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Induction of Accessory *Corpus luteum* by Gonadorelin in Relation to the Time of Treatment and the Follicle Size in Inseminated Cows

Radovan Doležel[1]*, *Cindy Chambert*[1], *Darja Musilová*[1], *Svatopluk Čech*[1], *Tomáš Páleník*[1,2]

[1]*University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic*

[2]*CEVA Animal Health Slovakia s.r.o., Bratislava, Slovak Republic*

**Corresponding author: dolezelr@vfu.cz*

ABSTRACT

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This study evaluated the occurrence of two *corpora lutea* (CL) and the pregnancy rate in cows treated with gonadorelin on days 5, 6 or 7 after insemination in relation to the day of treatment and the size of the largest follicle on the ovaries at the time of treatment. Cows bearing one CL on days 5 (n = 40), 6 (n = 48), and 7 (n = 47) after insemination with defined size of the largest follicle on the ovaries (small ≤ 9 mm, n = 34; medium 10–14 mm, n = 59; large 15–20 mm, n = 42) were included in the study. Gonadorelin was administered after examination (day 0) and ultrasonographic examination was repeated on days 14, 28, and around day 90. The occurrence of 2 CL vs 1 CL was higher in pregnant cows on day 14 as well as on day 28 (86 vs 14% and 82.5 vs 14%, n = 57). Pregnancy rates on day 28 and around day 90 were higher in cows bearing 2 CL (n = 57) vs cows bearing 1 CL (n = 54) (82.5 vs 18.5% and 79.0 vs 18.5%, $P < 0.001$). The occurrence of 2 CL on day 28 was higher in cows treated on day 5 compared to cows treated on day 6 or 7 after insemination (60.0 vs 33.3 or 36.2%, $P < 0.05$). No significant differences in the number of CL and pregnancy rate were found in relation to follicle size at the time of treatment. Our results showed the higher efficiency of accessory CL induction by gonadorelin administration in cows treated on day 5 vs those treated on day 6 or 7 post insemination, however efficiency of the treatment was not influenced by the size of the largest follicle on the ovaries.

Keywords: gonadotropin releasing hormone; *corpora lutea*; pregnancy rate; day of treatment; follicle

Embryo mortality caused by progesterone deficiency is considered to be an important cause of low reproductive performance in dairy cows. Increased metabolism in high yielding cows is accompanied by a higher rate of progesterone degradation and lower progesterone concentration in peripheral blood (Wiltbank et al. 2006; Robinson et al. 2008). Induction of accessory *corpus luteum* (CL) by gonadotropin releasing hormone (GnRH) represents one applicable method of increasing progesterone concentration during early pregnancy (Peters et al. 2000; Lopez-Gatius et al. 2006; Garcia-Ispierto and Lopez-Gatius 2012). Data about the efficiency of the treatment as well as the effect of accessory CL on reproductive performance are not however uniform. In our previous study we confirmed an increase of progesterone concentration in the presence of accessory CL. In addition, we found an unusually high pregnancy rate in cows bearing 2 CL, but conversely an extremely low pregnancy

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rate in cows bearing only 1 CL after the treatment (Musilova et al. 2014). For this reason we focused our attention on factors which could influence the efficiency of the accessory CL induction such as the time of treatment and the stage of follicular development at the treatment. Consequently, the occurrence of 2 CL and the pregnancy rate after gonadorelin administration in cows on days 5, 6 or 7 after insemination in relation to the day of treatment and the size of the largest follicle on the ovaries at the time of treatment were evaluated in this study.

