Taylor & Francis
Taylor & Francis Group

Journal of Applied Animal Research

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/taar20

Effect of equine chorionic gonadotropin on pregnancy rate in Brown Swiss cows under high altitude conditions

C. Mínguez & A. Calvo

JOURNAL OF APPLIED ANIMAL RESEARCH
2020, VOL. 48, NO. 1, 121–125
https://doi.org/10.1080/09712119.2020.1741373

Taylor & Francis
Taylor & Francis Group

OPEN ACCESS Check for updates

Effect of equine chorionic gonadotropin on pregnancy rate in Brown Swiss cows under high altitude conditions

C. Mínguez and A. Calvo

Departamento de Producción Animal y Salud Pública. Facultad de Veterinaria y Ciencias Experimentales, Universidad Católica de Valencia San Vicente Martir, Valencia, España

ABSTRACT

The objective of the present study was to evaluate the administration of Equine Chorionic Gonadotropin (eCG) in a protocol of fixed-time artificial insemination (FTAI) to increase the pregnancy rate in cows subjected to high altitude conditions. These conditions could generate a syndrome of right-sided heart failure and, as consequence, animals trend to have a low body score condition with a decrease in fertility. The research was conducted with 84 lactating cows (Brown Swiss) randomly into two groups with a body condition score between 2.5 and 3.5, allocated at 3100 m above sea level. In the FTAI protocol, 400 IU of eCG was administered ($n = 42$) or not ($n = 42$) at progesterone intravaginal device removal. A significant improvement in pregnancy rate was found with eCG treatment in those cows with low body condition score (BCS of 2.5, scale 1–5), while no effect was found in those cows with higher BCS (i.e. 3.0 and 3.5). In conclusion, the addition of 400 IU of eCG in an FTAI in cows under high altitude conditions and low body conditions can improve the fertility.

ARTICLE HISTORY
Received 12 January 2018
Accepted 5 March 2020

KEYWORDS
Altitude conditions; Brown Swiss; eCG; fixed-time artificial insemination; pregnancy rate

1. Introduction

The fertility rate is a very important parameter in the production of dairy cattle. However, this aspect has been reduced over the years as a result of genetic improvement in milk production (Royal et al. 2000). For this reason, in dairy cattle, it is necessary to include fertility as selection criterion in breeding programmes (Jorjani 2005) as well as to develop hormonal treatments to increase fertility (Friedman et al. 2014). As a result, in the last decade, numerous protocols of synchronized ovulation have been developed (Martínez et al. 2014).

The main characteristic of Ecuador and the Andean Region is its high altitude. The syndrome associated with altitude is called ‘altitude sickness’. Evidence of this sickness occurs at a modest elevation of 1600 m (Malherbe et al. 2012). The ‘altitude sickness’ produces the syndrome of right-sided heart failure due to the elevated blood pressure in the pulmonary arterial tree, generating a considerable drop in conception both in humans (Vitzthum et al. 2000) and in livestock species (Malherbe et al. 2012; Parraguez et al. 2014; Gonzalez-Bulnes et al. 2016). Brown Swiss herd is usual in this region, because it is more adapted to altitude conditions (Bartl et al. 2008).

Equine Chorionic Gonadotrophin (eCG) has been used in procedures of fixed-time artificial insemination (FTAI) in cattle to try to improve the fertility rate because it produces, among other effects, an increase of follicular growth (Murphy 2012; Núñez-Olivera et al. 2014). The application of 400 IU of eCG has shown that it can be a good treatment to increase fertility, especially in beef breeds (Menchaca et al. 2013; Nogueira et al. 2014); nevertheless, in dairy cattle results have been contradictory (Pulley et al. 2013).

Worldwide, experiments have been carried out using doses of 300–800 IU, obtaining different results. These unknowns in cattle do not diminish the usefulness and use of eCG in other species for the induction of folliculogenesis; the early onset of puberty, the resolution of anoestro and the induction of superovulation for embryo transfer (Murphy 2012).

The aim of this study was to evaluate the effect of administering 400 IU of eCG on a fixed-time artificial insemination protocol to increase the fertility of Swiss Brown dairy cows of different body condition scoring (BCS) kept in high altitude habitat.

2. Material and methods

2.1. Animals

Animals were reared and slaughtered in compliance with the regulations for the care and use of animals in research in the ‘Código Orgánico del Ambiente’ (ROS No 983, Ecuador).

