Follicular Development, Superovulation and Artificial Insemination In Embryo Donor Cattle

R.A. Godke, S.H. Pool and R.W. Rorie
Animal Science Department, LSU Agricultural Center
Louisiana State University, Baton Rouge, Louisiana 70803

Introduction.

Although significant advances have been made in embryo transplant technology, embryo transplant units still have to cope on a daily basis with persisting hurdles. One of these problems relates to superovulation methodology that results in consistent ova production within and across donor cows. Although research publications on the subject date back over 30 years, this problem remains as an annual topic of discussion at the professional and embryo transfer society meetings. It has now become painfully clear that our basic knowledge of primordial follicle activation, follicle recruitment, follicle receptor site interactions, endocrine involvement in follicular development and follicular growth dynamics is less than adequate. There is a need for research to develop workable physiological models so that we can better understand follicular growth and development.

A second problem relates to the artificial insemination (AI) of donor females to produce optimal fertilization rates across embryo collections. With thousands of donor cows superovulated and inseminated in the world over three years, it would seem that this problem should have been resolved by now, but unfortunately variability in fertilization rates and low viable embryo recovery still exists. These results suggest that basic research is also needed on oocyte maturation and fertilization before additional progress can be made in this area. It now appears that research efforts should focus on developing and understanding of how these physiological events occur **in situ** rather than conducting repetitive field trials hoping to uncover optimal superovulation and insemination schedules for donors in commericial embryo transplantation programs. It is agreed that this type of physiology research is often difficult, time consuming and costly. It has now become evident that a renewed effort is needed in basic research before further progress is made in harvesting the optimal number of transferable quality embryos from each donor female.

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Preparing Donor Cattle for Superovulation.

Preparation of donor females for superovulation is often overlooked as a cause for lowered ova production following gonadotropin treatment. Livestock producers have known for generations that poor (or improper) nutrition leads to lowered fertility, increased postpartum intervals and fewer offspring born in their breeding herds. Increasing the level of nutrition prior to the breeding season (flushing) has long been used as a management tool to increase the ovulation rates in sheep and swine early in the breeding season. Producers have long known that this management tool most often produces results when the breeding females are maintained on a marginal level of nutrition just prior to the dietary energy increase during the flushing interval.

In young dairy and beef females, it has been shown that a high level of nutrition increases follicle size and increases the number of follicles on the ovaries prior to the time of breeding. This correlates with producer observations that first estrus us usually exhibited at < 12 months of age in prepuberal heifers fed on a high plane of nutrition compared with first estrus at ≥ 12 months for herdmate heifers maintained on a low plane of nutrition during the same interval. In a variety of studies, it has been shown that feeding extra dietary energy to beef females the last 30 to 60 days prior to and immediately following calving produces a marked effect on shortening the postpartum interval and increases subsequent pregnancy rates. These classical studies, and many others, aid in establishing a close physiological relationship between proper nutrition and subsequent ovarian function in farm animals.

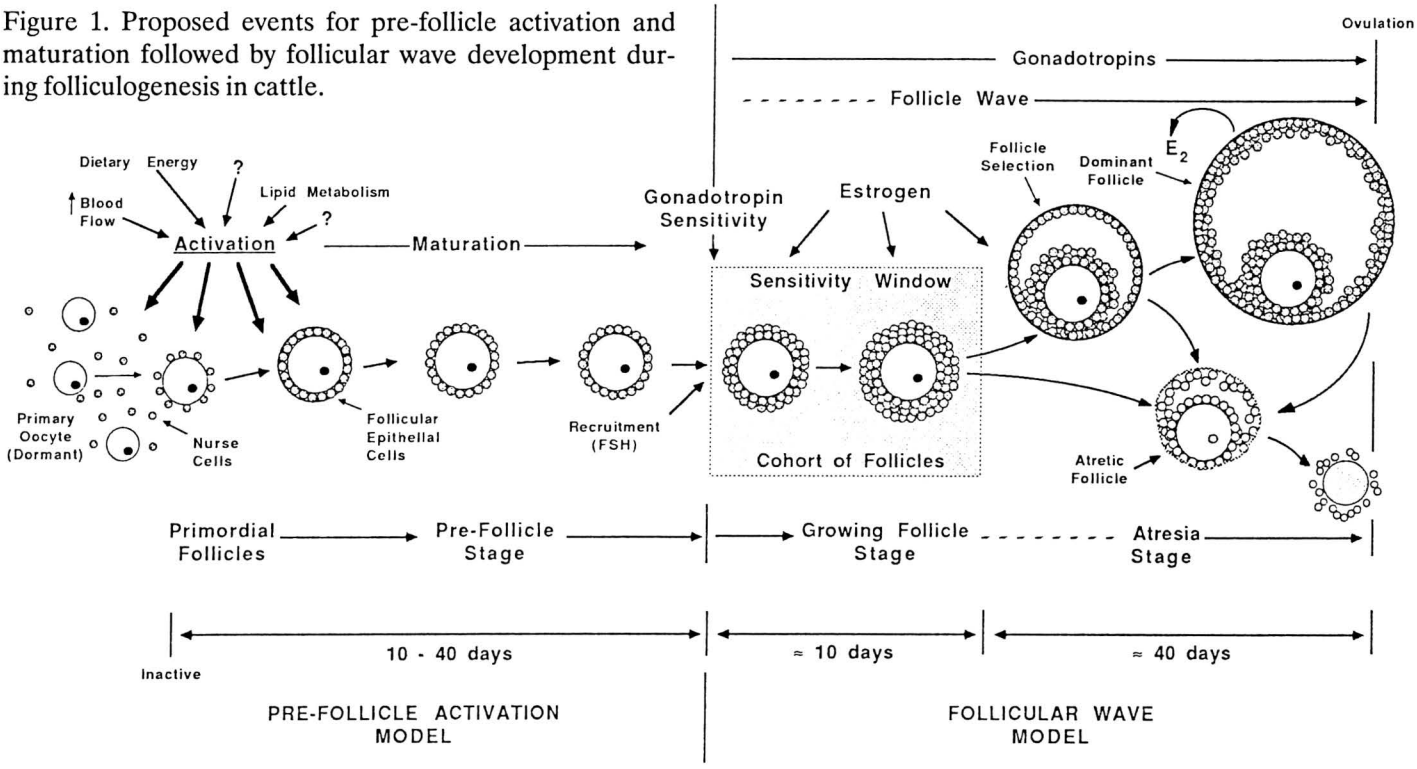
How then could the nutritional plane of donor animals affect the number of follicles and/or the size of follicles that develop during superovulation of donor cattle? Although the scientific literature does give some indirect clues for this, the answer is not completely clear. It was first noted in the late 1940's that the initial state of follicular development proceeds in the absence of pituitary gonadotropins. This finding has since been re-evaluated by researchers but has not been disputed. Today, it has been established that a cohort of young follicles (pre-follicles) begin developement into a follicular wave only after gonadotropin receptors become available on the attending follicular cells. This would suggest that the number of pre-