MATERIAL AND METHODS

Experimental animals. The trial was performed in four commercial dairy herds (250–600 cows; Holstein – farms A, B, or crossbreeds of Czech motley and Red Holstein – farms C, D; 7500–9500 l per lactation period) from May 2014 to June 2015. Cows were artificially inseminated (AI) in natural (farms C, D) or synchronized (farms A, B) estrus after a 45-day voluntary waiting period. Natural estrus was detected by visual observation with the aid of pedometers. Estrus synchronization protocol represented a shortened presynch-ovsynch (confirmation of CL + cloprostenol ($PGF_{2\alpha}$) – 10 days – lecirelin (GnRH) – 7 days – $PGF_{2\alpha}$ – 56 h – GnRH – 16–18 h – AI). Simple protocol after confirmation of CL ($PGF_{2\alpha}$ – 2 days – GnRH – 24 h – AI) was used rarely in re-breeders (≥ 4 inseminations).

Examination and treatment. Cows were examined, selected, and treated during regular fortnightly intervals within the framework of a reproductive health programme. Experimental cows were randomly selected on days 5, 6 or 7 after insemination. Reproductive organs in these cows were ultrasonographically examined by a real-time B-mode scanner equipped with 5–8 MHz linear-array rectal transducer Easi-Scan (BCF Technology Ltd, UK) and cows bearing 1 CL on the ovaries (n = 135) were included in the experiment (40 cows on day 5, 48 cows on day 6, and 47 cows on day 7 after insemination). The follicular population on the ovaries was assessed simultaneously and the cows were divided into 3 groups according to the diameter of the largest follicle (F) on the ovaries (S – small F ≤ 9 mm, n = 34; M – medium F 10–14 mm, n = 59; and L – large F 15–20 mm, n = 42). Using this method 9 groups were specified according to the day after insemination as well as the diameter of the largest follicle on the ovaries: groups 5/S (n = 14), 5/M (n = 20), 5/L (n = 6), 6/S (n = 13), 6/M (n = 19), 6/L (n = 16), 7/S (n = 7), 7/M (n = 20), and 7/L (n = 20). The numbers of cows in the groups varied because it was not easy to find all defined follicles in all defined terms, it was especially difficult to find large follicles on day 5 as well as only small follicles on day 7. All experimental cows were treated with gonadorelin (GnRH) (Ovarelin, Ceva Animal Health Ltd., UK; 0.10 mg intramuscularly) immediately after the 1st examination (day 0) with the aim of inducing accessory CL. Next, two ultrasonographical examinations were performed at 14-day intervals. The 2nd and the 3rd examinations with the target of determining the number of CL and the pregnancy diagnosis were performed on days 14 and 28. The last (4th) examination with the target of confirming pregnancy was performed ultrasonographically or by rectal palpation around day 90.

Evaluation. The efficiency of accessory CL induction was evaluated based on the occurrence of 1 or 2 CL on the ovaries and pregnancy rate after the treatment. The values were expressed in percentages.

A chi-squared test (χ^2-test) (or exact test as modification for low numbers) was used for statistical evaluation. The level for significant differences was determined as $P < 0.05$. Risk ratio (RR) and coefficient of association (CA) were determined in the case of a significant difference.

RESULTS

The occurrence of 2 CL as well as the pregnancy rate in all experimental cows was 42.2% (57/135) on day 28 after treatment (day of treatment – day 0). The occurrence of 2 CL was markedly higher compared to the occurrence of 1 CL in pregnant cows on days 14 as well as 28 (Table 1).

Pregnancy rates on day 28 and around day 90 were significantly higher in cows bearing 2 CL compared to cows bearing only 1 CL. Negligible pregnancy loss from day 28 to day 90 in cows bearing 2 CL (2 cows) was not significantly different in comparison with cows bearing 1 CL (no cow) (Table 2).

The occurrence of 2 CL on day 28 was significantly higher in cows treated on day 5 compared to cows treated on days 6 or 7 after insemination.

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Table 1. Occurrence of 1 and 2 *corpora lutea* (CL) in all experimental cows (gonadorelin administration on days 5–7 after insemination) and only in pregnant experimental cows on days 14 and 28 after treatment (in %)

	All experimental cows (n = 135)		Pregnant experimental cows (n = 57)	
	day 14	day 28	day 14	day 28
1 CL	29.6	40.0	14.0	14.0
2 CL	65.2	42.2	86.0	82.5

The pregnancy rate was not, however, influenced by the term of treatment (Table 3).

