

Advance Publication by J-STAGE
Journal of Reproduction and Development

Accepted for publication: March 25, 2020

Advanced Epub: April 7, 2020

1 Opinions & Hypotheses

2

3 Transfer of a single embryo versus drainage of subordinate follicles to prevent twin pregnancies

4 in dairy cows. Why not both?

5

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

7

8 ¹⁾ Transfer in Bovine Reproduction SLu, 22300 Barbastro, Spain.

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

10 ³⁾Department of Animal Science, University of Lleida, 25198 Lleida, Spain.

11

12 Running head: EMBRYO TRANSFER AND FOLLICULAR DRAINAGE

13

14

15 Correspondence: F. López-Gatius (email lopezgatiusf@gmail.com)

16

17

18

19 Abstract

20

21 In this study, we present two proposed approaches to prevent twin pregnancies in dairy cattle: 1)
22 single, *in vitro*-produced embryonic transfer into a recipient cow or 2) subordinate follicle
23 drainage at the time of insemination. Both procedures lead to improved embryonic survival. As
24 the use of sexed semen generates herd replacements and additional heifers, we propose the
25 transfer of a single female cattle embryo into cows that are not suitable for producing
26 replacements, and follicular drainage in lactating cows with genetic merit. This should eliminate
27 economic losses associated with twin pregnancies and increase cattle output of the herd.

28

29 Keywords: Double ovulation, Follicular co-dominance, CL function, Early fetal loss, Greenhouse
30 gas emissions

31

32 The problem of twin pregnancies in dairy herds

33

34 Twinning rates in dairy herds have increased considerably in parallel with milk production during
35 the last 30 years [1], possibly due to a higher double ovulation rate being associated with a high
36 level of milk production [2, 3]. Twin pregnancies, more frequent in older cows, may account for
37 25 % of all pregnancies on Day 60 of gestation in cows in their third lactation or more [4], and
38 are classified into bilateral (one fetus in each uterine horn: 44 %) and unilateral (both fetuses in
39 the same uterine horn, right or left: 56 %) [5]. Twin pregnancy is not desirable for the dairy cattle
40 economy [5–9]. The risk of pregnancy loss during the first trimester of gestation for twin-carrying
41 cows is three to seven times higher than that for cows carrying singletons [1], with an economic
42 burden estimated at \$ 97–\$ 225 per pregnancy depending on twin pregnancy laterality (unilateral
43 vs. bilateral), parity, and the days in milk when the twin pregnancy occurs [6]. This impact could
44 become even greater due to the incidence of abortion among pregnant cows during the second or
45 third trimester of gestation. In an extensive study on 1194 twin pregnancies, abortion was
46 recorded in 278 (23.3 %) cows before Day 260 of pregnancy: 7/522 (1.3 %) in bilateral and
47 271/672 (40.3 %) in unilateral pregnancies [5]. In this latter study, the presence of live twins was
48 determined by transrectal ultrasonography between 55 and 61 days of gestation. Furthermore,
49 losses after twin delivery in cows reaching parturition should be added to the economic impact of
50 twin pregnancies. Higher incidence of peripartum reproductive disorders, freemartins, stillbirths,
51 and calf mortality has been related to twin births [7–9]. Thus, both a higher culling rate and
52 reduced mean production lifespan (by 200 days) have been reported for cows delivering twins
53 versus singletons [7–9]. These are all cogent reasons to try to reduce the incidence of twin births.
54 Proposed approaches to prevent twin pregnancies are 1) the transfer of a single embryo to a non-
55 inseminated cow or 2) the follicular drainage of subordinate follicles at the time of insemination
56 [10].

57

58 Transfer of a single *in vitro*-produced embryo

59

60 Fertility rates for *in vitro*-produced (IVP) bovine embryos are lower than those achieved with *in*
61 *vivo*-derived embryos [11]. However, the global use of IVP embryos has increased over the past
62 twenty years, probably due to the increasing benefits and lower costs of IVP procedures [10].
63 Effectively, embryo transfer (ET) is considered the most effective mechanism for maximizing
64 fertility during heat stress, improving fresh IVP embryo pregnancy results comparable to artificial
65 insemination (AI) under heat stress conditions [12]. Treatment with GnRH on Day 5 post-estrus
66 increases the corpus luteum (CL) blood flow area, thus improving luteal function assessed on Day
67 7 at ET [13] and prompting additional corpora lutea formation [14]. This treatment improves
68 embryonic survival in IVP embryo recipients [14].

