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# Prediction of Superovulatory Response in Beef Cows Based on Serum Anti-Mullerian Hormone and Antral Follicle Number

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Prediction of Superovulatory Response in Beef Cows Based on Serum Anti-Mullerian Hormone  
and Antral Follicle Number

Prediction of Superovulatory Response in Beef Cows Based on Serum Anti-Mullerian Hormone  
and Antral Follicle Number

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Masters of Science in Animal Science

by

Keith Center  
Morehead State University  
Bachelor of Science in Animal Science, 2012

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This thesis is approved for recommendation to the Graduate Council.

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## ABSTRACT

A study investigated the use of Anti-Mullerian hormone (AMH) and/or follicle counts as a predictor of subsequent superovulatory response and embryo production in 79 beef cows. Before initiation of superovulation, ultrasonography was used to scan the ovaries of each donor cow to record the number of 3 to 5 mm follicles present, and a blood sample was collected for measure of serum AMH. At the time of embryo collection, the ovaries of donor cows were palpated to estimate the number of corpora lutea (CL) present on each ovary. Recovered embryos were evaluated for stage of development and morphological quality. Across cows, serum AMH ranged from 0.013 to 0.898 ng/mL, with a mean of 0.293 ng/mL. The distribution of AMH concentrations was divided into quartiles (AMH Q1 through Q4, with Q1 the lowest and Q4 the highest, ng/mL) for analysis. Donor cows in AMH Q4 had a greater ( $P < 0.001$ ) number of 3 to 5 mm follicles at the start of superovulation than did donors in either Q1 or Q2. At embryo collection, cows in AMH Q3 and 4 had more ( $P < 0.001$ ) palpable CL than cows in AMH Q1. The mean number of embryos recovered from donor cows in AMH Q4 was greater ( $P < 0.001$ ) than those recovered from cows in either AMH Q1 or 2, but similar to that of AMH Q3. The percentage of recovered embryos classified as transferrable, degenerate or unfertilized were similar ( $P \geq 0.275$ ) across AMH quartiles. Analysis indicated that AMH was positively correlated ( $P < 0.001$ ) with mean follicles ( $r = 0.458$ ), CL ( $r = 0.452$ ) and embryos recovered ( $r = 0.430$ ). In order to determine if follicle counts at the start of superovulation might also be predictive of subsequent superovulatory response, the distribution of follicle counts were divided into quartiles (F Q1 through Q4, with Q1 the lowest and Q4 the highest) for analysis. Donor cows with higher follicle counts (F Q3 and 4) at the start of superovulation had more ( $P < 0.001$ ) palpable CL at embryo collection than donor cows in F Q1 or 2. More ( $P < 0.001$ ) embryos were

recovered from cows with the highest follicle counts (F Q4) as compared with cows having lower (F Q1 and 2) follicle counts. The percentage of transferable embryos and unfertilized ova were similar ( $P \geq 0.688$ ) across follicle count quartiles. As was noted for AMH, mean number of follicles at the start of superovulation was positively correlated ( $P < 0.001$ ) with mean CL ( $r = 0.556$ ) and mean embryos ( $r = 0.423$ ) but not percentage of viable or degenerate embryos, or unfertilized oocytes ( $P \geq 0.153$ ). In conclusion, results confirm that relative AMH concentration was positively correlated with number of small antral follicles in the ovaries of cows and might be used to either predict superovulatory response or possibly adjust superovulatory regimen to improve superovulatory response. Antral follicle counts at the initiation of superovulatory treatments might be a more practical alternative to AMH for predicting superovulatory response. Further study is needed to determine the effectiveness of using either AMH concentration or follicle counts to adjust superovulatory regimens for improved response.

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*By wisdom a house is built and by understanding it is established. By knowledge the rooms are filled with precious and pleasant riches. A wise man is full of strengths, and a man of knowledge enhances his might, for by wise guidance you can wage war and in abundance of counselors*

*there is victory. Proverbs 24: 3-6*

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## LIST OF ABBREVIATIONS

All abbreviations in this thesis are also defined in the text.

AETA	American embryo transfer association
AFC	antral follicle count
AMH	anti-mullerian hormone
ART	assisted reproductive technology
BCS	body condition score
CIDR	controlled internal drug release
CL	corpus luteum
ET	embryo transfer
FSH	follicle stimulating hormone
GnRH	gonadotropin-releasing hormone
IETS	International embryo transfer society
IVF	in vitro fertilization
LH	luteinizing hormone
MIS	mullerian inhibiting substance
MOET	multiple ovulation and embryo transfer
PRID	progesterone-releasing intravaginal device
SRY	sex-determining region Y
TDF	testis-determining factor

## CHAPTER 1: LITERATURE REVIEW

### Sexual Differentiation and Discovery of Anti-Mullerian Hormone

German anatomist and physiologist, Johanness Muller (1801-1858) is credited with the first description of the Mullerian ducts, the anlagen of the uterus, oviducts and anterior vagina. The Wolffian ducts (named for German anatomist Caspar F. Wolff; 1733-1794) are male counterparts of the Mullerian ducts and differentiate into the vas deferens, epididymides and seminal vesicles (Teixeira et al., 2001). In females, the Wolffian ducts degenerate, giving rise only to the Mullerian ducts whereas in males, two processes must occur. First the Wolffian ducts must be stimulated and maintained to differentiate into the male reproductive tract by testosterone produced by Leydig cells in the testes. Secondly, the Mullerian ducts regress due to the action of Anti-Mullerian hormone (AMH; Munsterberg and Lovell-Badge, 1991).

Anti-Mullerian hormone is a homodimeric disulfide-linked glycoprotein in the transforming growth factor beta family, with a molecular weight of 140 kDa (La Marca and Volpe, 2006). Alfred Jost is credited for discovering AMH in the 1940s. By performing in vivo embryonic experiments in rabbits, Jost demonstrated the existence of what he called Mullerian "hormone inhibitrice" or "Mullerian inhibitor" (Teixeira et al., 2001). Anti-Mullerian hormone is responsible for establishing embryonic sexual dimorphism based on presence or absence of the testis-determining factor (TDF), also known as sex-determining region Y (SRY) (Tüttelmann et al., 2009). In the presence of a Y chromosome, the genital ridge will arise on the mesonephros and a testis will develop due to the action of the TDF. Leydig cells of the developing testes produce testosterone to maintain and develop the Wolffian ducts while AMH produced by Sertoli cells causes regression of the Mullerian ducts. If the Y chromosome is absent, a default

pathway is followed resulting in development of an ovary (Munsterberg and Lovell-Badge, 1991).