follicles available for a cohort of follicles is mediated or controlled by a separate, cortical level activation system, and not under the direct influence of circulating gonadotropins. This is further evidenced by the reports that indicate the pre-follicle activation process occurs in the ovaries of both hypophysectomized sheep and cattle. One possibility is that supplemental nutrition plays its primary role in follicle development by influencing the activation of the follicular epithelial cells around the dormant oocytes of the ovarian cortex. This activated follicular cell/primary oocyte complex (primary follicle) then slowly matures during a time course producing an activated per-follicle (Figure 1) for subsequent recruitment in a cohort of follicles that would be available for gonadotropin stimulation in a follicular wave.

Primordial follicle activation appears to be a continuous process starting shortly before or after birth and continuning throughout the life of the female. It has been proposed that every day one up to six activated follicles start to grow from the reserve of primordial follicles in cattle. The fate of most of these follicles during this developing process is atresia. These activated pre-follicles have also been shown to be available for follicular wave development during gestation in cattle. In recent studies in this laboratory, ovulatory-size follicles developed on the ovaries of gestating crossbred beef cows with twice daily 5-day FSH treatments during the first, the second and the third trimesters of pregnancy. Different accounts indicate that there are between 75,000 and 180,000 inactive oocytes in the ovaries of a heifer calf at birth. Several histological studies suggest that the number of these oocytes declines throughout the lifespan of the female, indicating there is a continuous process of primordial follicle activation and loss through atresia in each female. Although the number is greatly reduced from the time of birth, older cows (>10 years of age) still have thousands of dormant oocytes present in the cortical region of their ovaries. It has been estimated that <200 of the oocytes actually reach maturity and ovulate during a lifetime in the cow. Whether the decrease in oocytes throughout the life span of the cow is a true linear decline across time has not been clearly established. The greatest loss in number of oocytes per ovary has been reported to occur in cows after the fifth year of age.

Histological studies on bovine ovaries suggest that pre-follicles are in the activation process for 10 up to 40 days in females before they are available in the cohort of follicles for gonadotropin stimulation. Some studies have suggested that this activation process is even longer in some animals. Most bovine estrous cycles have two or three follicular waves of antral follicles, with each wave resulting in at least one dominant follicle. Recently, it has been shown that the recruitment of pre-follicles for a cohort is regulated by circulating FSH concentrations, follicular growth is modulated by FSH and LH, and selection of the dominant follicle within the wave is under the control of FSH, estradiol and possibly inhibin from the ovary. If activation and maturation of primordial follicles is a con-

Figure 1. Proposed events for pre-follicle activation and maturation followed by follicular wave development during folliculogenesis in cattle.

