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Administration of Human Chorionic Gonadotropin at Embryo Transfer Induced Ovulation of a First-Wave Dominant Follicle and Increased Progesterone and Transfer Pregnancy Rates

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Introduction

Embryo transfer (ET) has become more widespread in recent years as a way to improve cattle genetics. According to the annual statistical survey of the American Embryo Transfer Association, more than 200,000 fresh and frozen bovine embryos were transferred in 2008. But despite advancements in reproductive technologies that have occurred since ET was commercialized in the 1970s, industrywide pregnancy rates are only 62.4 and 56.9% for fresh and frozen-thawed ET, respectively. Using ET helps avoid problems from failed fertilization; however, fertilization failure has been characterized as a relatively unimportant factor of pregnancy loss. Approximately 10% of pregnancy failures resulted from fertilization failure and another 10% from failed embryo development. Approximately 20 to 25% of the pregnancy loss in an ET program could be characterized as early embryonic loss.

Use of supplemental progesterone has been shown to reduce early embryonic loss and enhance growth of the early embryo. Post-artificial insemination (AI) supplementation with progesterone also increased pregnancy rates. In contrast to those studies, use of a controlled intravaginal drug release (CIDR) insert to supplement progesterone in ET recipients post-transfer was not effective in reducing early embryonic loss.

Use of human chorionic gonadotropin (hCG) to stimulate ovulation of ovarian follicles to form accessory corpora lutea (CL) has been reported to increase circulating progesterone concentrations and increase pregnancy rates when administered during the post-breeding early luteal phase. More recent studies have determined that administration of 1,000 IU hCG was sufficient to ovulate a follicle. We recently administered 1,000 IU hCG to beef cows 7 days post-AI and observed formation of accessory CL and an increase in progesterone concentrations 14 days post-AI. Further, hCG administration to ET recipients at the dosage of 1,500 IU on either day 5 or 6 post-estrus has produced results ranging from no improvement to greater pregnancy rates than controls.

The objectives of this study were to: (1) monitor recipients for formation and retention of accessory CL, (2) determine if the circulating progesterone concentrations of pregnant recipients that received hCG were greater than those in control recipients, and (3) determine if hCG would reduce early embryonic loss (i.e., between transfer and first

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pregnancy diagnosis). We hypothesized that administration of hCG to recipients at ET would induce accessory CL, increase circulating progesterone concentrations, and reduce early embryonic loss.

Experimental Procedures

Mature beef cows at three locations (n = 719) received embryos approximately 7 days post-estrus if they had palpable CL. Fresh (n = 160) or frozen-thawed (n = 559) grade 1 or 2 embryos and transfers were made according to standard techniques as described by the International Embryo Transfer Society (IETS Manual, third edition, Champaign, IL). Embryo transfers were performed by 2 experienced veterinarians (Cross Country Genetics North, Inc., Westmoreland, KS) at Locations 1 and 2. Transfers at Location 3 were performed by a single experienced technician (Advanced Reproductive Associates, Daphne, AL). A single embryo was transferred to the uterine horn of the recipient ipsilateral to the CL. At the time of transfer, recipients alternately received either 1,000 IU hCG (1 mL, Chorulon; Intervet, Inc., Millsboro, DE) or 1 mL saline. Recipients were assigned a body condition score (BCS; 1 = thin and 9 = very fat). Pregnancy diagnoses were performed by transrectal ultrasonography (5.0 MHz linear-array transducer; Aloka 500 V; Corometrics Medical Systems, Inc., Wallingford, CT) at 35 and 65 days (mean) post-estrus.

Blood samples were collected from a coccygeal vessel at both pregnancy diagnoses in pregnant cows at Locations 1 and 2. Blood samples were refrigerated overnight and centrifuged the following morning. Blood serums were frozen at -4° F until the assay for progesterone was performed by radioimmunoassay.

Results and Discussion

Embryo transfer pregnancy rates did not differ among locations. Factors that significantly affected ET pregnancy rates at the first pregnancy diagnosis of 719 recipients are shown in Table 1. Treatment with hCG ($P=0.026$) at ET and transfer of fresh embryos ($P=0.016$) increased the likelihood of pregnancy at the first diagnosis. Further, recipients having BCS >5 at the time of transfer tended ($P=0.074$) to have greater pregnancy rates than recipients having BCS ≤5.

Positive additive effects of hCG treatment, BCS, and embryo type are illustrated in Figure 1. Within BCS class (≤5 vs. >5), recipients receiving fresh embryos always had greater transfer pregnancy rates, and hCG treatment produced greater transfer pregnancy rates in all embryo type-BCS classes except for cows of greater BCS receiving frozen embryos.