In cattle, freemartins are an example of abnormal sexual differentiation that occurs when a female is born twin to a male calf. During early gestation, the placentae of developing male and female fetuses fuse, resulting in common fetal circulation. Testosterone and AMH produced by the developing male testes enters female circulation to inhibit female reproductive tract development. Freemartins are first recognizable about 49 days of gestation, when the upper part of the Mullerian ducts regress and the presumptive ovaries stop growing (Jost et al., 1972). After birth, the female ovaries are often stunted and contain sterile seminiferous tubules (Jost et al., 1972). Portions of the Wolffian ducts, including the seminal vesicles and epididymis are present but no change is noted in the external genitalia (Jost et al., 1972).

### **Role of Anti-Mullerian Hormone in Females**

While the role of AMH in male sexual differentiation is well known, more recent studies have demonstrated that granulosa cells within follicles in the ovaries of females also produce AMH. In the male, a six week old fetal testis has no AMH expression, but AMH is expressed by eight and one-half weeks of development (Rajpert-De Meyts et al., 1999). Expression of AMH in the male increases after birth, then gradually declines until puberty. In the female, AMH expression is not detected in granulosa cells of pre-antral follicles in fetal ovaries until 36 weeks gestation (Rajpert-De Meyts et al., 1999). Production of AMH by granulosa cells continues until the end of ovarian activity at reproductive senescence (Josso et al., 2001). While antral follicles 2 to 5 mm in diameter produce more AMH due to high granulosa cell numbers, smaller follicles also contribute to serum AMH concentrations based on their larger numbers within ovaries (Van

Rooij et al., 2002). Therefore, the relative contribution of the different follicle stages to the final serum AMH concentration is unclear.

Anti-Mullerian hormone functions to regulate the recruitment of primordial follicles into the pool of growing follicles (i.e, folliculogenesis) and regulate the responsiveness of follicles to follicle stimulating hormone (FSH; Visser et al., 2006). Primordial follicles (resting stage) are formed within the ovaries of developing females during the first trimester of gestation. At birth, the number of follicles present in the ovaries of heifer calves is reported to range from 10,000 to 350,000 (Erickson, 1966). Studies utilizing AMH-deficient mice as a model show that such mice are fertile, but delete the number of primordial follicles in their ovaries earlier in life than control mice (Durlinger et al., 1999). Anti-Mullerian hormone limits the number of primordial follicles entering folliculogenesis at any given time by reducing their responsiveness to FSH (Visser et al., 2006). As antral-stage follicles grow beyond 4 to 5 mm in diameter, their AMH production declines, resulting in increased responsiveness to FSH. The antral follicle within the pool of growing follicles with greatest responsiveness to FSH is likely the one selected to become the dominant follicle.

### **Anti-Mullerian Hormone as a Predictor of Follicular Population and Fertility**

Anti-Mullerian hormone is highly correlated with the total number of healthy follicles within the ovaries (Visser et al., 2006). If AMH is to be used to characterize the follicular population within ovaries, the question arises as to when and how often AMH should be measured to accurately reflect that follicular population. Ireland et al. (2008) used ultrasonography to characterize beef heifers as having low, intermediate or high antral follicle counts, then measured AMH daily, from day 6 of the estrous cycle through day 2 of the subsequent cycle. Although AMH varied somewhat by day of estrous cycle, all measures of

AMH correctly characterized heifers into low, intermediate and high categories, regardless of day of the cycle when AMH was measured. Therefore, a single measurement of AMH concentration in serum can be used to determine the size of the ovarian reserve of follicles. After measure of AMH from day 6 through day 2 of the subsequent estrous cycle, Ireland et al. (2008) surgically removed one ovary from each heifer characterized as having either high and low antral follicle counts for further histological analysis. Heifers characterized as having low follicle counts were found to have 60% smaller ovaries and 80% fewer morphologically healthy follicles and oocytes, suggesting a link between follicle number and fertility.

Low concentrations of progesterone in circulation are associated with higher embryonic mortality in cattle (Mann and Lamming, 1999). Both beef and dairy cows characterized as having low antral follicle counts also have progesterone concentrations that are 40 to 50% lower during the luteal phase of the estrous cycle than similar cows having high antral follicular counts (Jimenez-Krassel et al., 2009). This study also reported that within animals, progesterone concentrations were similar (i.e., repeatable for cows with low versus high follicle counts) over 3 estrous cycles. In addition to impaired luteal function, there is evidence that oocyte quality is compromised in cows with low follicular counts. Ireland et al. (2009) recovered the ovaries from cattle with either high or low antral follicle counts on day 2 to 3 of the estrous cycle, and recovered the cumulus cells from the 3 largest follicles on each ovary. Measure of cathepsin B and S mRNA in cumulus cells found a 2 and 6 fold increase, respectively, in these mRNA transcripts in cumulus cells from animals with low follicle counts. Both of these mRNA transcripts are associated with reduced development of embryos to the blastocyst stage in vitro (Bettegowda et al., 2008).

Anti-Mullerian hormone has also been classified as the best predictive marker of the ovarian response to a stimulatory treatment, as defined by the number of oocytes collected in assisted reproductive technologies (ART) (Muttukrishna et al., 2004; Muttukrishna et al., 2005). Muttukrishna et al. (2004) collected blood samples on 69 women who were over the age of 38; 17 of which were canceled due to poor ovarian response. Blood samples were collected on day 5 or 6 of the follicular phase to investigate whether AMH could be a helpful tool in predicting the ovarian response to gonadotropin treatment. The average AMH concentration in blood was found to be lower for the canceled group than the completed group. Therefore, AMH was characterized as a predictive marker for the ovarian response in patients undergoing ovarian stimulation. To follow up study Muttukrishna et al. (2005) evaluated the relationship of AMH, inhibin B and antral follicle count (AFC) to ovarian response in women undergoing ovarian stimulation for in vitro fertilization (IVF). Ultrasonography was performed on day 3 to determine AFC and blood samples were collected for AMH, and inhibin B concentrations. Measurements of inhibin B and AFC were found to be positively associated with the number of oocytes collected, while AMH was the best predictor of patients who responded poorly to superovulatory treatment.

In addition to studies in humans, research has been done in livestock species regarding the potential use of AMH in assisted reproductive technologies and multiple ovulation and embryo transfer (MOET) programs. Monniaux et al. (2011) conducted an experiment using goats to establish any seasonal variation in AMH and AFC (during the breeding season, at the end of breeding season, and at the end of anestrus) and determine their effects on embryo production. Plasma was recovered for assay of AMH at three different time periods: before first FSH injection (T0), at time of insemination (TI) and at time of embryo collection (TEC) during

each season period. Plasma recovered during the spring (February to April) were found to increase in AMH concentration, while plasma recovered in the fall (August to October) slightly decreased during the last two weeks. Anti-Mullerian hormone at (T0) was found to have a high positive correlation with the number of embryos collected in all three season periods, with AMH expression highest in 1 to 5 mm follicles and expression practically lost in follicles greater than 8 mm. The AMH concentration at T0 was found to be a predictive marker for the number of embryos recovered, regardless of the season. While 1 to 5 mm follicles were found to be highly correlated with the total number of corpora lutea (CL) and embryos recovered, regardless of the season (Monniaux et al., 2011).

