The effects of follicle stimulating hormone dose administered to cattle embryo donors on embryo quality and in vitro development and plasminogen activator production

by
Mackenzie R. Gellings

A PROJECT

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Animal Sciences
(Honors Scholar)

Presented May 22, 2015
Commencement June 2015

AN ABSTRACT OF THE THESIS OF

Mackenzie R. Gellings for the degree of Honors Baccalaureate of Science in Animal Sciences presented on May 22, 2015. Title: The effects of follicle stimulating hormone dose administered to cattle embryo donors on embryo quality and in vitro development and plasminogen activator production

Abstract approved:

_____

Alfred R. Menino, Jr.

The relationship between dose of follicle stimulating hormone (FSH) used to superovulate cattle embryo donors and embryo viability as measured by in vitro development and plasminogen activator (PA) production was determined. Late morulae to blastocysts were collected non-surgically from 12 superovulated crossbred beef cows. Embryos were cultured for 8 d and at 24-h intervals embryos were evaluated for viability, stage of development and transferred to fresh culture medium. Conditioned medium was recovered and used for PA analysis. PA production was determined using a caseinolytic agar gel assay with human urokinase as the standard. Mean number of total ova and embryos recovered, transferrable embryos, degenerate embryos and unfertilized ova (UFOs) did not differ ($P>0.10$) between cows treated with 200 vs. 400 mg FSH. Likewise, mean percentage of embryos recovered, transferrable embryos, degenerate embryos and UFOs did not differ ($P>0.10$) between cows treated with 200 vs. 400 mg FSH. More embryos hatched ($P<0.05$) and developed sooner ($P<0.05$) to the expanded blastocyst stage recovered from cows superovulated with 200 mg compared to 400 mg FSH. Although not significantly different, PA production was twofold greater by embryos recovered from cows superovulated with 200 vs. 400 mg FSH. The poorer in vitro development observed by embryos recovered from cows treated with 400 mg FSH may be an indicator of in utero survival and the lower likelihood of pregnancy establishment.

Key Words: FSH, plasminogen activator, superovulation, embryo viability

Corresponding e-mail address: mackenzieraegellings@gmail.com

The effects of follicle stimulating hormone dose administered to cattle embryo donors on embryo quality and in vitro development and plasminogen activator production

by
Mackenzie R. Gellings

A PROJECT

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Animal Sciences
(Honors Scholar)

Presented May 22, 2015
Commencement June 2015

Honors Baccalaureate of Science in Animal Sciences project of Mackenzie R. Gellings presented on May 22, 2015.

APPROVED:

Alfred R. Menino, Jr., Mentor, representing the Department of Animal and Rangeland Sciences

---

Alexandria P. Snider, Committee Member, representing the Department of Animal and Rangeland Sciences

---

Dawn M. Sherwood, Committee Member, representing the Department of Animal and Rangeland Sciences

---

Matthew A. Kennedy, Committee Member, representing the Department of Animal and Rangeland Sciences

---

Toni Doolen, Dean, University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to readers upon request.

Mackenzie R. Gellings, Author

Table of Contents

List of Figures

Figure Page

List of Tables

Table Page

The effects of follicle stimulating hormone dose administered to cattle embryo donors on embryo quality and in vitro development and plasminogen activator production

Introduction

Plasminogen activators (PA) are serine proteases that activate plasminogen, a zymogen, to plasmin the primary enzyme involved in fibrinolysis. They are released by the bovine embryo, and other mammalian embryos, during blastocoelic expansion and hatching. Plasminogen activators have been speculated to have many roles in addition to clot breakdown including implantation, tissue remodeling and cell migration and the facilitation of hatching or shedding the zona pellucida. Menino and Williams (1987) reported that plasminogen activator activates plasminogen to plasmin which consequently degrades the zona pellucida, a glycoprotein surrounding mammalian embryos, to facilitate the hatching process.

Menino and Kaaekuahiwi (1990) determined a positive correlation between the amount of PA produced by an embryo and the percentage of embryos that completed the hatching process. This study also found that plasminogen activator production was correlated positively to cell number, cell stage and cell diameter, which strongly suggested plasminogen activator production was an indicator of embryo viability.

Determining the effect of follicle stimulating hormone (FSH) dose on the viability of bovine embryos, using PA as an indicator of viability, could enhance current technologies such as embryo transfer by allowing the transfer of embryos that have a higher probability of creating a pregnancy. If it could be proven that there is a correlation between FSH dose and embryo viability then techniques could be reevaluated to take advantage of this correlation. Techniques of determining embryo viability at the present include morphological evaluation of embryos in

culture; however this technique is not very accurate. Morphologically normal embryos are not certain to create a pregnancy as there could be other issues with embryo quality. Procedures that test the PA production of embryos before transfer could greatly increase the efficacy of determining embryo viability. Determining different FSH dosage techniques could also aid producers and technicians in increasing the amount of transferrable embryos they are able to collect from superovulated donor cows.

Embryo qualities that are used in the grading process include physical characteristics such as the shape of the embryo itself, color, number and compactness of cells, size or the perivitelline space, number of extruded and degenerated cells and the number and size of vesicles (Lindner and Wright, 1983).

The first objective of this research was to determine if there was an effect of FSH dose on the quantity and quality of embryos recovered from superovulated donor cows. The second objective of this research was to determine if FSH dose affected in vitro embryo development and PA production, a marker of embryo viability. We hypothesized embryos produced from the cows superovulated with 200 mg FSH would be more viable and produce more PA than embryos produced from cows superovulated with 400 mg FSH.