We did not find any significant differences in the number of CL or pregnancy rate in relation to the diameter of the largest follicle on the ovaries at the time of treatment (Table 4).

Similarly we did not find any significant differences in the number of CL or pregnancy rate in relation to the term of treatment and concurrently in relation to the follicle size at the time of treatment (groups 5S, 5M, 5L, 6S, 6M, 6L, 7S, 7M, and 7L).

DISCUSSION

We inducted accessory CL by gonadorelin on days 5–7 after insemination because the final phase of the 1st postovulatory follicular wave with a dominant follicle sensitive to luteinizing hormone (LH) on bovine ovaries can be presupposed at this time. Two or three follicular waves usually pass during estrous cycle in cows and the first one terminates about 7 days after ovulation (Price and Carriere 2004; Jaiswal et al. 2009). Therefore administration of GnRH or hCG with the aim of inducing accessory CL on days 5–7 (by the end of the 1st follicular wave) or on days 11–14 (by the end of the 2nd follicular wave) after insemination was performed most frequently (Peters 2005). Diskin et al. (2002) stated that the earliest term for induction of accessory CL by GnRH was day 5 because follicles on the ovaries were not sufficiently responsive to this treatment before this term. For this reason it was difficult to find large follicles on day 5 (6/135) or small follicles on day 7 (7/135) in our trial. Sartori et al. (2001) stated that follicles in cows reach the potential to ovulate after GnRH administration from a diameter of about 10 mm but higher doses of GnRH and a higher LH peak are necessary for the induction of ovulation in this follicle compared to larger follicles.

Geary et al. (2000) found ovulation in 66% cows treated with GnRH during estrous cycle. We found a similar occurrence of 2 CL on day 14 (65.2%) but a lower one on day 28 (42.2%) after gonadorelin administration. A higher occurrence of 2 CL on day 14 followed by a higher rate of CL regression between days 14 and 28 was found in cows which were diagnosed as non-pregnant on day 28 after treatment. The occurrence of 2 CL in

Table 2. Pregnancy rates on day 28 and around day 90 after insemination in experimental cows (gonadorelin administration on days 5–7 after insemination) bearing 1 or 2 *corpora lutea* (CL) after treatment (in %)

	Day 28	Day 90
1 CL (n = 54)	18.5^{a}	18.5^{b}
2 CL (n = 57)	82.5^{A}	79.0^{B}

$^{a:A}P < 0.001$, risk ratio (RR) = 4.64, coefficient of association (CA) = 0.64; $^{b:B}P < 0.001$, RR = 4.32, CA = 0.60

Table 3. Occurrence of 2 *corpora lutea* (CL) in cows on days 14 and 28 and pregnancy rate on day 28 after treatment (gonadorelin administration on days 5–7 after insemination) in relation to the term of treatment (days 5, 6 or 7 after insemination) (in %)

	Day 5 (n = 40)	Day 6 (n = 48)	Day 7 (n = 47)
2 CL (day 14)	77.5	60.4	59.6
2 CL (day 28)	60.0^{a}	33.3^{b}	36.2^{c}
Pregnancy (day 28)	45.0	41.7	40.4

$^{a:b}P < 0.05_{cor}$ (with correction), risk ratio (RR) = 1.8, coefficient of association (CA) = 0.27; $^{a:c}P < 0.05_{cor}$, RR = 1.7, CA = 0.24

Table 4. Occurrence of 2 *corpora lutea* (CL) in cows on days 14 and 28 and pregnancy rate on day 28 after treatment (gonadorelin administration on days 5–7 after insemination) in relation to the diameter of the largest follicle on the ovaries (small ≤ 9 mm, medium 10–14 mm, large 15–20 mm) (in %)

	Small (n = 34)	Medium (n = 59)	Large (n = 42)
2 CL (day 14)	70.6	59.3	69.1
2 CL (day 28)	44.1	40.7	42.9
Pregnancy (day 28)	47.1	42.4	38.1