### **Superovulation and embryo transfer**

The first successful embryo transfer (ET) was reported in 1890, when Walter Heape transferred two 4-cell Angora rabbit embryos into a bred Belgian doe that subsequently gave birth to four Belgian and two Angora offspring (Heape, 1891). It was not until 1950 that the first live ET calf was produced, by surgical transfer of an in vivo produced embryo recovered at slaughter (Willet et al., 1951). During the mid 1970s, development and/or refinement of non-surgical embryo collection and transfer techniques led to commercialization of the embryo transfer industry. According to the December 2013 report of the international embryo transfer society (IETS) Data Retrieval Committee ([http://www.iets.org/comm\\_data.asp](http://www.iets.org/comm_data.asp)), a total of 699,586 in vivo derived and 443,533 in vitro derived embryos were produced and available for transfer globally in 2013.

Over the past 4 decades, numerous research efforts have been directed toward improving superovulation regimens in cattle. Improvement has been made in the porcine FSH product most commonly used for superovulation. For several years, a crude porcine anterior pituitary extract

was used which contained varying amounts of both FSH and luteinizing hormone (LH). Variation in superovulatory response was attributed to batch-to-batch content of LH (Murphy et al., 1984). A study comparing the superovulatory response with a constant amount of FSH but varying amounts of LH reported that the number of fertilized and transferrable embryos recovered increased with decreasing LH content (Chupin et al., 1984). In fact, a later study (Looney et al., 1988) reported good superovulatory responses, with a high rate of fertilization and viable embryo production, when using recombinant bovine FSH alone (no exogenous LH) for superovulation of donor cows. A study comparing the superovulatory response of FSH preparations varying from pure FSH to a 1 to 1 ratio of LH to FSH indicated acceptable responses (total, fertilized and transferrable embryo production) occurred when the LH content was no more than 15 to 20% (Willmott et al., 1990). Currently, Follitropin-V is a widely used, purified FSH product for superovulation of cows that contains 16% LH.

For many years, superovulation was based on the donor's natural estrous cycle, with superovulatory treatment initiated between day 8 and 12 of the cycle. This required that donors be successfully synchronized within a few days of each other before superovulation could begin. A significant improvement in scheduling of donors occurred with the incorporation of supplemental progesterone in the superovulatory regimen. Goulding et al. (1994) compared the superovulatory response of beef heifers where superovulation treatment was initiated mid cycle versus after insertion of a progesterone-releasing intravaginal device (PRID) at varying times of the cycle. Results of the study showed that supplemental progesterone could be used to initiate superovulation at any stage of the cycle, simplifying scheduling of embryo donors. To avoid the need to synchronize the natural cycle of donors, controlled internal drug release (CIDR) progesterone inserts are currently used, with superovulation initiated 7 days after CIDR insertion.

The presence of a dominant follicle at the start of superovulation is known to reduce superovulatory response, due to suppression of the stimulatory effects of FSH by inhibin. This inhibition can be avoided by initiation of superovulation at follicular wave emergence. A method of timing initiation of superovulatory treatment with follicular wave emergence is to aspirate the 2 largest follicles present on the ovaries (i.e., follicle ablation) and initiate superovulatory treatment 2 days later (Baracaldo et al., 2000). However, this method requires transvaginal ultrasound-guided aspiration equipment that most ET practitioners do not have access or training to use. A procedure that has been shown to be as effective as follicle ablation is the use of steroid hormones to induce regression of the dominant follicle and emergence of a new follicular wave. With this method, embryo donors receive an injection of estradiol and progesterone in conjunction with a CIDR progesterone insert on day 0. On day 4, superovulation is initiated at follicular wave emergence (Baracaldo et al., 2000). In a superovulation protocol where a CIDR is inserted in conjunction with estradiol treatment on day 0 and FSH injections started on day 4, Bo et al. (2006) reported that delaying CIDR removal by 12 hours (from day 6.5 to day 7) and injecting gonadotropin-releasing hormone (GnRH) 12 to 24 hours after CIDR removal resulted in both synchronized ovulation and an increase in mean embryo produced. An advantage of the protocol was that it allowed for fixed-time insemination of donors, thus eliminating the need to detect estrus. The use of estradiol alone or in combination and progesterone to control follicular wave emergence is very effective. However, the use of steroids is prohibited in many countries.

While numerous improvements have been made in superovulatory regimens over the years, embryo production per donor has shown little progress. In 1994, members of the American Embryo Transfer Association (AETA) reported a mean number of embryos recovered

per donor of 5.5. Twenty years later, AETA and Canadian Embryo Transfer Association members reported a range of 5 to 7 embryos recovered per donor (Hasler, 2010). Donor response to gonadotropin treatment remains highly variable between individuals and difficult to predict (Rico et al., 2009). A major source of variability is the status of ovarian follicles at initiation of FSH treatment (Rico et al., 2009). Cows with few growing, small antral follicles subsequently have a poor ovulatory response to FSH treatment (Monniaux et al., 1983; Kawamata, 1994; Cushman et al., 1999). Recent studies indicate that AMH could be an endocrine marker to help predict superovulatory responses to treatments administered to cows for embryo production.

Monniaux et al. (2010) reported on a study where plasma was collected for measure of AMH before the start of superovulation treatment of Holstein cows, to determine if AMH had the potential to predict the number of embryos produced by individual cows. Between cows, AMH was found to range from 0.0011 to 0.5312 ng/mL. Cows with AMH greater than 0.2 ng/mL produced more embryos than cows having either 0.1 to 0.2 ng/mL or less than 0.1 ng/mL of AMH. In a recent study, Souza et al. (2015) reported that high producing dairy cows with AMH concentrations ranging from 0.1844 to 0.3733 ng/mL had a higher superovulatory response and more embryos recovered (14 versus 5 to 7) than cows with lower circulating AMH. These studies indicate that measure of AMH in blood of potential donor cows would be of value in identifying donors more likely to have a good response to superovulation. Prior knowledge of how a potential donor might respond to superovulatory treatment could also allow for modification of superovulatory protocols to improve their effectiveness.