Review of Literature

Plasminogen Activators

Plasminogen activators (PA) are serine proteases that activate plasminogen, a zymogen, to the active enzyme plasmin (Dano et al., 1985). Stickland et al. (1976) provided evidence into many different cell types that produce PA including; activated macrophages, neoplastic cells, ovarian granulosa cells and parietal endoderm. They proceeded to identify the trophoblasts of mouse embryos as the cells responsible for initiating synthesis of this enzyme. In the same study it was shown that the second phase of PA production is driven by the parietal endoderm (Strickland et al., 1976). Given that it is a protease produced and activated primarily by embryonic tissue, a conclusion can be drawn that it is an important part of embryo development.

This protease has been shown by multiple studies to have many functions that relate to embryo development, as well as other reproductive systems. PA production was shown to be closely correlated to the invasive period of mouse embryos in vivo, giving rise to the notion that PA production is important in the role of implantation of invasive embryo species (Stickland et al., 1976). Production of PA in swine has been correlated to involvement in tissue remodeling and tissue proliferation, specifically in the developing blastocyst (Fazleabas et al., 1983). PA production and conversion of plasminogen to plasmin in bovine embryos accelerated development to attain a particular cell stage, including hatching (Williams and Menino, 1987). Conversion of plasminogen to plasmin is a possible mechanism used by the expanding blastocyst to affect a sublysis or weakening of the zona pellucida to facilitate hatching as suggested by Domon et al. (1973) in the rodent. The PA produced by the bovine embryo during hatching has been identified as a urokinase-type PA (Berg and Menino, 1992).

Given all the roles of PA it seems like a simple step to using its enzymatic activity as an indicator of embryo viability. All the cells that are known to produce PA are of very diverse cell types but all function in two very similar ways; cell migration and/or destruction of extracellular matrix (Strickland et al., 1976). When PA is high in bovine embryos zona loss and endoderm migration is occurring. For this reason it is not unreasonable to suggest that PA is an important part of the interactions between cells and their surroundings and that the enzyme takes part in important embryonic processes such as remodeling and cellular migration.

Embryo Development and Viability

PA production seems to be an important factor in an embryo's ability to survive and continue to develop in the early parts of embryogenesis. To thrive in the uterine environment within its first few weeks of life, many processes need to take place within the developing embryo. Before fertilization occurs, fusion of the pronuclei (the egg and sperm) takes place within the ootid in the ampullary-isthmic junction of the oviduct. The ootid then becomes a zygote, developing from a single cell to a mass of cells which cannot be counted accurately. The embryo remains in the oviduct of the cow until d 4 or 5 and at this point the embryo has developed to a 16 to 32 cell stage morula. On d 4 or 5 in the cow the morula enters the uterus where it continues to develop. Cell differentiation takes place and the embryo forms a distinct inner cell mass, blastocoele and trophoblast to become an early blastocyst (Senger, 2005).

As the embryo continues to develop, more fluid fills the blastocoele and the inner cell mass and trophectoderm continue to mature. This rapid growth puts more and more pressure on the zona pellucida, which is a thick, translucent glycoprotein surrounding the embryo. At this stage the trophectoderm also starts to produce proteolytic enzymes, including PA, which weaken

the zona pellucida so that, eventually, it will rupture (Williams and Menino, 1987). Once the zona pellucida has ruptured and the embryo leaves its confines, the embryo is considered a hatched blastocyst.

The embryo then goes through other important processes, such as ensuring maternal recognition of pregnancy which occurs day 14 to 15 in the bovine embryo. The bovine embryo has not attached at this point in development, attachment to the endometrium will occur day 18 in the cow. More invasive embryo types exist, for example the mouse embryo which invades the endometrium rather than merely attaching itself to the endometrium. Rodent embryos specifically, however, use PA to aid in implantation in the endometrium, and where it has been speculated that bovine embryos facilitate attachment through another mechanism (Sherman et al., 1976). After this point in its development, sometime around day 18 to 22 in the bovine embryo, the embryo will attach itself to the uterine epithelium and continue to develop (Senger, 2005).

Viable embryos are ones that develop normally and can potentially create a pregnancy. There are several observable factors used to estimate viability of an embryo. Physical characteristics such as the shape of the embryo itself, color, number and compactness of cells, size of the perivitelline space, number of extruded and degenerated cells and the number and size of vesicles (Wright et al., 1983). The embryo's ability to stay structurally intact and not collapse in on itself in vitro is also an important indicator of viability. The timeline of development is also an important indicator; embryos that do not hatch within a normal timeframe are less likely to be viable.

Production of PA could also be used as an indicator of viability. The enzyme is integral in the facilitation of hatching by the weakening of the zona pellucida as the early blastocyst develops (Williams and Menino, 1987). PA is important in facilitating implantation and attachment of the hatched blastocyst to the wall of the endometrium by tissue disruption (Sherman et al., 1976). This enzyme is also vital in enabling tissue remodeling and tissue proliferation such as endodermal migration in the developing blastocyst (Fazleabas et al., 1983).

Follicle Stimulating Hormone

Follicle stimulating hormone (FSH) is a glycoprotein produced by the gonadotroph cells in the anterior lobe of the pituitary gland. The hormone targets the Sertoli cells of the male's testis and participates in continual function of these cells. In the female the hormone targets the ovary, and as its name implies, stimulates follicular growth and recruitment as well as synthesis of estradiol (Senger, 2005).

In the female, FSH is utilized in modern superovulation protocols. When embryo transfer began commercially in the 1970s equine chorionic gonadotropin (eCG) was used in superovulation of cattle. However, there are many problems associated with the continued use of eCG to superovulate cattle. The longer half-life of eCG causes prolonged stimulation of the ovary and abnormal hormone activity which results in reduced embryo quality. The LH activity in eCG also induced premature ovulation of the oocyte and it has also been shown that cattle quickly build up antibodies to the hormone, and so become less responsive to the treatment each time it is used (Bó and Mapletoft, 2014).

