ADMINISTRATION OF HUMAN CHORIONIC GONADOTROPIN TO EMBRYO TRANSFER RECIPIENTS INCREASED OVULATION, PROGESTERONE, AND TRANSFER PREGNANCY RATES

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Abstract

We hypothesized that administration of human chorionic gonadotropin (hCG) to recipients at embryo transfer (ET) would induce accessory corpora lutea (CL), increase circulating progesterone concentrations, and reduce early embryonic loss. At three locations, purebred and crossbred Angus, Simmental, and Hereford recipients (n = 719) were assigned alternately to receive i.m. 1,000 IU hCG or 1 ml saline (control) at ET. Fresh or frozen-thawed embryos were transferred on d 5.5 to 8.5 (median = d 7) of the estrous cycle to recipients having a palpable CL. Recipients received a body condition score (BCS) at ET. Pregnancy diagnoses occurred by transrectal ultrasonography 28 to 39 d (median = d 35) and reconfirmed 58 to 77 d (median = d 67) post-estrus. At one location (n = 108), ovaries were examined to count the number of CL at pregnancy diagnosis. More ($P < 0.001$) pregnant hCG-treated cows (69.0%) had multiple CL than pregnant controls (0%). Serum progesterone (ng/mL) determined at two locations (n=471) at both pregnancy diagnoses in pregnant cows was greater ($P \leq 0.05$) after hCG treatment than in controls (first: 8.1 ± 0.9 vs. 6.1 ± 0.8; second: 8.8 ± 0.9 vs. 6.6 ± 0.7), respectively. Transfer pregnancy rates were analyzed using logistic regression. Unadjusted pregnancy rates at the first diagnosis was 61.8 vs. 53.9% for hCG vs. controls. At the second diagnosis, pregnancy rates were 59.0 vs. 51.4%, respectively. Factors affecting pregnancy rates were treatment ($P = 0.03$), embryo type ($P = 0.02$), and BCS ($P = 0.08$). Odds ratios indicated that greater pregnancy rates occurred in recipients receiving hCG treatment, receiving a fresh embryo (66.3 vs.55.5%), and when BCS >5 vs. ≤5 (62.3 vs. 55.3%). We concluded that hCG at ET increased incidence of accessory CL, increased progesterone in pregnant recipients, and increased transfer pregnancy rates.

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Chapter 1: Review of Literature

1. Introduction

The report of the 2008 statistics from the American Embryo Transfer Association (AETA) indicated that more than 200,000 fresh and frozen bovine embryos were transferred by companies that participated in the survey (www.aeta.org). The 2006 report stated that pregnancy rates from the fresh and frozen transfers were 62.4 and 56.9%, respectively. It should be noted that the AETA believes that more than 90% of the rates are estimated because actually pregnancy data are difficult to acquire. Further, it is not common for the practitioner to be paid by the confirmed pregnancy.

Looney et al. (2006) stated that procurement and upkeep of recipient females is the most costly aspect of the embryo transfer (ET). A possible way to reduce the cost of ET would be to increase pregnancy rates so not as many recipients are required to produce a calf crop. One suggested application that may increase pregnancy rates would be to supplement the recipient with exogenous progesterone after ET. The potential for this method to increase pregnancy is the critical need for progesterone for establishment and maintenance of pregnancy.

Progesterone has been reported to have a positive effect of growth on the newly implanted embryo in numerous studies (Garrett et al., 1988; Mann et al., 1999; Mann et al., 2006). When progesterone was supplemented from d 2 to 5 post-breeding larger embryos were observed in the treated cows than in controls by d 15 post-breeding (Garrett et al., 1988). In addition, Mann et al. (2006) reported that cows supplemented with progesterone from d 5 to 9 post artificial insemination (AI) by using a progesterone-releasing intravaginal control internal drug release (CIDR) insert had longer conceptuses than control cows. It also was reported that

cows either supplemented or had naturally elevated progesterone had greater serum concentrations of interferon-tau (INF-τ) than control cows or cows having reduced concentrations of progesterone (Mann et al., 1999; 2006). In contrast to the previous reports, ET recipients whose plasma progesterone concentrations were measured at time of transfer (6.5 to 8.5 d post estrus) had similar transfer pregnancy rates as observed in recipients with less than 3.35 ng/mL and recipients that had greater than 4.5 ng/mL (Spell et al., 2001). This similarity in progesterone indicates that corpus luteum (CL) development and progesterone secretion are variable among individual cow and may change rapidly during the early luteal phase.

Mann et al. (1999) reported that INF-τ was first detected in the uterus 14 to 16 d post conception. Secretion of INF-τ is necessary to block the secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) that normally lyses the corpus luteum (CL) and ceases production of progesterone. Some cows seem to be unable to respond to the INF-τ even when multiple embryos are implanted, which should increase pregnancy establishment (Berg et al., 2010). In those cows, the uterine environment was not fit to establish pregnancy and was that way even before the time of transfer. Further, cows with lower circulating progesterone concentration had more pregnancy loss than in cows that maintained pregnancy (Mann and Lamming, 1999).

In addition to changes in the growth of the embryo, differences in the uterine environment have been observed in cows supplemented with progesterone post-breeding. Changes in the uterus are mostly attributed to secretion of more proteins in the presence of supplemental progesterone. These secretions are not the result of any additional or new proteins, but rather an increase in secretion of proteins that were already present in the uterine lumen, which are necessary for establishing pregnancy (Garrett et al., 1988). Recipient ewes supplemented with progesterone from d 4 to 9 of the estrous cycle had increased secretion of 10

of the 30 proteins that are present in the uterine environment (Ashworth and Bazer, 1989). Thatcher et al. (1994) reported that the uterine response to progesterone from that produced by the CL or by an exogenous source, provided an environment that is necessary for the development of the embryo and maintenance of pregnancy. This change in uterine environment via progesterone also allowed for embryos that are earlier in development than the uterus of the recipient to be as viable as embryos that are transferred in synchrony with the uterus of the recipient. This ability of the uterus to support embryos earlier in development was observed when ET recipients were treated with progesterone from d 1 to 4 of the cycle and then transferred a d 5 embryo to a cow that was at d 8 of the cycle (Geisert et al., 1991). Spell et al. (2001) observed that recipients classified in 12-h periods from −12 to +24 h of synchrony with the donor all had similar transfer pregnancy rates.

Enhancement of the uterine environment during early pregnancy of the cow has led to the hypothesis that pregnancy rates could be increased in cows with supplemental progesterone early in embryonic development. This hypothesis that supplemental progesterone is favorable for improved embryo development has led to many studies looking at effects of supplemental progesterone on pregnancy rates after either natural service or AI (Table 1.1). When those combined results were analyzed, progesterone supplementation from d 0 to 16 of the cycle, resulted in an average increase in pregnancy rate of 5.2% points (Table 1.2). Pregnancy rates were 10.3% points greater for cows treated with progesterone less than d 6 compared with those treated after d 6 of the cycle. Further, when control cows had conception rates < 50% were compared with controls having conception rates > 50%, cows that received supplemental progesterone had a much greater increase in fertility than control cows having conception rates < 50% (Table 1.2).

Improvement in early pregnancy rates is not the only positive effect on fertility that has been reported after progesterone supplementation in the bovine. Whereas improvement in pregnancy rates may have defined early embryonic loss, later losses also could be avoided by supplementing progesterone. Insertion of a progesterone-releasing intravaginal device (PRID) in pregnant cows between d 38 and 42 post-AI significantly reduced pregnancy loss up to d 90 of pregnancy from 12% in control cows to 5.3% in treated cows (Lopez-Gatius et al., 2004).

When looking at progesterone supplementation to ET recipients, the results were not as consistent as those reported for cows after AI. An increase of 12% points in conception rates were reported when ET recipients received a CIDR for 14 d post-ET, but this percentage did not differ significantly from that of controls because of inadequate replication (Looney et al., 2006). Corroborating results were not observed when recipients received a CIDR to supplement progesterone for 13 d post-ET because transfer pregnancy rates were actually less in supplemented cows compared with controls (65.3 vs. 69.6%; Purcell et al., 2005). These results in ET recipients fail to support those after AI where progesterone had a positive effect on the uterine environment and embryo development leading to improved pregnancy rates.

Another problem detected in cattle on high feed intake is increased metabolism of progesterone. In attempting to get ET recipients to appropriate body condition that would provide adequate nutrition for the cow and growing conceptus, increased rate of metabolism of progesterone could become a limiting factor leading to embryonic loss. In lactating dairy cows, ad libitum feed intake reduced circulating progesterone concentrations, even in the presence of more luteinizing hormone (LH) pulses (Wiltbank et al., 2006). Reduced progesterone concentration was attributed to increased liver blood flow and increased steroid metabolism

(Sangsritavong et al., 2002), and was thought to reduce fertility in lactating dairy cows (Wiltbank et al., 2006).

2. Structure and Function of Human Chorionic Gonadotropin (hCG)

Use of human chorionic gonadotropin (hCG) to improve fertility and induce ovulation is not a novel application because its use has been applied to those specific functions in cattle since the 1960's (Wiltbank et al., 1961). During that period of research the structure and function of hCG was not fully known, just the physiological effects that it had on the reproductive cycle of the cow. It was known that hCG was produced in pregnant human females and had ovulatory-inducing activity. It is produced beginning 11 d after fertilization in the pregnant human female by the trophoblastic cells of the rudimentary placenta, with peak concentrations detectable from 56 to 86 d and maintained at lesser concentrations throughout pregnancy (Knobil and Neill, 1998). Now it is known that hCG is a glycoprotein consisting of alpha and beta subunits, containing 92 and 145 amino acids, respectively. The alpha subunit is common to LH, follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH; Iles and Chard, 1993). The key difference among the three glycoproteins is that each hormone contains a unique beta subunit, thus endowing each hormone with different functional and binding capabilities.

The component that renders hCG capable of inducing ovulation is that 85% of the first 115 amino acids of its beta subunit are homologous with LH and amino acids 38 to 57 of the beta subunit are what binds to the LH receptor (LHR; Knobil and Neill, 1998). This structural component allows hCG to bind to the LHR on the theca and granulosa cells of the ovulatory follicle and the small luteal cells of the CL. Rao (1974) noted that hCG has a greater binding affinity than LH for the gonadotropin receptor of the bovine CL.

