Animal Reproduction Science 162 (2015) 88–94

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Comparative efficacy of exogenous eCG and progesterone on endogenous progesterone and pregnancy in Holstein cows submitted to timed artificial insemination

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ARTICLE INFO

Article history:
Received 4 February 2015
Received in revised form
23 September 2015
Accepted 26 September 2015
Available online 30 September 2015

Keywords:
Dairy cows
Serum progesterone
Conception rate
Late embryonic loss
Progesterone supplementation
Equine chorionic gonadotropin

ABSTRACT

The objectives were to evaluate the effects of the administration of either eCG or progesterone (P_4) alone or combined on endogenous P_4 concentrations and pregnancy per AI in lactating dairy cows. Cows received a P_4-releasing intravaginal device (PRID) and estradiol benzoate on D-8. The PRID was removed and a $PGF_{2\alpha}$ injection was given on D-3. An estradiol cypionate was given on D-2 and TAI was performed on D0. On D-2, cows were randomly allocated to treatments in a 2 × 2 factorial design: Control–saline solution on the D-2 and D+3 ($n = 104$), eCG – 400 IU eCG on D-2 ($n = 93$), P_4 – 600 mg of P_4 on D+3 ($n = 106$), and eCG + P_4 – 400 IU eCG on D-2 and 600 mg of P_4 on D+3 ($n = 95$). Blood samples were collected on days three, four, and thirteen and pregnancy diagnoses were performed at 32 and 46 days after AI. There was no interaction between eCG and P_4 injection. Cows treated with eCG and with P_4 injection had higher serum P_4 on Day +4. On Day +13 serum P_4 was lower in eCG-untreated primiparous cows (Interaction eCG × parity). Cows with serum $P_4 < 4.57$ ng/mL on Day +13 had lower probability to be pregnant on day 32. P/AI on days 32 and 46 and embryonic losses were not influenced by eCG and P_4 injection. In conclusion, the addition of 400 IU of eCG on D-2 and/or 600 mg of P_4 on D+3 to the present TAI protocol did not increase P/AI.

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1. Introduction

The causes of low fertility in high producing dairy cows are considered to be multi-factorial in nature (Chebel et al., 2004). Among the factors involved, low blood concentrations of progesterone (P_4) during first six days following artificial insemination (A.I.) have been considered as relevant (Lonergan, 2011). Many strategies aim at increasing blood concentrations of P_4 post AI, such as the enhancement of *corpus luteum* (CL) function (Pulley et al., 2013), the induction of accessory CLs (Stevenson et al., 2007), or by increasing endogenous P_4 through direct supplementation (Forro et al., 2012).

The use of eCG has been evaluated as a way to improve the fertility of dairy (Souza et al., 2009) and beef (Sá Filho

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http://dx.doi.org/10.1016/j.anireprosci.2015.09.014
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et al., 2010a,b; Sales et al., 2011) cows and heifers. Follicular development is stimulated by eCG, which results in larger and more steroidogenic CLs and in increased endogenous P_4 concentrations (Pulley et al., 2013). When administered at the time of P_4 intravaginal device removal in timed-AI (TAI) protocols, eCG increased (Marques et al., 2003; Sá Filho et al., 2010a) or tended to increase (Souza et al., 2009) serum concentrations of P_4 and P/AI (Sá Filho et al., 2010a,b; Pittí and Sagastume, 2010; Sales et al., 2011). However, other authors have not observed improvements in the fertility of cows treated with eCG (Ferreira et al., 2013).

The direct supplementation of P_4 after AI has also been evaluated as a strategy to increase conception rates (Lonergan, 2011). Exogenous P_4 administration through intra-vaginal releasing devices during the early luteal phase post AI increased blood concentrations of P_4 and conception rates (Macmillan and Peterson, 1993; Stevenson et al., 2007; Monteiro et al., 2014). Furthermore, P/AI increased in cows injected with P_4 after IA (Johnson, 1958 cited by Lonergan, 2011). In contrast, in a recent large study with lactating Holstein–Friesian cows, P_4 supplementation from day 4 to 9 after AI decreased P/AI by 12% (Parr et al., 2014). The moment when the treatment is imposed may be important. A meta-analysis of several P_4 supplementation studies indicated that treatment during the first week after AI increased P/AI, but not when injections were given in the second or third week after AI (Mann and Lamming, 1999).

