Research Report

Prostaglandin pretreatment enhances ova recovery in cattle

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Abstract

Despite ovarian superstimulation (superovulation) advantages in cattle over the last 40 years, fewer ova or embryos are recovered after uterine lavage than the number of corpora lutea (CL) on the ovaries. Uterine contraction agents given before collection may address this issue. We evaluated the effects of prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) treatment prior to embryo collection using a novel approach which allowed embryo donors to serve as their own control. After follicular wave synchronization and follicle stimulating hormone (FSH) treatment, embryos were collected from a uterine horn (control) and cows were given intramuscular PGF$_{2\alpha}$ (25 mg in Experiments 1 and 3, 6.25 mg in Experiment 2) prior to collection from the second uterine horn. Ova and embryos were collected 5 minutes after PGF$_{2\alpha}$ in Experiments 1 and 2, and after 1 hour in Experiment 3. Although the number of CL were similar, ova recovery rates were higher ($p < 0.05$) from the uterine horn collected after treatment.

Keywords: Superovulation, embryo recovery, bovine embryo collection

Introduction

In cattle, ovarian superstimulation (3 or more ovulations in an estrous cycle[1]) increases embryo recovery and on average about 11 total ova and 7 transferable quality embryos are recovered depending upon the type of FSH used and the type, breed, and age of donors.[2] Superstimulation is most effective when FSH treatment is initiated on the day of or after follicular wave emergence. Despite superstimulation advantages, number of ova or embryos recovered after uterine lavage is lower than the number of CL. In fact, embryo recovery rates can vary from 25-75% of the number of CL based on a variety of factors.[3] When embryo recovery is lower than the number of CL there are at least 2 reasons that are suspected: 1. the oocytes are not sufficiently expelled from the follicle/ovary or 2. the oocytes are released into the infundibulum and likely fertilized; however, the developing embryo becomes trapped in uterine folds resulting in economic and genetic losses. Stimulation of uterine contractions has the potential to alleviate the second challenge, by increasing embryo movement and minimizing the number of ova and embryos retained in the reproductive tract. Exogenous oxytocin and natural or synthetic PGF$_{2\alpha}$ analogues are widely used in cattle breeding to induce uterine contractions.[4] An immediate and substantial increase in the baseline tone of uterine contractions was observed after PGF$_{2\alpha}$ treatment and within 20 minutes cows exhibited or resumed spontaneous uterine activity patterns.[5] Not knowing embryo location at this stage of development, there is the potential for embryos to be retained in the uterus, especially if catheter placement is too far cranial.[6] Although, PGF$_{2\alpha}$ treatment 3 hours prior to collection did not increase embryo recovery rate,[7] altering treatment timing may be beneficial. Our objectives were to: 1. evaluate the effect of treatment with 25 or 6.25 µg of PGF$_{2\alpha}$ given 5 minutes prior to collection of the second uterine horn and 2. evaluate the effect of treatment with 25 µg of PGF$_{2\alpha}$ given 60 minutes prior to collection of the second uterine horn and recollection of the first uterine horn. With this approach, each cow served as its own control, with 1 uterine horn collected before and after PGF$_{2\alpha}$ treatment.

Materials and methods

Animal studies were approved by the Texas Tech University Institutional Animal Care and Use Committee (2022-1162).

Superovulation protocol

Nonpregnant (*Bos taurus*) cyclic embryo donor cows were selected after transrectal ultrasonographic examinations of the reproductive tract. Cows in Experiments 1, 2, and 3 (n = 7, n = 6, and n = 10, respectively) had a similar superovulation protocol. A new follicular wave was induced by ultrasound-guided transvaginal follicle ablation and PGF$_{2\alpha}$ treatment, immediately followed by vaginal insertion of a controlled internal drug release (CIDR; day 0). Intramuscular FSH (Folltropin, Vetoquinol USA; Fort Worth, USA) was given on the morning and evening of days 2-5 and intramuscular PGF$_{2\alpha}$ (25 µg dinoprost; Lutalyse HighCon, Zoetis, Parsippany, USA) was given on the evening of day 4 and

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morning of day 5; and CIDRs were removed on the morning of day 5. Regardless of estrous activity, cows were artificially inseminated in the morning and evening of day 6 with frozen/thawed semen from a single proven bull; intramuscular GnRH (100 µg; Cystorelin, Boerhinger Ingelheim, Duluth, USA) was given at first breeding.