Not very long after, it was discovered that a greater superovulatory response was achieved when a treatment with a pituitary extract containing FSH as well as luteinizing

hormone (LH) was used instead of eCG. Studies have shown that animals treated with eCG more frequently have abnormal endocrine profiles, specifically LH and progesterone, which have been associated with reduced ovulation and fertilization rates when compared to FSH treated animals (Bó and Mapletoft, 2014). Purified porcine FSH is now used in most modern superovulation protocols. To be effective, because of its short half-life, it must be given in twice daily injections. In two of the most common methods the injections are given over 4 to 5 days and can either be a consistent dose or a constantly decreasing dose with each day.

Studies focusing on the dosage of FSH given during superovulation protocols have suggested that embryo quality is adversely affected by higher dosages of the hormone. Sugano and Watanabe (1997) superovulated three groups of cattle with three differing dosages of purified porcine-FSH. Group 1 was given a decreasing dose, starting with a 4 mg dose, Group 2 was given a decreasing dose starting with a 6 mg dose and Group 3 was given two doses of 10 mg, in an attempt to reduce the amount of administrations. Three hundred and twenty embryos were recovered from all of the cattle after the superovulation protocols. Cattle from Group 1 produced an average of 6 embryos, the cattle from Group 2 produced 7 embryos on average and the cattle from Group 3 produced an average of 5 embryos. Most telling though was the quality of the embryos produced. Seventy-three percent of the embryos produced from Group 1 were considered high enough quality to be transferable, while only 63% and 47% were considered high enough quality from Groups 2 and 3, respectively. While higher concentrations of FSH produce a greater quantity of ovulations, this might in turn recruit follicles undergoing atresia and therefore produce embryos of lesser quality (Sugano and Watanabe, 1997).

Applications

The relationship between PA and embryo viability has been shown to have a positive correlation (Kaaekuahiwi and Menino, 1990). This relationship could be capitalized upon to improve the embryo transfer process in multiple species, especially in cattle. Modern techniques to analyze embryo quality before transfer are limited to visual quality grading which is fairly inefficient. An embryo that appears structurally and developmentally normal will not necessarily develop normally after a transfer. It has been acknowledged embryos producing higher levels of PA are more likely to undergo important processes such as hatching and cell migration (Kaaekuahiwi and Menino, 1990).

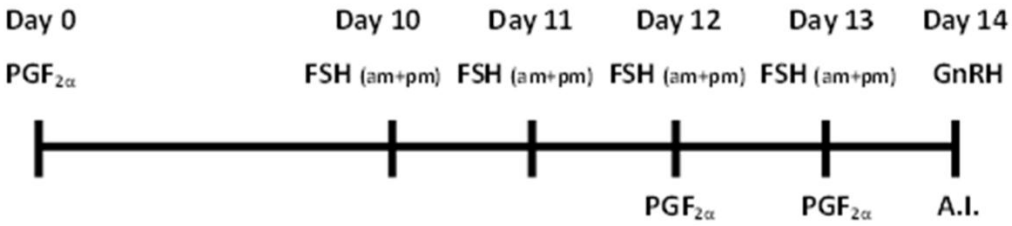
Studies have been done that show embryo quality is affected by FSH dosage. Embryo quality was negatively impacted by increased FSH doses for superovulating donor cattle (Sugano and Watanabe, 1997). A 10% decrease in transferrable embryos between the lower and higher doses of FSH can be observed. Although higher doses of FSH produce more ova, there are fewer fertilized ova and far more degenerate embryos compared to lower doses of FSH. Decreasing the dose of FSH used in commercial superovulation protocol would not only decrease the cost of these protocols but would also increase the efficiency of this procedure.

Materials and Methods

Embryo Collection and Culture

Twelve crossbred beef cows were separated into pairs for each trial. Cows began the estrous synchronization protocol with three injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Lutalyse®, Kalamazoo, MI), two 5 mL injections given on Day 0 and Day 12, followed by the final 2.5 mL injection on Day 13. Cows were then superovulated with twice daily intramuscular injections of FSH (Follitropin-V®, Athens, GA).

Each cow was given either a consistent dose of 5 mL per day or 2.5 mL per day. One cow was given a 2.5 mL dose of FSH in the morning and evening (400 mg per dose) which amounted to 5 mL per day, and 20 mL over the 4 days the injections were administered on Days 10, 11, 12 and 13. The other cow in the pair was given a 1.25 mL dose of FSH in the morning and evening (200 mg per dose) which amounts to 2.5 mL per day, and 10 mL over the 4 days the injections were administered on Days 10, 11, 12 and 13.



Estrous detection began 12 h after the last injection of FSH. All cows were artificially inseminated 3 times. Time of first breeding was dependent on when estrus was displayed, either at 12 or 24 h after the last injection of FSH. Cows were given 2 mL of gonadotropin releasing hormone (GnRH, Factrel®, Kalamazoo, MI) to insure ovulation after the first breeding.

Embryos were collected from cows 7 d after A.I. via a non-surgical flush of the uterus using Dulbecco's phosphate buffered saline containing 0.2% HTFCS and 10 ml/L of an antibiotic-antimycotic solution. The products of the uterine flushes were then examined for the presence of embryos using a dissecting microscope. Late morulae to blastocysts were recovered from the uterine flush by aspiration. Embryos were transferred into micro-drops, containing Ham's F-12 and 1.5% BSA, and evaluated for development and graded.

At 24-h intervals, for a total of 8 d, embryos were transferred to fresh culture medium and reevaluated for development. Fifteen microliters of conditioned medium were collected and frozen at -20^{o} C until assayed for PA. To correct for spontaneous activation of plasminogen due to the culture conditions, medium from drops not containing embryos were also recovered and frozen.