## **Conclusion**

After approximately 4 decades of practice, the bovine ET industry is well established in

many countries. Through numerous research efforts, improvements have been made in superovulatory products and regimens. Donor and recipient management have been simplified. Despite improvements, extreme donor-to-donor variability in superovulatory response remains a concern. Embryo production per donor has not changed in the past 20 years. The ability to predict superovulatory response and adjust regimens accordingly would be of great benefit to the ET industry. Research indicates that a single measure of circulating AMH accurately reflects the population of follicles within the ovaries. Animals with a greater number of follicles are those found to respond well to superovulatory treatments. Therefore, a study is proposed to investigate the use of AMH and/or follicle counts as a predictor of subsequent superovulatory response and embryo production in beef cows. In most instances, the client rather than the ET practitioner selects the potential embryo donor. If either or both AMH and antral follicle counts can accurately predict superovulatory responses, then protocols might be adjusted to improve the probability of success, regardless of the donor's potential.

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## **CHAPTER 2: ANTI-MULLERIAN HORMONE AS A PREDICTIVE ENDOCRINE MARKER FOR SUPEROVULATORY RESPONSE AND EMBRYO PRODUCTION IN BEEF CATTLE**

### **Abstract**

A study investigated the use of Anti-Mullerian hormone (AMH) and/or follicle counts as a predictor of subsequent superovulatory response and embryo production in 79 beef cows. Before initiation of superovulation, ultrasonography was used to scan the ovaries of each donor cow to record the number of 3 to 5 mm follicles present, and a blood sample was collected for measure of serum AMH. At the time of embryo collection, the ovaries of donor cows were palpated to estimate the number of corpora lutea (CL) present on each ovary. Recovered embryos were evaluated for stage of development and morphological quality. Across cows, serum AMH ranged from 0.013 to 0.898 ng/mL, with a mean of 0.293 ng/mL. The distribution of AMH concentrations was divided into quartiles (AMH Q1 through Q4, with Q1 the lowest and Q4 the highest, ng/mL) for analysis. Donor cows in AMH Q4 had a greater ( $P < 0.001$ ) number of 3 to 5 mm follicles at the start of superovulation than did donors in either Q1 or Q2. At embryo collection, cows in AMH Q3 and 4 had more ( $P < 0.001$ ) palpable CL than cows in AMH Q1. The mean number of embryos recovered from donor cows in AMH Q4 was greater ( $P < 0.001$ ) than those recovered from cows in either AMH Q1 or 2, but similar to that of AMH Q3. The percentage of recovered embryos classified as transferrable, degenerate or unfertilized were similar ( $P \geq 0.275$ ) across AMH quartiles. Analysis indicated that AMH was positively correlated ( $P < 0.001$ ) with mean follicles ( $r = 0.458$ ), CL ( $r = 0.452$ ) and embryos recovered ( $r = 0.430$ ). In order to determine if follicle counts at the start of superovulation might also be predictive of subsequent superovulatory response, the distribution of follicle counts were divided into quartiles (F Q1 through Q4, with Q1 the lowest and Q4 the highest) for analysis. Donor cows with higher follicle counts (F Q3 and 4) at the start of superovulation had more ( $P < 0.001$ )

palpable CL at embryo collection than donor cows in F Q1 or 2. More ( $P < 0.001$ ) embryos were recovered from cows with the highest follicle counts (F Q4) as compared with cows having lower (F Q1 and 2) follicle counts. The percentage of transferable embryos and unfertilized ova were similar ( $P \geq 0.688$ ) across follicle count quartiles. As was noted for AMH, mean number of follicles at the start of superovulation was positively correlated ( $P < 0.001$ ) with mean CL ( $r = 0.556$ ) and mean embryos ( $r = 0.423$ ) but not percentage of viable or degenerate embryos, or unfertilized oocytes ( $P \geq 0.153$ ). In conclusion, results confirm that relative AMH concentration was positively correlated with number of small antral follicles in the ovaries of cows and might be used to either predict superovulatory response or possibly adjust superovulatory regimen to improve superovulatory response. Antral follicle counts at the initiation of superovulatory treatments might be a more practical alternative to AMH for predicting superovulatory response. Further study is needed to determine the effectiveness of using either AMH concentration or follicle counts to adjust superovulatory regimens for improved response.

## **Introduction**

Anti-Mullerian hormone (AMH) also known as Mullerian Inhibiting Substance (MIS) is a homodimeric disulfide-linked glycoprotein belonging to the transforming growth factor- $\beta$  family, with a molecular weight of 140 kDa (Josso et al., 2001). In the male, AMH is expressed exclusively in the gonads, and causes regression of the Mullerian ducts during male fetal sexual differentiation (Jost et al., 1972). In the female, AMH is expressed within the granulosa cells of follicles (Vigier et al., 1984; Takahashi et al., 1986; Monniaux et al., 2008). Secretion of AMH is greatest in 2 to 5 mm follicles but smaller follicles also may contribute to serum AMH concentration (Van Rooij et al., 2002). Expression of AMH decreases as follicles grow and enlarge; expression is essentially lost when follicles reach 8 mm diameter or larger (Weenen et al., 2004).

In the ovary, AMH has an inhibitory effect on primordial follicle recruitment as well as on the responsiveness of growing follicles to follicle stimulating hormone (FSH) (Visser et al., 2006). Anti-Mullerian hormone concentration can be assessed in serum, but the relative contribution to the different follicle classes to the final serum concentration is unclear (Van Rooij et al., 2002). A single measurement of AMH concentration in serum is a useful tool to determine the size of the ovarian reserve of follicles in cattle (Ireland et al., 2008). Cattle that have low follicle numbers have diminished ovarian function, poor oocyte quality and suboptimal fertility (Ireland et al., 2009).

Anti-Mullerian hormone has also been classified as a good predictive marker of the ovarian response to follicular stimulation for oocyte retrieval and in vitro embryo production (Muttukrishna et al., 2004; Muttukrishna et al., 2005). Anti-Mullerian hormone has also been classified as an endocrine marker that could help predict superovulatory responses administered

to cows for embryo production (Rico et al., 2011). The present study was conducted to further investigate the use of AMH and/or follicle counts as a predictor of subsequent superovulatory response and embryo production in beef cows.

## **Materials and Methods**

**Animals and Superovulatory Treatments.** A total of 79 cows, including 31 Angus, 2 Chianina, 10 Polled Hereford, 1 Maine-Anjou, 15 Shorthorn, 3 Simmental, and 17 crossbred of various breeds were housed at the Food Animal Veterinary Services donor care facility located in Rensselaer, Indiana. The embryo donors ranged from yearling heifers to 13 year old cows. Donor body condition (BCS; 1 to 9 scale; Wagner et al., 1988) ranged from 4 to 8 and averaged about 6. The cows were superovulated for in vivo embryo production, with embryos recovered between April 28 and July 10, 2014. A total of 99 embryo collections were performed, with 20 of 79 donor cows collected twice. Depending upon scheduling, client preference and donor history, superovulatory treatment was initiated either during the luteal phase of cow's natural estrous cycle, or after insertion of a progesterone controlled internal drug release (CIDR) device.