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pregnant cows was, however, similar in both terms after treatment (86.0 vs 82.5%). Various terms of GnRH treatment after insemination have been tested for induction of accessory CL. MacMillan et al. (1986) administered GnRH on days 1–3, 4–6, 7–10, and 11–13 after insemination and they found the highest efficiency of the treatment on days 11–13. The occurrence of 2 CL was 15% in cows treated on days 0 and 12 (Lopez-Gatius et al. 2006) or 17.7% in cows treated on days 28–34 (Bech-Sabat et al. 2009) after insemination. Occurrences of 2 CL in cows treated on day 7 or on days 5–7 vs cows treated on day 13 or on days 11–13 after insemination were 54.2 or 43.9% vs 24.7 or 33.5% in our previous studies (Dolezel et al. 2014; Musilova et al. 2014). In this study we found a similar occurrence of 2 CL (42.2%) in the same period after treatment (day 28). Similarly Garcia-Ispierto and Lopez-Gatius (2012) found 2 CL in 50% of observed cows treated with GnRH on day 5 after insemination. We assigned experimental cows according to the particular day of the treatment and we found a higher occurrence of 2 CL in cows treated on day 5 in comparison with days 6 and 7 after insemination on days 14 and 28 after treatment. Significant differences were proved only on day 28 after treatment (60% vs 33.3 and 36.2%). We can speculate that the dominant follicle gradually loses functional dominance and it starts to be the only morphologically dominant follicle after day 5 post ovulation with decreased sensitivity to LH.

In spite of the fact that location, size or maintenance of accessory CL were not evaluated in this study we can state, based on our incomplete evidence, that the locations of 2 CL after treatment were ipsilateral as well as contralateral, CL were symmetric as well as asymmetric and both CL maintained on the ovaries until day 90 after insemination.

A follicle larger than 10 mm in diameter is usually considered to be a competent follicle which can respond to GnRH treatment by ovulation (Saint-Diziere et al. 2014). We considered a small follicle (≤ 9 mm in diameter) to be a follicle in the growth or selection phase of the follicular wave while medium (10–14 mm) and large (15–20 mm) follicles were considered to be follicles in the early and late (preovulatory) dominant phases of the follicular wave. The occurrence of 2 CL after GnRH treatment was significantly higher in inseminated cows bearing follicles 10–14 mm or 15–20 mm in diameter compared to cows bearing follicles ≤ 9 mm in diameter in our previous study (48.2 or 55.6% vs 6.7%; Musilova et al. 2014). We did not confirm the same relation in this trial. Strikingly there were not any significant differences in the occurrence of 2 CL in relation to the size of the largest follicle on the ovaries. The different efficiency of CL induction in relation to follicle size in this study compared to the previous study by our team can be partly caused by the different type of GnRH used in these experiments. Lecirelin was used in the previous study while gonadorelin was used in this study. Picard-Hagen et al. (2015) described a lower LH response after administration of gonadorelin compared to lecirelin or buserelin but ovulation responses were similar. An FSH response which could be important for smaller follicles was, however, not observed. A high incidence of ovulation after gonadorelin administration was also described by other authors (Martinez et al. 2003; Souza et al. 2009).

Data assessing the effect of induction of accessory CL by GnRH on pregnancy rates are not uniform. Some authors confirmed an increase of the conception rate by about 7–12% (Lopez-Gatius et al. 2006; Bech-Sabat et al. 2009; Mehni et al. 2012), but mainly in stressed cows in extremely unfavourable conditions. Other authors did not demonstrate any effect of the treatment (Bartolome et al. 2005; Szenci et al. 2006; Ergene 2012). In our experiment we found an extremely high conception rate (82.5%) in cows bearing 2 CL and on the other hand an extremely low conception rate (18.5%) in cows bearing only 1 CL on day 28 after induction of accessory CL by GnRH. Similar results were found in our previous studies. Pregnancy rates in cows bearing 2 CL vs cows bearing only 1 CL after treatment on day 7 (Dolezel et al. 2013) or on days 5–7 and 11–13 (Musilova et al. 2014) after insemination were 79.4 vs 12.5% or 100% and 86.7 vs 16.7% and 38.9%, but total pregnancy rates in treated as well as untreated (control) cows were similar. We did not establish control groups in this study. Nevertheless, the pregnancy rate in all experimental cows compared to the overall pregnancy rate in the farms during the same period was also similar (42.2 vs 41.8%). The effect of induced accessory CL by GnRH on maintenance of the pregnancy is not sufficiently documented as well. Usual rates of early embryo