Plasminogen Activator Assay

PA concentrations in the culture medium were determined using a modified caseinolytic assay described by Kaaekuahiwi and Menino (1990). Two percent non-fat dry milk was dissolved in a 0.38M Tris (hydroxymethyl)-aminomethane-0.10 M glycine buffer containing 0.195 g/l $CaCl_2 \bullet 2H_2O$, heated to 55°C, and mixed with an equivalent volume of 2% agarose (Sigma Chemical Co.). Fifteen milliliters of the casein-agar mixture was poured into the plastic plates and ten 4 mm diameter wells were cut in each casein-agar gel plate. Fifteen microliters of 120 µg/ml human plasminogen (Sigma Chemical Co., St. Louis, MO) was combined with 15 µl of recovered culture medium, or 0, 0.01, 0.05, 0.1, 0.5, 1, or 5 U/ml urokinase (Sigma Chemical Co., St. Louis, MO) as the standard and incubated for 15 minutes at 37^{o}C.

After incubation, 20 µl of the mixture was pipetted into wells in the casein-agar plates and incubated at room temperature for 24-h. Plates were fixed with 3% acetic acid for 15 minutes and rinsed in tap water. A digital electronic caliper was used to measure the diameter of the lytic zones. Concentrations of plasminogen activator in the culture media were determined from the standard curves of caseinolytic ring diameter by log urokinase concentration. By subtracting the amount of plasminogen activator in the medium without the embryo from the amount in the medium with an embryo for a 24-h period, the amount of PA produced by the embryo was determined.

Statistical Analysis

Differences in numbers and percentages of ova, embryos and UFOs recovered due to FSH dose were evaluated using the pooled t-test. Times required to develop to a particular cell stage were evaluated using one-way ANOVA. Difference in the percentages of embryos developing to the expanded, initiating hatching, hatched and attached blastocyst stages were determined by Chi-square analysis. Differences in PA production were determined using two-way ANOVA for repeated measures where the main effects were FSH, Time and the FSH X Time interaction.

Results

Mean numbers of ova recovered from cows treated with 200 or 400 mg FSH were 4.7 ± 2.3 and 8.8 ± 2.3 ova, respectively. Ova were not collected from two cows in the 200 mg FSH group hence Table 1 summarizes the results from the 4 and 6 cows in which ova were recovered. No differences ($P>0.10$) were observed in mean numbers of total ova, total embryos, transferrable embryos, degenerate embryos and UFOs recovered from cows treated with 200 vs. 400 mg FSH.

Similar to the results in Table 1, mean percentages of total embryos, transferrable embryos, degenerate embryos and UFOs recovered from cows treated with 200 vs. 400 mg FSH did not differ ($P>0.10$; Table 2).

Mean times required for embryos recovered from cows treated with 200 mg FSH to develop to the expanded, initiating hatching, hatched and attached blastocyst stages were 26, 58, 80 and 112 h, respectively (Figure 2). Mean times required for embryos collected from cows treated with 400 mg FSH to develop to the expanded, initiating hatching, hatched and attached blastocyst stages were 44, 68, 88 and72 h, respectively. Embryos recovered from cows treated with 200 mg FSH reached the expanded blastocyst stage sooner ($P<0.05$) than embryos recovered from cows treated with 400 mg FSH (Figure 2). Conversely, blastocysts developing from embryos collected from cows treated with 400 mg FSH attached to the plastic substratum sooner ($P<0.05$; Figure 2).

Percentages of embryos developing to the expanded, initiating hatching and attached blastocyst stages did not differ ($P>0.10$) between cows treated with 200 or 400 mg FSH (Figure

3). However more ($P<0.05$) embryos collected from cows superovulated with 200 mg FSH hatched compared to embryos recovered from cows treated with 400 mg FSH (Figure 3).

Although mean PA production by embryos collected from cows superovulated with 200 mg FSH was more than twofold greater (0.09 ± 0.2 IU/ml) compared to 400 mg FSH (0.04 ± 0.02 IU/ml), no significant difference was observed. Time however was a significant factor where PA production at 96 h was greater ($P<0.05$) than at 24, 48, 72, 120 and 192 h of culture (Figure 4). The FSH X Time interaction was not significant.

Figures and Graphs

Table 1. Mean numbers (± SE) of total ova and embryos, transferrable and degenerate embryos and unfertilized ova (UFOs) recovered from donor cows superovulated with 200 or 400 mg FSH.

	FSH Dose			
	n	200 mg	n	400 mg
Total Ova	4	7.0 ± 2.9	6	8.8 ± 2.3
Total Embryos	4	6.8 ± 3.0	6	7.3 ± 2.5
Transferable Embryos	4	5.5 ± 2.3	6	4.7 ± 1.6
Degenerates	4	1.3 ± 0.9	6	2.7 ± 1.1
UFOs	4	0.3 ± 0.3	6	1.5 ± 0.8

Table 2. Mean percentages (± SE) of total embryos, transferrable and degenerate embryos and unfertilized ova (UFOs) of the total number of ova recovered from donor cows superovulated with 200 or 400 mg FSH.

