

—Technology Report—

Effects of twin pregnancy prevention strategies such as GnRH dose and drainage of the smaller follicle on ovulation in dairy cows with two follicles of pre-ovulatory size in the same ovary

Irina GARCIA-ISPIERTO^{1, 2)} and Fernando LÓPEZ-GATIUS^{2, 3)}

¹⁾Department of Animal Science, University of Lleida, 25198 Lleida, Spain

²⁾Agrotecnio Centre; University of Lleida, 25198 Lleida, Spain

³⁾Transfer in Bovine Reproduction SLu, 22300 Barbastro, Spain

Abstract. We examined the effects of a single or 2.5-fold dose of dephereline [a gonadotropin-releasing hormone (GnRH) analogue] as well as the drainage of the smaller follicle at the time of insemination on ovulation in dairy cows with two follicles of pre-ovulatory size in the same ovary. The three study groups included 220 monovular cows (control), 110 non-drained cows with two follicles, and 110 cows with two follicles, of which one was drained. In each group, cows treated with a single dose or 2.5-fold dose of dephereline showed similar results following treatment. Ovulation failure of the non-drained follicle occurred in 29.1% of the drained cows, whereas ovulation occurred in 96% of the non-drained and control cows. Twin pregnancy was recorded in 19.4% of the pregnant non-drained cows with two follicles. In conclusion, the increased dephereline dose did not improve the ovulation rate in any group. Follicular drainage, however, prevented twin pregnancy in cows with two follicles, but also resulted in an increase in the non-drained follicle's rate of ovulation failure.

Key words: Double ovulation, Follicular co-dominance, Heat stress

(J. Reprod. Dev. 66: 485–488, 2020)

Twin births are not desirable in dairy cattle. Several ways have been proposed to prevent twin pregnancy, including transfer of a single embryo or drainage of the subordinate follicles [1, 2]. Puncture and drainage of the subordinate follicles while avoiding suction at the time of insemination has proved to be effective without resulting in a reduction in fertility [3–5]. This approach also has an additional benefit; it increases the number of corpora lutea derived from the drained follicles, thus, improving embryo survival in drained cows with two follicles of pre-ovulatory size (co-dominant follicles) [5]. However, it has been observed that puncture and drainage of the smaller follicle may induce failure of the larger non-drained follicle to ovulate. According to pooled data from our studies in cows with two co-dominant follicles (one in each ovary) [3–5], at least one follicle ovulated in 182 (94.3%) of the 193 non-drained cows, whereas there was an ovulation failure of the non-drained follicle in 39 (20.5%) of the 190 drained cows. No pregnancies were recorded in these 39 cows. This high incidence of ovulation failure in drained cows was offset by a slightly higher (non-significant) pregnancy rate in drained cows with an ovulating follicle compared to non-drained cows in which at least one follicle ovulated. In effect, pregnancy was recorded in 35.4% (64/181) of the non-drained ovulating cows, whereas 42.4% (64/151) of the drained ovulating cows

became pregnant. Further, in the non-drained cows, twin pregnancy was observed in 22 of the 64 pregnant cows (34.4%), whereas no twins were recorded in the drained cows [3–5]. Thus, in addition to preventing twin pregnancies, the technique of follicular drainage could improve fertility if the ovulation failure rate of the non-drained follicles could be lowered. It has been shown in a meta-analysis of 40 trials on 19,019 cows described in 27 published articles that the use of a 2.5x dose of gonadotropin-releasing hormone (GnRH) at the time of artificial insemination (AI) significantly increases the pregnancy risk [6]. Based on this, the objective of the present study was to examine the effects of a 2.5x dose of GnRH, given at the time of follicular drainage of the smaller follicle, on ovulation of the larger non-drained follicle in dairy cows with two co-dominant follicles. As this technique was developed for cows with a follicle of pre-ovulatory size in each ovary [3–5], this study was performed on cows with two co-dominant follicles in the same ovary.