3. Duration of Action of hCG

In the production setting, hCG has the approved label application in reproduction as a treatment for dairy cattle that are experiencing ovarian disorders, specifically follicular cysts. The definition of a follicular cyst is a follicle-like structure that is greater than 25 mm in diameter and is present for at least 10 d in the absence of a CL (De Rensis et al., 2008). The reason that hCG has the ability to be used for treatment of cysts is that it has a greater half-life or duration of action than LH once injected i.m. In humans, the half-life of hCG is 29 to 30 h (Knobil and Neill, 1998). Heifers that were injected with gonadotropin-releasing hormone (GnRH) had peak plasma concentrations of LH by 1 to 2 h before they returned to baseline by 6 h post-injection. When injections of hCG were administered, however, hCG was elevated in plasma for 10 h post-injection (Seguin et al., 1976). More recently, Schmitt et al. (1996) reported that hCG was still detectable in circulating blood plasma for 66 h when cows were injected with 3,000 IU hCG.

The reason for this greater half-life of hCG in circulation is in the chemical structure of the beta subunit. The beta subunit contains multiple sialic acid side chains that block the liver receptors from recognizing hCG. When the sialic acid was removed from the structure of hCG, however, hCG was metabolized by the liver in only a few minutes (Knobil and Neill, 1998).

A key benefit of the greater half-life of hCG is that it binds to the LHR for a longer duration than LH. Niswender et al. (1985) reported that ovine hCG-LHR complexes had less mobility than LH-LHR complexes. This concept explains the reduced internalization rate and explained the prolonged steroidogenic activity of hCG-LHR vs. the LH-LHR in the ovine luteal cell.

4. Physiological Effects of hCG

4.1. Ovarian Follicle

Through past research it has been reported that hCG binds to the LHR of the granulosa cell of the ovarian follicle and has the same action as that of LH (Rao, 1973; Iles and Chard, 1993; Cole, 1997). Through this common mode of action hCG is an effective treatment to cause ovulation of an ovarian follicle. Another unique feature of hCG is that it can bind to the LHR after injection and delivery to the ovary, whereas injection of GnRH must illicit a surge of LH from the anterior pituitary before affecting follicular function. Although a universal dose and time of the estrous cycle for the administration of hCG has not been standardized, many different scenarios are effective to ovulate ovarian follicles (Table 1.3).

The day of the estrous cycle, or more specifically, the maturity of associated ovarian follicle when hCG is administered, can determine whether the follicle will ovulate. In cattle, follicular growth occurs in 2 or 3 waves, with an ovulatory-size follicle generally present on d 5 to 10 and d 13 to 16 of the cycle. The follicle present at these times is somewhat similar to the follicle that undergoes spontaneous ovulation at d 0 of the estrous cycle. Price and Webb (1989) administered 1,500 IU hCG i.m. at four different periods of the estrous cycle in beef heifers: d 0, 1, 2, or 3; d 4, 5, 6, or 7; d 8, 9, 10, 12, or d 13; d 14, 15, or 16. During the early (d 4 to 7) and late luteal phase (d 13 to 16), 10 of 12 and 4 of 6 follicles ovulated, respectively. Most recently, administration of hCG during the first follicular wave (early luteal phase) is the most common and effective time to induce ovulation (Nishigai et al., 2001; Santos et al., 2001; Nishigai et al., 2002; Stevenson et al., 2007; Dahlen et al., 2010).

Many different doses from 500 to 5,000 IU hCG have been utilized in trials investigating ovulation of follicles (Tables 1.3 and 1.4). Differences in doses among studies make it difficult to interpret differences in the reported induced ovulation responses. Fortunately, two studies

have reported hCG dose titrations for ovulation induction in lactating beef (Burns et al., 2008) and dairy cattle (Buttrey et al., 2010). In these two studies 500, 1,000, 2,000, and 3,000 IU hCG were tested. In beef cattle (Burns et al., 2008), 500 IU hCG was found to be as effective as 100 μg GnRH, but the 1,000 IU hCG was recommended because of its relative ease of application (0.5 ml [500 IU] compared with 1.0 ml [1,000 IU]). Buttrey et al. (2010) reported that 1,000 IU hCG produced the greatest percentages of ovulations compared with 500, 2,000 and 3,000 IU (85.7% vs. 35.7, 56.3, or 69.2%), respectively.

4.2. Corpus Luteum

Effects of hCG are not limited to the ovulatory follicle alone. Because LHR are present on the small luteal cells (SLC) of the CL, hCG can alter CL function. When ewes were treated with hCG during the early luteal phase of the estrous cycle, an increased proportion of LLC to SLC and a heavier CL were detected compared with those in control ewes (Farin et al., 1988). Increase in the proportion of LLC to SLC indicated that hCG binds to the LHR of the SLC and caused further differentiation of SLC to LLC. The LLC produce 80% of the progesterone, so it is thought that progesterone biosynthesis would be greater after hCG treatment because of the increased proportion of LLC. Administration of hCG to cows on d 5 to 7 of the estrous cycle (early luteal phase) produced CL that were larger in diameter than the control cows by d 8 to 14 of the cycle (Rajamahendran and Sianangama, 1992; Nishigai et al., 2001; Santos et al., 2001). Greater serum progesterone concentrations also were observed in the hCG-treated cows than in control cows at 7 (Stevenson et al., 2007), 6 to 1l (Santos et al., 2001), or 14 d post treatment (Rajamahendran and Sianangama, 1992). This increase in progesterone could be attributed to ovulation of follicles and formation of new CL, growth of the existing CL, or both.

4.3. Estrous Cycle Duration

Administration of hCG to cattle during the luteal phase of the estrous cycle generally lengthened the estrous cycle. The cycle was extended when 1,000 to 10,000 IU were injected once between d 2 and 14 of the estrous cycle (Table 1.5), but no cycle extension occurred when hCG was administered on d 17 (Seguin, 1977).

4.4. Blood Flow

A lesser investigated aspect, but one that would be expected to change with the increased CL size and greater biosynthesis of progesterone, is blood flow to the CL under the influence of hCG. Beindorff et al. (2009) administered 3,000 IU hCG to cows on d 7 of the estrous cycle and measured immediate post-treatment luteal blood flow. Treated cows had a 51% increase in blood flow by 1 h post-hCG treatment and blood flow decreased to baseline thereafter. Progesterone concentrations also were increased by 31% after 1 h and 81% by 48 h after hCG administration, whereas no changes were detected in control cows. Although increased blood flow occurred after administration of hCG, it was not sustained and may indicate that increased blood flow was not the sole causative factor explaining the increased progesterone concentration (Beindorff et al., 2009).

4.5. Progesterone Secretion

Previously in this review, the function of progesterone in establishing and maintaining pregnancy was described as a critical component for pregnancy maintenance. Having elucidated how hCG induces ovulation of follicles to produce accessory CL, it must be noted that in cows having spontaneously ovulated multiple follicles, serum progesterone concentrations did not differ from that in cows that spontaneously ovulated but one follicle (Starbuck et al., 2004). In

contrast, studies in which hCG was administered and accessory CL were formed, progesterone concentrations were greater in the cows treated with 1,000 to 3,300 IU hCG once on d 4, 5, 6, 7, 8, or 9 of the cycle than in control cows (Rajamahendran and Sianangama, 1992; Fricke et al., 1993; Schmitt et al., 1996; Diaz et al., 1998; Santos et al., 2001; Nishagai et al., 2002, Stevenson et al., 2007; Dahlen et al., 2010). These differences in progesterone also were reported in cows treated with 1,000 IU hCG daily on d 2, 3, and 4 or daily from d 2 to 7 (Helmer and Britt, 1987; Veenhuizen et al., 1971) and 1,500 or 3,300 IU on d 5 of the estrous cycle (Walker et al., 2005; Ideta et al., 2003) when the ovulatory status was not monitored.

Increased biosynthesis of progesterone from CL present at the time of hCG administration likely occurs in the SLC because only SLC have a functional LHR. This increase is likely quite small because the main CL source of progesterone production is the LLC. As indicated previously, increased concentrations of progesterone when hCG is administered can be linked to many factors: multiple CL, larger CL, or both. The overall effect of hCG administration is increased total luteal volume and the ability of greater progesterone biosynthesis (Tables 1.6 to 1.8).

5. Profertility Effects of hCG

5.1. Artificial Insemination

Use of hCG as a treatment to improve pregnancy rates in cattle post-AI has been implemented using different doses and on varying days post-AI in both beef cows, dairy cows, and heifers. The basis for these studies was that hCG would ovulate a follicle during the luteal phase of the estrous cycle or early pregnancy, induce the formation of accessory CL, and lead to greater circulating progesterone concentrations. Doses ranging from 1,000 to 3,300 IU hCG

were administered from d 0 to 14 after estrus or post-AI (Table 1.9) Wiltbank et al. (1961) administered daily a dose of 1,000 IU from d 15 to 35 post AI. Variations in dose and day of administration led to pregnancy rates that varied from 33.6% (5.2% points greater than control; Stevenson et al., 2007) to 78% (40% points greater than control; Rajamahendran and Sianangama, 1992). The overall trend of many of the studies is that hCG improved pregnancy rates, but the differences were not always sufficiently large to produce a statistical significance at the $\alpha = 0.05$ level (i.e., most studies were statistically underpowered).

5.2. Embryo Transfer

Another application of using hCG to improve fertility is to stimulate ancillary CL formation in ET recipients (Table 1.10). Massey et al. (1983) administered 5,000 IU hCG to crossbred beef cows 5 d post-ET and reported a numerical increase in pregnancy rates compared with controls (53.6 vs. 47.9%). More recently, 1,500 IU hCG were administered to recipients on d 5 (Ideta et al., 2005) or d 6 (Nishgai et al., 2002) post-estrus. The authors hypothesized in both studies that hCG would induce the formation of an accessory CL and increase progesterone concentrations, thus producing increased pregnancy rates. The results from their trials for hCG vs. controls were: 66.6 vs. 69.8% and 67.5 vs. 45.0% (Ideta et al., 2005; Nishgai et al., 2002), respectively.