The study began in December 2016 and included 84 multiparous, cycling Swiss Brown cows, repeated breeders (≥ 2 negative AI), clinically healthy and in the lactation period. The cows were farmed at the ‘Centro de Excelencia Experimental Agropecuaria Burgay’ (2° 40′ 0″ South, 78° 56′ 0″ West) belonging to the ‘Gobierno Autónomo Descentralizado del Cañar (Ecuador)’. It has an altitude of 3100 m with a temperature average of 12.5°C and an annual rainfall average of 1180 mm. The farm

CONTACT C. Mínguez carlicosvillar@hotmail.com Departamento de Producción Animal y Salud Pública. Facultad de Veterinaria y Ciencias Experimentales, Universidad Católica de Valencia San Vicente Martir, Guillem de Castro 94, 46001 Valencia, España

has a conception rate to the first service of 59%. On the day treatment began, the cows underwent a gynaecological examination by external inspection, transrectal palpation and ultrasound (Aloka ProSound 2 Hitachi-Aloka Medical Ltda., Tokyo, Japan). Cows with ovarian or uterine pathologies were discarded from the experiment.

The cows had a milk production average of 16.8 ± 1.4 kg/day in 154.2 ± 16.6 days in lactation. The animals were among the second and fourth lactation and were between 4 and 6 years old with a BCS between 2.5 and 3.5 (560 ± 40 kg), which was calculated by using the 5-point system (1 = thin − 5 = obese) (Ferguson et al. 1994).

A total of 84 animals were distributed randomly in two experimental groups (42 per group) and were milked twice a day (every 12 h approximately). Both groups of animals grazed on a pasture cultivated with an association of gamine and legumes consisting of perennial ryegrass, red clover and Kikuyu grass. At the time of milking, all animals received a commercial supplement that allowed them to cover their nutritional needs according to the requirements described in the NRC (2001). In addition, they had fresh water ad libitum, and no health problems were observed during the experimental period.

2.2. Experimental design

In the FTAI protocol (Figure 1), the moment when animals were placed an intravaginal progesterone device of 1.38 mg (P4; CIDR®, Pfizer Cambridge, USA), as well as an intramuscular dose of 2 mg estradiol benzoate, (Fertigan®; Laquinsa, San Jose, Costa Rica) was designated as day 0 (D0). On day 7 (D7) an intramuscular dose of 0.5 mg of PGF2α (Lutalyse®; Zoetis Quito, Ecuador) was given.

On day 9 (D9), the intravaginal device was removed and cows received 1 mg of estradiol benzoate (Fertigan®; Laquinsa, San Jose, Costa Rica). Immediately after P4 withdrawal, a dose of 400 IU of eCG (Folligon®; Intervet Ecuador) was applied to cows of the 'Treated group' ('Non-Treated group' remained as the control group). On Day 11 (48 h after P4 withdrawal), all cows were inseminated by using straws of frozen semen (0.5 ml). Prior to the artificial insemination (AI), semen had been thawed at 37°C during 40 s (Bernardi et al. 2015). The cows were inseminated by the same experimented technician to avoid the operator effect. To avoid the influence of semen in the pregnancy rate (Anderson et al. 2004) the semen doses came from the same bull (Brown Swiss) being supplied by the 'Ministerio de Agricultura, Ganadería, Acuacultura y Pesca' (MAGAP, Ecuador). MAGAP guaranteed the minimum standard of motility and vigour in the semen by a computer-assisted sperm analysis (CASA) and flow cytometry (Vincent et al., 2012). Also, on the day of AI, the diameter of the largest follicle was determined from ovarian transrectal ultrasonography. Ovulation was considered when cows had the presence of a follicle with a diameter ≥ 8.5 mm on day 11 and the absence of this follicle 48 h later (D13) (Sá Filho et al. 2009).