Table 1 shows the effects of dephereline (GnRH analogue) dose and follicular drainage, at the time of AI, on ovulation failure, pregnancy, and twin pregnancy rates. In all the drained ovaries, the draining-induced luteal structure was easily discernible from either a normal corpus luteum (CL) (Fig. 1a) or a non-ovulated follicle (Fig. 1b). In all the animal groups, the 1x dephereline- or 2.5x dephereline-treated cows showed similar results following treatment. Ovulation failure of the non-drained follicle occurred in 32 of the 110 cows that underwent drainage (29.1%), at least one follicle ovulated in 106 (96.4%) of the 110 non-drained cows, and ovulation was recorded in 209 (96.3%) of the 220 control cows. No pregnancies were produced in cows experiencing ovulation failure, which included 32 drained cows, four non-drained cows in which both the follicles failed to ovulate, and 11 control cows. In

Received: March 1, 2020

Accepted: May 15, 2020

Advanced Epub: May 30, 2020

©2020 by the Society for Reproduction and Development

Correspondence: F López-Gatius (e-mail lopezgatiusf@gmail.com)

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: <https://creativecommons.org/licenses/by-nc-nd/4.0/>)

Table 1. Effects of dephereline (GnRH analogue) dose and follicular drainage at the time of artificial insemination (AI) on ovulation failure, pregnancy, and twin pregnancy rates

Number of follicles	Drainage *	Dephereline dose	Ovulation failure **	Pregnancy	Twins ***
One	No	1 dose (n = 110)	6 (5.5%) ^a	26 (23.6%)	0
		2.5x dose (n = 110)	5 (4.5%) ^a	27 (24.5%)	0
Two	No	1 dose (n = 55)	2 (3.6%) ^a	16 (29.0%)	3 (18.8%)
		2.5x dose (n = 55)	2 (3.6%) ^a	15 (27.3%)	3 (20.0%)
	Yes	1 dose (n = 55)	15 (27.3%) ^b	12 (21.8%)	0
		2.5x dose (n = 55)	17 (30.9%) ^b	13 (23.6%)	0

n = 440: 220 with one pre-ovulatory follicle and 220 cows with two follicles of pre-ovulatory size in the same ovary. * Drainage of the smaller follicle in cows with two ovulatory follicles of pre-ovulatory size in the same ovary. ** In cows with two follicles at the time of AI: for all follicles in the non-drainage group and for the non-drained follicle in the drainage group. *** In pregnant cows. Values with different superscripts differ significantly within columns when tested using the Tukey-Kramer test ($P < 0.05$).

