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Pregnancy rates after ewes were treated with estradiol-17β and oxytocin

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Summary

Ewes were assigned to the following treatments to determine whether estradiol-17β-oxytocin treatment affects luteal function and pregnancy rates on d 25: 1) diluent + saline (n = 26); 2) diluent + oxytocin (n = 25); 3) estradiol-17β + saline (n = 22); and 4) estradiol-17β + oxytocin (n = 24). On d 6 after expected estrus and mating, ewes received either i.v. injection of diluent or i.v. injection of 100 μg of estradiol-17β. Ten hours later, ewes received either i.v. injection of saline or i.v. injection of 400 USP units of oxytocin. Blood samples for progesterone assay were collected on d 6, d 7, d 8 (Period 1), d 16, d 18, d 20, d 22, and d 25 (Period 2). Transrectal ultrasonography on d 25 and progesterone concentrations were used to diagnose pregnancy. Neither estradiol-17β nor oxytocin affected pregnancy rates, and the estradiol-17β × oxytocin interaction was not significant. The pregnancy rate for diluent + saline was 61.5 percent; diluent + oxytocin, 76 percent; estradiol-17β + saline, 77.2 percent; and estradiol-17β + oxytocin, 62.5 percent. Progesterone concentration was greater ($P < 0.05$) in pregnant than in nonpregnant ewes (5.2 ± 0.3 ng/mL vs. 2.0 ± 0.6 ng/mL); the pregnancy status × period interaction was significant ($P < 0.01$); but estradiol-17β, oxytocin, and their interaction were not significant ($P > 0.05$). Treatment with estradiol-17β on d 6 after the expected onset of estrus and oxytocin 10 h later did not induce luteolysis or disrupt pregnancy in ewes.

Key Words: Sheep; Embryo Transfer; Estradiol; Oxytocin; Transcervical

Introduction

Surgical procedures have been used for at least 55 years to collect and transfer sheep embryos (Hunter et al., 1955; McKelvey et al., 1986; Nellenschulte and Niemann, 1992; Li et al., 2008). Sheep cervical anatomy makes nonsurgical embryo collection and transfer exceedingly difficult and considerably less effective than surgical procedures (McKelvey et al., 1986; Flohr et al., 1999; Wulster-Radcliffe et al., 1999; Anel et al., 2006; Candappa et al., 2009). Even though some of the surgical embryo transfer (ET) procedures are minimally invasive, they include analgesics, anesthetics, incisions, and sutures, and they require specialized training to perform.

Effective transcervical AI, embryo collection, and ET procedures for sheep may eliminate the need for surgical procedures. Sheep-specific transcervical procedures have been reported, but pregnancy rates after the procedures have been disappointing (Sayre and Lewis, 1997; Wulster-Radcliffe et al., 1999, 2004; Anel et al., 2006). Some of the procedures have included treatments to dilate the cervix and reduce the difficulty of manipulating a catheter through the cervix and into the uterus (Flohr et al., 1999; Wulster-Radcliffe et al., 2004; Anel et al., 2006; Candappa et al., 2009).

In one study, an i.v. injection of 100 µg of estradiol-17β on d 5 of an estrous cycle and an i.v. injection of 400 USP units of oxytocin 10 h later were used to dilate the cervix and permit atraumatic transcervical ET (Wulster-Radcliffe et al., 1999). Without the estradiol-17β-oxytocin treatment, the atraumatic transcervical ET procedure described in Wulster-Radcliffe et al. (1999) would not have been possible. Based on postmortem evaluations on d 14, the estradiol-17β-oxytocin treatment did not seem to reduce embryonic development, but the effects of the estradiol-17β-oxytocin treatment per se on later pregnancy rates were not determined (Wulster-Radcliffe et al., 1999). Thus, this experiment was conducted to determine whether treating ewes with estradiol-17β and oxytocin on d 6 and d 7, respectively, after the expected day of onset of estrus and mating would affect pregnancy rates on d 25.

Materials and Methods

Animals and Treatments

Estrus was synchronized using established procedures (Wulster-Radcliffe et al., 1999, 2004) during the autumn breeding season in a group of crossbred ewes (n = 97), that contained various combinations of white-faced and black-faced genetics. Ewes were mated naturally with rams immediately after progestogen withdrawal and i.m. injection of 400 IU of eCG (Sioux Biochemical, Sioux City, Iowa). Ewes were not checked for signs of estrus during any part of the experiment.