On day 39 (D28 after AI), a pregnancy diagnosis was performed by ultrasound observation. It is important to state that

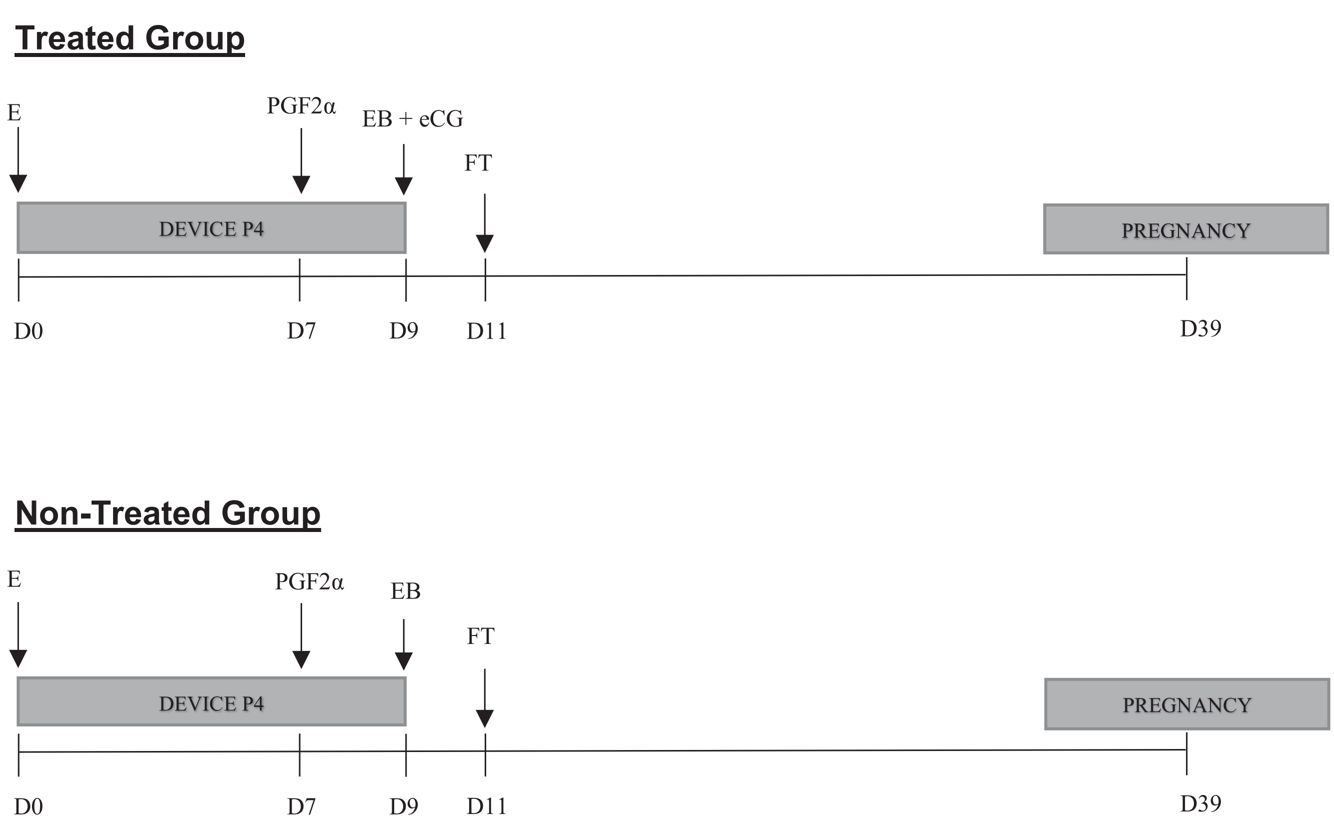

Figure 1. Schematic diagram of the experimental design for evaluating the effect of the administration of eCG in a fixed-time artificial insemination protocol (FTAI) in the fertility rate in lactating cows subjected to altitude conditions. P4 = 1.38 g of progesterone by intravaginal device; FTAI = fixed-time artificial insemination; EB = 2 mg. estradiol benzoate; eCG = 400 IU equine chorionic gonadotropin; D = day.

the survey data refer only to insemination on day 11 and do not include any subsequent AI. Conception rate was obtained by dividing the number of pregnant cows by the number of cows that ovulated. The pregnancy rate was obtained by dividing the number of pregnant cows by the number of treated cows.

2.3. Statistical analysis

Statistical analysis of the data was performed using a model with the interaction between treatment and BCS (D0). Data were analysed using the GLM package of the R project software (R project, version 3.1.2; University of California, Los Angeles, USA). In the package GLM, the option 'family = binomial' was used for ovulation, conception, and pregnancy rates, and 'family = normal' for follicular diameter. If *p-value* was lower than 0.05 the difference was assumed significant.