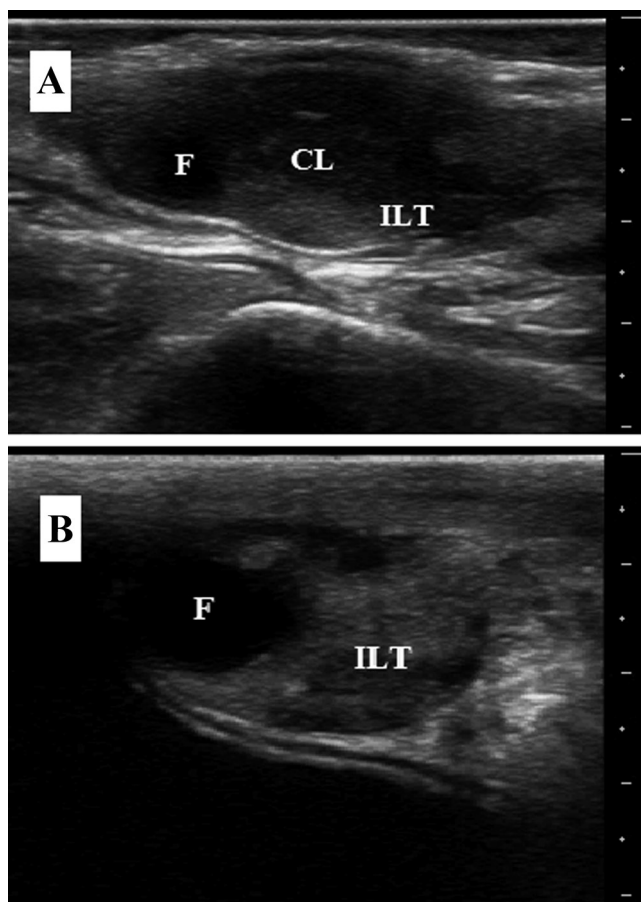


Fig. 1. Sonograms showing the largest area of the induced luteal tissue 7 days after follicular puncture and drainage alongside (A) the corpus luteum derived from the dominant follicle or (B) a pre-ovulatory size follicle. F, follicle; CL, corpus luteum; ILT, induced luteal tissue. Bar spacing: 10 mm.

the non-drained cows, double ovulation was observed in 65 cows (59.1%), while twin pregnancies were recorded in six (19.4%) of the 31 pregnant cows. No twins were recorded in the drained cows.

Mean milk production, days in milk at the time of AI, and number of lactations were 40.8 ± 8.9 kg, 128.5 ± 69.3 days, and 2.9 ± 1.7 lactations, respectively (mean \pm SD). No significant differences were detected in these values among the control, non-drainage, and drainage groups.

To our knowledge, the impact of an increased dose of the GnRH analogue dephereline, given in a fixed-time AI protocol, on the ovulation rate has not been examined previously. Dephereline treatment has a stronger and longer period of action than natural GnRH [7] and increases the ovulation rate under heat stress conditions [8]. However, the increased dephereline dose assessed here did not improve the ovulation rate in any group. Although the pregnancy rate was similar across the study groups, follicular puncture was related to a high incidence of ovulation failure of the non-drained follicle (29.1%), similar to what we have observed earlier in cows with a follicle of pre-ovulatory size in each ovary [3–5]. Presumably, local tissue injury might lead to a disturbance in the ovarian homeostasis and interfere with the physiological cascade, resulting in ovulation failure in these cows. Therefore, it seems that the drainage procedure either impairs ovulation or improves fertility in cows in which the non-drained follicle ovulates. More knowledge about the origin of follicles of pre-ovulatory size could shed some light on this issue. If co-dominant follicles arise from a follicular wave ipsilateral to the CL, this could produce a lower ovulatory response or higher fertility when the follicular wave occurs contralateral to the CL. The spatial relationship between the first-wave of the dominant follicle and the CL affects the conception rate in both lactating dairy cows and dairy heifers [9]. Moreover, treatment with human chorionic gonadotropin (hCG) at the time of insemination, which has a more direct action on the ovary than GnRH [10], could improve the chances of ovulation of the non-drained follicle. Further comprehensive studies are needed to confirm these assertions.

In the present study, the use of a simple transvaginal device proved valuable for follicular puncture and drainage of the smaller follicle at the time of synchronized AI in cows with two follicles of pre-ovulatory size in the same ovary. The risk of twin pregnancy was eliminated, while the results on ovulation failure and pregnancy were comparable with those obtained in cows with a follicle of pre-ovulatory size in each ovary [5].

As an overall conclusion, although the pregnancy rate was not

significantly different between the groups, follicular drainage prevented twin pregnancy in heat-stressed dairy cows with two large follicles in the same ovary. However, it also resulted in an increase in the rate of ovulation failure of the non-drained follicle.

Methods

Experimental animals

This study was performed over the period of May to September 2019 in a commercial dairy herd of Holstein-Friesian lactating dairy cows reared in north-eastern Spain (41.13 latitude, -2.4 longitude). Cows were included if they were healthy with a body condition score of 2.5–3.5 on a scale of 1 to 5 [11], produced more than 30 litres of milk per day and were free of detectable reproductive disorders and clinical diseases during the study period (days -5 to +28 from insemination). Cows were selected from groups synchronized for fixed-time insemination [12] treated with a GnRH agonist [dephe-reline: 100 µg gonadorelin acetate (6-D-Phe) i.m.; Gonavet Veyx, Ecuphar, Barcelona, Spain] along with the insertion of a controlled intravaginal progesterone-releasing device (CIDR, containing 1.38 g of progesterone; Zoetis Spain SL, Alcobendas, Madrid, Spain). The CIDR was left in place for 5 days; the animals were given cloprostenol (500 µg i.m.; PGF Veyx Forte, Ecuphar) on CIDR removal. Then, 24 h later, the cows received a second cloprostenol