Cows that were superovulated based on their natural cycle had superovulatory treatment initiated between day 8 and 12 of the estrous cycle. Cows received twice daily (10 to 12 hours apart), decreasing doses of porcine follicle stimulating hormone (FSH; Follitropin-V, Bioniche Animal Health, Belleville Ontario CA) over 4-day period. The cows were injected (i.m) with 50 mg (NIH units) of FSH twice daily the first 2 days, then 40 and 30 mg twice daily on days 3 and 4, respectively. On the morning of the fourth day of FSH treatment, cows received an injection of 2.5 cc (~ 312 µg) of Cloprostenol (Estrumate, Merck Animal Health, Summit, NJ) to induce luteal regression. After Cloprostenol treatment, cows were observed for onset of estrus.

Timing of insemination after detected estrus depended on the number of straws and type of frozen-thawed semen used. When a single straw of semen was used, insemination occurred 12 to 16 hours after detected estrus. When multiple straws of semen were used, insemination occurred within 12 hours of detected estrus, and again 6 to 8 hours later. When X- or Y-sorted semen was used, insemination occurred 16 and again at 24 hours after detected estrus, with 3 to 6 straws of semen used in total, depending on sperm concentration per straw.

Cows superovulated regardless of the day of the estrous cycle, received a 1.38 g progesterone insert (EAZI-Breed CIDR; Zoetis, Florham Park, NJ) of day 0, in conjunction with an injection (i.m.) of 2.5 mg estradiol 17 beta and 50 mg of progesterone. On day 4, superovulatory treatment was initiated following the same 4-day, descending dose protocol used for cows during their natural cycle. On the morning of the fourth day of FSH treatment, the CIDR was removed at the time of Cloprostenol injection. Insemination of cows after detected estrus depended on the type and number of straws of semen to be used, as described previously.

**Ultrasonography and Blood Collection.** Before the initiation of superovulation, ultrasonography (Aloka 500 V with 5.0 MHz linear transducer; Corometrics, Wallingford, CN) was used to scan the ovaries of each donor to record the number of 3 to 5 mm follicles present. Concurrent with ultrasonography, a 10 mL blood sample was collected via tail vein from each cow, using a BD Vacutainer SS Plus Blood Collection Tube, (Ref. No. 367985, Becton, Dickson, Franklin Lakes, NJ). Blood tubes were inverted several times to mix, and then allowed to clot for 30 minutes to 1 hour at room temperature. After clotting, the tubes were held on ice until centrifugation at 1,000 g for 10 minutes. Recovered serum was placed in 5 mL polypropylene tubes and stored in a chest freezer at -15 to -20 C until analysis for Anti-Mullerian hormone (AMH).

**Embryo Recovery and Evaluation.** Non-surgical embryo recoveries were performed ~ day 7 of the subsequent estrous cycle, using a Foley catheter and ViGRO complete flush medium (ViGRO™, Bioniche Animal Health, Pullman, WA). At the time of embryo collection, the ovaries of donor cows were palpated to estimate the number of corpora lutea (CL) present on each ovary. Recovered flush medium was filtered through a filter to reduce medium volume. The flush medium was then searched, using a stereomicroscope to recover embryos. Recovered embryos were evaluated for stage of development (morula, early blastocyst or blastocyst) and morphological quality (grade 1, 2, degenerate or unfertilized), using standards established by the International Embryo Transfer Society (IETS Manual, 3rd Ed., 1998).

**Analysis of Serum Anti-Mullerian Hormone.** Serum samples were removed from frozen storage and thawed over night at 4 C. A 500 µL aliquot of serum was removed for each tube and pipetted into cryogenic vials (Sumitomo Bakelite, Japan) and then re-frozen (-20 C). The serum samples were shipped on dry ice over night to the Texas A&M Veterinary Diagnostic Laboratory at College Station, TX. The diagnostic laboratory used a bovine AMH enzyme-linked immunosorbent assay kit (Bovine AMH ELISA AL-114; Ansh Labs, Webster, TX) to determine AMH concentration (ng/mL) in duplicate samples, following the assay kit manufacture's recommended procedures. The AMH assay had an analytical sensitivity of 0.011 ng/mL.

**Statistical Analysis.** Analysis was performed, using JMP Pro 10.0.0 statistical software (SAS Institute, Inc.). Variables considered in the analysis were embryo donor breed, superovulation protocol, AMH concentration, follicle and corpus luteum number, total, transferable, degenerate embryos, and unfertilized ova. Superovulation protocol had no effect ( $P \geq 0.293$ ) on number of corpora lutea, or the number of total, transferrable, degenerate embryos or

unfertilized ova, so was removed from the analysis. There were no breed differences ( $P = 0.321$ ) for serum AMH concentration. Frequency distribution was used to assign AMH concentration measured in serum samples to quartiles. Analysis of variance was then used to make comparisons between AMH quartiles for number of 3 to 5 mm follicles, number of corpora lutea at embryo collection, number of embryos recovered, and the percentages of transferrable and degenerate embryos, and unfertilized ova. Percent transferrable embryos were defined as the portion of total embryos recovered that were of grade 1 or 2 morphological quality. Percent degenerate embryos were defined as the portion of total embryos recovered that exhibited limited cleavage and/or were of poor morphological quality. Percent unfertilized ova were the portion of all recovered embryos/structures that exhibited no cleavage.

Frequency distribution was also used to assign follicle counts to quartiles. Analysis of variance was then used to make comparisons between follicle quartiles and number of corpora lutea at embryo collection, number of embryos recovered, and the percentages of transferrable and degenerate embryos, and unfertilized ova. Multivariate analysis was used to determine any correlations between AMH concentrations and number of 3 to 5 mm follicles, number of corpora lutea at embryo collection, number of embryos recovered, and the percentages of transferrable and degenerate embryos, and unfertilized ova. All values are expressed as the mean  $\pm$  SEM. Statistical differences were considered significant where  $P < 0.05$ .

Superovulation and embryo recovery was performed twice on 20 of 79 donor cows included in the study. To evaluate within donor, the repeatability of AMH, follicle and CL counts, and embryos recovered per superovulation, the distribution of the variables were assigned to quartiles and compared in a contingency table generated through Chi square analysis.

