Triennial Reproduction Symposium: Impediments to Fertility in Domestic Animals

9 The obstacle course to successful establishment of pregnancy in domestic livestock species. M. D. Utt and M. L. Day,* *Department of Animal Sciences, The Ohio State University, Columbus.*

Research in reproductive biology over the past century has provided valuable knowledge and technologies that have and will be used to increase the likelihood of creating a successful pregnancy in domestic livestock species. However, maximal reproductive efficiency has not been achieved in any of these species. Successful establishment of pregnancy is a complex series of interrelated events that can be impaired at the gamete level through fetal development. The gametes contain genetic information and other factors necessary for creating viable offspring, but genetic and/or developmental deficiencies of gametes and the gonadal environment in which they reside may influence embryonic and fetal development. When sperm are deposited in the female reproductive tract at an appropriate time relative to ovulation, fertilization rate is relatively high, and differences therein are related to compensable and uncompensable sperm traits and/or sperm transport. Following fertilization, developing embryos reside in the oviduct before moving into the uterus. The oviductal and uterine environments are influenced by estrogen and progesterone before and after ovulation, and embryonic development may be affected by fluctuations in concentrations of either or both steroids. Latent effects of estrogen and progesterone on the uterus may impact the lifespan of the corpus luteum (CL) and/or embryonic development through maternal recognition of pregnancy (MRP) and the preimplantation period. Regardless of species, MRP consists of embryonic-derived signals that inhibit destruction of the CL and consequently the conceptus. Defective interactions among the conceptus, uterus, and CL can subsequently lead to failure in MRP. After MRP, embryonic or fetal loss is still possible due to uterine deficiencies, environmental effects, or combinations thereof. Many of the aforementioned obstacles to pregnancy may be exacerbated by production and metabolic stress; therefore, lactating dairy cattle are an appropriate model to study the spectrum of factors that impede attainment of optimal reproductive efficiency.

Key Words: pregnancy, deficiency, embryo

10 Sperm characteristics that limit success of fertilization. W. L. Flowers,* *North Carolina State University, Raleigh.*

The theoretical relationship between male fertility and sperm characteristics resembles a positive, asymptotic equation. When numbers of spermatozoa are low, fertilization rates are low. As the number of sperm cells increase, so does fertility. This response gradually diminishes until a plateau is reached and additional spermatozoa do not affect male fertility. Variation clearly exists among males in how their fertility responds to increasing spermatozoa both in terms of the slope of the positive portion of the curve and the point at which it reaches a plateau. These differences in male fertility patterns often are attributed to differences in the quality of their sperm cells. Sperm characteristics can be qualitative or quantitative and measure individual or functional properties. Qualitative assessments such as motility and morphology deal with population dynamics, and as they increase so does fertilization rate to a point. Hence, qualitative characteristics probably are responsible for variations along the linear portion of male fertility curves. Quantitative evaluations such as amounts of plasma membrane proteins or acrosin activity estimate aspects of all sperm cells produced by males and most likely mainly influence the level at which fertilization rates reach their plateau. Individual and functional characteristics of sperm can be qualitative or quantitative and affect male fertility patterns accordingly. Intuitively, functional traits such as sperm binding should provide a better indication of fertilization than individual ones such as motility. However, correlations of both types with fertility are very similar. Reasons for this may be related to how characteristics of sperm cells are influenced by the female reproductive tract after insemination. For example, capacitation patterns of spermatozoa in some boars are different in vitro than in vivo. If this relationship holds true for other traits, development of tests that account for this variation are necessary to address a critical need for understanding sperm characteristics that limit fertility.