dose and were inseminated 50–56 h after CIDR removal. Both ultrasonography (a portable B-mode ultrasound scanner equipped with a linear 5–10 MHz transvaginal transducer) and manual rectal palpation were used to confirm if a cow was in estrus and ready for insemination [13]. Only cows with either a pre-ovulatory follicle or two unilateral follicles (two follicles in the same ovary) over 12 mm in diameter in the absence of a CL were included in the study. Further prerequisites for the animals to be included in the study were that they have a uterus that was highly tonic and contractile to touch and a vaginal discharge of copious clear fluid. As double ovulation has been shown to be related to the least possible size difference between the larger and smaller follicle, irrespective of the individual diameter of each follicle [14], only cows with a size difference of less than 2 mm between the two co-dominant follicles were included in the study.

Follicular drainage and experimental design

Cows with two co-dominant follicles were randomly assigned to the non-follicular drainage (n = 110) or follicular drainage (n = 110) groups. On the day of AI, monovular cows were assigned to a control group (n = 220) (Fig. 2). In the drainage group, the smaller follicle was punctured and drained with the help of a steel transvaginal cannula designed for follicular cyst puncture [5]. Immediately before follicular puncture, the cannula, vulva, and the perineal region of

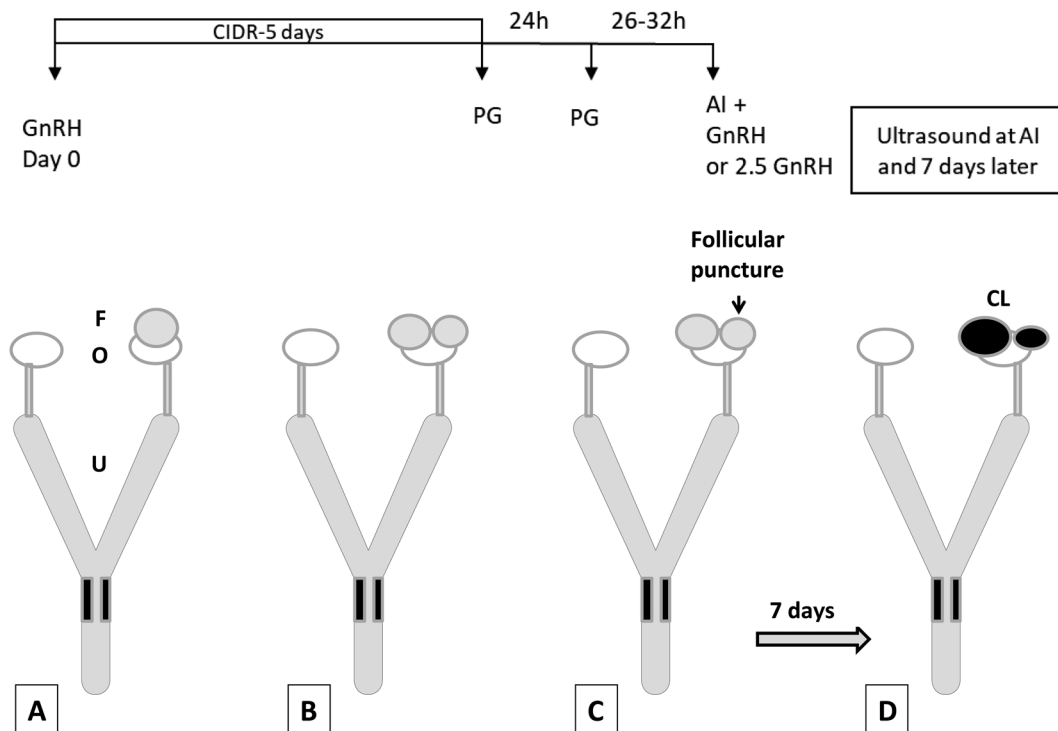


Fig. 2. Experimental design describing cows with two follicles of pre-ovulatory size in the same ovary undergoing timed artificial insemination (AI) (the protocol for timed AI has been described above). The study groups included: 220 monovular cows (A: control); 110 non-drained cows with two follicles (B: non-follicular drainage); and 110 cows with two follicles of which one was drained (C: follicular drainage). Puncture and drainage of the smaller follicle was performed at the time of AI in the drainage group (C). Ovulation was assessed on day 7 post-AI (D). A GnRH dose was administered at this time point to the group of drained cows (C). Pregnancy diagnosis was performed on day 28 post-AI. F, follicles; CL, corpora lutea; O, ovaries; U, uterus.