## **Results**

**Measure of AMH and its predictive value for superovulatory response.** Anti-Mullerian hormone measured in serum samples ranged from 0.013 to 0.898 ng/mL, with a mean of 0.293 ng/mL. The distribution of AMH concentrations was divided into quartiles (AMH Q1 through Q4, with Q1 the lowest and Q4 the highest ng/mL) for analysis. The range of AMH concentrations within each quartile are presented in Table 1. The assay failed to detect AMH in 3 serum samples, possibly due to either assay failure or concentrations below detection limits. Donor cows in AMH Q4 had a greater ( $P < 0.001$ ) number of 3 to 5 mm follicles at the start of superovulation than did donors in either Q1 or Q2. Cows in AMH Q3 were intermediate for mean number of follicles. At the time of embryo collection, cows in AMH Q3 and 4 had more palpable CL than cows in AMH Q1 ( $P < 0.001$ ). The mean number of CL for cows in AMH Q2 was intermediate and similar ( $P > 0.10$ ) to those in both AMH Q1 and 3. The mean number of embryos recovered for donor cows in AMH Q4 was greater ( $P < 0.001$ ) than those recovered from cows in either AMH Q1 or 2, but similar to that of AMH Q3. The percentage of recovered embryos that were classified as transferrable, degenerate or unfertilized were similar ( $P \geq 0.275$ ) across AMH quartiles. Multivariate analysis (Table 2) indicated that AMH was positively correlated ( $P < 0.001$ ) with mean follicles ( $r = 0.458$ ), CL ( $r = 0.452$ ) and embryos recovered ( $r = 0.430$ ).

**Predictive value of follicle number for superovulatory response.** The number of 3 to 5 mm follicles counted on the ovaries of donor cows ranged from 5 to over 30, with a mean of 16. In order to determine if follicle counts at the start of superovulation might be predictive of subsequent superovulatory response, the distribution of follicle counts were also divided into quartiles (F Q1 through Q4, with Q1 the lowest and Q4 the highest) for analysis (Table 3). Donor cows with higher follicle counts (F Q3 and 4) at the start of superovulation had more ( $P <$

0.001) palpable CL at embryo collection than donor cows in F Q1 or 2. More embryos were recovered from cows with the highest follicle counts (F Q4) as compared with cows having lower (F Q1 and 2) follicle counts ( $P < 0.001$ ). The number of embryos recovered from donors in F Q3 was intermediate and similar ( $P > 0.10$ ) to that of donors in F Q1, 2 and 4. The percentage of transferable embryos and unfertilized ova were similar ( $P \geq 0.688$ ) across follicle count quartiles. The mean percentage of degenerate embryos was greater for donor cows in F Q3 than any other follicle quartile ( $P = 0.002$ ).

As was noted for AMH, mean follicles at the start of superovulation was positively correlated ( $P < 0.001$ ; Table 2) with mean CL ( $r = 0.556$ ) and mean embryos ( $r = 0.423$ ) but not percentage of viable or degenerate embryos, or unfertilized oocytes ( $P \geq 0.153$ ). Other correlations noted were positive correlations between mean CL and embryos recovered ( $r = 0.887$ ;  $P < 0.001$ ), and between embryos collected and degenerate embryos ( $r = 0.236$ ;  $P = 0.021$ ). As might be expected, a significant negative correlation ( $r = -0.931$ ;  $P < 0.001$ ) existed between percentage viable and percent unfertilized embryos.

**Repeatability within donors.** Twenty of the donor cows used for the study were superovulated and had embryo collections performed twice. To evaluate repeatability within donor, AMH, follicle and CL counts and embryos recovered per collection were assigned to quartiles for comparison within donor cows (Table 4). Only a single serum AMH measure was available for one donor. Of the remaining 19 donors, 17 of 19 were found to be within the same or an adjacent quartile for AMH, from one superovulation and embryo collection until the next. For follicle counts, 18 of 20 cows were found to fall within the same or an adjacent quartile from one collection to the next. At embryo collection, 16 of 20 donors were within the same or an adjacent quartile for CL number, as were 18 of 20 donors for total embryo production.

## Discussion

Since the development of cattle superovulation and non-surgical embryo recovery in the 1970s, the unpredictability of the superovulatory response has remained a major obstacle. The variability between animals in ovulatory response to FSH-induced superovulation is mainly due to differences in ovarian activity at the time of treatment (Rico et al., 2009). Cows may ovulate from 0 to 40 follicles following a 4 to 5 day treatment with FSH (Rouillier et al., 1996). An average response to superovulation is 12 total and 6 transferable embryos, although 15 to 20 percent of donors will not respond to superovulation (Hasler, 2010). Cattle that are superovulated after reaching age 8 to 10 tend to produce fewer embryos. Individual variation among females remains the largest and least understood variable in superovulation. While some females consistently produce large numbers of embryos, other females of similar age, breed, weight, management, and etc. perform poorly.

The purpose of superovulation is to stimulate a number of small antral follicles to grow and mature, resulting in multiple ovulations. Therefore, the pool of small antral follicles available for stimulation should dictate the superovulatory response. Previous studies have shown a positive correlation between small antral follicle number and serum concentrations of AMH (Visser et al., 2006; Ireland et al., 2008). Furthermore, diminished ovarian reserves of follicles have been observed in cattle exhibiting low AMH concentrations (Ireland et al., 2010). The present study was conducted to investigate whether AMH and/or antral follicle numbers could be used as a predictor of superovulatory response and embryo production in donor cows.

Assay of AMH in the serum of 79 embryo donor cows in the present study found that AMH ranged from 0.013 to 0.898 ng/mL. Although different ranges have been reported in previous years. Souza et al. (2015) reported an AMH range from 0.00001 to 0.3743 ng/mL,

while Monniaux et al. (2010) reported AMH concentrations to range from 0.0011 to 0.5312 ng/mL and Rico et al. (2009) reported ranges in three different sessions, ( $T_0$ ) first injection of FSH, ( $T_E$ ) time of estrus and ( $T_L$ ) 7 days after estrus. Anti-Mullerian hormone concentrations ranged from 0.025 to 0.228 ng/mL at  $T_0$ , 0.049 to 0.359 ng/mL at  $T_E$  and 0.026 to 0.212 ng/mL at  $T_L$ , respectively. Compared to cows with lower AMH, cows with high AMH concentrations in serum had the highest follicular counts and ovulatory responses to superovulatory treatment. These results agree with data obtained from (Ireland et al., 2008; Rico et al., 2009; Ireland et al., 2010). Ireland et al. (2008) reported that AMH concentrations were approximately 6 and 2 fold greater in animals with higher follicular counts ( $39.61 \pm 2.3$ ) compared with low follicle counts ( $11.95 \pm 1.2$ ). Rico et al. (2009) indicated that cattle with high numbers of 3 to 7 mm follicles before the superovulatory treatment had higher AMH concentrations and resulted in a higher ovarian response. Furthermore, Ireland et al. (2010) classified follicle counts  $\geq 3$  mm into low ( $\leq 15$ ), intermediate (16 to 24) and high ( $\geq 25$ ) categories. Anti-Mullerian hormone concentrations were found to be 2 and 6 folds higher for cattle within the intermediate or high group.