	FSH Dose			
	n	200 mg	n	400 mg
Total Embryos	4	87 ± 12%	6	79 ± 11%
Transferable Embryos	4	68 ± 23%	6	55 ± 12%
Degenerates	4	19 ± 12%	6	23 ± 6%
UFOs	4	12 ± 12%	6	20 ± 11%

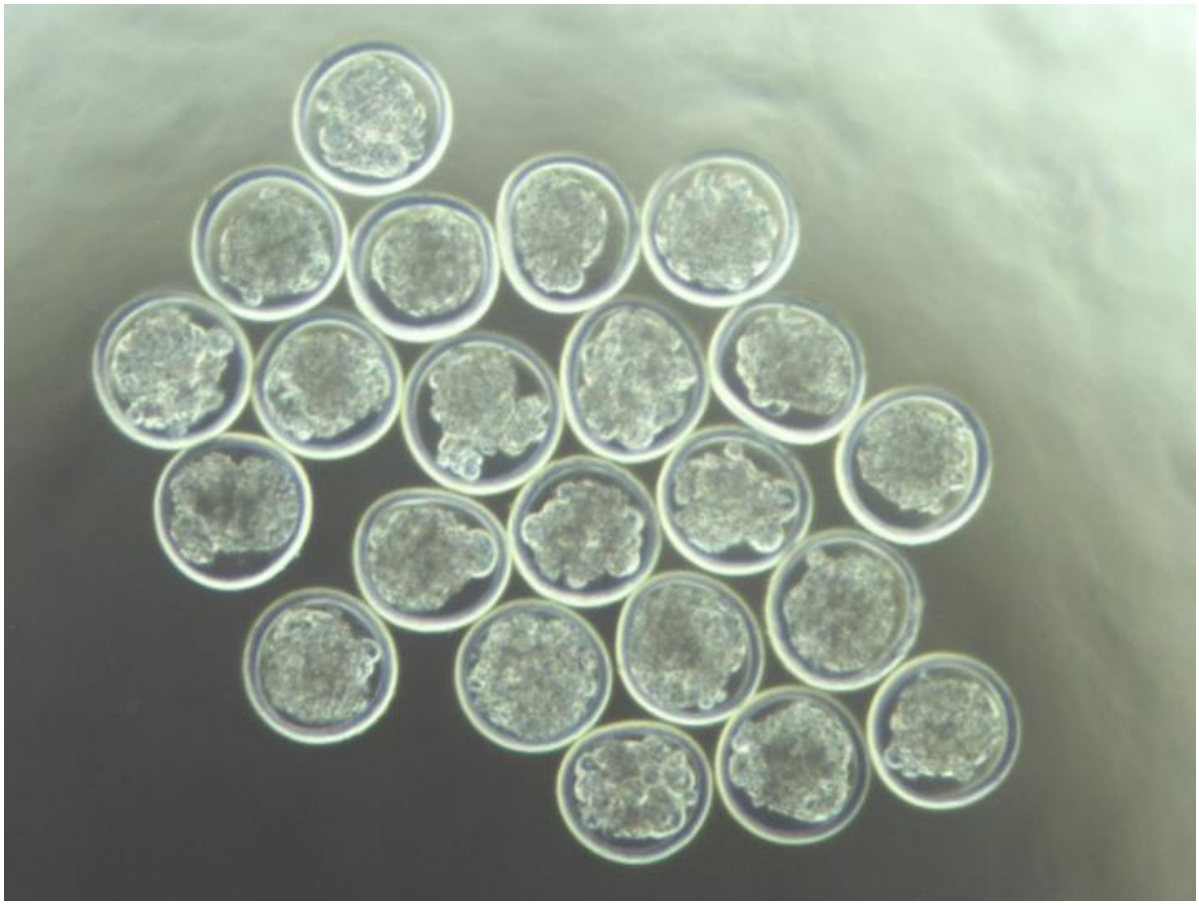


Figure 1. Embryos collected non-surgically from a donor cow superovulated with 200 mg FSH.