3. Results

Results of the different combinations 'Treatment x BSC' for ovulation, conception, and pregnancy rates are present in Table 1. Significant differences were found for conception rate and pregnancy rate against the interaction 'Non-Treated x BSC2.5'.

Diameter of the largest follicle does not differ among groups (Treated * BSC 2.5 = 10.24 ± 0.19 mm; Treated * BSC 3.0 = 10.19 ± 0.14 mm; Treated * BSC 3.5 = 10.18 ± 0.13 mm; Non-Treated * BSC 2.5 = 10.14 ± 0.16 mm; Non-Treated * BSC 3.0 = 10.21 ± 0.13 mm; Non-Treated * BSC 3.5 = 10.17 ± 0.14 mm).

4. Discussion

In this study, the improvement of pregnancy rates in dairy cows with a low BSC due to the application of eCG was observed.

The cases in which the application of eCG at the moment when the P4 device is removed in dairy cows shown beneficial effects in the conception rate are referred for animals in anoestrus (Bryan et al. 2010; García-Ispierto et al. 2012), with a low body condition (Souza et al. 2009), in old age (Bryan et al. 2010) or in heat conditions (Bó et al. 2008; Suplicy et al. 2012; De Rensis and López-Gatius 2014).

In dairy cattle, the absence of a positive conception rate effect by the use of eCG at a 400 IU dose has been reported in Souza et al. (2009) and Pulley et al. (2013) and in Ferreira et al. (2013).

Table 1. Ovulation, conception, and pregnancy rates of lactating Brown Swiss cows.

Interaction[a]	Proportion, *n* (%)		
	Ovulation rate[b]	Conception rate[c]	Pregnancy rate[d]
T * BSC 2.5	9/12 (75.0)[x]	5/9 (55.5)[x]	5/12 (41.6)[x]
T * BSC 3.0	16/21 (76.2)[x]	9/16 (56.2)[x]	9/21 (42.8)[x]
T * BSC 3.5	7/9 (77.8)[x]	4/7 (57.1)[x]	4/9 (44.4)[x]
NT * BSC 2.5	10/13 (76.9)[x]	4/10 (40.0)[y]	4/13 (30.7)[y]
NT * BSC 3.0	15/20 (75)[x]	8/15 (53.4)[x]	8/20 (40.0)[x]
NT * BSC 3.5	7/9 (77.8)[x]	4/7 (57.1)1[x]	4/9 (44.4)[x]

[a]T = cows received treatment 400 IU eCG on day 9; NT cows non-received treatment; BSC = body condition scores assessed on a 1 to 5 scale.
[b]Percentage of cows ovulating after PGF2α compared with all cows treated.
[c]Percentage of cows pregnant to FTAI compared with cows that ovulated.
[d]Percentage of cows pregnant to FTAI compared with all cows treated.
[x y]Values in the same row with the same superscript do not differ significantly (significant difference at $\alpha = 0.05$).

On the other hand, applying a high eCG dose (800 IU) two days after the beginning of the FTAI protocol reduces the conception rate (Kenyon et al. 2012). Regarding postpartum (a single dose of 600 UI between days 9 and 15 postpartum), Freick et al. (2017) did not observe a significant increase in the reproductive performance.

Similarly, Rostami et al. (2011) observed that application of 500 UI of eCG 6 days after birth in primiparous Holstein cows quickly resumed ovarian activity.

There are more studies regarding the application of eCG on beef cattle. In Angus, Martínez et al. (2014) reported an improvement in the conception ratio after the application of 400 IU of eCG on day 2 and day 7 (when P4 device was removed). Gaievski et al. (2015), in Nellore and in Simmental × Nelore, included a dose of 400 IU of eCG at day 10 in an FTAI. In Nellore, Sá Filho et al. (2010a) applied the same dose at the moment in which the P4 device was removed with a positive effect on pregnancy rates.

However, some studies demonstrated an improvement in pregnancy rates of postpartum cows after the application of eCG in the FTAI with a lower BCS (Baruselli et al. 2004; Sá Filho et al. 2010b). Tortorella et al. (2013) in cross beef cows, applying 400 UI of eCG two days prior removal of the progesterone intravaginal device in cows in anoestrus obtained an increase in the diameter of the dominant follicle, total luteal volume, concentration of serum progesterone and conception rate. In Nellore, the eCG + Temporary Weaning protocol was efficient in primiparous cows (Campos et al. 2013; Souza et al. 2016).

