)	31.2^{bx} (73/ 234)	23.1^{b} (64/ 277)	23.6^{b} (95/ 403)	27.9^{b} (97/ 347)	32.2^{a} (123/ 382)	24.3^{b} (238/ 981)	28.6^{b} (291/ 1019)	20.2^{b} (273/ 1355)	23.7^{b} (336/ 1419)

^{a,b,c} Superscripts within the same column indicate significant differences at $p < 0.05$ (a > b > c); ^{x,y} Superscripts within the same row indicate significant differences at $p < 0.05$ (x > y).

Throughout the whole period under consideration, both groups exhibited a high proportion of heat detection rate; however, there were a few minor deviations during the summer season, when both groups had lower values of this parameter ($P < 0.05$). On the other hand, the heat detection rate for the untreated group from the 18 periods tested at 21-day intervals was smaller in two-period intervals and greater in four-period intervals ($P < 0.05$) when compared to the treated group ($P < 0.05$). The cumulative heat detection rate was similar in both groups ($P > 0.05$) (Table 2).

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The conception rate was found to be highest ($P < 0.05$) in both groups during the months of January to half of May and October to December, while the lowest values ($P < 0.05$) were observed during June to October. When comparing the two groups (G5 and G0), G5 showed high values in three periods and lowest values in four periods ($P < 0.05$) from the 18 periods tested. However, the cumulative conception rate was found to be similar in both groups ($P > 0.05$) (Table 2).

The study found that the pregnancy rate was higher ($P < 0.05$) in both groups during January to mid-May and from October to December. In the G0 group, the pregnancy rate was found to be highest ($P < 0.05$) compared to the G5 group in four time periods from 18 taken into account. However, the cumulative pregnancy rate was similar in both groups ($P > 0.05$) (Table 2).

Table 2. Heat detection rate, pregnancy rate, and conception rate in G0 vs. G5 group.