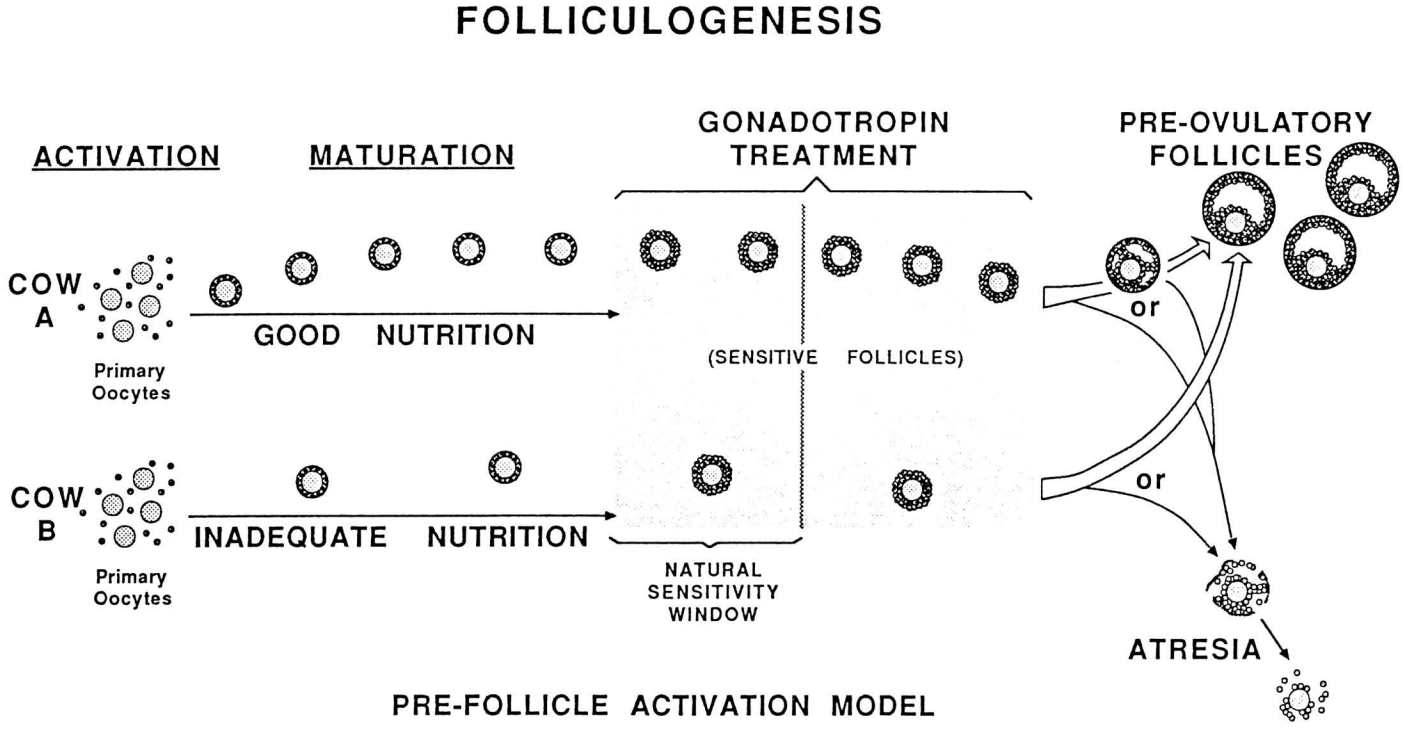


tiunous process requiring a set maturation interval before they become gonadotropin sensitive, how then would increasing the plane of nutrition enhance the number of follicles produced during superovulation in donor cattle? It appears that the process of activating primordial follicles to become activated pre-follicles has a unique relationship with dietary energy intake in the female. If dietary energy is increased, an increased number of cortical pre-follicles should be activated and become available for gonadotrpin stimulation at the time of follicular wave development. If more pre-follicles were available for each cohort of follicles, then exogenous gonadotropins could stimulate more ovulatory-size follicles to develop during the hormone treatment schedule. If this is the case, then the effect of increased energy in the diet weeks before gonadotropin treatment can be visualized for donor females in the following pre-follicle activation model (Figure 2). Differentiation and maturation of each of these pre-follicles is a part of a dynamic process likened to that of a slow, consistent moving conveyor belt in the cortical region of the ovary. Since activation of each primordial follicle is an individual event, each pre-follicle on the conveyor belt would be at a different stage of maturation before becoming gonadotropin sensitive.

The pre-follicle activation model may also help explain how pre-breeding season dietary flushing could increase ovulation rates reported in multiple ovulating farm animals. The frequency of pre-follicle activation in the ovarian cortex appears to be an important variable in the sequence of physiological events, more so than the DNA-controlled growth and maturation of these pre-follicles. The interval needed for follicular cell growth and pre-antral follicle maturation gives all indications of being consistent within each female and appears to have genetic rather than nutritional implications. This model is also based on the assumption that pre-follicle activation is a self-contained system within each ovary. If this is the case, dietary energy would have a similar effect on each ovary, making more pre-follicles available for the cohort of follicles at the time of gonadotropin stimulation. This would then result in a similar number of ovulatory-size follicles from each ovary being available for ovulation. This is known to occur under natural conditions in swine and in FSH-stimulated sheep, goats and cattle.

Therefore, based on these assumptions, proper nutrition of donor cattle 40 to 60 days prior to hormone treatment could ultimately increase the number of gonadotropin-dependant antral follicles available for ovulation. It would seem logical then that the number of pre-follicles available for superovulation would be more important than the dose levels of gonadotropins used for superovulation. The number of pre-follicles available for gonadotropin stimulation could explain why the number of ovulatory-size follicles and CL plateau in donor cattle even though the daily dose level of FSH is doubled or even tripled in the treatment schedule.

Figure 2. An example of how nutrition may subsequently affect the number of follicles that develop in each ovary of donor cattle during superovulation treatment.



A Canadian study in 1981 has shown that high energy rations fed to multiparous cows increased the mean ovulation rate after gonadotropin treatment compared with similarly treated cows fed low energy rations. In a superovulation study at this station, 50 English breed beef donors were divided into two groups; half the females were fed a ration with extra dietary energy while on native pastures for 60 days prior to superovulation. The remaining donors were maintained on native grass pastures and supplemented only with hay and mineral *ad libitum* prior to superovulation. Following FSH treatment, the donors supplemented with extra dietary energy had significantly more CL (15.8) and medium to large-size follicles (6.8) per donor at the time of collection compared with herdmate donors maintained on a marginal nutrition level (9.6 CL and 3.2 follicles) the last 60 days before superovulation.

In another nutritional study conducted at Auburn University, supplemental feeding of monensin sodium to donor cows prior to superovulation was reported to increase ovulation rates over that of donors in the control group. Studies at Texas A&M University have shown that cows fed 200 mg/head/day of monensin and then injected with FSH and HCG had ovaries twice the size and had more follicles ovulate than cows that received no monensin supplementation. These researchers have suggested that monensin feeding produces a positive effect on nutritional efficiency and subsequent reproductive performance in cattle by altering the availability of short chain fatty acids in the bovine rumen. Although it is proposed herein that nutrition has an effect on pre-follicle activation in the cortex of the ovary, other nutritional involvement (e.g. glucose, insulin, fatty acids, proteins, growth factors) has been associated with stimulating hypothalamic centers, altering pituitary gonadotropin secretion and antral follicle development at the ovary level. These components cannot be overlooked when developing an overall model for superovulation in the cow.

Although most of the evidence that nutrition may subsequently affect donor ova production is indirect in nature, it appears that there is more than a casual relationship between the number of follicles produced and the variability in response to gonadotropins within and across donor cows. Since pre-follicle activation is a slow and continuous process, it could easily be overlooked as a factor in donor superovulation responses.

Superovulatory Treatments

Various gonadotropin preparations have been used for follicular stimulation in donor cattle over the years. The most notable of these are pregnant mare's serum gonadotropin (PMSG) and natural follicle stimulating hormone (FSH) products. Often availability of the agent and/or governmental restrictions have dictated which of these two gonadotropin preparations are used in commercial embro transfer situations. PMSG, although a very effective stimulating agent, apparently has some drawbacks that have reduced its popularity in recent years. The complaints most often registered following the use of PMSG include: hypertrophy of the ovarian and uterine tissues, its extended half life in the circulation causes over-stimulation of the ovary, asynchronous ovulation patterns, variable fertilization rates and a lower percentage of transferable embryos per collection. Whether all these problems can be directly attributed to PMSG remains to be established. There does appear to be variability in acceptance of PMSG for use in cattle. Many transplant units outside the United States have used it with good success for years.