the cow were washed with a disinfectant solution. Ovaries bearing co-dominant follicles were positioned adjacent to the vaginal wall by rectal manipulation. The end of the cannula (outer cannula sleeve: 1.2 cm outer diameter/50 cm length) was introduced into the dorsal vaginal fornix, to the left or right of the cervix, depending on the side of the ovary to be punctured [5]. The ovary with the co-dominant follicles was then positioned transrectally against the flat end of the cannula sleeve such that the follicle to be punctured was separated only by the vaginal wall. The inner cannula (0.8 cm outer diameter/49.5 cm length), fitted with a sterile 18 G 25-mm long needle, was inserted into the ovary and the vaginal wall was subsequently pierced in a cranial direction through the fornix, following which the needle was introduced into the follicular antrum, as previously described [3]. No suction was applied. Cows underwent AI immediately after follicle puncture. Cows were inseminated by the same technician using frozen-thawed semen from one bull. Ovulation was recorded as the presence of a CL, assessed 7 days post-AI. To improve luteal function of the draining-induced CL, cows in the drainage group received an additional GnRH dose on day 7 post-AI [4]. Pregnancy diagnosis was performed by ultrasonography 28 days post-AI. All procedures were approved by the ethics committee on animal experimentation of the University of Lleida (license number CEEA.06-01/12).

A clear negative effect of heat stress on the reproductive performance of lactating dairy cows has been described in the time period of May to September in our geographical area in north-eastern Spain [15, 16]; ovulation failure has been found to increase dramatically during this period [17, 18]. Thus, this study was performed over this warm period to increase the chances of ovulation failure in a pre-ovulatory follicle. Since dephereline increases ovulation and pregnancy rates [8, 19] and has been shown to be especially more active than natural GnRH under heat stress conditions [8], all cows received either a single or 2.5x dose of dephereline at the time of AI, to promote ovulation.

Statistical analyses

Differences in the rates of different reproductive parameters between the groups were identified using Tukey-Kramer multiple comparison tests. Possible inter-group differences in terms of milk production, days in milk at the time of AI, and number of lactations were tested using student's *t*-test.

Acknowledgments

The authors thank Ana Burton for her assistance with the English translation. This study received financial support from the project '01.02.01 de Transferència Tecnològica del Programa de desenvolupament rural de Catalunya 2014-2020' (Number 19005).