Serum progesterone concentrations in pregnant cows were greater ($P<0.05$) in hCG-treated recipients than recipients treated with saline at the time of the first (8.1 ± 0.9 vs. 6.1 ± 0.8 ng/mL) and second pregnancy diagnosis (8.7 ± 0.9 vs. 6.5 ± 0.7 ng/mL). Ovaries of pregnant recipients (n = 59) from Location 1 were monitored for the number of luteal structures at the time of both pregnancy diagnoses. All occurrences of accessory CL at the time of pregnancy diagnosis (20 of 29; 68.9%) on day 32 were detected in the hCG treatment. Nineteen multiple ovulating cows each had 1 accessory CL, and 1 cow formed 2 accessory CL. The proportion of recipients having 1 or more accessory CL was greater ($P<0.001$) after hCG treatment (68.9%) than after saline (0%).

In summary, administration of hCG to ET recipients at the time of transfer increased the incidence of accessory CL, increased serum progesterone concentrations, and increased transfer pregnancy rates. Assuming equal viability of embryos transferred to cows receiving hCG or saline, the increased transfer pregnancy rates were interpreted to indicate that increased progesterone resulting from hCG-induced ovulation reduced early embryonic losses after transfer of embryos to recipients. As expected, pregnancies were more likely in recipients that received fresh embryos than in cows that received frozen-thawed embryos. The tendency for cows in better body condition (BCS >5) to have greater transfer pregnancy rates reiterates the importance of properly managing the nutrition program for the recipient herd. Monitoring BCS could help predict the success of an ET. The positive effects of hCG treatment helped improve transfer rates resulting from transfer of fresh embryos and improved BCS of recipients.

Implications

Administering 1,000 IU hCG (1 mL Chorulon) to ET recipients at the time of ET increased transfer pregnancy rates.

Table 1. Factors affecting embryo transfer pregnancy rates

Item	n	Pregnancy rate, %	AOR (95% CI)[a]	P-value
Treatment				
Saline	358	53.9	Referent	
hCG	361	61.8	1.40 (1.04-1.89)	0.026
Embryo type				
Frozen	559	55.5	Referent	
Fresh	160	66.3	1.57 (1.08-2.27)	0.016
BCS				
≤5	454	55.3	Referent	
>5	265	62.3	1.33 (0.97-1.82)	0.074

[a] AOR = adjusted odds ratio; CI = confidence interval. An example of how to interpret an odds ratio: Treatment with hCG was 1.4 times more likely to increase transfer pregnancy rates than saline (referent).

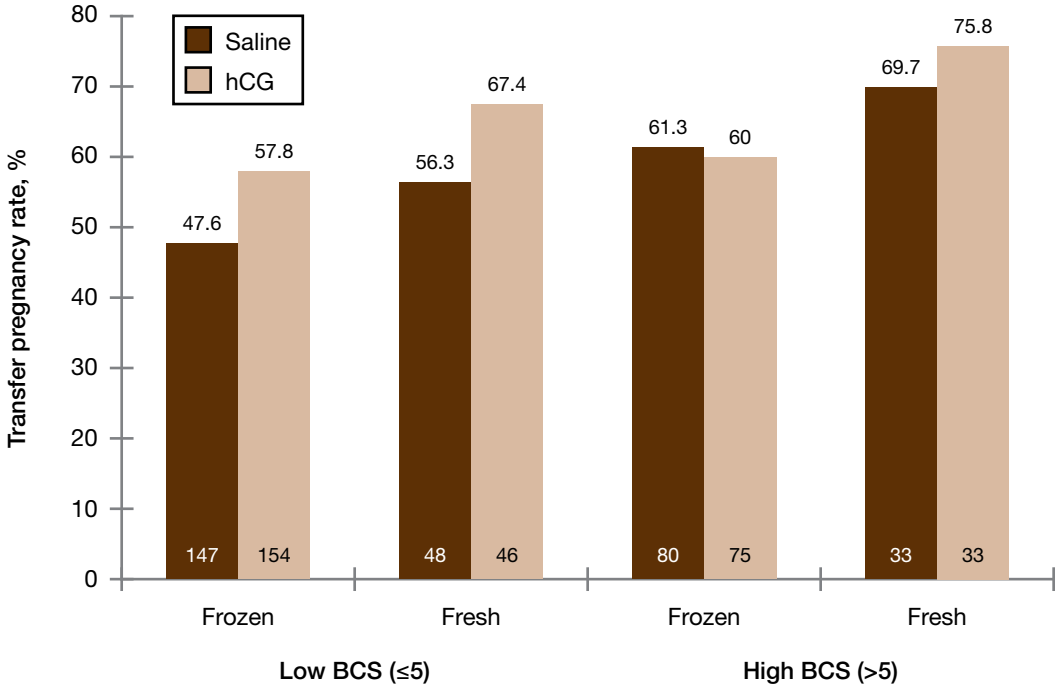

Figure 1. Additive effects of hCG treatment at embryo transfer, body condition score (BCS), and type of embryo transferred on transfer pregnancy rates in beef cow recipients. Numbers in bar boxes represent the number of recipients treated with either saline or 1,000 IU hCG at embryo transfer.