A natural FSH preparation (FSH-P®) is the most often used gonadotropic agent for stimulating supplemental follicular growth in beef and dairy donors in North America. Treatment dose schedules vary in some degree from one embryo transplant unit to the next. Although most units feel their treatment schedule has the edge on their competitors, they often complain their results remain variable for successive treatments within and across donors. Studies are presently underway to evaluate laboratory-purified FSH preparations and a new genetically engineered pure FSH product. Preliminary results are encouraging from several trials, but the final results are not in yet.

In recent years, the length (days) of FSH treatment and the total dose of FSH per donor has tended to decrease. In a 1986 study evaluating 2,048 beef donor collections at Granada Genetics in Marquez, Texas, a 28-mg total dose of FSH-P administered twice daily over a 4-day interval produced the greatest number of good quality embryos per donor collection. When total dose administered per donor either increased or decreased from the 28-mg dose level, the mean number of good quality embryos decreased per donor.

Exogenous FSH has been reported to have a shorter half life in the blood stream than PMSG, so it is usually administered twice daily at 10- to 12- hour intervals in cattle donors. It is interesting to note, however, that one study has shown that FSH admininistered once daily to beef donors has produced ovulation rates that were not significantly different from those of donors given FSH (from the same lot number) on a twice-a-day treatment schedule. In a study conducted at this laboratory, a single 50-mg dose of FSH (given in one injection) on the first treatment day produced similar ovulation rates in beef donors to those given the same total dose of FSH over a 5-day interval with a twice-a-day injection schedule. These findings tend to cast some doubt on the basic thinking behind our reason for twice daily FSH treatment schedules in donor cattle. Recent reports have suggested that the circulating FSH level required for stimulating gonadotropin-dependent follicles needs only to be proportional to the receptor site

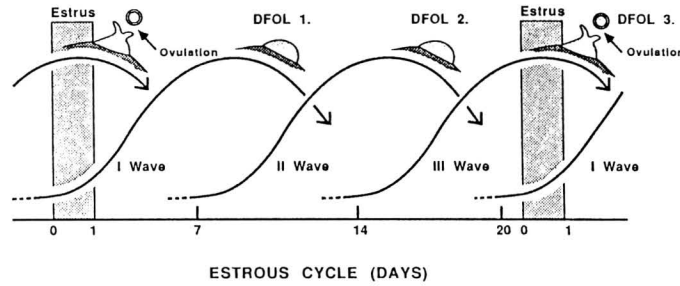
population of the follicles within that follicular wave, and FSH above that level is wastage. It is becoming evident that studies on the availability of FSH and LH receptors at the ovarian level are needed to enhance our understanding of superovulation of cattle.

The FSH to LH ratio and biological activity of the commercial FSH preparations has caused much concern to those in the commercial embryo transplant business. In recent years, FSH:LH ratios have been blamed for the inconsistent ovulation rates across donors. However, when ovulation rates have been compared for donors across lot numbers (batches) from one commercial FSH source in several studies, the results showed that there was no significant difference in ovulation rates across either lot numbers of FSH-P® or the laboratory animal-derived biological activity units of those lots. Efforts are now being made to field test various purified and recombinant-DNA FSH products on dairy and beef donors, again in hopes of reducing individual animal-to-animal variability in response. In several recent studies, priming females with norgestomet implants prior to FSH-P treatment has given good stimulatory responses in both dairy and beef donors. This approach appears to be very effective on the transitional postpartum beef donor cow. The question persists, however, as to whether individual donor variability will be greater than the resulting effect of any of the exogenous treatments used.

Recent studies using ultrasonography at Cornell, Michigan State University and the University of Wisconsin have helped to verify the follicular wave pattern during the estrous cycle of cows. Although it has been shown that cyclic females may have two follicular waves, most of the cows have three follicular waves per estrous cycle (Figure 3). In each follicular wave there is at least one gonadotropin-dependent dominant follicle that is capable of producing estrogen. Evidence now indicates that the dominant follicle is involved in suppressing the remaining follicles in the follicualr wave. At the recent American Embryo Transfer Association Meetings in Reno, Nevada, Dr. Roy Fogwell suggested that proper timing of donor gonadotropin stimulation during the estrous cycle may be more important for successful superovualtion that was once realized. For maximum follicle development during the cycle, it would seem logical that exogenous gonadotropins should be administered to donors when the dominant follicle is not suppressing the remaining follicles of the wave. For this reason, it may be important to identify follicular growth patterns of each donor female via ultrasound before starting the superovulation schedule.

It has been proposed that exogenous gonadotropins do not increase the number of follicles available from the pre-follicle pool for development but rather work primarily to rescue existing gonadotropin-sensitive follicles of the wave that normally would become atretic. In a study at this station, beef donor cows treated twice daily with FSH

Figure 3. An example of the follicular wave pattern during the estrous cycle in cattle. Most cattle have three follicular waves resulting in three dominant follicles (DFOL) during each estrous cycle. (Baird, 1987; Sirois and Fortune, 1988; Fogwell, 1988).



starting at the onset of natural estrus exhibited a second estrus 36 to 72 hours after the first estrus and produced numerous supplementary ovulatory-size follicles. Stimulating supplemental follicle development during estrus in cattle would tend to support the hypothesis that FSH-stimulated follicles of the wave can be rescued prior to normal follicle loss during or in the days follwing estrus. It should not be overlooked, however that exogenous gonadotropins may also increase the natural sensitivity window so that additional maturing pre-follicles could be added to the developing follicular wave. A wider than normal ovarian sensitivity window could help explain why multiple ovulations occur in litter bearing animals.