Definitely, there is enough evidence in the literature that the inclusion of eCG in an FTAI does not improve the fertility rate in cattle with a good BCS. Binelli et al. (2009) mentioned that the eCG effectiveness varies with the BCS and Nogueira et al. (2014) observed that the BCS had a positive effect on the probability of pregnancy using eCG in an FTAI. Ferreira et al. (2013) indicated that the differences observed in the inclusion of eCG in FTAI might be explained by the corresponding inequalities in BCS. Also, Sales et al. (2011) and Pinheiro et al. (2009) showed that in animals with a good BCS, the eCG treatment does not promote an increase in pregnancy rates.

5. Conclusion

The addition of 400 IU of eCG in an FTAI protocol in Brown Swiss cattle under high altitude conditions and low BCS could improve the pregnancy rate.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

C. Mínguez http://orcid.org/0000-0002-4262-3279
A. Calvo http://orcid.org/0000-0002-4995-2841

References

Andersson M, Taponen J, Koskinen E, Dahlbom M. 2004. Effect of insemination with dose of 2 or 15 million frozenthawed spermatozoa and semen deposition site on pregnancy rate in dairy cows. Theriogenology. 61:1583–1588. doi: 10.1016/j.theriogenology.2003.09.006.

Bartl K, Goméz CA, García M, Aufdermauer T, Kreuzer M, Hess HD, Wettstein HR. 2008. Milk fatty acid profile of Peruvian Criollo and Brown Swiss cows in response to different diet qualities fed at low and high altitude. Arch Anim Nutrition. 62:468–484. doi:10.1080/17450390802453450.

Baruselli PS, Reis EL, Marques MO, Nasser LF, Bó GA. 2004. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. Anim Reprod Sci. 82:479–486. doi:10.1016/j.anireprosci.2004.04.025.

Bernardi SF, Di Prinzio M, Maglione D, Rinaudo A, Marini PR. 2015. Efecto del protocolo de descongelación de semen sobre el porcentaje de preñez en bovinos lecheros. Revista Veterinaria. 26:27–32. ISSN (on line) 1669-6840.

Binelli M, Machado R, Bergamaschi MACM, Bertran CM. 2009. Manipulation of ovarian and uterine function to increase conception rates in cattle. Animal Reproduction. 6:125–134.

Bó GA, Cutaia LE, Souza AH, Baruselli PS. 2008. Actualización sobre protocolos de IATF en bovinos de leche. Proceedings of the 3th International Symposium on Animal Reproduction Applied, Londrina, Brazil, pp 95–110.

Bryan MA, Bo GA, Heuer C, Emslie FR. 2010. Use of equine chorionic gonadotrophin in synchronised AI of seasonal-breeding, pasturebased, anoestrous dairy cattle. Reprod Fertil Dev. 22:126–131. doi:10.1071/RD09225.

Campos JT, Marinho LSR, Lunardelli PA, Morotti F, Seneda MM. 2013. Resynchronization of estrous cycle with eCG and temporary calf removal in lactating *Bos indicus* cows. Theriogenology. 80:619–623. doi:10.1016/j.theriogenology.2013.05.029.

De Rensis F, López-Gatius F. 2014. Use of equine chorionic gonadotropin to control reproduction of the dairy cow: a review. Reprod Domest Anim. 49:177–182. doi:10.1111/rda.12268.

Ferguson JD, Galligan DT, Thomsen N. 1994. Principal descriptors of body condition score in Holstein cows. J Dairy Sci. 77:2695–2703. doi:10.3168/jds.S0022-0302(94)77212-X.

Ferreira RM, Ayres H, Sales JNS, Souza AH, Rodrigues CA, Baruselli PS. 2013. Effect of different doses of equine chorionic gonadotropin on follicular and luteal dynamics and P/AI of high-producing Holstein cows. Anim Reprod Sci. 140:26–33. doi:10.1016/j.anireprosci.2013.04.014.

Freick M, Passarge O, Weber J. 2017. Lack of effects of an equine chorionic gonadotropin (eCG) administration between days 9 and 15 postpartum on reproductive performance in a Holstein dairy herd. Reprod Domest Anim. 52:429–436. doi: 10.1111/rda.12928.