Period	G0	G5	G0	G5	G0	G5
	Heat detection rate %		Pregnancy rate %		Conception rate %	
19.12 - 8.01	66.7^{a} (94/141)	68.9^{a} (144/209)	29^{ab} (40/138)	34.5^{a} (70/203)	43^{by} (40/93)	54.3^{ax} (70/129)
9.01 - 29.01	72.9^{a} (105/144)	66.3^{ab} (120/181)	29.2^{ab} (40/137)	30.6^{a} (52/170)	39.2^{bc} (40/102)	44.1^{b} (52/118)
30.01 - 19.02	71.9^{a} (105/146)	67.2^{a} (117/174)	29.3^{ab} (41/140)	23.2^{b} (39/168)	39.4^{b} (41/104)	39^{b} (39/100)
20.02 - 12.03	74.7^{a} (109/146)	71.8^{a} (125/174)	35^{a} (50/143)	35.1^{a} (59/168)	47.6^{by} (50/105)	55.7^{ax} (59/106)
13.03 - 2.04	72.5^{a} (87/120)	70.6^{a} (113/160)	37.6^{ax} (44/117)	25.5^{by} (40/157)	52.4^{abx} (44/84)	41.7^{by} (40/96)
3.04 - 23.04	70.8^{a} (85/120)	68.8^{a} (110/160)	33.6^{ab} (39/116)	33.1^{ab} (51/154)	47.6^{b} (39/82)	53.7^{a} (51/95)
24.04 - 14.05	64.3^{aby} (74/115)	72.7^{ax} (104/143)	35.1^{a} (40/114)	29.6^{ab} (42/142)	57.1^{a} (40/70)	50^{a} (42/84)
15.05 - 4.06	57.4^{bx} (62/108)	49.3^{cy} (66/134)	19.2^{c} (20/104)	12.1^{c} (16/132)	32.8^{c} (20/61)	32^{bc} (16/50)
5.06 - 25.06	64.2^{ab} (70/109)	59.2^{b} (87/147)	21.9^{b} (23/105)	16.6^{c} (23/144)	33.3^{c} (23/69)	30.7^{c} (23/75)
26.06 - 16.07	53.3^{by} (65/122)	60.5^{bx} (89/147)	16.7^{bc} (19/114)	16.6^{c} (24/145)	31.1^{c} (19/61)	28.2^{c} (24/85)
17.07 - 6.08	68.1^{abx} (92/135)	57.1^{by} (96/168)	30.3^{ax} (40/132)	15.9^{cy} (26/164)	45.5^{bx} (40/88)	28.3^{cy} (26/92)
7.08 - 27.08	67.5^{ab} (85/126)	65.2^{ab} (118/181)	26.9^{bx} (32/119)	11.9^{cy} (21/177)	38.1^{bcx} (32/84)	19.3^{dy} (21/109)
28.08 - 17.09	71.1^{ax} (101/142)	61.2^{by} (123/201)	25.4^{b} (34/134)	26.3^{b} (50/190)	34.3^{bcy} (34/99)	43.5^{bx} (50/115)
18.09 - 8.10	72.8^{a} (126/173)	73.9^{a} (145/196)	26.2^{b} (44/168)	25^{b} (46/184)	34.9^{bc} (44/126)	33.6^{bc} (46/137)
9.10 - 29.10	73.2^{ax} (145/198)	64.6^{aby} (128/198)	28.5^{abx} (55/193)	22^{by} (42/191)	42.6^{b} (55/129)	35.9^{bc} (42/117)
30.10 - 19.11	72.7^{a} (160/220)	74.3^{a} (162/218)	27.6^{ab} (59/214)	29.7^{ab} (62/209)	39.1^{bc} (59/151)	42.2^{b} (62/147)
20.11 - 10.12	69^{a} (156/226)	72.8^{a} (155/213)	29.5^{ab} (64/217)	32.7^{ab} (67/205)	46^{b} (64/139)	46.9^{ab} (67/143)
11.12 -31.12	77.7^{a} (164/211)	74.9^{a} (152/203)	36.3^{a} (74/204)	29.7^{ab} (58/195)	50.7^{abx} (74/146)	39.7^{bcy} (58/146)
Cumulative	69.8 (1885/2702)	67.2 (2154/3207)	29.1 (758/2609)	25.4 (788/3098)	42.3 (758/1793)	40.5 (788/1944)

^{a,b,c} Superscripts within the same column indicate significant differences at $p < 0.05$ (a > b > c); ^{x,y} Superscripts within the same row indicate significant differences at $p < 0.05$ (x > y).

The study show that conception rates were higher in nulliparous dairy cows at 2 and +4 artificial inseminations (AIs) in the G5 group compared to the G0 group ($P < 0.05$). For lactating cows, the

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conception rate was higher for primiparous cows with 3 AIs, secundiparous repeat breeder dairy cows, and all cows at 2 AIs from the G0 group compared to the G5 group (P < 0.05). Conversely, the 3+ lactation repeat breeder dairy cows showed a higher conception rate in the G5 group compared to the G0 group (P < 0.05). The total AI conception rates were similar in the G0 compared with G5 group (P > 0.05) (Table 3).

Table 3. The distribution of the conception rate according with the number of AIs in G0 vs. G5 group.