Key Words: livestock, fertility, spermatozoa

11 The ovarian follicular reserve in ruminants: What regulates its formation and size? J. E. Fortune,* M. Y. Yang, and J. J. Allen, *Cornell University, Ithaca, NY.*

Primordial follicles have an oocyte arrested in first prophase of meiosis and a layer of flattened granulosa cells. These nongrowing follicles comprise the follicular reserve, the source of growing follicles until reproductive senescence. Indirect evidence indicates that the size of the follicular reserve in adult women is a measure of their reproductive potential. Therefore, it is of interest to elucidate the mechanisms that regulate follicle formation and factors that may enhance or diminish the size of the primordial pool. In species such as cattle, sheep, and humans, follicle formation and the establishment of the follicular reserve occur during fetal life. We use fetal bovine ovaries as a model to study the regulation of follicle formation and acquisition of the capacity to activate (initiate growth). Our results suggest a critical role for fetal ovarian steroids in the timing of follicle formation and differentiation of newly formed primordial follicles to a stage where meiosis has arrested in prophase I and ability to activate has been acquired. Current studies are focused on the regulation of fetal ovarian steroidogenesis by gonadotropins, steroids, and growth factors. We hypothesize that factors that interfere with production and/or action of fetal steroids may affect the size of the follicular reserve and perhaps the reproductive potential of the ensuing adult. Because domestic animals may be exposed to forage containing estrogenic compounds and to other environmental estrogens, the effects of endocrine disrupting chemicals on follicle formation and capacity to activate are potentially important. Nutrition may be another influence on the size of the follicular reserve. In adult cattle, the number of follicles in follicular waves has been correlated with their fertility and nutritional restriction during the first 110 d of bovine pregnancy was associated with fewer follicles in waves (vs. controls) when daughters reached maturity. Although there is much to be learned about the regulation of follicle formation in cattle and its relationship to fertility, this stage of ovarian development may be very important to adult reproductive potential.

Key Words: ovarian follicle, ovary, cattle

12 Influence of follicle characteristics at ovulation on early embryo survival. T. W. Geary*[1], M. F. Smith[2], M. D. MacNeil[1], M. L. Day[3], G. A. Bridges[4], G. A. Perry[5], F. M. Abreu[3], J. A. Atkins[2], K. G. Pohler[2], E. M. Jinks[3], and C. A. Roberts[1], *[1]USDA-ARS, Fort Keogh, Miles City, MT, [2]Division of Animal Sciences, University of Missouri, Columbia, [3]Department of Animal Science, Ohio State University, Columbus, [4]Department of Animal Science, University of Minnesota, Grand Rapids, [5]Department of Animal and Range Sciences, South Dakota State University, Brookings.*

Reproductive losses are significant in domestic animals and represent huge economic inefficiencies in livestock production. Across livestock species, embryonic mortality is the greatest contributor to reproductive losses. In most livestock species, early pregnancy diagnosis is an impediment to making significant progress to alleviate these losses. Perhaps unfairly, the female of each livestock species receives the blame for the majority of pregnancy failures. Research has focused on production of an optimal follicle that leads to ovulation of a highly fertile oocyte and production of steroids responsible for preparation of the female reproductive tract for pregnancy. Most studies have used hormonal manipulation of the estrous cycle and ovulation to identify factors affecting pregnancy success. Physiological maturity of the ovulatory follicle includes numerous reproductive processes and events obligatory for pregnancy establishment and maintenance. In sows, mares, and beef and dairy cows, oocytes derived from larger follicles were more competent, as assessed by in vitro development, than oocytes from smaller follicles. In cattle, ovulatory follicle size was highly correlated with serum estradiol concentration, and estradiol supplementation at AI improved pregnancy rates of cows ovulating smaller follicles. Greater ovulatory follicle size and more rapidly growing follicles at GnRH-induced ovulation were positively associated with fertilization success; while ovulatory follicle size, but not follicle growth rate, was positively associated with recovery of a viable embryo on d 7 in beef cows. In mares, age related changes in follicular signaling appear to play a large role in oocyte quality and fertility. This presentation focuses on follicular characteristics involved in early embryo survival, including follicle/oocyte maturation as affected by ovulatory follicle age, size, hormonal environment, and so on, and their effects on pregnancy establishment and maintenance. There is a strong relationship between the ovulatory follicle and steroid production related to early embryo development and pregnancy establishment.