5.3. Pregnancy Loss

Another application of hCG would be to reduce early embryonic loss (Table 1.11). This is assessed as pregnancies are lost from one pregnancy diagnosis to a later diagnosis. When hCG was administered either d 26 (beef cattle; Burns et al., 2008) or d 33 d post-AI (dairy cattle; Buttrey et al., 2010), a reduction in embryo losses was not observed compared with control cows

from the day of treatment to d 60 to 70 of pregnancy. In contrast, Santos et al. (2001) reported a tendency for hCG to reduce pregnancy loss from d 45 to 90 post-AI (4.9 % for hCG vs. 13.5% for control), but overall loss did not differ from that which included the entire period from d 28 to 90 post-AI.

6. Summary

Use of ET in cattle is becoming a rather sizable industry with many more producers utilizing this technology to improve the genetic quality of their cattle. With the increase in number of ET, several treatments have been attempted to increase progesterone concentrations in the recipients post-ET to possibly increase transfer pregnancy rates. The uterine environment seems to be altered by supplemental progesterone to better complement the growing embryo. Progesterone supplementation has been shown to increase pregnancy rates when used in most post-AI applications, but the results are less conclusive when used in ET recipients. Another avenue to increase progesterone concentrations is to administer hCG to induce ovulation and the formation of an accessory CL. This potentiality exists because hCG has similar actions as LH in that it binds to the LHR and induces ovulation of the dominant follicles. Circulating plasma or serum progesterone concentrations are increased after formation of accessory CL, increase in CL size, or both, which leads to greater biosynthesis of progesterone by the LLC. When hCG was administered post-AI, pregnancy rates were increased. In the few studies where hCG was administered on d 5, 6, or 7 of the estrous cycle in conjunction with ET, conflicting results exist for resulting transfer pregnancy rates. Action of hCG to induce ovulation of a dominant follicle, formation of accessory CL, and biosynthesize more progesterone is the basis of the present study. The hypothesis for the present research was that administration of hCG to recipient cows at the time of ET would induce accessory CL, increase circulating progesterone concentrations,

and reduce early embryonic loss between transfer and the first pregnancy diagnosis at approximately d 30 to 32 post-estrus.

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Table 1.1. Results of studies in which cattle were supplemented with progesterone after insemination.

Reference	Start day[a]	Treatment		Control		Effect[c]
		%[b]	n	%[b]	n	%
Herrick (1953)	0	5.0	20	35.0	20	30.0
Dawson (1954)	4	16.7	18	46.8	47	30.1
Wiltbank et al. (1956)	3	29.9	67	41.8	67	11.9
Johnson et al. (1958)	2-9	37.7	69	70.0	70	32.3
Sreenan and Diskin (1983)	5	45.0	20	73.7	19	18.7
Sreenan and Diskin (1983)	10	61.1	167	65.4	156	4.3
Diskin and Sreenan (1986)	5	40.0	65	47.5	61	7.5
Robinson et al. (1989)	5	30.0	30	60.7	28	30.7
Robinson et al. (1989)	10	30.0	30	59.3	27	29.3
Walton et al. (1990)	5	57.1	14	68.0	25	10.9
Macmillan et al. (1991)	10-16	67.0	628	64.3	493	–2.7
Macmillan et al. (1991)	14	63.6	472	64.0	514	0.4
Macmillan et al. (1991)	4	66.3	466	74.6	461	8.3
Stevenson and Mee (1991)	13	42.4	92	50.0	36	7.6
Van Cleef et al. (1991)	7	53.6	155	57.9	159	4.3
Larson and Butler (1995)	3	34.9	63	47.8	67	12.9
Mann et al. (1998)	10	53.3	135	56.0	134	2.7

Adapted from Mann and Lamming (1999).

[a] 0 = day of insemination.

[b] Percentage of the number (n) pregnant.

[c] Treatment minus control.

Table 1.2. Overall analysis of supplemental progesterone data from Table 1.1.

Group	Control % (n)	Treatment % (n)	Change in conception rate[a]	P-value
All	58.1 (1459/2511)	63.3 (1508/2384)	5.20%	< 0.001
Start < 6 d[b]	54.6 (406/743)	64.9 (503/775)	10.30%	NS[c]
Start > 6 d[b]	61.1 (1026/1679)	62.5 (949/1519	1.40%	< 0.001
Control <50%[d]	34.4 (124/362)	53.6 (207/386)	19.30%	< 0.001
Control >50%[d]	62.7 (1334/2129)	65.4 (1294/1978)	2.70%	NS

Adapted from Mann and Lamming (1999).

[a] Treatment minus control.

[b] Progesterone treatment started either < 6 d or > 6 d after insemination.

[c] Nonsignificant.

[d] Control pregnancy rates were either < 50% or > 50%.

Table 1.3. Ovulation incidence in dairy cattle administered hCG.

Reference	Animal type	n	hCG dosage (IU)	Treatment day[a]	Ovulation rate (%)
Buttrey et al. (2010)	Dairy cows	14	500	22 to 40 d P-AI	5/14 (36)
		14	1,000	22 to 40 d P-AI	12/14 (86)
		16	2,000	22 to 40 d P-AI	9/16 (56)
		13	3,000	22 to 40 d P-AI	9/13 (69)
Diaz et al. (1998)	Dairy heifers	6	1,000 i.v., 2,000 i.m.	5	6/6 (100)
Howard and Britt (1990)	Dairy heifers	16	5,000	10	13/16 (81)
Rajamahendran and Sianangama (1992)	Dairy cows	8	1,000	0 d P-AI	0/8 (0)
		9	1,000	7 d P-AI	7/9 (78)
		9	1,000	14 d P-AI	4/9 (44)
Santos et al. (2001)	Dairy cows	203	3,300	5 d P-AI	175/203 (86)
Schmitt et al. (1996)	Dairy heifers	5	3,000	5	5/5 (100)
		6	3,000	5	5/6 (83)
		5	3,000 + 8 µg GnRH	5	5/5 (100)
Sianangama and Rajamahendran (1996)	Dairy cows	12	1,000	7	12/12 (100)
Stevenson et al. (2007)	Dairy cows	40	3,300	4, 5, 6, 7, 8, or 9	24/40 (60)
		40	100 µg GnRH	4, 5, 6, 7, 8, or 9	31/40 (78)

[a] Day of the estrous cycle unless specified; P-AI = post artificial insemination.

Table 1.4. Ovulation incidence in beef cattle administered hCG.

Reference	Animal type	n	hCG dosage (IU)	Treatment day[a]	Ovulation rate (%)
Burns et al. (2008)	Beef cows	11	500	Random days of cycle	4/7 (57)
		10	1,000	Measure all F >5	6/9 (66)
		7	2,000		2/3 (66)
		7	3,000		3/6 (50)
Dahlen et al. (2010)	Beef cows	33	1,000	7 d P-AI	17/33 (52)
Fricke et al. (1993)	Beef cows	5	1,500 iv	6	5/5 (100)
Nishigai et al. (2001)	Beef cows	4	1,500	0	0/4 (0)
		5	1,500	5	5/5 (100)
Nishigai et al. (2002)	Beef cows	12	1,500	1	0/12 (0)
		12	1,500	6	11/12 (92)
Price and Webb (1989)	Beef heifers	19	1,500	0, 1, 2, or 3	2/19 (11)
		12	1,500	4, 5, 6, or 7	10/12 (83)
		20	1,500	8, 9, 10, 12, or 13	7/20 (35)
		6	1,500	14, 15, or 16	4/6 (66)
Wiltbank et al. (1961)	Beef heifers	39	1,000	15 to 35 d P-AI	23/39 (59)

[a] Day of the estrous cycle unless specified; P-AI = post artificial insemination.

Table 1.5. Duration of estrous cycles after post estrus hCG administration.

Reference	Animal type	n	hCG dosage (IU)	Treatment day[a]	Cycle length	P-value[b]
Diaz et al. (1998)	Dairy heifers	6	2,000, 1,000 i.v.	5	22.9	
		7	Control		22.1	
Helmer and Britt (1987)	Dairy heifers	9	1,000	2, 3, 4	20.8	0.01
		9	Control		19.6	
Howard and Britt (1990)	Dairy heifers	4	5,000	10	25.5	0.05
		4	Control		20.5	
Howard et al. (1990)	Dairy cows/heifers	4	10,000	10	25	0.1
		4	Control		20.7	
Price and Webb (1989)	Beef heifers	12	1,500	Once any day	1+ CL = 21.3[c]	NS[e]
		9	1500	Once any day	1 CL = 19.2[d]	NS
		4	Control		20.2	
Nishigai et al. (2001)	Dairy cows	4	1,500	5	26	
		4	Control		23	
Rajamahendran and Sianangama (1992)	Dairy cows	8	1,000	0	19.7	
		9	1,000	7	20.7	
		9	1,000	14	23.4	
		8	Control		21.4	
Seguin et al. (1977)	Dairy heifers	6	10,000	10	26.8	0.01
		6	Control		19.3	
		6	10,000	15	24.2	0.01
		6	Control		19.8	
		6	10,000	17	20.3	NS
		6	Control		20.2	
		5	10,000	10 or 11	25.8	0.01
		5	100 µg GnRH	10 or 11	20.2	NS
		4	Control		20.3	

[a] Day of the estrous cycle.

[b] Different from control.

[c] Treated animals with more than one CL.

[d] Treated animals with one CL.

[e] Nonsignificant.

Table 1.6. Progesterone concentrations of dairy cows administered hCG.