AIs and C.R.	Nulliparous %		1st lact. %		2nd lact. %		3+lact.%		All cows. %		Total %	
	G0	G5	G0	G5	G0	G5	G0	G5	G0	G5	G0	G5
1 AIs. C.R.	61.8 (295/ 477)	60.5 (376/ 622)	54.3 (208/ 383)	57.8 (119/ 206)	44 (92/ 209)	43.9 (137/ 312)	41.4 (129/ 312)	41 (128/ 312)	47.5 (429/ 904)	46.3 (384/ 830)	52.4 (724/ 1381)	52.3 (760/ 1452)
2 AIs. C.R.	50.8^{y} (100/ 197)	59.4^{x} (148/ 249)	47.9 (89/ 186)	40 (38/ 95)	44.1 (56/ 127)	32.6 (56/ 172)	42.5 (74/ 174)	36.3 (69/ 190)	45^{x} (219/ 487)	35.7^{y} (163/ 457)	46.7 (319/ 684)	44.1 (311/ 706)
3 AIs. C.R.	56.7 (59/ 104)	61.5 (67/ 109)	52.9^{x} (54/ 102)	42.4^{y} (28/ 66)	38.5 (30/ 78)	37 (40/ 108)	33.3 (29/ 87)	40.4 (46/ 114)	42.3 (113/ 267)	39.6 (114/ 288)	46.4 (172/ 371)	45.6 (181/ 397)
4+ AIs. C.R.	34.5^{y} (30/ 87)	58.7^{x} (37/ 62)	40.7 (33/ 81)	40 (34/ 85)	38.3^{x} (31/ 81)	28^{y} (35/ 125)	26.4^{y} (24/ 91)	35.6^{x} (31/ 87)	34.8 (88/ 253)	33.7 (100/ 297)	34.7 (118/ 340)	38.1 (137/ 360)
Cumulative AIs. C.R.	54.7 (473/ 865)	58.7 (612/ 1043)	49.6 (373/ 752)	45.8 (207/ 452)	40.2 (199/ 495)	36.5 (262/ 717)	37.1 (246/ 664)	37.6 (264/ 703)	42.8 (818/ 1911)	39.2 (733/ 1972)	46.5 (1291/ 2776)	46.1 (1345/ 2915)

^{x,y} Superscripts within the same row indicate significant differences at $p < 0.05$ ($x > y$); C.R. - conception rate.

4. Discussion

The timing of an increase in P4 following ovulation plays a crucial role in establishing and maintaining pregnancy **[12,24]**. When P4 is supplemented during early embryo development, it increases the expression of genes associated with the synthesis of triglycerides and transport of glucose, which are used as sources of energy for the embryo **[25]**. The P4 concentrations in the early stages of pregnancy or the preimplantation phase have been raised by inducing an aCL by administering gonadotropin-releasing hormone and human chorionic gonadotropin during the luteal phase after AI in dairy cows **[5]**. Cows on the fifth day of their estrous cycle usually have a dominant follicle that can ovulate in response to either LH **[26,27]** or GnRH/hCG administration **[5,23,28]**. According to Peters **[29]**, administration of GnRH between days 5–7 and/or days 11–14 after insemination is commonly practiced. In our study, we used the term 5 days after AI, as during this time, the dominant follicular waves are expected **[30–32]**.

Multiple studies conducted by López-Gatius et al. **[33]**, Villarroela et al. **[34]**, Larson et al. **[35]**, Bech-Sàbat et al. **[36]**, Mehni et al. **[37]**, and Pilz et al. **[38]** have demonstrated that dairy cows have a higher concentration of P4 and conception rate when the induction of aCL is performed. However, there is no large-scale study that has assessed the effect of this treatment on the interestrus interval. There is no apparent effect of GnRH treatment on the interestrus interval for the nulliparous cows. The majority of cows in both groups exhibited an 18-25 day cycle interval (P < 0.05). However, primiparous cows showed a higher proportion of 18-25 days cycle intervals compared to multiparous cows who had a dominant 26-35 days interestrus interval. When comparing both groups (G0 vs. G5), it is surprising to note that a larger percentage of the untreated cows have interestrus intervals of 18-25 days, while a higher proportion of the treated cows have 26-35 day intervals. This may suggests that the GnRH treatment given in day 5 after AI has the potential to prolong the cycle. We do not know whether the longer interestrus interval in the G5 group resulted from pregnancy and subsequent early embryonic mortality or from the increase in the luteal phase of the cycle. Apparently, the magnitude of the extende cycle increase when injections of GnRH agonist were given up to day 16 of the cycle **[39]**. In the study of Cunha et al. **[18]**, administration of hCG on day 7 after

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AI disrupted the natural luteolysis and ovulation, which are critical physiological events that regulate cycle lenght. According to our study, using GnRH analog on the day 5 after AI can extend the cycle lenght. This works by interrupting the normal luteolysis process, which breaks down the CL. It's important to note that approximately half of cows treated in this manner develop two CLs **[4]**. These Cls have different ages, with the second CL regressing later for non-pregnant cows, resulting in a prolonged luteal phase.