Friedman E, Voet H, Reznikov D, Wolfenson D, Roth Z. 2014. Hormonal treatment before and after artificial insemination differentially improves fertility in subpopulations of dairy cows during the summer and autumn. J Dairy Sci. 97:7465–7475. doi:10.3168/jds.2014-7900.

Gaievski FR, Lamb GC, Weiss RR, Bertol MAF, Segui MS, Abreu ACMR Kozicki LE. 2015. Gonadotropin releasing hormone (GnRH) and equine chorionic gonadotropin (eCG) improve the pregnancy rate on protocols for timed-artificial insemination in beef cattle. Vet e Zootec. 22:471–480.

García-Ispierto I, López-Helguera I, Martino A, López-Gatius F. 2012. Reproductive performance of anoestrous high-producing dairy cows improved by adding equine chorionic gonadotrophin to a progesterone-based oestrous synchronizing protocol. Reprod Domest Anim. 47:752–758. doi:10.1111/j.1439-0531.2011.01954.x.

Gonzalez-Bulnes A, Astiz S, Parraguez V, Garcia-Contreras C, Vazquez-Gomez M. 2016. Empowering translational research in fetal growth restriction: sheep and swine animal models. Curr Pharm Biotechnol. 17:848–855.

Jorjani H. 2005. Preliminary report of Interbull pilot study for female fertility traits in Holstein populations. Interbull Bull. 33:34–44.

Kenyon AG, Lopes JG, Mendonca LGD, Lima JR, Bruno RGS, Denicol AC, Chebel RC. 2012. Ovarian responses and embryo survival in recipient lactating Holstein cows treated with equine chorionic gonadotropin. Theriogenology. 77:400–411. doi:10.1016/j.theriogenology.2011.08.014.

Malherbe CR, Marquard JD, Legg E, Cammack K, ÓTool D. 2012. Right ventricular hypertrophy with heart failure in Holstein heifers at elevation of 1.600 meters. Veterinary Sci. 24:867–877. doi:10.1177/1040638712453580.

Martínez MF, Tutt D, Quirke LD, Tattersfield G, Juengel JL. 2014. Development of a GnRH-PGF2α-progesterone-based synchronization protocol with eCG for inducing single and double ovulations in beef cattle. J Anim Sci. 92:4935–4948. doi:10.2527/jas.2013-7512.

Menchaca A, Núñez R, Wijma R, Pintos CG, Fabini F, Castro T. 2013. Como mejorar la fertilidad de los tratamientos de IATF en vacas *Bos taurus*. In Proceeding 10th Simposio Internacional de Reproducción Animal IRAC. 4-6 July, Cordoba (Argentina).

Murphy BD. 2012. Equine chorionic gonadotropin: an enigmatic but essential tool. Anim Reprod. 9:223–230.

Nogueira É, Batista DSN, Costa Filho LCC, Dias AM, Silva JCB, Ítavo LCV. 2014. Pregnancy rate in lactating *Bos indicus* cows subjected to fixed-time artificial insemination and treated with different follicular growth inducers. Rev Bras Zootec. 43:358–362. doi:10.1590/S1516-35982014000700003.

NRC. 2001. Nutrient requirements of dairy cattle. Natl Acad Sci, Washington, DC.

Núñez-Olivera R, de Castro T, García-Pintos C, Bó G, Piaggio J, Menchaca A. 2014. Ovulatory response and luteal function after eCG administration at the end of a progesterone and estradiol based treatment in postpartum anestrous beef cattle. Anim Reprod Sci. 146:111–116. doi:10.1016/j.anireprosci.2014.02.017.

Parraguez V, Diaz F, Cofré E, Urquieta B, De Los Reyes M, Astiz S, Gonzalez-Bulnes A. 2014. Fertility of a high-altitude sheep model is compromised by deficiencies in both preovulatory follicle development and plasma LH availability. Reprod Domest Anim. 49:977–984. doi:10.1111/rda.12417.

Pinheiro VG, Souza AF, Pegorer MF, Satrapa RA, Ereno RL, Trinca LA, Barros CM. 2009. Effects of temporary calf removal and eCG on pregnancy rates to timed-insemination in progesterone-treated postpartum Nellore cows. Theriogenology. 71:519–524. doi:10.1016/j.theriogenology.2008.08.018.

Pulley SL, Wallace LD, Mellieon HI, Stevenson JS. 2013. Ovarian characteristics, serum concentrations of progesterone and estradiol, and fertility in lactating dairy cows in response to equine chorionic gonadotropin. Theriogenology. 79:127–134. doi:10.1016/j.theriogenology.2012.09.017.