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mortality, late embryo/early fetal mortality, and late abortion in cows are 20–45%, 8–17.7%, and 1–4%, respectively (Humblot 2001). Insignificant lower late embryo/early fetal mortalities in the presence of accessory CL have been described by Bartolome et al. (2006), Bech-Sabat et al. (2009), and Musilova et al. (2014). We did not find significant differences in pregnancy loss between treated cows bearing 1 or 2 CL in this trial either.

CONCLUSION

The results showed the higher efficiency of accessory CL induction by administration of gonadorelin in cows treated on day 5 compared to cows treated on days 6 or 7 after insemination, however efficiency of the treatment was not influenced by the size of the largest follicle on the ovaries. Pregnancy rate was markedly higher in cows bearing 2 CL compared to cows bearing 1 CL after the treatment.

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REFERENCES

Bartolome J.A., Sozzi A., McHale J., Swift K., Kelbert D., Archbald L.F., Thatcher W.W. (2005): Resynchronization of ovulation and timed insemination in lactating dairy cows. III. Administration of GnRH 23 days post AI and ultrasonography for non pregnancy diagnosis on day 30. Theriogenology, 63, 1643–1658.

Bartolome J.A., Kamimura S., Silvestre F., Arteche A.C.M., Trigg T., Thatcher W.W. (2006): The use of desloreline implant (GnRH agonist) during the late embryonic period to reduce pregnancy loss. Theriogenology, 65, 1443–1453.

Bech-Sabat G., Lopez-Gatius F., Garcia-Ispierto I., Santolaria J.P., Serrano B., Nogareda C., de Sousa N.M., Beckers J.F., Yaniz J. (2009): Pregnancy patterns during the early fetal period in high producing dairy cows treated with GnRH or progesterone. Theriogenology, 71, 920–929.

Diskin M.G., Austin E.J., Roche J.F. (2002): Exogenous hormonal manipulation of ovarian activity in cattle. Domestic Animal Endocrinology, 23, 211–228.

Dolezel R., Bartonek J., Musilova D., Cech S., Palenik T. (2013): Effect of GnRH administration on day 7 after insemination on ovarian activity and pregnancy in dairy cows. Reproduction in Domestic Animals, 48 (Suppl. 1), 109.

Dolezel R., Musilova D., Bartonek J., Cech S., Palenik T. (2014): Factors influencing induction of accessory corpus luteum by GnRH in inseminated cows. Reproduction in Domestic Animals, 49 (Suppl. 3), 64.

Ergene O. (2012): Progesterone concentrations and pregnancy rates of repeat breeder cows following postinsemination PRID and GnRH treatments. Turkish Journal of Veterinary and Animal Sciences, 36, 283–288.

Garcia-Ispierto I., Lopez-Gatius F. (2012): Effects of GnRH or progesterone treatment on day 5 post-AI on plasma progesterone, luteal blood flow and leucocyte counts during the luteal phase in dairy cows. Reproduction in Domestic Animals, 47, 224–229.

Geary T.W., Downing E.R., Bruemmer J.E., Whittier J.C. (2000): Ovarian and estrous response of suckled beef cows to the Select Synch estrous synchronization protocol. Professional Animal Scientist, 16, 1–5.

Humblot P. (2001): Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. Theriogenology, 56, 1417–1433.