Uterine lavage and embryo collection

Nonsurgical embryo collection was performed on day 13 using a closed, gravity-flow uterine horn lavage system. Collected ova and embryos were counted and embryos were evaluated microscopically by a single technician for quality grade and stage of development following International Embryo Technology Society criteria.[8]

Prostaglandin treatment

In Experiments 1 and 2, a uterine horn was collected first, and then the catheter was placed in the opposite uterine horn, the stylet was removed, and intramuscular $PGF_{2\alpha}$ was given. In Experiment 3, the catheter was removed after the first uterine horn collection and the second uterine horn was collected 60 minutes after $PGF_{2\alpha}$ treatment. In Experiment 1, the right uterine horn was collected first. After the catheter was placed in the left uterine horn, 25 mg of $PGF_{2\alpha}$ was given and collection was performed after 5 minutes. In Experiment 2, the right uterine horn was collected first and 6.25 mg of $PGF_{2\alpha}$ was given after placement of the catheter in the left uterine horn. After 5 minutes, the left uterine horn was lavaged. In Experiment 3, 6 cows had the right uterine horn collection first and 4 cows had the left uterine horn collected first (assigned randomly). Catheters were removed and all cows received 25 mg of $PGF_{2\alpha}$ and were released into a holding pen. After 60 minutes, cows returned to the chute, the catheter was placed, and the second uterine horn was lavaged. It is unknown if the exogenous $PGF_{2\alpha}$ or a combination of endogenous and exogenous $PGF_{2\alpha}$ caused release of additional ova from the first uterine horn; therefore, the uterine horn that was collected prior to $PGF_{2\alpha}$ was recollected.

Embryo evaluation, cryopreservation, thawing, and in vitro culture

Embryos were evaluated under a stereomicroscope (80 x magnification) to determine if they were of transferable quality, unfertilized, or degenerate.[8] In Experiment 3, the cryotolerance of a subset of the transferable embryos was evaluated by cryopreservation of treated or control embryos (1.5 M ethylene glycol [EG]) using a slow cool (ramp = 0.5°C/minute) approach after holding at –6.0°C for 8 minutes in EG media. Embryos were plunged into liquid nitrogen at –32.5°C and stored until thawed. Embryos were thawed in air for 5 seconds, and then in a 30°C water bath for 30 seconds. Embryos were removed from straws, rinsed twice in holding media and placed individually into 5 µl culture droplets containing commercially available in vitro culture media (IVF Bioscience, Falmouth, UK). Embryos were cultured under mineral oil in a 5% O_2, 5% CO_2 and 90% N_2 fully humidified gas environment for 48 hours. Embryos were evaluated at cryopreservation, thaw, and after 24 and 48 hours of culture.[8]

Data analysis

Recovery rates were analyzed via testCompareR analysis (compares 2 binary values using paired data; each cow served as its own control). Significance was set at $p < 0.05$. Analyses were performed on R Statistical Software (R version 4.2.1).[9] The number of transferable, degenerated, and unfertilized ova were counted for each cow from each uterine horn; CL were counted via transrectal ultrasonography after embryo collection. Recovery rates were calculated by dividing the number of recovered ova by the number of CL. Number of total ova, transferable embryos from each uterine horn and CL from each ovary were analyzed via ANOVA. Contingency tables were constructed for recovery outcomes. Ova recovery for each CL alone was the observational unit with a binary outcome recorded (recovered ova or not). Stage of embryo development and quality of postthaw embryos were analyzed with ANOVA.

Results

In Experiment 1 (Table 1), the average percentage of ova recovered from the uterine horn relative to the number of CL present on the control ovary was higher ($p < 0.05$) after PGF_{2a} treatment (73.7 ± 7.2%) compared to pretreatment controls (51.1 ± 7.4%). There was no significant difference between sides in the number of CL or transferable embryos.

Similarly, in Experiment 2 (Table 2), the average percentage of ova recovered from the uterine horn relative to the number of CL present on the control ovary was higher ($p < 0.05$) after PGF_{2a} treatment (87.8 ± 5.1%) compared to pretreatment controls (52.4 ± 7.8%). There was no significant difference between sides in the number of CL or transferable embryos.

