J. Dairy Sci. 89:2056–2064

Embryo Survival from Gossypol-Fed Heifers after Transfer to Lactating Cows Treated with Human Chorionic Gonadotropin

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ABSTRACT

Objectives were to determine the effects of gossypol exposure during early embryo development on embryonic survival after transfer of frozen and thawed embryos to lactating dairy cows treated with human chorionic gonadotropin (hCG). Holstein cows (n = 269) were either treated or not treated with 3,300 IU of hCG on d 5 of the estrous cycle and received an embryo collected from heifers fed or not fed gossypol. Embryo donor heifers consumed either 0 or 12 g/d of free gossypol for 76 d prior to embryo collection, resulting in mean plasma gossypol concentrations of 0 and 7.38 μg/mL, respectively. Embryos were transferred on d 7 of the estrous cycle and pregnancy diagnosed 21 and 35 d later. Progesterone was analyzed in plasma collected on d 5 and 12 of the estrous cycle. Treatment with hCG increased the total luteal area on d 12 (818.0 vs. 461.1 mm^2) because of increased number of corpora lutea (2.0 vs. 1.0) and increased area of the original corpora lutea (522.7 vs. 443.5 mm^2). Plasma progesterone concentrations were similar between treatments on d 5, but increased by d 12 in hCG-treated cows (6.46 vs. 4.78 ng/mL). Pregnancy rates on d 28 and 42 were not affected by hCG. However, after transfer into lactating cows, embryos collected from heifers not fed gossypol resulted in higher pregnancy rates at 28 d (33.3 vs. 23.1%) and 42 d (29.6 vs. 20.2%) of gestation compared with embryos collected from heifers fed gossypol. Our data suggest that the negative effects of gossypol on fertility are mediated by changes in embryo viability in spite of similar grade quality at transfer.

Key words: gossypol, human chorionic gonadotropin, embryo survival, dairy cow

INTRODUCTION

Cottonseed is commonly fed to cattle as a source of protein, fat, and fiber, but it contains gossypol, a toxic yellow pigment (a polyphenolic binaphthyl dialdehyde) found primarily in the pigment glands of the seed (Ponday and Thejappa, 1975). Gossypol binds to the lipid portion of cell membranes (Reyes et al., 1984) and disrupts mechanisms of energy generation (Abou-Donia and Dieckert, 1974). It has been shown to decrease anion transport and glucose uptake and increase free radical generation (Reyes et al., 1984; Kanwar et al., 1990), which may affect early embryo development in vitro (Zirkle et al., 1988; Brocas et al., 1997). Feeding different amounts of gossypol from different types of cottonseeds raised total plasma gossypol concentrations above 5 μg/mL in lactating dairy cows (Santos et al., 2003) and Holstein heifers (Villaseñor et al., 2003). The increase in plasma gossypol negatively affected fertility of lactating dairy cows (Santos et al., 2003) and embryo quality in vivo (Randel et al., 1996) and in vitro (Brocas et al., 1997). Furthermore, Villaseñor et al. (2003) observed that, despite similar grade quality of embryos collected on d 5 after AI, those from heifers fed gossypol had fewer blastomeres and retarded development in vitro. Therefore, embryos collected from heifers fed gossypol may lead to lower fertility after embryo transfer.

Luteal activity has been associated with capacity of embryos to secrete IFN-τ and block the luteolytic cascade (Mann and Lamming, 2001). Administration of human chorionic gonadotropin (**hCG**) during the early luteal phase induced ovulation of the first-wave dominant follicle, led to the formation of a functional accessory corpus luteum (**CL**), increased progesterone concentration in plasma (Diaz et al., 1998), and increased pregnancy rates in beef cows after embryo transfer (Nishigai et al., 2002) and in dairy cows after AI (Santos et al., 2001). Therefore, hCG may improve embryo survival in recipient dairy cows by enhancing luteal function.

We hypothesized that embryos collected from gossypol-fed donor heifers would result in lower embryonic survival after transfer into recipient lactating dairy cows, and that treatment of recipient cows with hCG would enhance luteal function and improve pregnancy rates. The objective of this study was to determine the effect of exposure to gossypol during early embryo development in donor heifers on embryonic survival after transfer of frozen and thawed embryos to lactating dairy cows treated with hCG on d 5 of the estrous cycle.

Received November 30, 2005.
Accepted January 17, 2006.
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MATERIALS AND METHODS

Animals, Housing, and Feeding

The University of California–Davis Institutional Animal Care and Use Committee approved all procedures involving animals. Two hundred sixty-nine lactating Holstein cows (93 primiparous and 176 multiparous) from a high-producing commercial dairy farm located in the Central Valley of California were enrolled in the study at 70 ± 3 d postpartum (study d 0) from August 2002 to February 2003, and the study was completed in April of 2003. The lactating herd size during the study was 880 cows and the 3.5% FCM rolling-herd average was 11,590 kg/cow.