69

70 Puncture and drainage of subordinate follicles at the time of insemination

71

72 Puncture and drainage without suction of subordinate follicles—either ultrasound-guided [15, 16]
73 or by using a simple transvaginal device [17]—at the time of insemination has proved efficient to
74 eliminate the risk of twin pregnancy without reducing fertility. Only bi-ovular cows with a size
75 difference of less than 2 mm between the two follicles were included in these studies [15–17].
76 This technique increases the incidence of additional drained follicle-derived corpora lutea. The
77 function of the drainage-induced CL was improved with GnRH treatment on Day 7 post-drainage
78 [16, 17]. This treatment improved embryonic survival in drained cows [17]. It should be noted
79 here that an ultrasound-guided training before follicular puncture should be considered by
80 inseminators. Furthermore, a potential problem related to the technique is the fact that the smaller
81 follicle is not always the subordinate follicle at the time of insemination. More extensive studies
82 are thus needed that take into account the ranges between the dominant and drained subordinate
83 follicle diameter.

84

85 The use of sexed semen helps twin prevention strategies

86

87 The use of sexed semen has been traditionally recommended only for heifers [18, 19], as
88 pregnancy rates are reduced in cows [20, 21]. Although its usage is low (< 5%) within the AI
89 market [22], it generates herd replacements and additional heifers [23]. Sexed sperm have been
90 successfully used in *in vitro* fertilization procedures [24, 25] so that embryos of a desired sex may
91 be transferred.

92

93 Concluding remarks

94

95 In herds where sexed semen is used in heifers thus providing sufficient herd replacements, the
96 strategies proposed to prevent twin pregnancies could increase herd profitability in a number of
97 ways:

98

99 - By conducting both, the embryo transfer of a single cattle embryo to cows that are not suitable
100 for producing replacements and the follicular drainage in lactating cows with genetic merit, the
101 economic losses associated with twin pregnancies should be prevented.

102 - Following both procedures, induced additional corpora lutea [14, 17] will reduce the risk of
103 pregnancy loss [26].

104 - Use of female cattle embryos or sexed semen, should reduce the incidence of male calf-related
105 dystocia, improving animal health. Gestation of a female calf has also been related to increased
106 milk production [27, 28].

107 - Introducing ET into the breeding program should improve the fertility of older cows under heat
108 stress conditions [12].

109 - Compared with the use of conventional semen, sexed semen used in heifers and follicular
110 drained parous cows should expedite herd expansion and increase the sale value of calves.

111 - While increasing cattle output from a dairy herd, greenhouse gas emissions will be lower
112 compared with beef cow herds, and land use will be more efficient [23].

113

114 Acknowledgments

115

116 The authors thank Ana Burton for assistance with the English translation. This study received
117 financial support from procedure “01.02.01 de Transferència Tecnològica
118 del Programa de desenvolupament rural de Catalunya 2014-2020” (Number 19005).

119

120

121 References

122

- 123 1. López-Gatius F, Andreu-Vázquez C, Mur-Novales R, Cabrera VE, Hunter RHF. The
124 dilemma of twin pregnancies in dairy cattle. A review of practical prospects. *Livest Sci* 2017;
125 197: 12-16.
- 126 2. Fricke PM, Wiltbank MC. Effect of milk production on the incidence of double ovulation in
127 dairy cows. *Theriogenology* 1999; 52: 1133-1143.
- 128 3. López-Gatius F, López-Béjar M, Fenech M, Hunter RHF. Ovulation failure and double
129 ovulation in dairy cattle: risk factors and effects. *Theriogenology* 2005; 63: 1298-1307.
- 130 4. Garcia-Ispuerto I, López-Gatius F. The effects of a single or double GnRH dose on pregnancy
131 survival in high producing dairy cows carrying singletons or twins. *J Reprod Dev* 2018; 64:
132 523-527.
- 133 5. Garcia-Ispuerto I, López-Gatius F. Abortion in dairy cattle with advanced twin pregnancies:
134 Incidence and timing. *Reprod Domest Anim* 2019; 54(Suppl 4): 50-53.
- 135 6. Mur-Novales R, López-Gatius F, Fricke PM, Cabrera VE. An economic evaluation of
136 management strategies to mitigate the negative effect of twinning in dairy herds. *J Dairy Sci*
137 2018; 101: 8335-8349.
- 138 7. Nielsen M, Schukken YH, Scholl DT, Wilbrink HJ, Brand A. Twinning in dairy cattle: A
139 study of risk factors and effects. *Theriogenology* 1989; 32: 845–862.