Attempts at synchronizing ovulation or altering ovulation rates in donor cattle with either GnRH or HCG preparations at induced estrus have given inconsistent results over the years. Incorporating ovulatory hormones into the superovulation procedure has all but discontinued since prostaglandins have been added to the hormone treatment schedule for cattle. Success has recently been reported using various anti-PMSG antisera to aid in controlling the PMSG stimulatory response. At present, ovary stimulation research in farm animals is revolving around hormone immunization of donors and using an anti-inhibin approach to superovulation.

Over the years of testing and evaluation of different gonadotropic agents, dose levels and injection schedules, it has become apparent that individual animal-to-animal variability across donors is likely the major contributor to inconsistent ovulation rates in gonadotropin-treated donor cattle. Furthermore, the use of continuous intravenous FSH infusion on donor cattle over a 5-day treatment interval has not solved the variability associated with superovulation. This is not to discount that other factors such as donor pre-treatment steroid hormone levels, breed type and season of the year (in hot, humid regions) could have potentiating affects on the superovulatory response of donors, but such factors appear to be secondary in the overall

problem relating to donor variability.

Indirect evidence suggests that each ovary responds to exogenous gonadotropins as an individual entity, and this correlates with the number of pre-follicles available for stimulation and the number of receptors sites available for gonadotropins in each ovary. Recent evidence suggests that ovarian inhibin production is likely the indirect control for modulating stimulatory activity of both ovaries. It would appear that basic research on follicular wave cycles, receptor site production and oocyte activiation seems to be more in order at this time, rather than seeking the elusive optimal FSH or PMSG treatment schedule that fits all donor cows.

Artifical Insemination Schedules for Donor Cattle

A survey of the scientific literature reveals that the fertilization rates for superovulated donor cattle usually range from 50 to 85%. Ovulation has been reported to occur over an 8- to 12-hour period in gonadotropin-treated donor cattle. This interval may even be longer with abundant follicle development. Multiple inseminations have been implemented for superovulated donors to offset the variation in timing of ovulation and to insure sperm cell availability for fertilization of multiple ova in the oviducts. This assumption has led to AI schedules for donors that called for two units or more of semen at 12-hour intervals three and four times at induced estrus for individual donor animals. Even with these insemination schedules, fertilization rates remain variable within and across donor females.

In recent years, the approach to inseminating donors has been re-evaluated in controlled experiments. In one study at this station, beef donors were inseminated with frozen-thawed semen of either high or low quality from either dairy or beef bulls at 12, 24, 36 or 48 hours after the onset of standing estrus. The controls were inseminated with two units of the same semen at 12, 24, 36 and 48 hours after the onset of estrus. The highest overall fertilization rate (78.7%) and transferable embryo recovery rate (6.7 embryos per donor) resulted when donors were inseminated once at 24 hours after the onset of estrus with two units of semen, and these parameters were not significantly different than those of the control donor animals, each inseminated four different times with two units of semen (Figures 4-6). The lowest fertilization rate (19.2%) and transferable embryo recovery rate (0.2 embryos per donor) resulted when donors were inseminated once with two units of semen 48 hours after the onset of estrus. It is interesting to note that mean embryo degeneration rate at the time of collection was 6%, 19%, 41% and 96% for donor

Figure 4. Relationship of embryo parameters (fertilized embryo rate, embryo degeneration rate and transferable embryo rate) in FSH–treated beef donor females inseminated at different intervals following gonadotropin-induced estrus (onset of estrus = 0 hours). (Schiewe *et al.*, 1987)

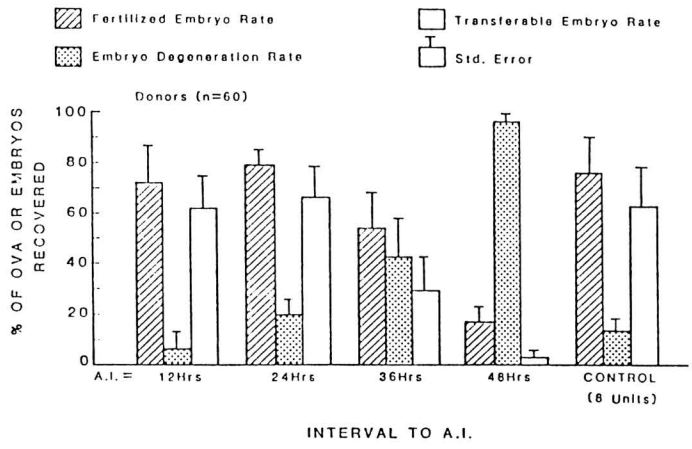


Figure 5. The effect of semen quality on fertilized embryo rate and transferable embryo rate of beef donor females inseminated with either high quality or low quality semen at different intervals at induced estrus (onset of standing = 0 hours). (Schiewe *et al.*, 1987)

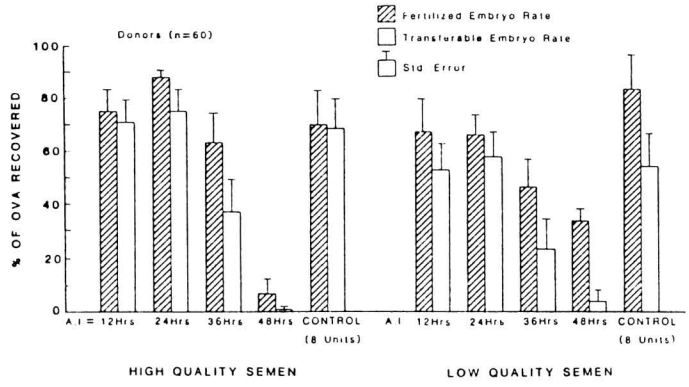
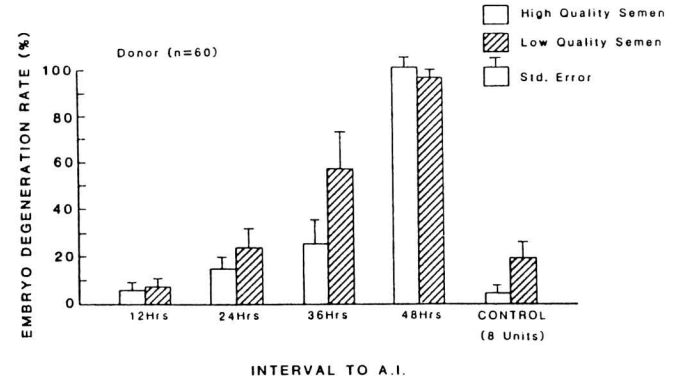


Figure 6. The effect of semen quality on embryo degeneration rate of beef donor females inseminated with either high quality or low quality semen at different intervals at induced estrus (onset of standing estrus = 0 hours). (Schiewe *et al.*, 1987)



cows inseminated once with two units of semen at 12, 24, 36 and 48 hours, respectively. This pattern strongly suggests that timing of donor insemination after the onset of estrus may greatly affect the percentage of viable embryos at the time of collection. When individual insemination times for donors were evaluated in this study, high quality semen consistently produced more favorable results than when low quality semen was inseminated across similar donor cows.