In Experiment 3 (Tables 3 and 4), the average percentage of ova recovered from the uterine horn relative to the number of CL present on the control ovary was higher ($p < 0.01$) after PGF_{2a} treatment (83.0 ± 5.5%) compared to pretreatment controls (59.1 ± 7.5%). There was no significant difference between sides in the number of CL. There were fewer ($p < 0.05$) ova collected from the initial collection of the first uterine horn (pretreatment; 2.6 ± 0.7) compared to the second uterine horn (posttreatment: 3.9 ± 1.7). There were an additional 1.0 ± 0.3 ova collected when the first uterine horn was recollected (posttreatment) for a similar total number of ova for the first (3.6 ± 0.5) and second uterine horn

Table 1. Mean ± SEM (Experiment 1) ova recovery rates, number of transferable embryos and CL (n = 7)

Treatment Group	Number of ova	Ova recovery (%)	Transferrable embryos	Number of CL
Control	47	51.1^{b} ± 7.4	1.4 ± 0.4	6.7 ± 1.0
$PGF_{2\alpha}$	38	73.7^{a} ± 7.2	2.1 ± 0.8	5.4 ± 0.7

^{a,b}within a column, means with different superscripts differed ($p < 0.05$)

Table 2. Mean ± SEM (Experiment 2) ova recovery rates, number of transferable embryos and CL (n = 6)

Treatment Group	Number of ova	Ova recovery (%)	Transferrable embryos	Number of CL
Control	42	$52.4^{b} \pm 7.8$	1.5 ± 0.4	7.0 ± 1.3
$PGF_{2\alpha}$	41	$87.8^{a} \pm 5.1$	3.8 ± 1.5	6.8 ± 1.4

^{a,b}within a column, means with different superscripts differed ($p < 0.05$)

Table 3. Mean ± SEM (Experiment 3) ova recovered from each collection (n= 10)

Uterine horn	Collection	Total ova	Transferable	Degenerated	Unfertilized ova
First	Initial	2.6 ± 0.7^{a}	1.5 ± 0.5	0.9 ± 0.3	0.2 ± 0.2
	Recollection	$1.0 \pm 0.3^{\#}$	0.7 ± 0.3	0.2 ± 0.1	0.1 ± 0.1
Second		3.9 ± 1.7^{b}	2.9 ± 1.0	0.8 ± 0.3	0.5 ± 0.3

^{a,b}within a column, means with different superscripts differed ($p < 0.05$)
$^{\#}$combined number of ova from the first uterine horn 2 collections were not different from the second uterine horn

Table 4. Mean ± SEM (Experiment 3) postthaw in vitro developmental dynamics (48 hours) of embryos exposed to exogenous $PGF_{2\alpha}$

Group	Postthaw grade*	Postthaw stage*	24 hour grade	24 hour stage	48 hour grade	48 hour stage
Control (n = 12)	1.7 ± 0.3	4.1 ± 0.1	1.7 ± 0.3	6.2 ± 0.4	1.6 ± 0.3	6.8 ± 0.5
$PGF_{2\alpha}$ (n = 14)	2.0 ± 0.2	4.1 ± 0.4	1.9 ± 0.2	5.9 ± 0.3	1.5 ± 0.2	7.4 ± 0.4

*Grade 1= excellent, 2 = fair, 3 = poor, Stage 4 = morula, 5 = early blastocyst, 6 = full blastocyst, 7 = expanding blastocyst, 8 = hatching blastocyst

(3.9 ± 1.7). Interestingly, 70% of the additional ova collected on recollection of the first uterine horn were of transferable quality (Table 3), although this was not due to treatment effects. Further, after cryopreservation in EG, thawing, and in vitro culture (48 hours) the transferable quality embryos exposed in utero to exogenous $PGF_{2\alpha}$ had similar developmental dynamics to embryos not exposed to exogenous $PGF_{2\alpha}$ (Table 4).

Discussion

Our hypothesis was $PGF_{2\alpha}$ treatment stimulates uterine contractions and enhances ova and embryo recovery rate. In Experiments 1 and 2, the uterine horn that was collected 5 minutes after $PGF_{2\alpha}$ had significantly higher recovery rate compared to the control for both 25 and 6.25 mg treatments. Experiment 3 also had significantly higher recovery after 1 hour. Uterine contractions increased 220% 11-20 minutes after $PGF_{2\alpha}$ treatment in early diestrus Holstein cows;[10] similarly, intrauterine pressure was increased by various prostaglandin analogues.[11] Effect of 4 intravenous PGE_2 treatments on intrauterine pressure and motility in healthy dairy cows was evaluated;[12] mean amplitude and intrauterine pressure were significantly different after PGE_2 but the frequency of pressure waves did not differ. Consequently, PGE_2 treatment increased uterine pressure by modulating shape and amplitude of the pressure curve, but did not affect the number of pressure waves.[12] In mares oxytocin (another smooth muscle stimulator) is often given to aid in the emptying of the uterus during embryo collection.[13]