Reference	Animal type	n	hCG dosage (IU)	Treatment day	Sample day	P4 (ng/ml)	P-value[b]
Buttrey et al. (2010)	Dairy cows	14	100 µg GnRH	22 to 40 d P-AI	7 d post trt	5.3 ± 0.8	0.03
		14	500	22 to 40 d P-AI	7 d post trt	4.3 ±0.7	0.03
		14	1000	22 to 40 d PAI	7 d post trt	5.4 ± 1.0	0.03
		16	2000	22 to 40 d PAI	7 d post trt	5.8 ± 0.7	0.03
		13	3000	22 to 40 d PAi	7 d post trt	5.1 ± 0.8	0.03
		14	Control		7 d post trt	1.7 ± 1.5	
Rajamahendran and Sianangama (1992)	Dairy cows	8	1,000	at AI	Cycle d 18	5.3 ± 1.0	NS[e]
		9	1,000	7 d P-AI	Cycle d 18	7.4 ± 0.9	<0.05
		9	1,000	14 d P-AI	Cycle d 18	7.2 ± 0.8	<0.05
		8	Control		Cycle d 18	4.9 ± 0.8	
Santos et al. (2001)	Dairy cows	203	Prim 3,300[d]	5 d P-AI	11-16 d P-AI	23.57	0.02
			Control	5 d P-AI	11-16 d P-AI	14.83	
			Multi 3,300[e]	5 d P-AI	11-16 d P-AI	14.07	NS
			Control	5 d P-AI	11-16 d P-AI	12.87	
Sianangama and Rajamahendran (1992)	Dairy cows	20	1,000	at AI	18 d P-AI	8.6 ± 1.6	NS
		20	1,000	7 d P-AI	18 d P-AI	12.8 ± 1.2	NS
		20	1,000	14 d P- AI	18 d P-AI	10.7 ± 1.8	NS
		19	Control		18 d P-AI	6.4 ± 1.6	
		29	1,000	at AI	21 d P-AI	4.1 ± 1.4	NS
		32	1,000	7 d P-AI	21 d P-AI	13.4 ± 1.3	<0.01
		29	1,000	14d P-AI	21 d P-AI	10.3 ± 1.3	<0.01
		31	Control		21 d P-AI	5.7 ± 1.4	
Stevenson et al. (2007)	Dairy cows	40	1,500	4, 5, 6, 7, 8, or 9 d P-AI	7 d post trt	> than control	< .001
		41	Control		7 d post trt		

[a] Day of the estrous cycle unless otherwise specified; P-AI = post artificial insemination.

[b] Different from control.

[c] Nonsignificant.

[d] Primiparous: cows in first lactation.

[e] Multiparous: cows in 1+ lactations.

Table 1.7. Progesterone concentrations of dairy heifers administered hCG.

Reference	Animal type	n	hCG dosage (IU)	Treatment day[a]	Sample day[a]	P4 (ng/ml)	P-value[b]
Ideta et al. (2005)	Dairy heifers	32	1,500	5	15	5.34 ± 1.29	< 0.05
		33	Control		15	3.54 ± 1.16	
		32	1,500	5	5-15[c]	4.12 ± 1.33	< 0.05
		33	Control		5-15	2.52 ± 1.25	
Diaz et al. (1998)	Dairy heifers	6	3,000	5	9	15.3	<0.01
		7	Control		9	11.1	
			3,000	5	15	20.4	<0.01
			Control		15	12.9	
Howard and Britt (1990)	Dairy heifers	16	5,000	10	16	17.6 ± 1.7	0.05
		16	Control		16	10.7 ± 1.9	
Schmitt et al. (1996)	Dairy heifers	5	3,000	5	8-16[d]	18 ± 1.2	< 0.001
		4	Control		8-16	9.7 ± 1.2	

[a] Day of the estrous cycle.

[b] Different from control.

[c] Difference in progesterone from d 5 to 15.

[d] Difference in progesterone from d 8 to 16.

Table 1.8. Progesterone concentrations of beef cattle administered hCG.

Reference	Animal type	n	hCG dosage (IU)	Treatment day[a]	Sample day[a]	P4 (ng/ml)	P-value[b]
Dahlen et al. (2010)	Beef cows	32	1,000	7 d P-AI	14	6.8 ± 0.4	<0.05
			Control		14	5.4 ± 0.5	
		252	1,000	7 d P-AI	33	7.7 ± 0.3	< 0.05
			Control		33	5.8 ± 0.3	
Fricke et al. (1993)	Beef cows	5	1,500	6	8	4.9	< 0.05
		5	Control		8	3.0	
			1,500	6	13	9.9	<0.01
			Control		13	6.0	
Funston et al. (2005)	Beef heifers	165	3,300	8 d P-PGF	14 d P-PGF	8.6	< 0.01
		109	Control		14 d P-PGF	4.6	
		160	3,300	9 d P-PGF	14 d P-PGF	11.2	< 0.01
		101	Control		14 d P-PGF	5.6	
Walker et al. (2005)	Beef heifers	48	3,300	5 d P-AI	7 d P-AI	2.4	< 0.05
			Control		7 d P-AI	1.4	
		48	3,300	5 d P-AI	8 d P-AI	2.6	< 0.05
			Control		8 d P-AI	1.4	

[a] Day of the estrous cycle unless otherwise specified; P-AI = post artificial insemination; P-PGF = post prostaglandin $F_{2\alpha}$ injection.

[b] Different from control.

Table 1.9. Artificial insemination pregnancy rates of cattle administered hCG.

Reference	Animal type	n	hCG dosage (IU)	Treatment day[a]	Pregnancy rates (%)	P-value[b]
Dahlen et al. (2010)	Beef cows	254	1,000	7 d P-TAI	56.3	0.09
		252	Control		50.0	
Funston et al. (2005)	Beef heifers	165	3,333	8 d P-PGF	65.0	NS[c]
		160	Control		70.0	
		109	3,333	9 d P-PGF	61.0	0.1
		101	Control		50.0	
		90	3,333	6 d P-AI	62.0	NS
		90	Control		59.0	
Rajamahendran and Sianangama (1992)	Dairy cows	8	1,000	at AI	50.0	NS
		9	1,000	7 d P-AI	78.0	NS
		9	1,000	14 d P-AI	44.0	NS
		8	Control		38.0	
Santos et al. (2001)	Dairy cows	203	3,300	5 d P-AI	38.2	<.008
		203	Control		32.0	
Schmitt et al. (1996)	Dairy heifers	122	3,000	5 d P-AI	65.0	NS
		121	Control		63.0	
Shabankareh et al. (2009)	Dairy cows	329	3,000	5 d P-AI	43.2	0.001
		338	Control		30.2	
Sianangama and Rajamahendran (1992)	Dairy cows	49	1,000	At AI	47.0	NS[c]
		56	1,000	7 d P-AI	62.0	<0.05
		49	1,000	14 d P-AI	55.0	<0.05
		50	Control		40.0	
Stevenson et al. (2007)	Dairy cows	714	3,300	Once d 4-9 P-AI	33.6	0.05
		711	CIDR	7 d start 4-9 d P-AI	32.7	0.075
		719	100 µg GnRH	Once d 4-9 P-AI	28.1	NS
		708	Control		28.3	
Wiltbank et al. (1961)	Beef heifers	39	1,000	15-35 d P-AI	69.0	NS
		41	Control		63.0	

[a] Day of the estrous cycle unless otherwise specified; P-AI = post artificial insemination; P-PGF =: post prostaglandin $F_{2\alpha}$ injection.

[b] Different from control.

[c] Nonsignificant.

Table 1.10. Embryo transfer pregnancy rates in cattle administered hCG.

Reference	Animal type	n	hCG dosage (IU)	Treatment day[a]	Pregnancy rates (%)	P-value[b]
Ideta et al. (2005)	Dairy heifers	69	1,500	5	66.6	NS[c]
		71	8 µg GnRH[e]	5	75.0	NS
		89	Control		69.8	
Massey et al. (1983)	Beef cows	399 total[d]	5,000	5 d P-ET	53.6	NS
			Control		47.9	
Nishigai et al. (2002)	Beef cows	40	1,500	1	42.5	NS
		40	1,500	6	67.5	<0.05
		40	Control		45.0	

[a] Day of the estrous cycle unless otherwise specified; P-ET = post embryo transfer

[b] Different from control.

[c] Nonsignificant.

[d] Number of control and treatment animals not specified.

[e] Burserelin.

Table 1.11. Pregnancy loss in cattle administered hCG.

Reference	Animal type	n	hCG dosage (IU)	Treatment day[a]	Interval of loss[a]	Pregnancy loss (%)	P-value[b]
Burns et al. (2008)	Beef cows	180	1,000	26 d P-AI	33-68 d P-AI	5.0	NS
		164	100 µg GnRH	26 d P-AI	33-68 d P-AI	6.1	NS
		169	Control		33-68 d P-AI	5.9	
Buttrey et al. (2010)	Holstein cows	379	1,000	33 d P-AI	39-67 d P-AI	6.6	NS
		379	100ug	33 d P-AI	39-67 d P-AI	6.6	NS
		357	Control		39-67 d P-AI	3.6	
Santos et al. (2001)	Holstein cows	93	3,300	5 d P-AI	28-45 d P-AI	11.8	ns NS
		79	Control		28-45 d P-AI	6.3	
		82	3,300	5 d P-AI	45-90 d P-AI	4.9	0.11
		74	Control		45-90 d P-AI	13.5	
		93	3,300	5 d P-AI	28-90 d P-AI	16.1	NS
		79	Control		28-90 d P-AI	19.0	

[a] Day of the estrous cycle unless otherwise specified; P-AI = post artificial insemination.

[b] Different from control; NS: not significant.

Chapter 2: Administration of human chorionic gonadotropin to embryo transfer recipients increased ovulation, progesterone, and transfer pregnancy rates

Abstract

We hypothesized that administration of human chorionic gonadotropin (hCG) to recipients at embryo transfer (ET) would induce accessory corpora lutea (CL), increase circulating progesterone concentrations, and reduce early embryonic loss. At three locations, purebred and crossbred Angus, Simmental, and Hereford recipients (n = 719) were assigned alternately to receive i.m. 1,000 IU hCG or 1 ml saline (control) at ET. Fresh or frozen-thawed embryos were transferred on d 5.5 to 8.5 (median = d 7) of the estrous cycle to recipients having a palpable CL. Recipients received a body condition score (BCS) at ET. Pregnancy diagnoses occurred by transrectal ultrasonography 28 to 39 d (median = d 35) and reconfirmed 58 to 77 d (median = d 67) post-estrus. At one location (n = 108), ovaries were examined to count the number of CL at pregnancy diagnosis. More ($P < 0.001$) pregnant hCG-treated cows (69.0%) had multiple CL than pregnant controls (0%). Serum progesterone (ng/mL) determined at two locations (n=471) at both pregnancy diagnoses in pregnant cows was greater ($P \leq 0.05$) after hCG treatment than in controls (first: 8.1 ± 0.9 vs. 6.1 ± 0.8; second: 8.8 ± 0.9 vs. 6.6 ± 0.7), respectively. Transfer pregnancy rates were analyzed using logistic regression. Unadjusted pregnancy rates at the first diagnosis was 61.8 vs. 53.9% for hCG vs. controls. At the second diagnosis, pregnancy rates were 59.0 vs. 51.4%, respectively. Factors affecting pregnancy rates were treatment ($P = 0.03$), embryo type ($P = 0.02$), and BCS ($P = 0.08$). Odds ratios indicated that greater pregnancy rates occurred in recipients receiving hCG treatment, receiving a fresh

embryo (66.3 vs.55.5%), and when BCS >5 vs. ≤5 (62.3 vs. 55.3%). We concluded that hCG at ET increased incidence of accessory CL, increased progesterone in pregnant recipients, and increased transfer pregnancy rates.