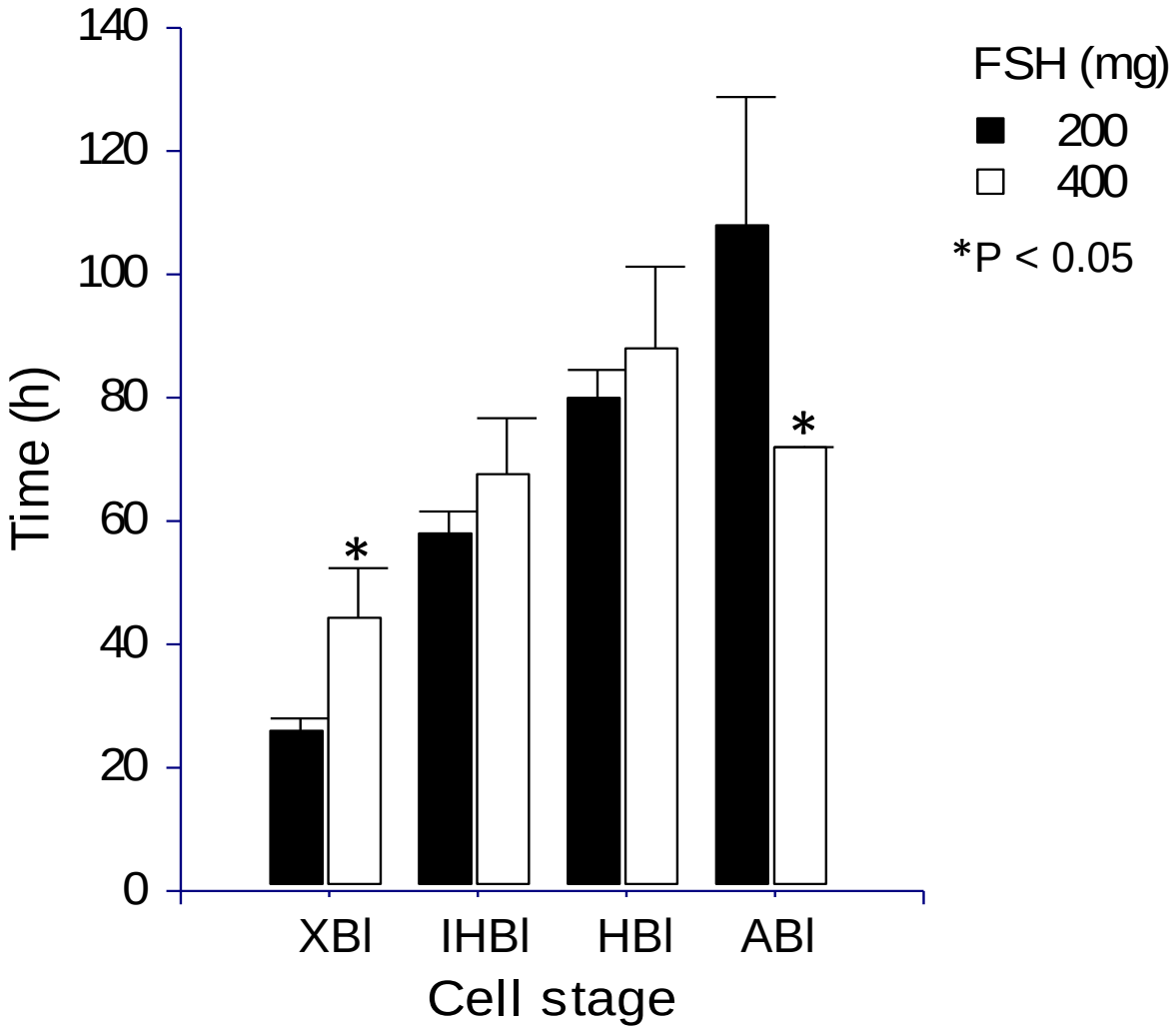


Figure 2. Times (h) required for embryos recovered from cows treated with 200 or 400 mg FSH to develop to the expanded (XBl), initiating hatching (IHBl), hatched (HBl) and attached blastocyst (ABl) stages.

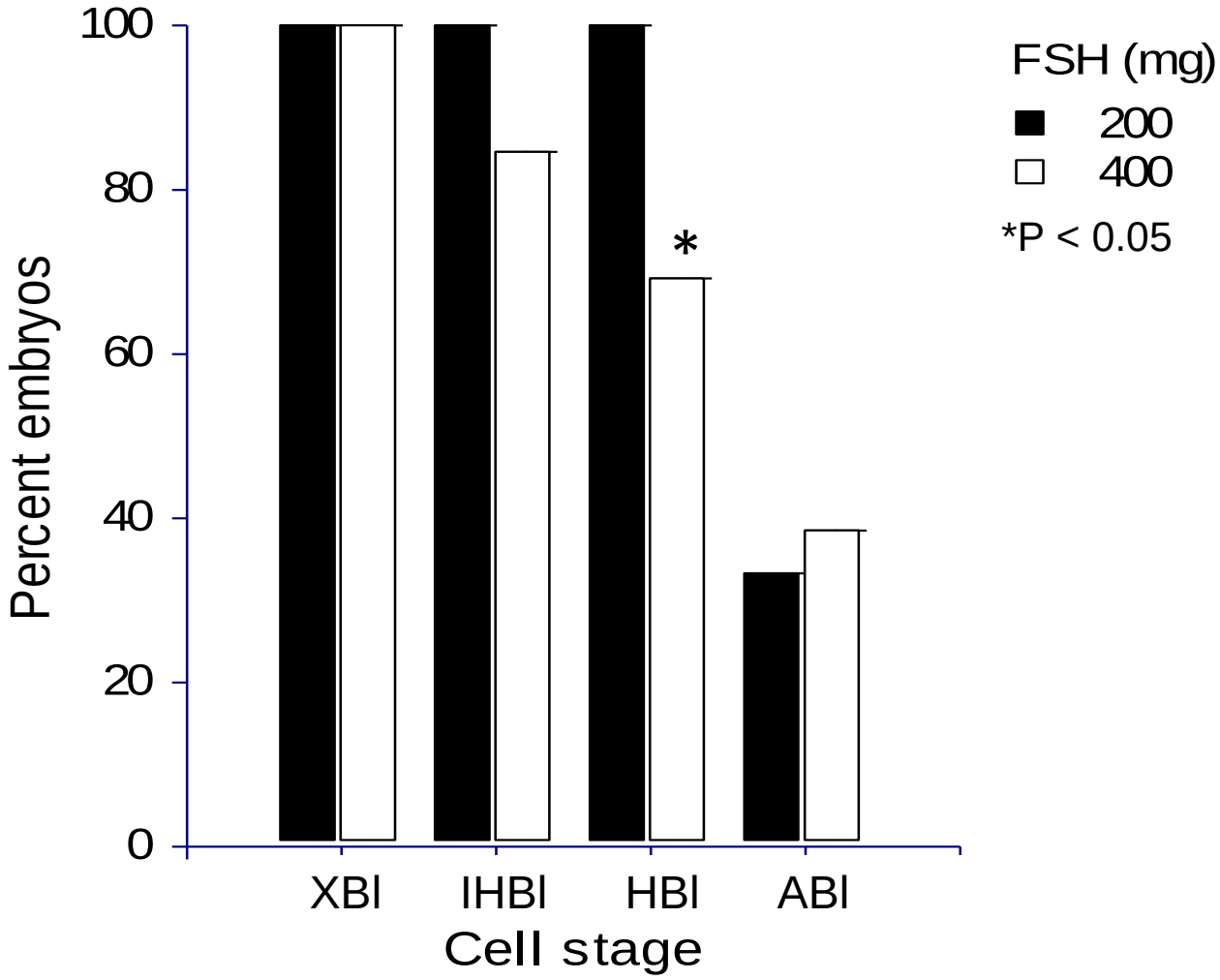


Figure 3. Effects of superovulating donor cows with 200 or 400 mg FSH on embryo development to the expanded (XBl), initiating hatching (IHBl), hatched (HBl) and attached blastocyst (ABl) stages.