Timing of $PGF_{2\alpha}$ injection may also have an impact on recovery. Embryo recovery rates were compared in 334 superovulated cows and heifers that received $PGF_{2\alpha}$ 3 hours prior to embryo collection.[7] Although $PGF_{2\alpha}$ treatment did not improve embryo recovery rate,[7] expected cow-to-cow variation could have attributed to the difference.[7] In our study, cow-to-cow variation was eliminated by using the same cow with a uterine horn serving as the control prior to the second uterine horn serving as the treatment. The optimal time between $PGF_{2\alpha}$ treatment and embryo collection is unknown; however, uterine contraction after intramuscular $PGF_{2\alpha}$ treatment did not begin for 6-8 minutes[10] which may be influenced by route of delivery. In Experiment 3 cows were released from the chute after collection of the control uterine horn and brought back for collection of the treated horn and recollection of the first horn ~ 1 hour later. Still, embryo recovery was significantly increased and an additional embryo on average was recovered from the recollected horn. A double uterine flushing method was used to increase recovery rate; however, the exact mechanism was not dicussed.[3] Although cumbersome, DPBS was infused through the catheter and the plunger was closed with a disposable 5 ml syringe for 30 minutes before recovery of DPB;[3] the double flush did improve embryo recovery rates compared to the original lavage.[3]

Many other strategies have been implemented to improve embryo recovery rates when collecting embryos from cows. Autologous platelet rich plasma was injected into the bovine ovary before gonadotropin treatment increased the number of follicles responsive to ovarian superstimulation and improved embryo recovery.[14] One ovary was injected whereas the other ovary and uterine horn served as control; significantly more embryos recovered from the treated side compared to the control.[14] Further, catheter placement may also be important, as uterine horns collected with a deep catheter placement (~ 7 cm caudal to the tip of uterine horn) had significantly higher lower recovery compared to the uterine horn collected with shallow (~ 5 cm cranial to the beginning of the uterine bifurcation) placement, indicating the general location of the embryos within the uterine horns.[15]

Many interrelated factors could affect embryo recovery rates, including technician and technical issues such as failure to sufficiently fill the uterine horn with collection media, incomplete recovery of the media at each lavage, and failure to correctly identify embryos in the collection vessel. Animal factors such as genetics and characteristics (age, body condition, health, ovarian conditions, or follicular dominance), and the population of follicles are important factors to consider as well. Extrinsic factors such as the use of different commercial preparations of FSH, dosage, route of application, breeds of cattle and season are equally important.[16,17] Farm management has been reported to have the largest effect upon the number and quality of recovered embryos.[18] These results highlight the importance of proper animal management in a successful embryo transfer program.[18]

Some limitations should be considered when interpreting the results a study of this type. First, the donor cow population was heterogeneous, comprising animals of varying breeds, body sizes, ages, and unknown fertility histories. These factors are known to influence ovarian supestimulation response and embryo recovery rates, and their variability may have introduced confounding effects. Additionally, limited sample sizes may have led to the elevated variability among donors in response to FSH and overall embryo recovery. The embryo recovery process itself presents another source of variability, as the skill and technique of the veterinarians performing embryo collections can directly impact recovery success. Embryo collections in this study were performed by American Embryo Transfer Association practitioners to limit variability among collections. Furthermore, CL counts (to estimate ovulatory response) were assessed solely via transrectal ultrasonography in this project. Rectal palpation to estimate CL counts is commonly used in the field, but it lacks precision and can be influenced by the experience and interpretation of the technician. Finally, although outside the scope of our hypothesis, it is important to note, that there was likely some endogenous $PGF_{2\alpha}$ release associated with uterine lavage and manipulation. Effects of this endogenous prostaglandin and the combination with exogenous $PGF_{2\alpha}$, were not evaluated and are unknown. Increasing the number of replications, standardizing donor characteristics, and improving the accuracy of CL assessments would strengthen future studies that may involve using embryo collection media infused with $PGF_{2\alpha}$.

In conclusion, despite ovarian superstimulation advantages and the utilization by the industry, limited progress has been made in improving embryo recovery. Using a novel approach where each donor cow served as its own control, we documented that $PGF_{2\alpha}$ treatment enhanced ova recovery rate. Although there was no overall difference in the number of transferable embryos prior to and after treatment, some of these additional embryos recovered after $PGF_{2\alpha}$ treatment were transferable. Further research is required to fully understand the effects of $PGF_{2\alpha}$ treatment on the embryo developmental dynamic after transfer and endogenous $PGF_{2\alpha}$ release effect on embryo recovery.

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Authors' contribution and agreement

JL: methodology, investigation, data curation, writing original draft**;** **LO:** investigation, data curation, writing original draft; **SH:** data curation; and **JG:** conceptualization, methodology, investigation, validation, data curation, funding. Authors have read and approved the final submission.

Conflict of interest

None to report.

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