Thus, strategies to optimize circulating concentrations of P_4 during selected reproductive periods my enhance management tools for improving reproductive efficiency of lactating dairy cows (Wiltbank et al., 2014). The combination of eCG and injectable P_4 may have additive effects that increase blood concentrations of P_4 after AI and ensuing P/AI. The use of a single high dose of injectable P_4 (600 mg) should be evaluated since it may reduce animal handling, compared to the use of lower doses (100 mg) for several days (Garrett et al., 1988).

Therefore, the objectives were to evaluate the supplementation effects of either 400 IU of eCG two days prior AI or 600 mg of injectable P_4 three days after AI alone or combined on serum concentration of P_4 and P/AI in lactating dairy cows subjected to TAI protocol.

2. Material and methods

2.1. Animals and management

The experiment was conducted between March and July 2013 in two commercial dairy herds kept in a free-stall system, located in southeastern Brazil. Three hundred twenty-nine (329) Holstein cows were used, in which 398 protocols were performed (237 in primiparous and 161 in pluriparous). Cows averaged 27.7 ± 0.35 kg milk/day, 137 ± 4.66 days in milk (DIM), 3.0 ± 0.02 units of body condition score (BCS), and 3.0 ± 0.11 services per conception. Of the total protocols performed, 166 were in cows that received at least one $_r$bST injection previously to the protocol and 255 were in cows that had at least one CL on the first day of the protocol.

On both farms, cows were subjected to three daily milking and fed a total ration based on corn silage, cornmeal, citrus pulp, soybean meal, and a vitamin and mineral mix, formulated to meet or exceed the nutritional requirements of lactating dairy cows. Rations were in line with the 2001-NRC recommendations (National Research Council, 2001). Within each farm, the cows were managed as a single group, and there was no difference in the nutritional management or in any other management practice. Experimental procedures were approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Lavras (UFLA, protocol 037/12).

2.2. Milk production and body condition assessment

The milk record closest to the beginning of the synchronization protocol was used to evaluate the response to treatments. The interval from milk test and the beginning of the protocol was 7.1 ± 0.2 days.

Body condition scores were assigned by three independent evaluators through visual observation using a scale from 1 to 5 (1 – thin and 5 – obese) according to Wildman et al. (1982), on the first day of the protocol. The average BCS from the three evaluators was considered for analysis.

2.3. Experimental design

Cows with ≥35 DIM, reproductively sound (free of uterine infections, follicular cysts, and adhesions around the ovaries and/or uterus), with BCS ≥ 2.25, and no hoof problems were assigned to hormonal treatments. At 137 ± 4.66 DIM (D-8) cows received 2 mg of estradiol benzoate (Sincrodiol®, Ouro Fino Saúde Animal, Cravinhos, Brazil), i.m., and an intravaginal device containing 1 g of P_4 (Sincrogest®, Ouro Fino Saúde Animal, Cravinhos, Brazil). On D-3, cows received 0.530 mg of cloprostenol (Sincrocio®, Ouro Fino Saúde Animal, Cravinhos, Brazil), i.m. On D-2, the intravaginal device was removed and 1 mg of estradiol cypionate (ECP®, Pfizer Saúde Animal, São Paulo, Brazil) was injected (Fig. 1). Artificial insemination (AI) was performed 48 h after the progesterone device withdrawal (D0).

On D-2 the cows were randomly allocated within each farm to a 2×2 factorial design based on the administration of eCG on D-2 and P_4 on D+3, as follows: (1) Control: saline solution (2 mL, i.m.) on D-2 and on D+3 ($n = 104$); (2) eCG: 400 IU (i.m.) of eCG (SincroeCG, Ouro Fino Saúde Animal, Cravinhos, Brazil) on D-2 and saline solution (i.m.) on D+3 ($n = 93$); (3) P_4: saline solution (i.m.) on D-2 and 600 mg (i.m.) of injectable progesterone (Sincrogest Ouro Fino Saúde Animal, Cravinhos, Brazil) on D+3 ($n = 106$); (4) eCG + P_4: 400 IU (i.m.) of eCG on D-2 and 600 mg (i.m.) of injectable progesterone on D+3 ($n = 95$).

2.4. Blood sampling and progesterone analysis

Blood samples were collected from a subgroup of cows to quantify serum progesterone immediately before (D+3, $n = 15$/group) and 24 h after (D+4, $n = 15$/group) the P_4 injection and 13 days after AI (D+13, Control: $n = 39$, eCG: $n = 39$, P_4: $n = 38$ and eCG + P_4: $n = 38$). Samples were collected from different cows on each day of collection. Samples were obtained by coccygeal venipuncture

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