Treatments were in a 2 × 2 factorial array. Ewes were assigned to one of four randomized treatments: 1) diluent + saline (n = 26); 2) diluent + oxytocin (n = 25); 3) estradiol-17β + saline (n = 22); and 4) estradiol-17β + oxytocin (n = 24). During the afternoon of d 6 after the expected onset of estrus and mating (i.e., 48 h after progestogen withdrawal; Cline et al., 2001), approximately half of the ewes received a 5-mL i.v. injection of diluent (50 percent ethanol:50 percent sterile, isotonic saline), and the remaining ewes received an i.v. injection of 100 µg of estradiol-17β (Sigma Chemical Co., St. Louis, Mo.) in 5 mL of diluent. At 10 h after the initial injections (i.e., morning of d 7), approximately half of the diluent-treated and half of the estradiol-17β-treated ewes received 20-mL i.v. injections of sterile, isotonic saline, and the remaining diluent-treated and estradiol-17β-treated ewes received i.v. injections of 400 USP units of oxytocin (20 mL; Vedco, St. Joseph, Mo.; Khalifa et al., 1992; Wulster-Radcliffe et al., 1999). Injections were via a jugular vein. Estradiol-17β and oxytocin were administered on d 6 and d 7, respectively, because sheep embryos are commonly transferred during this portion of the estrous cycle (Li et al., 2008).

Blood Sampling

On d 6, d 7, d 8, d 16, d 18, d 20, d 22, and d 25 after the expected onset of estrus and mating, heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, N.J.) were used to collect approximately 10 mL of jugular venous blood from each ewe. Blood samples were placed in an ice bath immediately after collection and stored there until approximately 1 h after the end of each collection period. Plasma was then harvested after centrifugation and stored in polypropylene tubes at -20°C.

Blood samples were collected just before injections were administered on d 6 and d 7. Blood samples were not collected between d 9 and d 15 because progesterone concentrations were expected to be similar between pregnant and nonpregnant ewes during those days (deNicolo et al., 2009).

Pregnancy Diagnosis

Transrectal ultrasonography 25 d after the expected onset of estrus and progesterone concentrations was used to diagnose pregnancy. An Aloka 500 V instrument equipped with a 5.0 MHz linear array transducer (Corometrics Medical Systems, Inc., Wallingford, Conn.) was used. Ewes were restrained in dorsal recumbency in a Poldenvale Commodore cradle (Premier Sheep Supplies, Washington, Iowa) during the ultrasound procedure. A ewe was considered pregnant if 1) an embryo was visible in an ultrasound image, 2) a heartbeat could be visualized, and 3) progesterone concentrations on d 16 through d 25 were greater than 2.0 ng/mL. A heartbeat could be detected in all embryos that were visualized. A ewe was considered nonpregnant if 1) an embryo was not visible in an ultrasound image and 2) progesterone concentrations on d 16 through d 25 were less than 2.0 ng/mL. Lambing data could not be collected, as the ewes in this study were scheduled to be sold as excess. However, the decision to sell a ewe was not based on the lack of reproductive soundness. In addition, approximately 20 percent of the ewes can be expected to lose embryos or fetuses after d 25, and these losses could compromise the ability to determine the short-term effects of the estradiol-17β and oxytocin treatments (Dixon et al., 2007).

Progesterone Assay

A solid-phase RIA [^{125}I] progesterone (Diagnostic Products, Los Angeles, Calif.) was used to quantify progesterone in all plasma samples (Seals et al., 2002; Lewis, 2003; Wulster-Radcliffe et al., 2003; Lewis and Wulster-Radcliffe, 2006).

Statistical Methods

Procedures for analyzing categorical

data were used to determine the effects of treatment on pregnancy rates, and general linear-model procedures were used to determine the effects of treatment on progesterone concentrations (SAS, Cary, N.C.). The model used to determine the effect of treatment on pregnancy rate included terms for estradiol-17β, oxytocin, and the estradiol-17β × oxytocin interaction.

The initial model used to analyze the progesterone data included terms for estradiol-17β, oxytocin, pregnancy status (i.e., pregnant or nonpregnant), period (i.e., Period 1 = d 6, d 7, and d 8; Period 2 = d 16, d 18, d 20, d 22, and d 25), all interactions, and error terms. Data were then sorted according to pregnancy status, and similar models were used to determine the effects of treatment and period on progesterone concentrations in pregnant ewes and nonpregnant ewes.