1. Introduction

In recent years use of embryo transfer (ET) has become more widespread as an application to enhance genetic improvement of a herd. According to the annual statistical survey of the American Embryo Transfer Association (AETA), more than 200,000 fresh and frozen bovine embryos were transferred in 2008 (www.aeta.org). Even with the advancement in reproductive technologies in ET since its commercialization in the 1970's, AETA reported in 2006 that industry-wide pregnancy rates are only 62.4% and 56.9% for both fresh and frozen-thawed embryo transfers. By utilizing ET, problems from failed fertilization are avoided, however, fertilization failure has been characterized as a relatively unimportant factor of pregnancy loss. A review by Mann and Lamming (1999) reported that pregnancy failure resulting from lack of fertilization was only 10% with another 10% of loss resulting in failed development of the embryo. 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 (Lopez-Gatius et al., 2004) and enhance growth of the early embryo (Garrett et al., 1988; Mann et al., 2006). Supplementation of progesterone post-AI also increased pregnancy rates (Mann and Lamming, 1999). 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 (Looney et al., 2006; Purcell et al., 2005).

Use human chorionic gonadotropin (hCG) to stimulate ovulation of ovarian follicles to form accessory corpora lutea (CL) has been reported to increase the circulating progesterone concentrations (Rajamahendran and Sianangama, 1992; Funston et al., 2005; Stevenson et al., 2007) and increase pregnancy rates (Santos et al., 2001; Stevenson et al., 2007; Shabankareh et al., 2009) when administered during the post-breeding early luteal phase. More recent studies (Burns et al., 2008, Buttrey et al., 2010) have determined that administration of 1,000 IU hCG was sufficient to ovulate a follicle. Dahlen et al. (2010) administered 1,000 IU hCG to beef cows 7 d post-AI and observed formation of accessory CL and an increase in progesterone concentrations 14 d post-AI. Further, hCG administration to ET recipients at the dosage of 1,500 IU on either d 5 or 6 post-estrus has produced varied results (greater pregnancy rates than control vs. no improvements; Nishigai et al., 2002; Ideta et al., 2005).

The objectives of the present 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 in control recipients; and 3) determine if hCG reduces early embryonic loss (between transfer and first 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.

2. Materials and Methods

The Kansas State University (Manhattan, KS) Institutional Animal Care and Use Committee approved all procedures involving cows in this study.

2.1. Experimental Design

Mature beef cows at three locations (n = 719) received embryos approximately 7 d post-estrus if a CL was palpated per rectum. Fresh (n = 160) or frozen (n = 559) thawed, grade 1 or 2 embryos were transferred using standard techniques according to the International Embryo Transfer Society (Champaign, IL). Embryo transfers were performed by two experienced veterinarians (Cross Country Genetics North, Inc., Westmoreland, KS USA) at Locations 1 and 2. Transfers at Location 3 were performed by experienced technicians at Southern Cattle Company (Marianna, FL USA). 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 USA) or 1 mL saline (Figure 1). Recipients were assigned a body condition score (BCS; 1= emaciated and 9 = obese; Richards et al., 1986). Pregnancy diagnoses were performed by transrectal ultrasonography (5.0 MHz linear-array transducer; Aloka 500 V; Corometrics Medical Systems, Inc., Wallingford, CT USA) at 35 and 65 d (mean) post-estrus. Various characteristics of the three locations were summarized in Table 1.

2.2. Location 1

During winter months before the experiment when native grass pasture was dormant recipient cattle were offered brome hay (9 kg DM) and wet corn gluten feed (2.7 kg DM). On May 1 cattle were pastured on native tallgrass range. Mature Angus, Simmental, and Hereford cows were used as recipients for 108 transfers performed in six replicates in April and May of 2008 and 2009 in Manhattan, KS. Recipients were synchronized using a Select Synch + CIDR protocol. Cattle received an injection of GnRH (100 µg i.m.; Cystorelin, Merial, Duluth, GA) and a CIDR (controlled internal drug release; Eazi-Breed CIDR, Pfizer Animal Health, New

York, NY) insert at d 0. At d 7 the CIDR was removed and $PGF_{2\alpha}$ (25 mg i.m.; Lutalyse, Pfizer Animal Health) was administered and estrus was detected. Embryos were transferred to recipients 5.5 to 8.5 d post-estrus. Recipients were given a CL quality score by the ET technician : 1 (CL had palpable diameter > 10 mm and firm to moderately firm) or 2 (CL had palpable diameter ≤ 10 mm, and/or soft in texture; Spell et al., 2001). One technician performed all but 26 transfers. Pregnancy was diagnosed 31 and 59 d post-estrus and ovarian structures were determined at the time of pregnancy diagnosis. Location and number of CL were recorded in pregnant cows. Coccygeal vessel blood samples were collected from all cows at ET and at both pregnancy diagnoses in pregnant cows only for later determination of serum progesterone concentrations.

2.3. Location 2

Mature Angus, Hereford, and Charolais crossbred cows were used as recipients for 363 transfers in five replicates from November 2008 to April 2009 in Manhattan, KS. Cattle were fed daily a total mixed ration consisting of ground hay (11.3 kg DM), corn silage (5.4 kg DM), and dried distillers grain (2.3 kg DM). Estrus was synchronized in recipients using the 7-11 Synch protocol. Cows were fed melengestrol acetate (0.5 mg per head per day; MGA, Pfizer Animal Health) for 7 d and received an injection of $PGF_{2\alpha}$ on d 7. On d 11 GnRH was administered; on d 18 $PGF_{2\alpha}$ was administered and estrus was detected. Embryos were transferred to recipients 5.5 to 8.5 d post-estrus. The recipients were given a CL quality score by the ET technician as described for cows at Location 1. Cattle not having a CL at the time of transfer or did not express estrus were resynchronized and used at later transfer dates. Cattle found not pregnant at the first pregnancy diagnoses were used as recipients at later transfer dates.

Estrus was synchronized by using the Select Synch + CIDR protocol as described for Location 1. One technician performed all transfers. All recipient cows received i.m. 500 mg flunixin meglumine before transfer (Purcell et at., 2005). Pregnancy was diagnosed 34 to 39 and 71 to 77 d post-estrus in four transfer groups and only at 68 to 69 d post-estrus in one group. Blood samples were collected at transfer from all cows and from only pregnant cows at both pregnancy diagnoses for later determination of serum progesterone concentrations.

2.4. Location 3

Mature Angus and Angus crossbred cows were used as recipients for 248 transfers during a period of 5 d (December 2009) in Marianna, FL. Estrus was synchronized by using CO-Synch + CIDR protocol. Cattle received an injection of GnRH and a CIDR insert on d 0; at d 7 the CIDR was removed and $PGF_{2\alpha}$ was administered; at 48 h after $PGF_{2\alpha}$ injection, GnRH was administered. Embryos were transferred to the uterine horn ipsilateral to a palpable CL as determined by the technician 7 d after GnRH. One technician performed all transfers. Pregnancy was diagnosed at 33 and 55 d (mean) post-GnRH. Recipients were maintained on Bahia grass pasture with ad libitum access to a commercial mineral supplement. When pastures were dormant, cows were provided ad libitum access to Bahia and Bermuda grass hay and a commercial mineral supplement.

2.5. Radioimmunoassay of Progesterone

Blood samples were collected from a coccygeal vessel at time of transfer and at pregnancy diagnoses in pregnant cows at Locations 1 and 2. Blood samples were refrigerated overnight and centrifuged the following morning. Blood sera were frozen at –20° C until assay was performed. Concentrations of progesterone were quantified by radioimmunoassay (RIA;

Skaggs et al., 1986) in samples. Intra- and inter-assay CV for eight assays were 4.7 and 5.8%, respectively, for a pooled serum sample that averaged 3.95 ± 0.11 ng/mL (n=22).

2.6. Statistical Analyses

Analysis of first pregnancy diagnosis data (0 = not pregnant vs. 1 = pregnant) was accomplished by logistic regression (procedure LOGISTIC; SAS Inst. Inc., Cary, NC). The initial model included location (n = 3), breed (n = 5), lactation status (dry vs. lactating), treatment (hCG vs. saline), embryo type (fresh vs. frozen-thawed), donor, sire, and BCS (≤ 5 and >5). The final model was produced by a backward stepwise elimination based on the Wald statistic criterion when $P < 0.10$. The final model for first pregnancy diagnosis included treatment, embryo type, and BCS. Adjusted odds ratios were calculated for profile likelihood and the Wald statistic was used to calculate 95% confidence limits.

Progesterone concentrations in pregnant cows at the time of pregnancy diagnoses were analyzed by using a general linear model (procedure GLM; SAS Inst. Inc.). The model consisted of treatment (saline vs. hCG), location (1 and 2), breed (Angus, Angus cross, Hereford cross, other crosses, and Simmental), lactation status (dry vs. lactating), embryo type (fresh vs. frozen), BCS (≤ 5 and >5), number of times transferred (1 and >1), and interactions of treatment by herd, lactation status, embryo type, and BCS. Differences among least squares means were detected by the method of least-significant difference only when F-tests were significant ($P \leq 0.05$).