A difficulty is using AMH as a predictor of superovulatory response is in how to classify a donor cow by AMH. Studies differ in the reported cut-off values for identifying AMH concentration as low, moderate or high. Rico et al. (2012) reported AMH concentrations ranging from 0.005 to 0.244 ng/mL where cattle below 0.087 ng/mL identified as poor responders to superovulation and having less than 15 large follicles near the time of estrus. Guerreiro et al. (2014) classified AMH into high and low categories with  $0.2 \pm 0.01$  ng/mL being low and  $0.4 \pm 0.02$  ng/mL being high. Ribeiro et al. (2014) classified AMH into three categories, where low ranged from 0.01 to 0.14 ng/mL, intermediate 0.141 to 0.45 ng/mL and high 0.451 to 3.19800 ng/mL. Differences in cut-off points between studies may be related to several factors, including

differences in measure of superovulatory response (large follicle counts near estrus; Rico et al., 2012 versus CL counts on the day of embryo collection; Souza et al., 2015), different AMH assays, and different methods used to collect plasma as reported by Rico et al. (2012). Therefore, cows were classified into quartiles of circulating AMH concentration rather than by specific concentrations in blood. Donor cows within the highest quartile (Q4) for AMH had more 3 to 5 mm follicles present in their ovaries at the start of superovulation than did donors in the lowest quartile (Q1). Overall, mean follicle number at the start of superovulatory treatment was positively correlated ( $r = 0.458$ ;  $P < 0.001$ ) with AMH. Rico et al. (2009) reported a similar correlation ( $r = 0.79$ ;  $P < 0.001$ ) between AMH and number of antral follicles detected via ultrasonography before the start of superovulatory treatment.

Donor cows within Q4 for AMH had more CLs and produced more embryos at embryo collection than donor cows in the lowest 2 quartiles (Q1 and 2). As might be expected, mean corpora lutea was positively correlated ( $r = 0.887$ ;  $P < 0.001$ ) with mean embryos recovered. However the number of embryos or structures classified as transferrable, degenerate or unfertilized were similar among AMH quartiles and were not correlated with AMH. Souza et al. (2015), who divided superovulated dairy cows into quartiles based on circulating AMH, reported results similar to the current study in that total number of embryos recovered was greater for donors in the highest AMH quartile versus the lowest. Also in agreement with the current study, Souza et al. (2015) reported that AMH quartile had no effect on the percentage of transferrable or fertilized embryos.

Fertilization rate can vary depending on semen quality and concentration, insemination timing and technique, and inherit fertility of the bull. Embryo morphological quality after fertilization can be influenced by the same factors, as well as uterine environment. Although

superovulating cattle does produce more offspring from superior genetics, repeated treatment with high doses of gonadotropins can alter follicular development, oocyte maturation, ovulation and sperm transport. These abnormalities may disrupt the normal fertilization and/or embryo development process to result in an increased number of unfertilized oocytes and poor quality embryos (Kafi and McGowan, 1997). Perhaps, it is not surprising that level of circulating AMH had little measurable effect on fertilization rate or embryo morphological quality.

Assay for AMH is relatively expensive and limited in availability to most embryo transfer practitioners. However, many practitioners have access to ultrasonography and often evaluate ovarian structures before superovulation. As an alternate to AMH, the number of 3 to 5 mm follicles present in the ovaries at initiation of superovulatory treatment was evaluated as a predictor of superovulatory response. As with serum AMH, donors were assigned to quartiles for comparison. Results suggest that follicle counts would be of value for predicting subsequent superovulatory response. Donor cows in the highest quartile for 3 to 5 mm follicles also had more embryos at collection than cows in the lowest two quartiles. In a previous study using ultrasound to determine antral follicle count, Ireland et al. (2007) reported that cows with high follicle numbers also had more embryos recovered and more transferable embryos.

As previously stated, there is great donor-to-donor variation in superovulatory response and embryo production (Kafi and McGowan, 1997). Empirical evidence suggests that there is more consistency within donors. A limited number of donors in the present study were superovulated and embryo recoveries performed twice. While AMH concentration varied within donor, 17 of 19 donors were categorized within the same or adjacent quartile for AMH prior to superovulation. Likewise follicle counts within donors were within the same or an adjacent quartile at subsequent superovulations. These results confirm that there is some consistency

within donors, and that at least over a short (~ 3 month) period of time, a single AMH assay can be used as a potential predictor of superovulatory response.

Currently, embryo donor history such as breed, age, weight, BCS, and parity are used to adjust the superovulatory regimen in an effort to improve embryo production. While improvements have been made in hormonal treatment and synchronization protocols (Hasler, 2014) the mean number of transferrable embryos recovered per donor animal has remained relatively constant for the past 40 years. Many commercial embryo transfer (ET) programs across the country are limited in options because donors and service sires are often chosen by the owner, and many times procedures are done on farm and mostly out of the control of practitioners (Hasler, 2010). The ability to make adjustments to superovulatory regimens based on predicted superovulatory response of individual donors would be of great benefit to the ET industry moving forward. The study confirms that relative AMH level is positively correlated with number of small antral follicles in the ovaries of cows and might be used to either predict superovulatory response or possibly adjust superovulatory regimen to improve superovulatory response. Antral follicle counts at the initiation of superovulatory treatments might be a more practical alternate to AMH for ET practitioners to use in predicting superovulatory response. Further study is needed to determine the effectiveness of using either AMH assay or follicle counts to adjust superovulatory regimens for improved response.

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**Table 1. Quartile categorization of AMH concentrations as predictor of superovulatory outcomes.**

Item	Quartile of AMH concentration				P-value
	Q1	Q2	Q3	Q4	
AMH, ng/mL	0.013 - 0.068	0.069 - 0.263	0.264 - 0.363	0.364 - 0.898	
No. of donors	26	22	24	24	
No. of follicles	13.46 ± 0.91 <sup>b</sup>	14.95 ± 0.98 <sup>b</sup>	16.79 ± 0.94 <sup>ab</sup>	19.33 ± 0.94 <sup>a</sup>	0.001
No. of CL	11.62 ± 1.54 <sup>c</sup>	13.68 ± 1.67 <sup>bc</sup>	17.58 ± 1.60 <sup>ab</sup>	20.54 ± 1.60 <sup>a</sup>	0.001
No. of embryos	9.77 ± 1.76 <sup>b</sup>	9.36 ± 1.91 <sup>b</sup>	15.50 ± 1.83 <sup>ab</sup>	20.13 ± 1.83 <sup>a</sup>	0.001
Transferable %	69.32 ± 6.62	57.06 ± 7.08	58.75 ± 6.62	51.05 ± 6.62	0.275
Degenerate %	5.52 ± 2.52	7.89 ± 2.69	9.40 ± 2.52	9.87 ± 2.52	0.614
Unfertilized %	25.16 ± 6.73	35.06 ± 7.19	31.85 ± 6.73	39.09 ± 6.73	0.519

<sup>a,b,c</sup> Numbers within rows with unlike superscripts differ ( $P \leq 0.05$ ).