Results and Discussion

The results of this experiment indicate that neither estradiol-17 nor oxytocin administered on d 6 and d 7, respectively, after the expected onset of estrus and mating affected pregnancy rates on d 25, and the estradiol-17β × oxytocin interaction was not significant. The overall pregnancy rate was 69.1 percent (67/97), which was similar to the d 25-pregnancy rates in Dixon et al. (2007). The pregnancy rate for diluent + saline was 61.5 percent (16/26); diluent + oxytocin, 76 percent (19/25); estradiol-17β + saline, 77.2 percent (17/22); and estradiol-17β + oxytocin, 62.5 percent (15/24). Embryos that were transferred after estradiol-17β-oxytocin-induced cervical dilation on d 6 of the estrous cycle appeared to survive and develop until they were evaluated postmortem on d 14 of pregnancy (Wulster-Radcliffe et al., 1999). However, experiments must be conducted to determine whether embryos that are transferred transcervically after estradiol-17β and oxytocin treatment will survive to term.

Pregnancy status affected ($P < 0.05$) progesterone concentrations (pregnant, 5.2 ± 0.3 ng/mL of plasma vs. nonpregnant, 2.0 ± 0.6 ng/mL of plasma), and the pregnancy status × period interaction was significant ($P < 0.01$; Table 1). However, estradiol-17β treatment, oxytocin treatment, and the estradiol-17β × oxytocin interaction were not significant ($P > 0.05$; Table 1). In pregnant ewes, the concentration of progesterone during Period 1 (4.9 ± 0.3 ng/mL) was less ($P < 0.01$) than it was during Period 2 (5.4 ± 0.4 ng/mL). In nonpregnant ewes, progesterone concentrations were less ($P < 0.01$) during Period 2 than they were for all ewes during Period 1 and for pregnant ewes during Period 2. Progesterone concentrations in nonpregnant ewes had decreased ($P < 0.05$) to less than 1.0 ng/mL by d 16, and they remained less than 1.0 ng/mL through d 25. The differences in progesterone concentrations in pregnant and nonpregnant ewes and reduced progesterone concentrations in nonpregnant ewes on d 16 through d 25 after the expected onset of estrus and mating, compared with d 6 through d 8, are consistent with data from numerous other experiments (Lewis et al., 1981; Wulster-Radcliffe et al., 1999; deNicolo et al., 2009).

Considering nonpregnant ewes only, the estradiol-17β × oxytocin × period interaction was significant ($P < 0.01$; Table 2). During Period 2, progesterone concentrations were greater ($P < 0.05$) in ewes that were treated with estradiol-17β and oxytocin (1.7 ng/mL) than they were in ewes in the other groups (0.5 ng/mL).

The data indicating that use of estradiol-17β, oxytocin, or the combination of estradiol-17β and oxytocin do not reduce progesterone concentrations are consistent with the results of a previous experiment in which luteal-phase ewes were treated with estradiol-17β, oxytocin, or the combination of estradiol-17β and oxytocin (Wulster-Radcliffe et al., 1999). The results of the current experiment and Wulster-Radcliffe et al. (1999) are also consistent with the results of a study to determine whether exogenous oxytocin administered s.c or intraluteally at various times during the estrous cycle or pregnancy altered, luteal function in ewes (Milvae et al., 1991). Milvae et al. (1991) found that exogenous oxytocin did not reduce the duration of the estrous cycle in nonpregnant ewes and did not interrupt pregnancy in

Table 1. Progesterone concentrations (ng/mL) in jugular plasma from ewes that had been diagnosed as pregnant or nonpregnant[a]

Day	Pregnant (n = 67)	Nonpregnant (n = 30)
6	4.7	3.7
7	4.6	3.7
8	5.5	4.2
16	6.5	0.9
18	5.9	0.5
20	5.0	0.5
22	4.5	0.8
25	5.0	0.9