Difference in accessory CL formation after hCG for cows at Location 1 was determined by Chi-square (SAS Inst. Inc.) using the Cochran-Mantel-Haenszel statistic option.

3. Results

3.1. Pregnancy Rates

Embryo transfer pregnancy rates at first and second diagnoses and pregnancy loss are shown in Table 2.1. The first and second pregnancy rates varied across locations; however, no difference was detected among locations. Factors that significantly affected ET pregnancy rates at the first diagnoses for 719 transfers are shown in Table 2.2. Treatment with hCG ($P = 0.03$) at time of transfer and transfer of fresh embryos ($P = 0.02$) increased the likelihood of pregnancy at the first diagnosis. The recipients having BCS > 5 at the time of transfer tended ($P = 0.07$) to have greater pregnancy rates than recipients having BCS ≤ 5.

Treatment had a positive influence on pregnancy transfer rates at two of the three locations (Figure 2.2). Consistently across all three locations transfer of fresh embryos produced ($P < 0.05$) greater pregnancy rates than transfer of frozen-thawed embryos (Table 2.3). Overall, ET pregnancy rates in recipients having BCS > 5 were numerically greater ($P = 0.07$) than in recipients having BCS ≤ 5. This advantage was evident for cows at Locations 1 and 2, whereas at Location 3, recipients with BCS ≤ 5 had numerically greater pregnancy rates than recipients with BCS > 5 (57.7 vs. 53.4%).

3.2. Pregnancy Loss

Across the three locations pregnancy loss from first to second pregnancy diagnosis was variable (Table 2.4). Overall, pregnancy loss was small at all locations (4.6%) and no difference was detected among locations. Those factors significantly affecting pregnancy rates at first pregnancy diagnoses (treatment and embryo type) did not alter pregnancy loss (Table 2.4).

3.3. Progesterone Concentrations

Serum progesterone concentrations of pregnant recipients at locations 1 and 2 at the first and second pregnancy diagnoses are shown (Table 2.5). At both periods after hCG treatment, pregnant hCG-treated recipients had greater ($P < 0.05$) serum progesterone concentrations than recipients treated with saline. Although embryo type (fresh or frozen) influenced pregnancy rates (Table 2.4), recipients to which were transferred either fresh or frozen embryos had similar serum progesterone concentrations at both of the pregnancy diagnoses.

At the time of the first pregnancy diagnosis, progesterone tended to be greater ($P < 0.10$) in the pregnant recipients with a BCS >5 than in recipients with BCS ≤ 5. This tendency was not maintained through the second diagnosis. Number of previous embryo transfers either affected or tended to affect progesterone concentrations at the first and second pregnancy diagnosis, respectively (Table 2.5). Recipients that became pregnant after a single transfer had greater progesterone concentrations ($P < 0.05$) than recipients that had previously received an embryo.

3.4. Accessory CL Formation and Retention

Ovaries of pregnant recipients (n = 59) from Location 1 were monitored for the number and location of luteal structures at the time of both pregnancy diagnoses. All occurrences of accessory CL at the time of pregnancy diagnosis (20 of 29) were detected in the hCG treatment (Figure 2.3). Proportion of recipients having accessory CL was greater ($P < 0.001$) after hCG treatment than after saline (69 vs. 0%, respectively).

Between the first and second pregnancy diagnoses, two of the saline-treated recipients lost their pregnancy. In the hCG treatment, four recipients had luteal regression of one CL each while maintaining their pregnancy, whereas one hCG cow had luteal regression and lost her

pregnancy. Among the four recipients who had luteal regression and maintained their pregnancy, one cow had a CL on both ovaries and the CL regressed that was contralateral to the uterine horn that had received the embryo (Figure 2.4A). In another cow a CL was in both ovaries, had the CL regress that was ipsilateral to the horn bearing the transferred embryo (Figure 2.4B). Two cows each had 2 CL ipsilateral to the horn to which the embryo was transferred and one of the two CL regressed (Figure 2.4C).

4. Discussion

Timing of treatment of the ET recipients with 1,000 IU hCG at the time of ET was applied to ovulate a first wave dominant follicle. Many studies report that hCG will ovulate a follicle in the early luteal phase of the estrous cycle (Price and Webb, 1989) or early luteal phase post-AI (Stevenson et al., 2007; Dahlen et al., 2010). The focus of the study was to determine if formation of an accessory CL would increase circulating progesterone concentrations and enhance early embryonic survival before the first pregnancy diagnosis. Because embryos used for ET are assumed to be viable, any improvement in transfer pregnancy rate would reflect improved embryo survival between the day of ET and pregnancy diagnosis on median d 35.

In a limited number of experiments, hCG has been administered to ET recipients with a range of doses (1,500 to 5,000 IU) and days (d 5 post estrus to 7 d post transfer) with mixed results (Massey et al., 1983; Nishgia et al., 2002; Ideta et al., 2005). The study of Nishgia et al. (2002) used a 50% larger dose of hCG than in our study (1,500 vs. 1,000 IU, respectively), but at similar times (d 6 post-estrus vs. median d 7 post-estrus). Both studies showed improved pregnancy rates compared with the control. In contrast to these results, neither Massey et al. (1983) nor Ideta et al. (2005) detected differences between hCG-treated cows and controls when treatment of 5,000 IU on d 7 post-ET and 1,500 IU on d 5 of the cycle were administered,

respectively. As shown in previous studies, hCG increased pregnancy rates when administered to dairy cows post-AI (Sianangama and Rajamahendran, 1992; Santos et al., 2001; Stevenson et al., 2007; Shabankareh et al., 2009).

Other factors found to be either significant or having a tendency to influence pregnancy rates at the first pregnancy diagnosis were embryo type (fresh vs. frozen) and the BCS of the recipient (≤ 5 vs. >5). Improved pregnancy rates in recipients that received a fresh embryo rather than a frozen-thawed embryo are consistent with results from earlier studies in both dairy (Chebel et al., 2008) and beef cattle (Spell et al., 2001; Purcell et al., 2005).

Relationships of BCS or level of nutrition with fertility are not novel. Randel (1990), suggested that inadequate prepartum or postpartum nutrition reduced pregnancy rates. Further, he recommended that BCS of cows should be ≥ 5 at calving. In other studies, beef cows having greater body condition at calving led to greater pregnancy rates and fewer days until resumption of luteal activity (Richards et al., 1986; Selk et al., 1988). Dairy cows with greater body condition at breeding had greater pregnancy rates (Moreira et al., 2000; Santos et al., 2001). In addition, dairy cows with greater body condition at breeding (≥ 3.5, 1 = emaciated and 5 = obese) had fewer days open from calving to confirmation of pregnancy (Lopez-Gatius et al., 2003).

Maintenance of the recipient herd is the most costly aspect of ET. Management of the recipient is more important than management of the donor because of the necessity of the recipient to maintain a pregnancy to term (Looney et al., 2006; Jones and Lamb, 2008). The ideal BCS or range in BCS for a recipient at the time of transfer has not been recommended. It is recommended, however, that recipients should be on an increasing plane of nutrition or in positive energy balance at the time of transfer (Looney et al., 2006; Stroud and Hasler, 2006;

Jones and Lamb, 2008). Mapletoft et al. (1986) reported that dairy cow recipients with a BCS of 2 and 3 (1= emaciated and 5 = obese) had greater pregnancy rates than recipients with BCS ≤ 1 and ≥ 4. Results of the present study concur with the latter results where thinner recipients had poorer transfer rates, however; fatter than average recipients did not have poorer results in our study.

Ability of hCG to ovulate a first-wave dominant follicle was monitored in cows at Location 1. Cows that received hCG at the time of transfer and later found to be pregnant formed an accessory CL 69% of the time. Ovulation success was consistent with the results from suckled beef cows treated with hCG post-AI in which 17 of 33 (51.6%) cows ovulated in response to 1,000 IU hCG on d 7 post-AI (Dahlen et al., 2009). Other researchers detected more than 60% of the dairy cattle ovulated after administration of hCG during the early luteal phase post-AI (Santos et al, 2001; Stevenson et al., 2007).

Although size of original or accessory CL was not measured in our study, other researchers have reported that the original CL in cows treated with hCG was larger than that in controls 3 to 7 d post treatment (Rajamahendran and Sianangama, 1992; Nishigai et al., 2001; Santos et al., 2001; Stevenson et al., 2007). Human chorionic gonadotropin has LH activity and is able to bind to the LH receptor of the small luteal cell (SLC). When ewes were treated with hCG on d 5 or 7.5 of the estrous cycle, Farin et al. (1988) observed an increased ratio of large luteal cell (LLC) to SLC by d 10 of the cycle. Because the LLC produce 80% of the progesterone, it is logical to expect greater progesterone biosynthesis in larger CL or in cows having multiple CL.

Recipients that were administered hCG at the time of transfer had greater serum progesterone concentrations at both the first and second pregnancy diagnosis than control cows.

This is consistent with findings in suckled beef cows administered hCG 7 d post-AI when greater progesterone concentration was detected on d 33 post-AI (Dahlen et al., 2010). In other studies, hCG-treated cows had greater concentrations of progesterone than controls when blood samples were collected 2 to 10 d post-treatment (Funston et al., 2005; Ideta et al., 2005; Walker et al., 2005; Stevenson et al., 2007; Buttrey et al., 2010). The significant increase in progesterone was not associated with greater pregnancy rates in all studies (Funston et al., 2005; Ideta et al., 2005; Dahlen et al., 2010). Starbuck et al. (2004) observed that cows spontaneously forming two CL did not have greater progesterone concentrations than cows with one CL and cows having two CL had more pregnancy loss from 5 to 9 wk.

Increased progesterone biosynthesis may be considered the most important result when administering hCG to form accessory luteal tissue. Supplementation of progesterone increases the growth of the embryo post-AI (Garrett et al., 1988; Mann et al., 2006). Another benefit of increasing progesterone concentration in circulation is the modification of the uterine environment. Garrett et al. (1988) observed an increase in the number of uterine-derived proteins necessary for establishment of pregnancy in cattle treated with exogenous progesterone. In the current study, it is possible that greater progesterone concentrations in hCG-treated recipients led to greater pregnancy rates because of changes in the uterine environment or via direct stimulation of embryo growth.