Other studies at this station and in Denmark have shown that one unit of good quality semen at 12, 18 or 24 hours after the onset of estrus has given comparable fertilization rates to superovulated donors inseminated once with two units of semen or with multiple inseminations at 12-hour intervals at induced estrus. Most commercial embryo transplant units today have decreased the number of inseminations and the number of units of semen used per superovulated donor. In summary, a reduction in the number of services per donor would conserve valuable, high cost semen and reduce possible handling stress of donor animals.

RELATED REFERENCES

Baird, D.T. A model for follicular selection and ovulation: Lessons for superovulation. J. Steriod Biochem. *27*:15 (1987). Biggers, J.D. Metabolism of the oocyte. *In*: Oogenesis. University Park Press, Baltimore, pp. 241-252 (1972). Bindon, B.M., Piper, L.R., Cahill, L.P., Driancourt, M.A. and O'Shea, T. Genetic and hormonal factors affecting superovulation. Theriogenology *25*:53 (1986). Boland, M.P., Nancarrow, C.D., Hoskinson, R.M., Murray, J.D., Scaramuzzi, R.J., Radford, H.M., Avenell, J.A. and Bindon, B.M. Superovulatory response in cows following immunization against testosterone and treatment with bovine follicular fluid and PMSG. Theriogenology *23*:185 (1985). Brambell, F.W.F. Ovarian changes. *In*: Marshall's Physiology of Reproduction. Longmans, Green & Co., New York, NY, pp. 397-542 (1956). Britt, J.H. Current concepts of folliculogenesis and endocrinology. Embryo Transf. *3(4)*:1 (1988). Britt, J.H. and Holt, L.C. Endocrinological screening of embryo donors and embryo transfer recipients. A review of research with cattle. Theriogenology *29*:198 (1988). Bushmich, S.L., Randel., R.D. and McCartor, M.M. Effect of dietary monensin on ovarian response following gonadotrophin treatment in prepuberal heifers. J. Anim. Sci. *51*:692 (1980). Callesen, H., Greve, T. and Hyttel, P. Preovulatory endocrinology and oocyte maturation in superovulated cattle. Theriogenology *25*:71 (1986). Choudary, J.B., Gier, H.T. and Marion, G.B. Changes in bovine vesicular follicles. J. Anim. Sce. *27*:472 (1968). Corah, L.F. Nutrition and its effect on reproductive performance of beef cattle. Proc. National Extension-Industry Invitational Workshop on Beef Cattle Reproduction Management. (October), Oklahoma City, Oklahoma, pp. 20-37 (1978). Dhondt, D., Boulters, R., Spincemaille, J., Coryn, M. and Vanderplassche, M. The control of superovulation in the bovine with a PMSG-antiserum. Theriogenology *9*:529 (1978). Donaldson, L.E. Cattle breed as a source of variation in embryo transfer. Theriogenology *21*:1013 (1984). Donaldson, L.E. Dose of FSH-P as a source of variation in embryo production from superovulated cows. Theriogenology *22*:205 (1984). Donaldson, L.E. Estimation of superovulation response in donor cows. Vet. Rec. *117*:33 (1985). Donaldson, L.E. Effect of insemination regimen on embryo production in superovulated cows. Vet. Rec. *117*:35 (1985). Donaldson, L.E. and Perry, B. Embryo production by repeated superovulation of commercial donor cows. Theriogenology *20*:163 (1983). Draincourt, M.A., Gibson, W.R. and Cahill, L.P. Follicular dynamics throughout the oestrus cycle in sheep: A review. Reprod. Nutr. Develop. *25*:1 (1985). Dufour, J.J., Adelakoun, V. and Matton, P. Limited multiple ovulation in heifers fed high plane of nutrition before PMSG stimulation and steroid hormone concentrations in single, twin and multiple ovulations. Theriogenology *15*:433 (1981). Dufour, J.J., Whitmore, H.L., Ginther, O.J. and Casida, L.E. Identification of the ovulating follicle by its size on different days of the estrous cycle in heifers. J. Anim. Sci. *34*:85 (1972). Dunn, T.G. Relationship of nutrition to successful embryo transplantation. Theriogenology *13*:27 (1980). Dunn, T.G., Ingalls, J.E., Zimmerman, D.R. and Wiltbank, J.N. Reproductive performance of 2-year-old Hereford and Angus heifers as influenced by pre- and post-calving energy