- 140 8. Bicalho RC, Cheong SH, Galvao KN, Warnick LD, Guard CL. Effect of twin birth calvings
141 on milk production, reproductive performance, and survival of lactating cows. *JAVMA* 2007;
142 231: 1390-1397.
- 143 9. Andreu-Vázquez C, Garcia-Ispuerto I, Ganau S, Fricke PM, López-Gatius F. Effects of
144 twinning on the subsequent reproductive performance and productive lifespan of high-
145 producing dairy cows. *Theriogenology* 2012; 78: 2061-2070.
- 146 10. López-Gatius F, Hunter RHF. Preventing twin pregnancies in dairy cattle, turning the odds
147 into reality. *Livest Sci* 2019; 229: 1-3.
- 148 11. Sartori R, Prata AB, Figueiredo ACS, Sanches BV, Pontes GCS, Viana JHM, Pontes JH,
149 Vasconcelos JLM, Pereira MHC, Dode MAN, Monteiro Jr PLJ, Baruselli PS. Update and
150 overview on assisted reproductive technologies (ARTs) in Brazil. *Anim Reprod* 2016; 13: 300-
151 312.
- 152 12. Hansen PJ. Reproductive physiology of the heat-stressed dairy cow: implications for fertility
153 and assisted reproduction. *Anim Reprod* 2019; 16: 497-507.
- 154 13. Kanazawa T, Seki M, Ishiyama K, Araseki M, Izaike Y, Takahasi T. Administration of
155 gonadotropin-releasing hormone agonist on Day 5 increases luteal blood flow and improves
156 pregnancy prediction accuracy on Day 14 in recipient Holstein cows. *J Reprod Dev* 2017; 63:
157 389-399.
- 158 14. García-Guerra A, Sala RV, Carrenho-Sala L, Baez GM, Motta JCL, Fosado M, Moreno JF,
159 Wiltbank MC. Postovulatory treatment with GnRH on Day 5 reduces pregnancy loss in
160 recipients receiving an in vitro produced expanded blastocyst. *Theriogenology* 2020; 141: 202-
161 210.
- 162 15. López-Gatius F, Hunter RHF. Puncture and drainage of the subordinate follicles at timed
163 artificial insemination prevents the risk of twin pregnancy in dairy cows. *Reprod Domest Anim*
164 2018; 53: 213-216.
- 165 16. López-Gatius F, Garcia-Ispuerto I, Serrano-Pérez B, Balogh OG, Gabor G, Hunter RHF.
166 Luteal activity following follicular drainage of subordinate follicles for twin pregnancy
167 prevention in bi-ovular dairy cows. *Res Vet Sci* 2019; 124: 439-443.

- 168 17. Garcia-Ispuerto I, López-Gatius F. Improved embryo survival following follicular drainage
169 of subordinate follicles for twin pregnancy prevention in bi-ovular dairy cows. *J Reprod Dev*
170 2020; 66: 93-96.
- 171 18. Seidel Jr GE, Schenk JL, Herickhoff LA, Doyle SP, Brink Z, Green RD, Cran DG.
172 Insemination of heifers with sexed sperm. *Theriogenology* 1999; 52: 1407-1420.
- 173 19. Newton JE, Hayes BJ, Pryce JE. The cost-benefit of genomic testing of heifers and using
174 sexed semen in pasture-based dairy herds. *J Dairy Sci* 2018; 101: 6159-6173.
- 175 20. DeJarnette JM, Nebel RL, Marshall CE, Moreno JF, McCleary CR, Lenz RW. Effect of sex-
176 sorted sperm dosage on conception rates in Holstein heifers and lactating cows. *J Dairy Sci*
177 2008; 91: 1778-1785.
- 178 21. DeJarnette JM, Nebel RL, Marshall CE. 2009. Evaluating the success of sex-sorted semen in
179 US dairy herds from on farm records. *Theriogenology* 2009; 71: 49–58.
- 180 22. Seidel GE Jr. Update on sexed semen technology in cattle. *Animal* 2014; 8: 160–164.
- 181 23. Holden SA, Butler ST. Review: Applications and benefits of sexed semen in dairy and beef
182 herds. *Animal* 2018; 12S1: s97-s103.
- 183 24. Lu KH, Cran DG, Seidel GE Jr. In vitro fertilization with flow-cytometrically-sorted bovine
184 sperm. *Theriogenology* 1999; 52: 1393-1405.
- 185 25. Hamano K, Li X, Qian X, Funauchi K, Furdade M, Minato Y. Gender preselection in cattle
186 with intracytoplasmically injected, flow cytometrically sorted sperm heads. *Biol Reprod* 1999;
187 60: 1194-1197.
- 188 26. López-Gatius F. Factors of a noninfectious nature affecting fertility after artificial
189 insemination in lactating dairy cows. A review. *Theriogenology* 2012; 77: 1029-1041.
- 190 27. Hinde K, Carpenter AJ, Clay JS and Bradford BJ. Holsteins favor heifers, not bulls: biased
191 milk production programmed during pregnancy as a function of fetal sex. *PLoS One* 2014; 9:
192 e86169.
- 193 28. Hess MK, Hess AS, Garrick DJ. The effect of calf gender on milk production in seasonal
194 calving cows and its impact on genetic evaluations. *PLoS ONE* 2016; 11: e0151236.
- 195