Key Words: follicle, fertility, embryo survival

13 Deficiencies in the uterine environment and failure to support embryo development. G. A. Bridges,* *University of Minnesota, Grand Rapids.*

Pregnancy failure in cattle can result from failure to fertilize the oocyte or embryonic loss during gestation. Although fertilization failure occurs, embryonic mortality has a greater contribution to pregnancy failure. A variety of factors contribute to embryonic loss and it may be exacerbated in certain animals including high-producing lactating dairy cows and in some cattle in which estrous synchronization and timed AI was performed. Asynchronous follicular dynamics during timed AI protocols can induce ovulation of immature follicle that produce insufficient estradiol and a corpus luteum that potentially produces reduced progesterone concentrations compared with cows induced to ovulate mature follicles. In lactating dairy cows, circulating concentrations of steroids are reduced due to elevated feed intake causing increased liver blood flow and increased steroid catabolism in the liver. In each of these classes of animals that have decreased steroid concentrations, fertility is reduced compared with females that ovulate mature follicles at timed AI or to nonlactating dairy animals. Recent research in beef cows induced to ovulate immature follicles and lactating dairy cows suggest that deficient uterine function is the primary factor responsible for infertility in these animals. Failing to provide adequate concentrations of estradiol before ovulation results in prolonged effects on expression and localization of uterine genes and proteins that participate in regulating uterine functions during early gestation. Furthermore, progesterone concentrations during early gestation affect embryonic growth, interferon-tau production, and uterine function. Therefore, an inadequate uterine environment induced by insufficient steroid concentrations before and after ovulation could cause early embryonic death either directly by failing to provide an adequate uterine environment for embryo survival, adhesion, and implantation, or indirectly by failing to support appropriate embryonic growth which could lead to decreased conceptus size and malfunction of the process of maternal recognition of pregnancy.

Key Words: fertility, uterus, steroids

14 Interactions of the embryo, uterus and corpus luteum for sustenance of embryos. T. R. Hansen,* A. Q. Antoniazzi, J. J. Romero, R. L. Ashley, and R. C. Bott, *Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, Colorado State University, Fort Collins.*

Interferon-tau (IFNT) from the ruminant conceptus disrupts oxytocin signaling and pulsatile release of prostaglandin F2α (PGF) in the endometrium through prevention of upregulation of the estradiol receptor, and consequently oxytocin receptor, which occurs during luteolysis. Microarray analysis revealed upregulation of several hundred genes in bovine endometrium and peripheral blood cells, and in ovine corpus luteum (CL) in response to early pregnancy that were mostly interferon-stimulated genes (ISG). For this reason, we hypothesized that IFNT not only protects pregnancy through paracrine action on release of endometrial PGF, but also has endocrine action through induction of resistance of the CL to lytic actions of PGF during early pregnancy. Antiviral activity, an indicator for type I IFN, increased in uterine vein blood on d 15 of pregnancy in sheep and was blocked through preadsorption with anti-IFNT antibody, providing evidence that IFNT was present in uterine vein blood. A specific (no cross-reactivity with other type I or II IFN) and sensitive (100 pg/mL) radioimmunoassay for IFNT was developed and used to detect IFNT in uterine vein blood from d 15–16 pregnant sheep. Global mass spectrometry was used to demonstrate presence of IFNT in uterine vein serum from a d 15 pregnant ewe. The ISG were upregulated in CL by d 14 of pregnancy and were localized primarily to large luteal cells, but also to small luteal cells in ewes. Culture of isolated ovine large and small luteal cells as well as mixed luteal cells with low (100 pg/mL) levels of IFNT caused upregulation of ISG. Endocrine delivery of IFNT (200 µg/d) using miniosmotic pumps into the uterine vein for 7 d (d 10–17 of estrous cycle) caused a delay in return to estrus beyond 32 d. Finally, systemic delivery (subcutaneous or jugular vein) of 20 µg of IFNT/d using miniosmotic pumps starting on d 10 of the estrous cycle, followed 24 h later by a challenge with PGF injection resulted in protection of the CL through d 13 based on serum progesterone profiles. It is inferred from these studies that IFNT protects early pregnancy in ruminants through paracrine action on the endometrium and also through endocrine action on the CL and peripheral blood cells in context of enhancing luteal resistance to PGF and the maternal ability to combat viral infections, respectively. USDA-NIFA-AFRI 2011-67015-20067.