intake. J. Anim. Sci. *29*:719 (1969). Elsden, R.P., Nelson, L.D. and Seidel, Jr., G.E. Superovulating cows with follicle stimulation hormone and pregnant mare's serum gonadotropin. Theriogenology *9*:17 (1978). Erickson, B.H. Development and radioresponse of the prenatal bovine ovary. J. Reprod. Fertil. *11*:97 (1966). Erickson, B.H. Development and senescence of the postnatal bovine ovary. J. Anim. Sci. *25*:800 (1966). Erickson, B.H., Reynolds, R.A. and Murphree, R.L. Ovarian characteristics and reproductive performance of the aged cow. Biol. Reprod. *15*:555 (1967). Fogwell, R.L. Ovarian follicular growth in cattle: Physiology and pharmacology. Proc. 7th Annual Convention American Embryo Transfer Association. (October 27-29). Reno, Nevada pp. 119-131 (1988). Foote, R.H. and Onuma, H. Superovulation, ovum collection, culture and transfer: A review. J. Dairy Sci. *53*:1681 (1970). Fortune, J.E., Sirois, J. and Quirk, S.M. The growth and differentiation of ovarian follicles during the bovine estrous cycle. Theriogenology *29*:95 (1988). Fortune, J.E. and Quirk, S.M. Regulation of steroidogenesis in bovine preovulatory follicles. J. Anim. Sci. (Suppl. 2) 66:1 (1988). Greve, T. Bovine egg transplantation in Denmark. Ph.D. Dissertation. Royal Veterinary and Agricultural University, Copenhagen, Denmark, pp. 146-157 (1981). Greve, T., Callesen, H. and Hyttel, P. Endocrine profiles and egg quality in the superovulated cow. Nord. Vet. Med. *35*:408 (1983). Hafez, E.S.E., Sugie, T. and Gordon, I. Superovulation and related phenomena in the beef cow. I. Superovulatory responses following PMS and HCG injections. J. Reprod. Fertil. *5*:359 (1963). Henricson, B. and Rajakoski, E. Studies of oocytogenesis in cattle. Cornell Vet. *49*:494 (1959). Hill, K.G., Bondioli, K.R. and Looney, C.R. Use of norgestomet implants in conjuction with follicle stimulating hormone (FSH) for superovulation of donor cattle. Theriogenology *25*:160 (1986). Hill, K.G., Looney, C.R., Schiewe, M.C. and Godke, R.A. Effect of different lot numbers of follicle stimulating hormone (FSH-P) on superovulation response of donor cattle. Theriogenology *21*:241 (1984). Hill, K.G., McFarland, C.W., Rorie, R.W., Viker, S.D. and Godke, R.A. A single 50 mg injection of follicle stimulating hormone (FSH) for superovulation of embryo donor cattle. Theriogenology *23*:196 (1985). Hill, Jr., J.R., Lamond, D.R., Henricks, D.M., Dickey, J.F. and Niswender, G.D. The effect of undernutrition on ovarian function and fertility in beef heifers. Biol. Reprod. *2*:78 (1970). Hisaw, F.L. Development of the Graafian follicle and ovulation. Physiol. Rev. *27*:95 (1947). Hofliger, H. The ovary of the bovine at different periods of life with special consideration to its functional histology. Acta Anatomica (Suppl. 5) *3*:1 (1947). Hseuh, A.J., Adashi, E.Y., Jones P.B.C. and Welsh, T.H. Hormonal regulation of the differentiation of cultured ovarian granulosa cells. Endocrinol. Rev. *5*:76 (1984). Ireland, J.J. Control of follicular growth and development. J. Reprod. Fertil. (Suppl.) *34*:39 (1987). Ireland, J.J., Coulson, P.B. and Murphree, R. L. Follicular development during four stages of the estrous cycle of beef cattle. J. Anim. Sci, *49*:1261 (1979). Kim, H.N., Rorie, R.W., Youngs, C.R., White, K.L. and Godke, R.A. The use of anti-PMSG antibodies with PMSG for superovulating beef cattle. Theriogenology *27*:243 (1987). Lambeth, V.A., Root, R.G., Kreider, J.L. and Godke, R.A. Induced follicular stimulation in beef cows during behavioral estrus with FSH-P. J. Anim. Sci. (Suppl. 1) *45*:18 (1977). Laster, D.B. Follicular development in heifers infused with follicle-stimulating hormone. J. Reprod. Fertil. *28*:285 (1972). Leathem, J.H. Nutritional effects on hormone production. J. Anim. Sci.(Suppl. 1) *25*:68 (1966). Lerner, S.P., Thayne, W.V., Baker, R.D., Henschen, T., Meredith, S., Inskeep, E.K., Dailey, R.A., Lewis, P.E. and Butcher, R.L. Age, dose of FSH and other factors affecting superovulation in Holstein cows. J. Anim. Sci. *63*:176 (1986).