Jaiswal R.S., Singh J., Marshall L., Adams G.P. (2009): Repeatability of 2-wave and 3-wave patterns of ovarian follicular development during the bovine estrous cycle. Theriogenology, 72, 81–90.

Lopez-Gatius F., Santolaria P., Martino A., Deletang F., De Rensis F. (2006): The effects of GnRH treatment at the time of AI and 12 days later on reproductive performance of high producing dairy cows during the warm season in northeastern Spain. Theriogenology, 65, 820–830.

MacMillan K.L., Taufa V.K., Day A.M. (1986): Effects of an agonist of GnRH (Buserelin) in cattle. III. Pregnancy rates after a post-insemination injection during metoestrus or dioestrus. Animal Reproduction Science, 11, 1–10.

Martinez M.F., Mapletoft R.J., Kastelic J.P., Carruthers T. (2003): The effects of 3 gonadorelin products on luteinizing hormone release, ovulation, and follicular wave emergence in cattle. Canadian Veterinary Journal, 44, 125–131.

Mehni S.B., Shabankareh H.K., Kazemi-Bonchenari M., Eghbali M. (2012): The comparison of treating Holstein dairy cows with progesterone, CIDR and GnRH after insemination on serum progesterone and pregnancy rates. Reproduction in Domestic Animals, 47, 131–134.

Musilova D., Bartonek J., Cech S., Palenik T., Dolezel R. (2014): Induction of accessory corpus luteum in cows

doi: 10.17221/46/2016-CJAS

by gonadotropin-releasing hormone administrated after insemination. Acta Veterinaria Brno, 83, 107–111.

Peters A.R. (2005): Veterinary clinical application of GnRH – questions of efficacy. Animal Reproduction Science, 88, 155–167.

Peters A.R., Martinez T.A., Cook A.J.C. (2000): A meta-analysis of studies of the effect of GnRH 11–14 days after insemination on pregnancy rates in dairy cattle. Theriogenology, 56, 637–647.

Picard-Hagen N., Lhermie G., Florentin S., Merle D., Frein P., Gayrard V. (2015): Effect of gonadorelin, lecirelin, and buserelin on LH surge, ovulation, and progesterone in cattle. Theriogenology, 84, 177–183.

Price C.A., Carriere P.D. (2004): Alternate two- and three-follicle wave interovulatory intervals in Holstein heifers monitored for two consecutive estrous cycles. Canadian Journal of Animal Science, 84, 145–147.

Robinson R.S., Hammond A.J., Wathes D.C., Hunter M.G., Mann G.E. (2008): Corpus luteum–endometrium–embryo interactions in the dairy cow: underlying mechanisms and clinical relevance. Reproduction in Domestic Animals, 43, 104–112.

Saint-Dizier M., Legendre A.C., Driancourt M.A., Chastant-Maillard S. (2014): The induction of a secondary corpus luteum on day 12 post-ovulation can delay the time of luteolysis in high-producing Holstein cows. Reproduction in Domestic Animals, 49, 920–925.

Sartori R., Fricke P.M., Ferreira J.C.P., Ginther O.J., Wiltbank M.C. (2001): Follicular deviation and acquisition of ovulatory capacity in bovine follicles. Biology of Reproduction, 65, 1403–1409.

Souza A.H., Cunha A.P., Silva E.P.B., Gumen A., Ayres H., Guenther J.N., Wiltbank M.C. (2009): Comparison of gonadorelin products in lactating dairy cows: efficacy based on induction of ovulation of an accessory follicle and circulating luteinizing hormone profiles. Theriogenology, 72, 271–279.

Szenczi O., Takacs E., Sulon J., de Sousa N., Bechers J.F. (2006): Evaluation of GnRH treatment 12 days after AI in the reproductive performance of dairy cows. Theriogenology, 66, 1811–1815.

Wiltbank M., Lopez H., Sartori R., Sangsritavong S., Gumen A. (2006): Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology, 65, 17–29.

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