Rostami B, Niasari-Naslaji A, Vojgani M, Nikjou D, Amanlou H, Gerami A. 2011. Effect of eCG on early resumption of ovarian activity in postpartum dairy cows. Anim Reprod Sci. 128:100–106. doi:10.1016/j.anireprosci.2011.09.006.

Royal MD, Darwash AO, Flint APF, Webb R, Woolliams JA, Lamming GE. 2000. Declining fertility in dairy cattle: Changes in traditional and endocrine parameters of fertility. Anim Sci. 70:487–501. doi:10.1017/S1357729800051845.

Sá Filho MF, Ayres H, Ferreira RM, Marques MO, Reis EL, Silva RC, Rodrigues CA, Madureira EH, Bó GA, Baruselli PS. 2010b. Equine chorionic gonadotropin and gonadotropin-releasing hormone enhance fertility in a norgestomet-based, timed artificial insemination protocol in suckled Nelore (*Bos indicus*) cows. Theriogenology. 73:651–658. doi:10.1016/j.theriogenology.2009.11.004.

Sá Filho OG, Meneghetti M, Peres RFG, Lamb GC, Vasconcelos JLM. 2009. Fixed-time artificial insemination with estradiol and progesterone for *Bos indicus* cows II: strategies and factors affecting fertility. Theriogenology. 72:210–218. doi:10.1016/j.theriogenology.2009.02.008.

Sá Filho MF, Torres-Junior JR, Penteado L, Gimenes LU, Ferreira RM, Ayres H. 2010a. Equine chorionic gonadotropin improves the efficacy of a progestin-based fixed-time artificial insemination protocol in Nelore (*Bos indicus*) heifers. Anim Reprod Sci. 118:182–187. doi:10.1016/j.anireprosci.2009.10.004.

Sales JNS, Crepaldi GA, Girotto RW, Souza AH, Baruselli PS. 2011. Fixed-time AI protocols replacing eCG with a single dose of FSH were less effective in stimulating follicular growth, ovulation, and fertility in suckled anestrus Nelore beef cows. Anim Reprod Sci. 124:12–18. doi:10.1016/j.anireprosci.2011.02.007.

Souza ALB, Segui MS, Kozicki LE, Weiss RR, Abreu A, Bertol MAF, Oliveira DMA. 2016. Impact of equine Chorionic Gonadotropin associated with temporary Weaning, Estradiol Benzoate, or Estradiol Cypionate on Timed artificial insemination in Primiparous *Bos Indicus* Cows. Braz Arch Biol Technol. 59. doi:10.1590/1678-4324-2016150389

Souza AH, Viechnieski S, Lima FA, Silva FF, Araujo R, Bo GA, Wiltbank MC, Baruselli PS. 2009. Effects of equine chorionic gonadotropin and type of ovulatory stimulus in a timed-AI protocol on reproductive responses in dairy cows. Theriogenology. 72:10–21. doi:10.1016/j.theriogenology.2008.12.025.

Suplicy M, Suplicy Filho H, Kozicki LE, Breda JC, Weiss RR, Segui MS. 2012. Reduction of the interval calving-conception by use of hormonal protocols and fixed-time artificial insemination dairy cows. Archives Veterinary Sci. 17:57–62. doi:10.5380/avs.v17i3.21676.

Tortorella R, Ferreira R, Tonellotto Dos Santos J, Silveira de Andrade Neto O, Barreta MH, Oliveira JF. 2013. The effect of equine chorionic gonadotropin on follicular size, luteal volume, circulating progesterone concentrations, and pregnancy rates in anestrous beef cows treated with a novel fixed-time artificial insemination protocol. Theriogenology. 79:1204–1209. doi:10.1016/j.theriogenology.2013.02.019.

Vincent P, Underwood SL, Dolbec C, Bouchard N, Kroetsch T, Blondin P. 2012. Bovine semen quality control in artificial insemination centers. Anim Reprod. 9:153–165. doi: 10.1002/9781118833971.ch74.

Vitzthum VJ, Ellison PT, Sukalich S, Caceres E, Spielvogel H. 2000. Does hypoxia impair ovarian function in Bolivian women indigenous to high altitude? High Alt Med Biol. 1:39–49. doi:10.1089/152702900320676.