[a] Ewes received one of four randomized treatments: 1) diluent + saline (n = 26); 2) diluent + oxytocin (n = 25); 3) estradiol-17β + saline (n = 22); and 4) estradiol-17β + oxytocin (n = 24). On d 6 after the expected onset of synchronized estrus and mating, each ewe received an i.v. injection of 5-mL of diluent or 100 µg of estradiol-17β in 5 mL of diluent. At 10 h after the initial injection, each ewe received an i.v. injection of either 20 mL of sterile, isotonic saline or 400 USP units (20 mL) of oxytocin. Jugular blood samples were collected from all ewes on d 6 d, d 7, d 8 (i.e., Period 1), d 16, d 18, d 20, d 22, and d 25 (i.e., Period 2) after the expected onset of estrus and mating. Transrectal ultrasonography 25 d after the expected onset of estrus and progesterone concentrations were used to diagnose pregnancy. Over all ewes, pregnancy status affected ($P < 0.01$) progesterone concentrations, and the pregnancy status × period interaction was significant ($P < 0.01$). The estradiol-17β treatment, oxytocin treatment, and estradiol-17β × oxytocin interaction were not significant. Considering pregnant ewes only, period affected ($P < 0.01$) progesterone concentrations (Period 1, 4.9 ± 0.3 ng/mL; Period 2, 5.4 ± 0.4 ng/mL), but neither the estradiol-17β treatment, oxytocin treatment, nor any of the interactions were significant.

Table 2. Progesterone concentrations (ng/mL) in jugular plasma from ewes that had been diagnosed as nonpregnant[a]

Day	Diluent + saline (n = 10)[b]	Diluent + oxytocin (n = 6)	Estradiol-17β + saline (n = 5)	Estradiol-17β + oxytocin (n = 9)
6	3.4	3.8	4.5	3.0
7	3.8	4.2	3.9	3.2
8	4.2	4.8	4.4	3.4
16	1.3	0.6	0.8	1.2
18	0.3	0.2	0.2	1.4
20	0.2	0.2	0.2	2.2
22	0.2	0.3	1.2	1.6
25	0.8	0.8	0.2	1.9

[a] Ewes received one of four randomized treatments: 1) diluent + saline (n = 26); 2) diluent + oxytocin (n = 25); 3) estradiol-17β + saline (n = 22); and 4) estradiol-17β + oxytocin (n = 24). On d 6 after the expected onset of synchronized estrus and mating, each ewe received an i.v. injection of 5-mL of diluent or 100 µg of estradiol-17β in 5 mL of diluent. At 10 h after the initial injection, each ewe received an i.v. injection of either 20 mL of sterile, isotonic saline or 400 USP units (20 mL) of oxytocin. Jugular blood samples were collected from all ewes on d 6, d 7, d 8 (i.e., Period 1), d 16, d 18, d 20, d 22, and d 25 (i.e., Period 2) after the expected onset of estrus and mating. Transrectal ultrasonography 25 d after the expected onset of estrus and progesterone concentrations were used to diagnose pregnancy. The estradiol-17β × oxytocin × period interaction was significant ($P < 0.01$), but none of the other interactions were significant.
[b] Number of ewes that were diagnosed as nonpregnant.

ewes that were mated before oxytocin treatments were commenced. The authors of one study asserted that exogenous oxytocin administered on d 1 to d 7 of the estrous cycle of ewes was luteolytic (Hatjiminaoglou et al., 1979), but this seems to be the only report of such an effect of oxytocin. Indeed, another study indicates that exogenous oxytocin may prolong luteal function in ewes (Sheldrick, 1992). Thus, one should not expect exogenous oxytocin on d 7 of the estrous cycle or pregnancy to induce luteolysis in ewes.

By contrast, exogenous estradiol-17β increased uterine secretion of $PGF_{2\alpha}$ in ewes (Ford et al., 1975), and exogenous estradiol-17β can be luteolytic in ewes. The luteolytic doses of estradiol-17β have ranged from 125 µg to 750 µg and have been administered i.m. in vegetable oil on d 9 and d 10 or d 11 and d 12 of the estrous cycle (Stormshak et al., 1969; Hawk and Bolt, 1970; Kittok and Britt, 1977; Sheldrick, 1992). Treatment of ewes i.m. with 250 µg or 750 µg of estradiol-17β in corn oil on d 1 and d 2, d 3 and d 4, or d 5 and d 6 of the estrous cycle did not affect luteal weight or morphology (Hawk and Bolt, 1970). Thus, a single i.v. injection of 100 µg of estradiol-17β on d 6 after the expected onset of estrus and mating should not be luteolytic, and this assertion is consistent with the results of the current experiment.

Conclusions

Treatment of ewes with 100 µg of estradiol-17β on d 6 and 400 USP units of oxytocin 10 h later, on d 7, after the expected onset of estrus, which should dilate the cervix and reduce the difficulty of manipulating a catheter through the cervix and into the uterus for ET, did not induce luteolysis or disrupt early pregnancy in this study. However, studies are needed to determine whether transcervical ET after estradiol-17β-oxytocin treatment on d 6 and d 7, respectively, will produce acceptable lambing rates.

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