Cows were housed in freestall barns equipped with fans and sprinklers that were activated during the hot months of the year when environmental temperature rose above 26°C. Cows were enrolled during periods of heat stress (August to September 2002) or thermoneutral temperature (October 2002 to February 2003). The mean (± SD) daily average and daily maximum temperatures for the heat stress and thermoneutral periods were 25.2 ± 2.8°C and 34.4 ± 4.0°C, and 12.7 ± 3.9°C and 18.5 ± 5.3°C, respectively. Daily average temperatures ranged from 18.3 to 29.4°C and from 2.8 to 26.1°C for the heat-stress and thermoneutral periods, respectively.

Primiparous and multiparous cows were housed in the same barn, but in separate pens throughout the study and were fed the same diet as a TMR, twice daily, to meet or exceed the dietary requirements for a lactating cow weighing 680 kg and producing 45 kg of 3.5% FCM (NRC, 2001). Cows were milked twice daily and production was measured for individual cows once monthly during the official California DHIA milk test performed by the DHIA laboratory in Hanford, CA. Milk yields during the first 3 mo postpartum were used to assess the effects of milk yield on reproductive responses. All recipient cows had their BCS evaluated using a 5-point (1 = thin to 5 = fat) scoring system (Ferguson et al., 1994) at study enrollment.

Diets, Superovulatory Treatments, Embryo Collection, and Freezing

Holstein heifers (n = 81) were randomly assigned to consume either 0 or 12 g/d of free gossypol during 76 d before embryo collection, which resulted in mean plasma gossypol concentrations of 0 and 7.38 μg/mL. Heifers were housed in open corrals, and diets were offered as component-fed, with concentrate fed once daily separately from the forage. The forage component of the diet, a blend of alfalfa and wheat hay (2:1), was offered for ad libitum intake. Concentrates were offered at 2.2 kg of DM/heifer per day and were formulated to be isonitrogenous and isocaloric, but to vary in the amount of free gossypol content by adding cracked Pima cottonseed (*Gossypium barbedense*). Heifers in the no-gossypol treatment (n = 40; 0 g/d of free gossypol) received a concentrate containing (DM basis) 44.0% almond hulls, 30.0% soybean meal, 15.0% beef tallow, 6.7% monensin supplement to supply 200 mg of monensin, and 4.3% mineral and vitamin supplement. Heifers in the gossypol treatment (n = 41; 12 g/d of free gossypol) received a concentrate containing (DM basis) 55.0% cracked Pima cottonseed, 10.0% steam-flaked corn, 16.0% almond hulls, 5.0% soybean meal, 3.0% beef tallow, 6.7% monensin supplement to supply 200 mg of monensin, and 4.3% mineral and vitamin supplement. Diets were offered to meet the nutrient requirements of Holstein heifers weighing 400 kg and gaining 0.7 kg/d (NRC, 2001), considering the daily average forage intake of 4.5 kg/heifer based upon daily group intake.

Concentrates and hays were sampled weekly, dried at 55°C for 48 h, ground in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA), frozen, and later analyzed for DM at 105°C, as well as OM, ether extract, CP, NDF, and ADF. The composition of the concentrates for the no-gossypol and gossypol treatments were, respectively, 21.3 and 21.5% CP, 17.9 and 18.8% ether extract, 12.4 and 19.9% ADF, and 3.45 and 3.54 Mcal of ME/kg (NRC, 2001). Cottonseed was analyzed for free and total gossypol content in decorticated seeds as described previously (Mena et al., 2001). Pima cottonseed contained 1.10% total gossypol and 1.03% free gossypol, with a ratio of 47.1 to 52.9% plus and minus isomers, respectively.

A blood sample (20 mL) was collected from each heifer the day before uterine flushing by puncture of the median coccygeal vein or artery using heparinized Vacutainer (Becton Dickinson and Co., Franklin Lakes, NJ) tubes. Blood tubes were immediately placed in ice and transported to the laboratory within 1 h of collection. Tubes were centrifuged at 2,000 × *g* for 10 min at 10°C for plasma separation. The plasma was then frozen at –25°C and later analyzed for total gossypol and gossypol isomers by HPLC as described previously (Mena et al., 2001).

Six days after being observed in estrus, all heifers received a controlled intravaginal drug releasing (**CIDR**) insert containing 1.38 g of progesterone (EAZI-BREED, Pfizer Animal Health, New York, NY) and an i.m. injection of 2 mg of estradiol benzoate (β-estradiol 3-benzoate, E-8515, Sigma Chemical Co., St. Louis, MO) 24 h later. The superovulatory treatment was initiated 4 d after the injection of estradiol benzoate with decreasing, twice-daily doses of FSH (300 mg/heifer, Folltropin-V, Vetrepharm Inc., Canada) during 4 d. Two