References

1. López-Gatius F, Hunter RHF. Preventing twin pregnancies in dairy cattle, turning the odds into reality. *Livest Sci* 2019; **229**: 1–3. [CrossRef]
2. López-Gatius F, García-Ispuerto I. Transfer of a single embryo versus drainage of subordinate follicles to prevent twin pregnancies in dairy cows. Why not both? *J Reprod Dev* 2020; **66**: 287–289. [CrossRef]. [Medline]
3. López-Gatius F, Hunter R. Puncture and drainage of the subordinate follicles at timed artificial insemination prevents the risk of twin pregnancy in dairy cows. *Reprod Domest Anim* 2018; **53**: 213–216. [Medline] [CrossRef]
4. López-Gatius F, García-Ispuerto I, Serrano-Pérez B, Balogh OG, Gábor G, Hunter RHF. Luteal activity following follicular drainage of subordinate follicles for twin pregnancy prevention in bi-ovular dairy cows. *Res Vet Sci* 2019; **124**: 439–443. [Medline] [CrossRef]
5. García-Ispuerto I, López-Gatius F. Improved embryo survival following follicular drainage of subordinate follicles for twin pregnancy prevention in bi-ovular dairy cows. *J Reprod Dev* 2020; **66**: 93–96. [Medline] [CrossRef]
6. Morgan WF, Lean IJ. Gonadotrophin-releasing hormone treatment in cattle: a meta-analysis of the effects on conception at the time of insemination. *Aust Vet J* 1993; **70**: 205–209. [Medline] [CrossRef]
7. Busch W. Experiences on the use of prostaglandin-F2-alpha in the control of reproduction and the therapy of reproductive disorders in cattle. *Tierarztl Umsch* 1986; **41**: 497–503.
8. García-Ispuerto I, De Rensis F, Pérez-Salas JA, Nunes JM, Pradés B, Serrano-Pérez B, López-Gatius F. The GnRH analogue dephereline given in a fixed-time AI protocol improves ovulation and embryo survival in dairy cows. *Res Vet Sci* 2019; **122**: 170–174. [Medline] [CrossRef]
9. Miura R. Physiological characteristics and effects on fertility of the first follicular wave dominant follicle in cattle. *J Reprod Dev* 2019; **65**: 289–295. [Medline] [CrossRef]
10. De Rensis F, López-Gatius F, García-Ispuerto I, Techakumpu M. Clinical use of human chorionic gonadotropin in dairy cows: an update. *Theriogenology* 2010; **73**: 1001–1008. [Medline] [CrossRef]
11. Edmondson AJ, Lean IJ, Weaver CO, Farver T, Webster G. A body condition scoring chart for Holstein dairy cows. *J Dairy Sci* 1989; **72**: 68–78. [CrossRef]
12. García-Ispuerto I, López-Gatius F. Effects of different five-day progesterone-based fixed-time AI protocols on follicular/luteal dynamics and fertility in dairy cows. *J Reprod Dev* 2014; **60**: 426–432. [Medline] [CrossRef]
13. López-Gatius F. Factors of a noninfectious nature affecting fertility after artificial insemination in lactating dairy cows. A review. *Theriogenology* 2012; **77**: 1029–1041. [Medline] [CrossRef]
14. López-Gatius F, García-Ispuerto I, Serrano-Pérez B, Hunter RHF. The presence of two ovulatory follicles at timed artificial insemination influences the ovulatory response to GnRH in high-producing dairy cows. *Theriogenology* 2018; **120**: 91–97. [Medline] [CrossRef]
15. López-Gatius F. Is fertility declining in dairy cattle? A retrospective study in northeastern Spain. *Theriogenology* 2003; **60**: 89–99. [Medline] [CrossRef]
16. García-Ispuerto I, López-Gatius F, Bech-Sabat G, Santolaria P, Yáñez JL, Nogareda C, De Rensis F, López-Béjar M. Climate factors affecting conception rate of high producing dairy cows in northeastern Spain. *Theriogenology* 2007; **67**: 1379–1385. [Medline] [CrossRef]
17. López-Gatius F, López-Béjar M, Fenech M, Hunter RHF. Ovulation failure and double ovulation in dairy cattle: risk factors and effects. *Theriogenology* 2005; **63**: 1298–1307. [Medline] [CrossRef]
18. López-Gatius F, Hunter R. Clinical relevance of pre-ovulatory follicular temperature in heat-stressed lactating dairy cows. *Reprod Domest Anim* 2017; **52**: 366–370. [Medline] [CrossRef]
19. Böhme H, Bach S, Busch W, Letz W. The induction of ovulation in the spontaneous estrous in cattle by Gonavet "Berlin Chemie". *Reprod Domest Anim* 1988; **23**: 201–207.