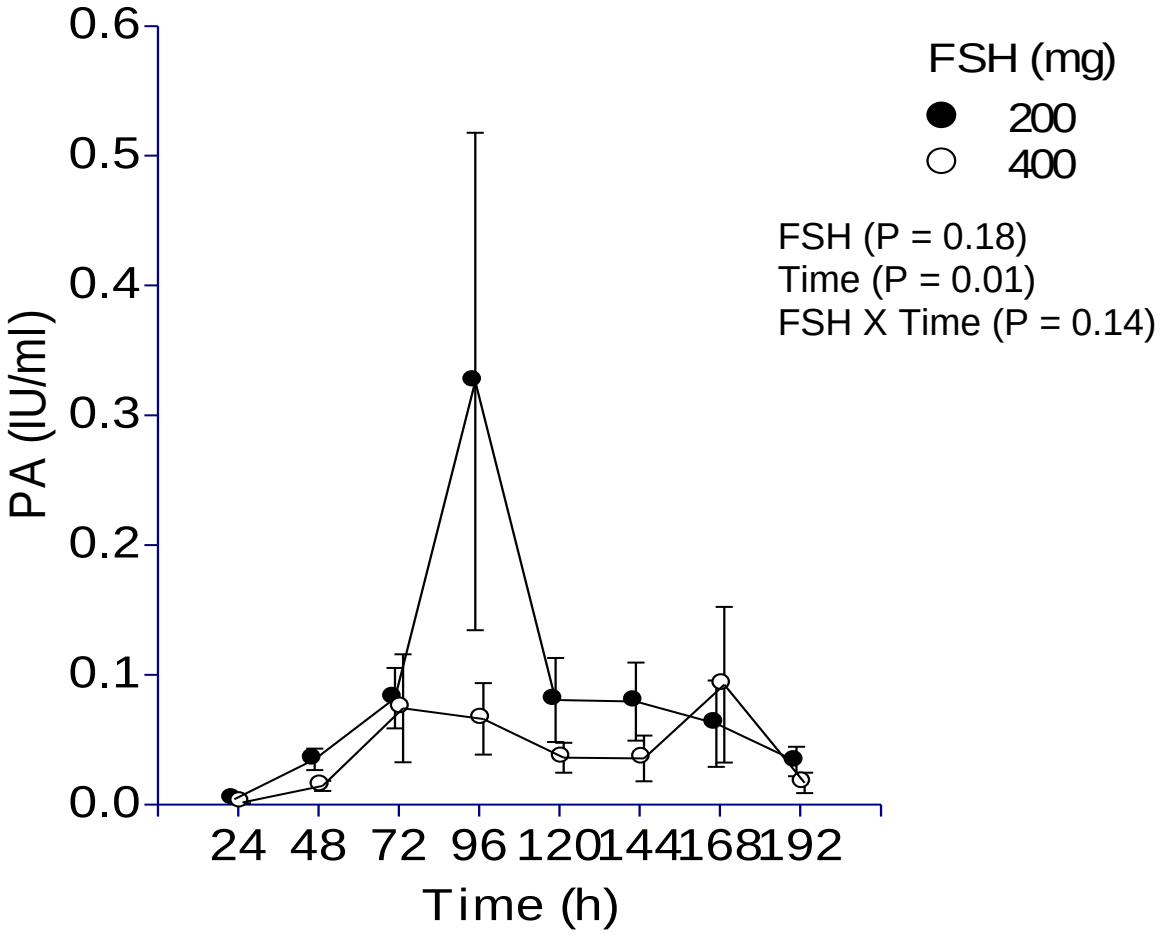


Figure 4. Plasminogen activator (PA) activity (IU/ml) in conditioned medium from embryos recovered from donor cows superovulated with 200 or 400 mg FSH.

Discussion

The percentage of transferable embryos collected from cows treated with the lower dose of FSH were similar to the number of transferrable embryos collected by Sugano and Watanabe (1996) and Pawlyshyn et al. (1986). In the 1996 study by Sugano and Watanabe three different dosing schemes were used: a high dose of 6 mg 2Xs day, a low dose of 4 mg 2Xs day and a third dose of 10 mg 1X day. Of the embryos collected in the study, 73% produced from the 4 mg dosed cows were considered transferrable whereas 63% and 47% of the embryos recovered from the 6 and 10 mg dosed cows, respectively, were considered transferrable (Sugano et al., 1997). Pawlyshyn et al. (1986) examined four different dosing schemes: 50%, 100%, 150% and 200% doses. Similarly to the study by Sugano et al. (1997), a decrease in transferrable embryos was observed as the dose of FSH increased from 55% transferrable in the 50% dose to 1% transferrable in the 200% dose (Pawlyshyn et al., 1986). Ovulatory response increased as the dosage increased; however, increased dosage negatively impacted transferability of the embryos.

Our results were very similar to both studies. The average percentage of transferrable embryos for the low dose cows receiving 200 mg FSH was 68% whereas the average percentage of transferrable embryos for the cows receiving 400 mg FSH was 55%. Although the ova and embryo recovery data were not statistically significant a trend was apparent where embryo quality was negatively affected by increased FSH dosage. Numbers of total ova and embryos recovered were greater for the high dosed cows however more transferrable embryos and less degenerate embryos and UFOs were associated with the low dosed cows. Similarly, percentages of total embryos and transferrable embryos were greater and percentages of degenerate embryos and UFOs were less in the low dosed cows.