Lindsell, C.E., Rajkumar, K., Manning, A.W., Emery, S.K., Mapletoft, R.J. and Murphy, B.D. Variability in FSH:LH ratios among batches of commercially available gonadotropins. Theriogenology *25*:167 (1986) Looney C.R. Superovulation in beef females. Proc. 5th Ann. Amer. Embryo Transf. Assoc. Mtgs. (October 18-21), Ft. Worth, Texas. pp. 16-29 (1986)Looney, C.R., Bondioli, K.R., Hill, K.G. and Massey, J.M. Superovulation of donors cows with bovine follicle-stimulating hormone (bFSH) produced from recombinant DNA technology. Theriogenology *29*:271 (1988). Looney, C.R., Boutte, B.W., Archbald, L.F. and Godke, R.A. Comparison of once daily and twice daily FSH injections in superovulation of beef cattle. Theriogenology *15*:13 (1981). Lussier, J.G., Matton, P. and Dufour, J.J. Growth rates of follicles in the ovary of the cow. J. Reprod. Fertil. *81*:301 (1987). Maree, C. Observations on the influence of high levels of feeding on the ovarian activity and fertility in dairy cows. J. South African Vet. Assoc. *51*:167 (1980). Mauleon, P. and Mariana, J.C. Oogenesis and folliculogenesis. *In*: Reproduction in Farm Animals. H.H. Cole & P.T. Cupps (Eds.), Academic Press, NewYork, NY, pp. 175-215 (1977). Maxwell, D.P., Massey, J.M. and Kraemer, D.C. Timing ovulations in the superovulated bovine. Theriogenology *2*:97 (1978). McClure, T.J. Malnutrition and infertility of cattle in Australia and New Zealand. Australian Vet. J. *44*:134 (1968). Monniaux, D., Chupin, D. and Saumande, J. Superovulatory responses of cattle. Theriogenology *19*:55 (1983). Moor, R.M., Cahill, L.P. and Stewart, F. Ovarian stimulation or egg production as a limiting factor of egg transfer. Proc. 9th Internat'l Congr. Anim. Reprod. Artif. Insem. *2*:43 (1980). Moor, R.M., Kruip, Th.A.M. and Green, D. Intraovarian control of folliculogenesis: Limits to superovulation. Theriogenology *21*:103 (1984). Moseley, W.M., McCarter, M.M. and Randel, R.D. Effects of monesin on growth and reproductive performance of beef heifers. J. Anim. Sci *45*:961 (1977). Munyakazi, L., Denniston, R.S., Wiemer, K.E., Price, R.H., Humes, P.E. and Godke, R.A. Estimating pregnancy rates from the number of embryos collected and transferable quality embryos for Holstein donor females from a single herd unit. Theriogenology *31*:231 (1989). Murphy, B.D., Mapletoft, R.J., Manns, J. and Humphrey, W.D. Variability in gonadotropin preparations as a factor in the superovulatory response. Theriogenology *21*:117 (1984). Ortuno, A.M. and Carson, R.L. The effect of dietary monensin sodium upon superovulation and embryo viability from mature cows. Theriogenology *23*:743 (1985). Pederson, T. Follicle kinetics in the ovary of the cyclic mouse. Acta Endocrinol. *64*:304 (1970). Pierson, R.A. and Ginther, O.J. Ultrasonography of the bovine ovary. Theriogenology *21*:495 (1984). Pierson, R.A. and Ginther, O.J. Follicular populations during the estrous cycle in heifers. I. Influence of day. Anim. Reprod. Sci. *14*:165 (1987). Pinney, D.O., Pope, L.S. and Stephens, D.F. Effect of alternate low-high or high-low winter feed levels on growth and reproduction of replacement heifers. Oklahoma Agricultural Experiment Station Misc. Publ. *67*:77 (1962). Rajakoski, E. The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical and left-right variations. Acta Endocrinol. (Suppl.) *52*:6 (1960). Randel, R.D., Harrison, L.M. and Hansen, T.R. Effects of monensin upon response to FSH-P in sexually mature crossbred heifers and Brahman cows. Texas A&M University Beef Cattle Res. Rep. pp. 78-80 (1981). Reid, J.T. Effect of energy intake upon reproduction in farm animals. J. Dairy Sci, (Suppl.) *43*:103 (1960). Richards, J.S. Maturation of ovarian follicles: Actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. Physiol. Rev. *60*:51 (1980). Ryan, D.P., Blakewood, E.G., Swanson, W.F., Rodriguez, H. and Godke, R.A. The use of follicle stimulating hormone (FSH) to stimulate follicle development for *in vitro* fertilization during the first trimester of pregnancy in cattle. Theriogenology 33:(Submitted). Ryan D.P., Rodriguez, H., Casey, P.L. and Godke, R.A. The use of follicle stimulating hormone to stimulate follicular development during the second trimester of pregnancy in cattle. J. Dairy Sci. *72*:2220 (1989). Saumande, J. and Chupin, D. Superovulation: A limit to egg transfer in cattle. Theriogenology *7*:141 (1977). Savio, J.D., Keenan, L., Boland, M.P. and Roche, J.F. Pattern of growth of dominant follicles during the estrous cycle of heifers. J. Reprod. Fertil. *83*:663 (1988). Scaramuzzi, R.J., Turnbull, K.E. and Nancarrow, C.D. Growth of Graafian follicles in cows following luteolysis induced by the prostaglandin F_2 analogue, cloprostenol. Australian J. Bio. Sci. *33*:63 (1980). Schalles, R.R., Kiracofe, G. and Smith, E.F. Flushing Cows. Kansas State University Cattleman's Rep. pp. 6-7 (1976). Schiewe, M., Looney, C.R., Hill, K.G., Johnson, C.A. and Godke, R.A. Transferable embryo recovery rates following different artificial insemination schedules in beef donor cattle. Theriogenology *28*:395 (1987). Schiewe, M., Voelkel, S.A. and Godke, R.A. Artificial insemination of superovulated beef cattle with a single insemination of frozen semen. Theriogenology *23*:228 (1985). Schneider, Jr., H.F., Castleberry, R.S. and Griffin, J.L. Commercial aspects of bovine embryo transfer. Theriogenology *13*:73 (1980). Sirois, J. and Fortune, J.E. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biol. Reprod. *39*:308 (1988). Spicer, L.J. and Echternkamp, S.E. Ovarian follicular growth, function and turnover in cattle: A review. J. Anim. Sci. *62*:428 (1986). Staigmiller, R.B. and England, B.G. Folliculogenesis in the bovine. Theriogenology *17*:43 (1982). Viker, S.D., Hembry, F.G. and Godke, R.A. Care and management of the beef herd for embryo transfer. C.A. and F.E. King Workshop - Reproductive Management of Beef Cattle. University of Arkansas, Fayetteville (December) Spec. Rep. No. 119, pp. 60-69 (1984). Warren W.W., Bercovitiz, A.B., Kreider, J.L. and Godke, R.A. Follicular stimulation in beef heifers with continuous FSH infusion. J. Anim. Sci.*41*:384 (1975). Wasserman, P.M. Oogenesis: Synthetic events in the developing mammalian egg. *In* :Mechanism and Control of Animal Fertility. J.F. Hartmann (Ed.), Academic Press, New York, NY, pp. 1-46 (1983). Wiltbank, J.N., Rowden, W.W., Ingalls, J.E., Gregory, K.E. and Koch, R.M. Effect of energy level on reproductive phenomena of matue Hereford cows. J. Anim. Sci, *21*:219 (1962). Wiltbank, J.N., Rowden, W.W., Ingalls, J.E. and Zimmerman, D.R. Influence of postpartum emergy level on reproductive performance of Hereford cows restricted in energy intake prior to calving. J. Anim. Sci. *23*:1049 (1964). Ying, S. -Y. Inhibins, activins and follistatins: Gonadal proteins modulating the secretion of follicle stimulating hormone. Endocrinol. Rev. *9*:267 (1988). Zamboni, L. Ultrastructure of mammalian oocytes and ova. Biol. Reprod. (Suppl.) *2*:44 (1970).