Key Words: corpus luteum, endometrium, pregnancy

15 Limitations in uterine and conceptus physiology that lead to fetal losses. J. L. Vallet,* *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE.*

Conceptus losses in livestock occur throughout gestation. Clearly, the uterus and embryo/placenta/fetus play interconnected roles in these losses, the details of which depend on the period of gestation and the species. Studies in sheep and pigs have indicated that the uterine glands are essential for full fertility, based on experiments where gland development was reduced through the use of exogenous hormones. In sheep and cattle, normally the uterus is well able to support more than a single fetus, although these species differ in the consequences of multiple births. When 2 conceptuses are present, the placentas of cattle often anastomose, putting one fetus at risk if the other is lost. One likely reason this does not occur in sheep is because sheep embryos undergo intrauterine migration, similar to pigs. In pigs, the equidistant separation of conceptuses is likely to be essential for optimizing conceptus survival, as is the simultaneous and uniform elongation of blastocysts that occurs during the time of maternal recognition of pregnancy. Other studies in pigs have indicated that the size of the uterus influences litter size and therefore fetal losses. In response to crowded intrauterine conditions in the pig, increased conceptus losses begin to occur between d 30 and 40 of pregnancy, and further losses occur sporadically during later gestation. There is evidence that improved fetal erythropoiesis can reduce these losses. Reports also indicated that profound changes in placental development occur under crowded intrauterine conditions that may contribute to losses during late gestation. Reductions in placental stroma formation may compromise the ability of the pig placenta to adapt to reduced uterine space. Consistent with this, both hyaluronan and hyaluronidase activity are decreased in the placentas of small compared with large fetuses. These results suggest that improvements in placental stroma formation could improve placental ability to compensate for reduced intrauterine space, resulting in increased placental function and reduced fetal losses during late gestation. USDA is an equal opportunity provider and employer.

Key Words: placenta, erythropoiesis, hyaluronan

16 The spectrum of factors that impede pregnancy in dairy cows. R. L. A. Cerri*[1], J. E. P. Santos[2], W. W. Thatcher[2], and J. L. M. Vasconcelos[3], *[1]University of British Columbia, Vancouver, BC, Canada, [2]University of Florida, Gainesville, [3]Sao Paulo State University, Botucatu, SP, Brazil.*

The fertility problem in dairy cows is complex and multifactorial but can be divided into 2 major categories: 1) failure to submit cows for insemination, and 2) extensive embryonic and fetal losses. Numerous factors influence these 2 categories (e.g., heat stress, energy balance, cow comfort, health, ovarian dynamics and uterine environment). In the past 10 yr, however, the trend in daughter pregnancy rate has been positive based on USDA data through 2010. This improvement is likely a direct effect of the intensive use of timed AI (TAI) programs, which improved submission rates and, to some extent, pregnancy per AI because of physiological optimization of the reproductive programs. Recently, the use of activity monitors in North American herds has improved submission rates, thereby reducing pharmacological interventions. Although fertilization rate is relatively high (80%), only around 40% of fertilized embryos result in a pregnancy at 60 d after insemination. Early and late embryonic losses are still an unsolved problem in lactating dairy cows and are partly explained by changes in ovarian and uterine physiology. More time between follicle emergence to ovulation concomitantly with decreased concentrations of circulating progesterone and estradiol, reduce embryo quality and create a suboptimal uterine environment that negatively affects pregnancy. Metabolic and health disorders also play major roles as they affect around 40 to 50% of cows in early lactation. Pregnancy per AI exceeds 50% in cows not affected by any clinical periparturient disorder. A pool of knowledge has been acquired regarding the interactive cellular and molecular mechanisms of the conceptus and endometrium within the periattachment period. However, additional research is needed to unveil the effects of steroid and metabolic hormones, health disorders, and among-cow differences in molecular mechanisms pivotal to successful pregnancy. Genetic selection and applied and basic technologies to improve submission rates and reduce pregnancy losses, without affecting milk production, will likely remain at the forefront to improve reproductive efficiency in modern dairy cows.

Key Words: dairy cows, estrous detection, pregnancy loss