Embryo development is associated with concentrations of progesterone and the ability of the conceptus to secrete the antiluteolytic hormone, interferon-τ (Mann et al., 1999). Exogenous progesterone has been shown to stimulate embryo development (Thatcher et al., 1994). Conception failure is coincident with less than normal concentrations of progesterone as early as d 6 after insemination (Thatcher et al., 2001). In general, blood concentrations of progesterone

rise earlier and achieve greater concentrations in pregnant than in nonpregnant cows (Thatcher et al., 2001).

The greater progesterone concentration of recipients that became pregnant to transfer after one transfer in comparison with recipients that failed to conceive to earlier transfers could be explained by the fertility of the recipient. Looney et al. (2006) reported that when recipients were transferred to up to three times that pregnancy rates decreased after each subsequent transfer. From the first to second transfer, a 12% decrease occurred and from the second to third transfer a 12% decrease also occurred.

Although formation of an accessory CL is important, its retention could be as important for pregnancy maintenance. At Location 1 where the ovaries were scanned at both pregnancy diagnoses for the incidence of accessory CL, only one of five cows in the hCG treatment with CL regression was associated with pregnancy loss. Among the four cows that maintained their pregnancy, two had CL regression from 2 CL to 1 CL ipsilateral to the original CL and one cow had regression contralateral to the original CL. Stevenson et al. (2008) reported that more CL regression occurred in pregnant cows (> d 30 of pregnancy) when the induced CL was contralateral rather than ipsilateral to the original CL. One cow in the present study lost the original CL between d 30 and 60 of pregnancy, whereas the induced contralateral CL was maintained. This observation differs from a report in which the induced contralateral CL alone 28 to 54 d post-conception was unable to maintain a pregnancy (Bridges et al., 2000). In contrast, Starbuck et al. (2006) reported that after d 54 of pregnancy, an induced contralateral CL did maintain a pregnancy to term.

Increased ET pregnancy rates of 7.9% from the administration of hCG to recipients should lead to increased profits for the ET program. When treating 100 cows with hCG, an

approximate 8% increase in pregnancy rates would provide 8 additional calves. The dosage used in this study, 1,000 IU, cost $2.62 (price quoted 6/10/2010, Agtech Inc., Manhattan, KS USA) per recipient. Administration of 1,000 IU hCG to 100 recipients would cost: 100 recipients x $2.62/ dose = $262.00. The cost of each of the 8 additional calves would be: $262 / 8 calves = $32.75. The cost of each additional calf, $32.75, would be considered inexpensive when considering the costs associated with maintaining a recipient.

5. Conclusions

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. As expected, pregnancies of recipients to which were transferred fresh embryos were more likely than in cows to which were transferred frozen-thawed embryos. The tendency for cows in better body condition (> 5) to have greater conception rates reiterates the importance of properly managing the nutrition program for the recipient herd. Monitoring BCS could be a factor to predict the success of an ET.

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Table 2.1. Herd characteristics of beef cows in Locations 1, 2, and 3.

	Location		
Traits	1	2	3
Embryo transfer dates	4/14/08, 5/5/08, 5/27/08, 4/16/09, 5/9/09, 5/29/09	11/20/08, 12/12/08, 1/6/09, 1/27/09, 4/25/09	12/14/09-12/18/09
Breeds	Purebred Angus, Simmental, and Hereford	Crossbred Angus, Hereford, and Charolais	Purebred and crossbred Angus
Herd location	Manhattan, KS	Manhattan, KS	Marianna, FL
Number of transfers	108	363	248
First pregnancy diagnosis (d)			
Mean	31.4	36.5	33.4
Median	32	36	33
Range	28-34	34-39	31-35
Second pregnancy diagnosis (d)			
Mean	59.4	74.0	55.4
Median	60	75	53
Range	58-62	68-77	53-57
Body condition score[a], mean ± SD	4.8 ± 0.6	5.4 ± 0.9	5.4 ± 0.7
Embryo transfer pregnancy rates (%)			
First diagnosis	54.6	60.1	56.0
Second diagnosis	51.9	56.7	54.5
Pregnancy loss[b]	5.1	5.5	2.9

[a] Assessed at the time of transfer.

[b] Loss between first and second pregnancy diagnosis.

Table 2.2. Significant factors affecting embryo transfer pregnancy rates.

Item	N	Pregnancy rate (%)	Odds ratio	95% confidence limit	P value
Treatment					
Saline	358	53.9	Referent		0.0265
hCG	361	61.8	1.403	1.040 – 1.893	
Embryo type					
Frozen	559	55.5	Referent		0.016
Fresh	160	66.3	1.565	1.081 – 2.266	
BCS					
≤ 5	454	55.3	Referent		0.0739
> 5	265	62.3	1.330	0.973 – 1.817	

Table 2.3. Other factors affecting embryo transfer pregnancy rates at three locations.

	Location			
Item	1	2	3	Overall
		% (n)		
Embryo type				
Fresh	56.5 (23)	67.0 (100)	70.3 (37)	66.3[a] (160)
Frozen	54.1 (85)	57.4 (263)	53.6 (211)	55.5[b] (559)
BCS				
≤ 5	50.6 (89)	55.5 (209)	57.7 (156)	55.3[c] (454)
> 5	73.7 (19)	66.2 (154)	53.4 (92)	62.3[d] (265)

[a,b] Mean percentages having different superscript letters within item differ ($P \leq 0.05$).

[c,d] Mean percentages having different superscript letters within item differ ($P < 0.10$).

Table 2.4. Factors affecting pregnancy loss from first to second diagnosis.

	Location			
Item	1	2	3	Overall
		% (n)		
Treatment				
Saline	6.7 (30)	5.0 (100)	3.2 (63)	4.7 (193)
hCG	3.4 (29)	6.0 (117)	2.6 (76)	4.5 (222)
Embryo type				
Fresh	0.0 (13)	3.0 (67)	3.8 (26)	2.8 (106)
Frozen	6.5 (46)	6.7 (150)	2.7 (113)	5.2 (309)
BCS				
≤ 5	6.7 (45)	7.8 (115)	6.1(90)	5.2 (250)
> 5	0.0 (14)	2.9 (102)	1.1 (49)	1.9 (165)

Table 2.5. Progesterone concentrations at first and second pregnancy diagnosis in pregnant recipients at Locations 1 and 2[x].

Item	First diagnosis	Second diagnosis
	ng/mL (n)	
Treatment		
Saline	6.1 ± 0.8 [a] (129)	6.5 ± 0.7 [a] (122)
hCG	8.1 ± 0.9 [b] (145)	8.7 ± 0.9 [b] (137)
Embryo type		
Fresh	7.5 ± 0.8 (86)	7.8 ± 0.7 (77)
Frozen	6.7 ± 0.7 (186)	7.4 ± 0.7 (182)
BCS		
≤ 5	6.6 ± 0.8 [c] (163)	7.4 ± 0.7 (146)
> 5	7.6 ± 0.8 [d] (111)	7.8 ± 0.7 (113)
Number of transfers[y]		
= 1	7.8 ±0.7 [c] (244)	8.4 ± 0.6 [a] (236)
> 1	6.4 ± 1.0 [d] (30)	6.8 ± 0.9 [b] (23)

[a,b] Means having different superscript letters within item differ ($P \leq 0.05$).

[c,d] Means having different superscript letters within item differ ($P < 0.10$).

[x] Blood samples were not collected in one replicate of Location 2 at first pregnancy diagnosis.

[y] Number times a recipient had previously received an embryo.