**Table 2. Correlations between AMH, follicle counts, and embryo production of superovulated donor cows.**

Variable 1	Variable 2	Correlation	P-values
Anti-Mullerian hormone	Mean # follicles	0.458	0.001
	Mean # corpora lutea	0.452	0.001
	Mean # embryos	0.430	0.001
	Percent viable	-0.126	0.231
	Percent degenerate	0.195	0.061
	Percent unfertilized	0.052	0.621
Mean # follicles	Mean # corpora lutea	0.556	0.001
	Mean # embryos	0.423	0.001
	Percent # viable	-0.037	0.724
	Percent degenerate	0.147	0.153
	Percent unfertilized	-0.018	0.857
Mean # corpora lutea	Mean # embryos	0.887	0.001
	Percent viable	-0.008	0.942
	Percent degenerate	0.191	0.063
	Percent unfertilized	-0.064	0.539
Mean # embryos	Percent viable	0.011	0.919
	Percent degenerate	0.236	0.021
	Percent unfertilized	-0.098	0.341
Percent viable	Percent degenerate	-0.184	0.073
	Percent unfertilized	-0.931	0.001
Percent unfertilized	Percent degenerate	-0.188	0.067

**Table 3. Quartile categorization of follicle counts as a predictor of superovulatory response and embryo production.**

Item	Quartile of follicle counts				P-value
	Q1	Q2	Q3	Q4	
No. of donors	26	35	20	18	
Follicle range	5 - 12	13 - 17	18 - 20	21 - 30	
No. of CL	10.65 ± 1.40 <sup>b</sup>	13.51 ± 1.20 <sup>b</sup>	19.15 ± 1.59 <sup>a</sup>	23.33 ± 1.68 <sup>a</sup>	0.001
No. of embryos	9.62 ± 1.79 <sup>b</sup>	11.57 ± 1.55 <sup>b</sup>	16.50 ± 2.04 <sup>ab</sup>	20.00 ± 2.16 <sup>a</sup>	0.001
Transferable %	58.20 ± 6.44	64.77 ± 5.63	54.51 ± 7.53	58.46 ± 7.97	0.716
Degenerate %	4.82 ± 2.23 <sup>b</sup>	6.71 ± 1.95 <sup>b</sup>	17.09 ± 2.61 <sup>a</sup>	5.38 ± 2.76 <sup>b</sup>	0.002
Unfertilized %	36.99 ± 6.44	28.53 ± 5.63	28.39 ± 7.54	36.16 ± 7.97	0.688

<sup>a,b</sup> Numbers within rows with unlike superscripts differ ( $P \leq 0.05$ ).

**Table 4. Repeatability of embryo donors for AMH, follicle and corpus luteum counts and embryo production.**

Embryo Donor	Superovulation and embryo collection		Superovulation and embryo collection		Superovulation and embryo collection		Superovulation and embryo collection	
	<u>1</u> AMH quartile	<u>2</u> AMH quartile	<u>1</u> Follicle quartile	<u>2</u> Follicle quartile	<u>1</u> CL quartile	<u>2</u> CL quartile	<u>1</u> Embryo quartile	<u>2</u> Embryo quartile
1	1	1	1	2	1	2	3	3
2	3	4	2	3	2	3	3	4
3	3	4	2	3	2	2	2	2
4	4	4	2	3	2	3	3	3
5	3	4	1	2	1	2	1	2
6	1	1	1	2	1	1	1	1
7	1	3	2	3	3	3	3	4
8	1	3	2	3	1	2	1	2
9	4	4	4	4	4	4	4	4
10	*	2	2	3	2	2	1	2
11	1	1	2	2	1	1	1	1
12	3	4	3	3	2	4	3	4
13	1	1	1	2	1	2	2	2
14	2	2	1	2	1	2	1	1
15	3	4	3	4	3	4	2	4
16	3	4	1	4	4	4	3	4
17	3	4	4	4	4	4	4	4
18	2	2	3	4	1	4	1	1
19	1	2	1	1	1	3	1	3
20	1	1	2	4	2	4	3	3

\*Anti-Mullerian hormone could not be detected in one blood sample from donor 10.

### CHAPTER 3: CONCLUSION

Research presented in this thesis examined physiological variations and clinical applications of Anti-Mullerian hormone (AMH) in various breeds of cattle. In addition, antral follicle counts (AFC) were evaluated via ultrasonography to evaluate the correlation among AMH, AFC, total number of corpora lutea (CL), total number of embryos recovered in vivo, and the percentage of unfertilized oocytes. The primary goal of this review was to focus on AMH expression in the cow; nevertheless, some of the derived information in this document was accommodated from other species but not limited to humans, goats and rodents.

Anti-Mullerian hormone is one of the most important hormones in reproductive physiology because of the role the hormone plays in sexual differentiation during fetal development. To date, AMH has been compared to AFC which represents the total number of follicles detected using ultrasonography although, is it possible a large proportion of atretic follicles, do not contribute to circulating AMH concentrations. Therefore, one could theorize that AMH concentrations are a reflection on the number of follicles being recruited in each follicular wave, although it would be difficult to detect as new recruited follicles are being overlapped by larger regressing follicles. Nevertheless, AMH appeared to be a useful tool on the prediction of total number of antral follicles and embryos in the cow.

The variation in AMH concentration and AFC in cows tended to reflect ovarian reserve and follicular function. Results in this study indicated that follicle and embryo numbers are impaired with low AFC or low AMH concentration and that higher AFC and AMH resulted in higher follicle and embryo numbers, respectively. Therefore, measuring the concentration of AMH as well as determining the number of antral follicles should be an essential part in the

reproductive examination of cattle as it provides valuable information about superovulatory response and could help embryo transfer practitioners predict how well a cow will respond to superovulatory treatments throughout her reproductive lifespan.

In conclusion, circulating AMH concentration and AFC were highly associated with superovulatory response and the total number of embryos produced in individual cows. If commercial AMH assays were to become available it could become a valuable practical method for improving the efficiency of multiple ovulation and embryo transfer (MOET) programs in beef cattle herds not only in America but across the world.