The extended luteal phase negatively impacted reproductive performance in Holstein cows, leading to lower pregnancy rates and a longer calving to conception interval **[40]**. Thus, we aimed to assess the impact of the extended luteal phase, generated by the treatment with GnRH in the day 5 after AI, on the estrus detection rate, conception rate and pregnancy rate. Surprisingly, the estrus detection rates were higher in both groups with only minor variations, particularly during the summer. This, confirm a high management standard of the herd which is clearly reflected in the estrus behavior detection of the dairy cows. According to the activity monitoring system, 95% of cows that were identified to be in estrus ovulated, whereas only 5% did not ovulate within seven days of the induction of luteolysis. This means that 95% of cows that the activity monitoring system identified as estrus ovulated **[41]**. The low rate of detecting estrus in dairy cows during the summer is a well-known problem **[42]**. In our study, the treatment with GnRH on day 5 after AI, or the extended luteal phase generated by this treatment, does not seem to have any effect on this issue.

During the period from half of May to October, which is the hottest period in Romania, both groups experienced lower pregnancy and conception rates. However, when we evaluated the pregnancy rates and conception rates based on the number of AIs, the G5 group showed an improvement in reproductive activity for nulliparous dairy cows at 2 and +4 AIs and for the repeat breeder dairy cows at +3 lactations. This is consistent with our previous study **[1]**, which showed that administering this therapy 7-14 days after AI improved reproductive activity in repeat breeder cows. This finding indicates that administering gonadorelin during earlier and latter in the luteal phase may increase the survival of embryos in repeat breeders heifers and dairy cows. Also, this study clarify the aspect regarding the fesability of using this therapy which, in accordance with our two studies it is not suitable for the herd. In some cases, this therapy has a negative effect. Additionally, we have to consider that the treatment cost for the G5 group was approximately $6,462 per herd per year. However, we recommend implementing this therapy 7-14 days after AI in repeat breeder dairy cows. It is conclusive, can be done once a week, and has a significant economic impact on the dairy farm **[1]**.

The results of the current study can be explained by the presence of repeat breeder dairy cows with low genetic merit for fertility. These cows were more likely to exhibit consistent and substantial differences in circulating concentrations of P4 during the luteal phase, and also showed altered ovarian and hormonal phenotypes that are associated with poor reproductive performance. The most noticeable differences were the inconsistent and modest variations in follicular wave dynamics among cows with varying fertility potential **[43]**. Therefore, cows that have inferior genomic merit for fertility are the ones that benefit from this therapy, as it addresses their fertility issues.

According to a study by Sitko et al. **[43]**, the size of the dominant follicle in fertile cows was 7% larger than in infertile cows, suggesting a correlation between differences in CL volume and follicle size. Additionally, fertile cows had a 15-25% higher concentration of circulating P4 than infertile ones **[43]**. High concentrations of P4 during the late luteal phase of the ovulatory wave have been associated with better oocyte quality **[44]** and pregnancy rates **[45,46]**. As a result, the infertility of repeat breeder cows may be related to low P4 concentrations during early stages of embryo development or poor oocyte quality during the late luteal phase **[43]**.

5. Conclusions

In summary, infertile cows can be treated with GnRH during the luteal phase following AI. We recommend administering this therapy 7-14 days after AI in repeat breeder dairy cows, as it can have a significant economic impact on the dairy farm. This is because an aCL can compensate for low

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progesterone levels, thereby reducing the risk of embryo loss and pre-term labor. Therefore, implementing this strategy on the farm is feasible only for cows with low genetic merit for fertility.

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