There have yet to be any studies following FSH dosing all the way to percentage of successful pregnancies but there have been a few studies connecting embryo quality to pregnancy rate. Lindner and Wright (1983) superovulated and collected embryos from multiple donor cows to determine the effect of embryo quality on pregnancy rates. Embryos were graded as excellent, good, fair or poor based on morphological and developmental characteristics and were transferred into synchronized recipients. A total of 783 embryos were transferred and 308 pregnancies resulted. Pregnancy rates were 45%, 44%, 27% and 20% for excellent, good, fair and poor embryos, respectively (Lindner and Wright, 1983). Excellent and good quality embryos resulted in much higher pregnancy rates than their fair and poor counterparts.

Besides grading, PA production is also an effective measure of viability. Menino and Williams (1987) and Kaaekuahiwi and Menino (1990) both correlated PA production positively to embryo quality. Menino and Williams (1987) determined that physiologic levels of plasminogen in the culture medium accelerated hatching in vitro. Their results suggested that the activation of plasminogen to plasmin by PA was a possible mechanism to facilitate hatching, a necessary process for embryo development (Menino and Williams, 1987). Kaaekuahiwi and Menino (1990) determined from embryo culture and PA analysis that as embryonic development increased embryos produced more PA. PA production in culture was positively correlated to developmental stage and embryonic cell number (Kaaekuahiwi and Menino, 1990). Both of these studies linked PA production to embryo viability.

Using PA as an indicator of viability, we determined that an increased FSH dosage negatively affected embryo quality and viability. Excellent and good quality embryos were cultured from cows dosed with 200 or 400 mg FSH. Embryos from the higher dosed cows produced less PA than embryos from the 200 mg dosed cows. Embryos recovered from the 200

mg dosed cows also exhibited a prominent spike in PA at 96 h whereas embryos from the 400 mg dosed cows displayed a small spike in PA production at 168 h of culture. Most significant however was 100% of the embryos collected from the 200 mg dosed cows hatched whereas only 69% of the embryos from the 400 mg dosed cows completed hatching.

In conclusion, the results suggest embryos recovered from cows superovulated with a lower dose of FSH were more likely to advance to more developed cell stages and produce more PA than embryos recovered from cows treated with 400 mg FSH. Producers using embryo transfer in their herds should consider using reduced dosages of FSH for superovulating donor cows because embryo viability after collection is compromised with the higher doses.

Literature Cited

Berg, D.A. and Menino, A.R. 1992. Bovine embryos produce a urokinase-type plasminogen activator. Molecular Reproduction and Development 31:14-19

Blondin, P., Coenen, K., Guilbault, L.A. and Sirard, M.A. 1996. Superovulation can reduce the developmental competence of bovine embryos. Theriogenology 46:1191-1203

Bó, G.A. and Mapletoft, R.J. 2014. Historical perspectives and recent research on superovulation in cattle. Theriogenology 81: 38-48

Breuel, K.F., Baker, R.D., Butcher, R.L., Townsend, E.C., Inskeep, E.K., Dailey, R.A. and Lerner, S.P. 1991. Effects of breed, age of donor and dosage of follicle stimulating hormone on the superovulatory response of beef cows. Theriogenology 36 (2):241-255

Dano, K., Andreasen, P.A., Grondahl-Hnasen, J., Kristensen, P., Nielsen, L.S. and Skriver, L. 1985. Plasminogen activators, tissue degradation, and cancer. Adv. Cancer Res. 44:139

Domon, M., Pinsker, M.C. and Mintz, B. 1973. Thiocyanate assay for sublytic change in the zona pellucida of the mouse egg. Biol. Reproduction 9:246-253

Fazleabas, A.T., Geisert, R.D., Bazer, F.W. and Roberts, R.M. 1983. Relationship between release of plasminogen activator and estrogen by blastocysts and secretion of plasmin inhibitor by uterine endometrium in the pregnant pig. Biology of Reproduction 29:225-238

Hasler, J. 2013. Forty years of embryo transfer in cattle: A review focusing on the journal Theriogenology, the growth of the industry in North America, and personal reminisces. Theriogenology 81:152-169

Hiraizumi, S., Nishinomiya, H., Oikawa, T., Sakagami, N., Sano, F., Nishino, O., Kurahara, T., Nishimoto, N., Ishiyama, O., Hasegawa, Y. and Hashiyada, Y. 2014. Superovulatory response in Japanese Black cows receiving a single subcutaneous porcine follicle-stimulating horone treatment or six intramuscular treatments over three days. Theriogenology 83:466-473

Kaaekuahiwi, M.A. and Menino, A.R. 1990. Relationship between plasminogen activator production and bovine embryo development in vitro. Journal of Animal Science.

Lindner, G.M. and Wright, R.W. 1983. Bovine embryo morphology and evaluation. Theriogneology 20:407

Menino, A.R. and Williams, J.S. 1987. Activation of plasminogen by the early bovine embryo. Biology of Reproduction 36:1289-1295

Pawlyshy, V., Lindsell, C.E., Braithwaite, M. and Mapletoft, R.J. 1986. Superovulation of beef cows with FSH-P: a dose response trial. Theriogenology 25(1):179

Sherman, M.I., Strickland, S. and Reich, E. 1976. Differentiation of early mouse embryonic and teratocarcinoma cells in vitro: plasminogen activator production. Cancer Research 36: 4208-4216

Strickland, S., Sherman, M.I. and Reich, E. 1976. Plasminogen activator in early embryogenesis: enzyme production by trophoblast and parietal endoderm. Cell 9: 231-240

Sugano, M., and Watanabe, S. 1997. Use of highly purified porcine FSH preparation for superovulation in Japanese black cattle. J. Vet. Med. Sci. 59(3): 223-225