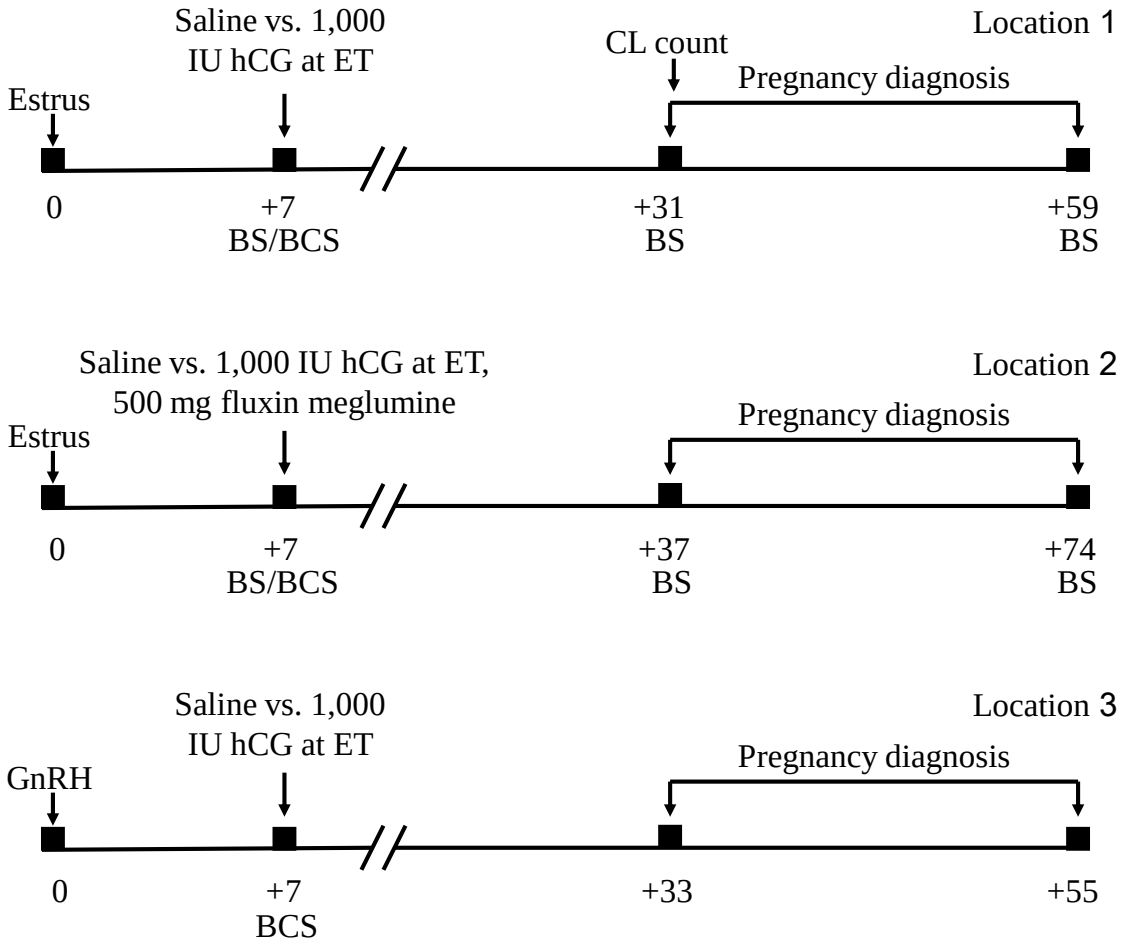

Figure 2.1. Experimental design

Location 1. Mature cows detected in estrus (d 0) and possessing a palpable CL at d 7 received an embryo (n = 108) during six different replicates. Cows were alternatively administered 1 ml saline or 1,000 IU hCG at time of transfer and assigned a body condition score (BCS). Pregnancy was diagnosed on average 31 and 59 d post-estrus and ovaries in pregnant cows were assessed for corpora lutea by transrectal ultrasonography (US). Blood samples (BS) were collected at transfer and at both pregnancy diagnoses in pregnant cows only. *Location 2*. Mature cows detected in estrus (d 0) and possessing a palpable CL at d 7 received an embryo (n = 365) during five different replicates. Cows were alternatively administered 1 ml saline or 1,000 IU hCG at time of transfer and assigned a BCS. All recipient cows received i.m. 500 mg flunixin meglumine before transfer. Pregnancy was diagnosed on average 37 and 74 d post-estrus in 4 transfer groups and at 68 d in one group. Blood samples were collected at transfer and at each pregnancy diagnosis from pregnant cows. *Location 3*. Mature cows were synchronized using a CO-Synch + CIDR protocol with GnRH 48 h after CIDR removal. Embryos were transferred 7 d after GnRH to cows possessing a palpable CL (n = 278) during five different replicates. Cows were alternatively administered 1 ml saline or 1,000 IU hCG at time of transfer and assigned a BCS. Pregnancy was diagnosed at 33 and 55 d post GnRH.

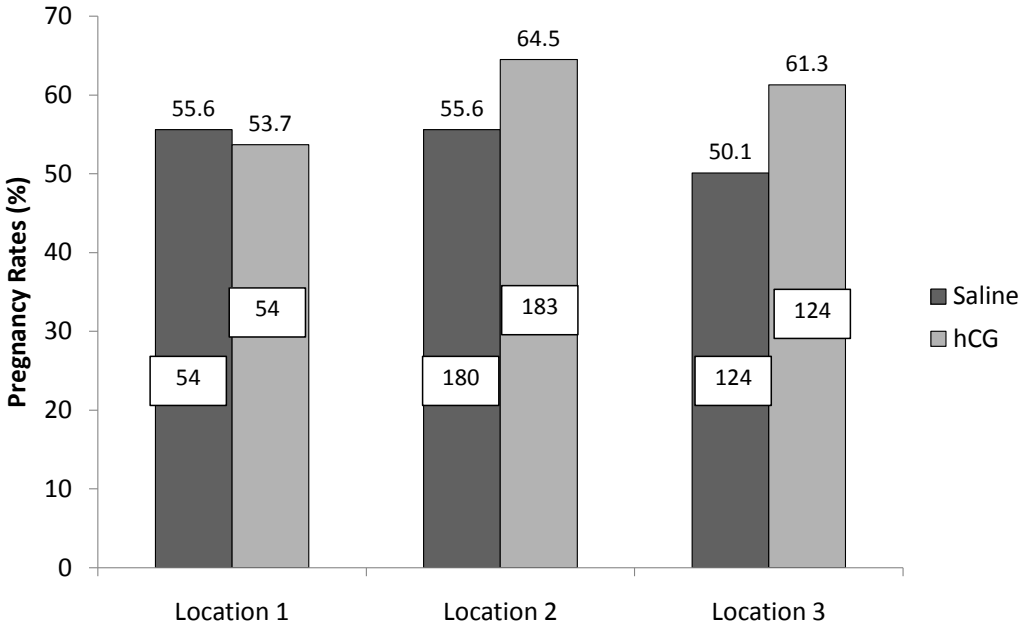

Figure 2.2. Treatment effects on embryo transfer pregnancy rates at first diagnosis (median d 32, 36, and 33) at three locations.

Numbers in bar boxes represent the number of recipients treated with either saline or 1,000 IU hCG at embryo transfer.

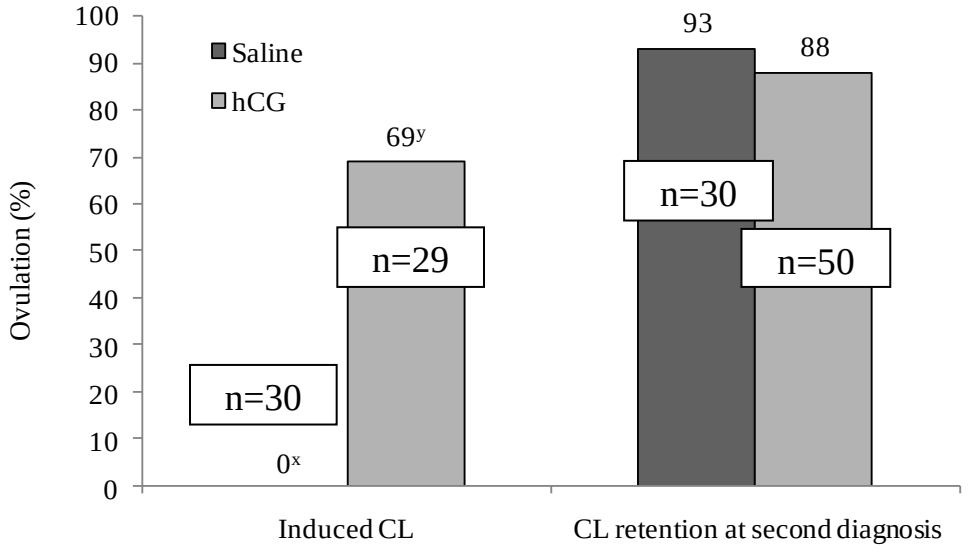

Figure 2.3. Incidence of multiple ovulation assessed at pregnancy diagnosis in response to treatment at ET and retention of CL at second pregnancy diagnosis.

Presence of multiple CL were detected by transrectal ultrasonography in pregnant cows at each of the two pregnancy diagnoses.

^{x,y} $P < 0.001$

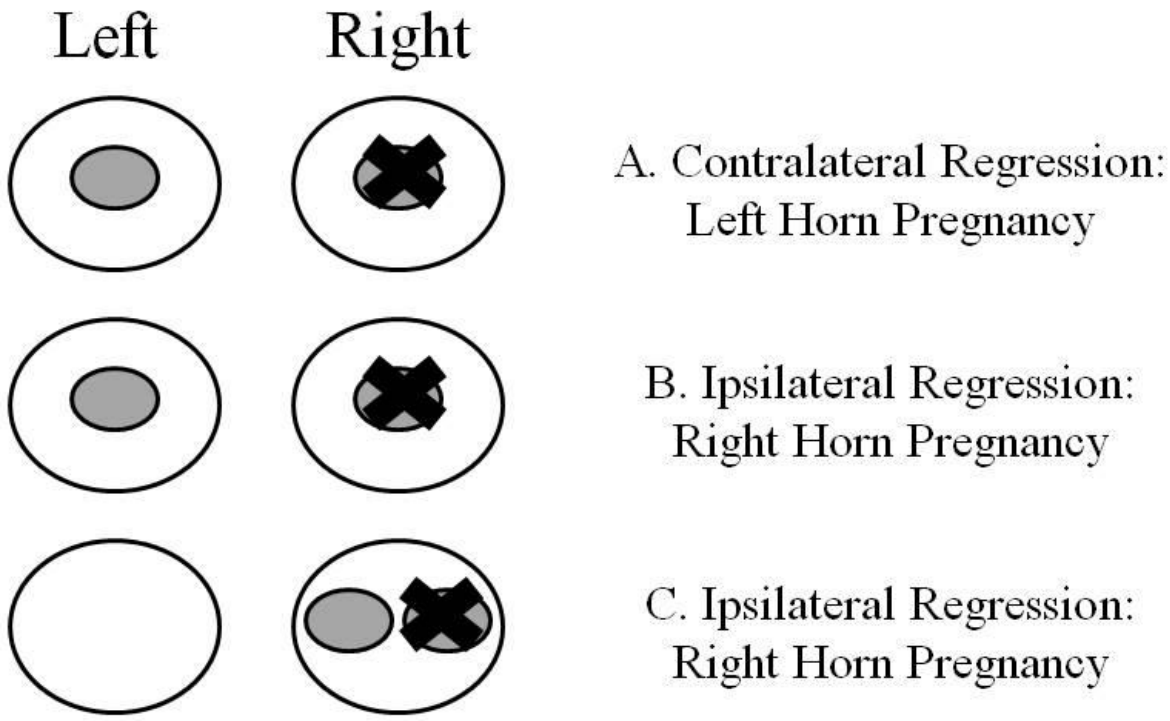

Figure 2.4. Luteal regression in pregnant recipients from first to second pregnancy diagnosis.

The figure describes changes in ovaries that occurred in the four recipients that experienced luteal regression. The large circle represents the ovary, the small circle represents the CL present at the first pregnancy diagnosis, and the x represents the CL that had regressed by the second pregnancy diagnosis. *A. Contralateral regression (n=1):* An embryo was transferred to the left horn and the induced CL regressed on the right ovary by the time of the second pregnancy diagnosis. *B. Ipsilateral regression (n=1):* An embryo was transferred to the right horn, and at the second pregnancy diagnosis, the original CL (right ovary) had regressed and the induced CL (left ovary) was retained. *C. Ipsilateral regression (n=2):* An embryo was transferred to the right horn and at first pregnancy diagnosis two CL were present (one induced CL plus the original CL). At the second diagnosis one of two CL had regressed.