

Physiology and Endocrinology: Sperm-Oviduct Interactions in Livestock and Poultry

531 Evidence that oviduct secretions influence sperm function: a retrospective view for livestock. G. J. Killian*, *The Pennsylvania State University, University Park.*

The mammalian oviduct has long been recognized as an organ essential to the success of reproduction. Bovine, ovine, porcine and equine animal models have offered clear advantages for oviduct study related to gamete physiology, fertilization and early embryo development. Livestock species are amenable to surgical alteration of the reproductive tract, estrous cycle manipulation, gamete cryopreservation, artificial insemination as well as in vitro fertilization and embryo production. Although most reproductive technology developed for livestock was intended to benefit production animal agriculture, these techniques are a treasure trove of tools for researchers to better understand how the oviduct influences gamete function. Oviduct secretions obtained from in vitro tissue cultures or via indwelling oviduct catheters have been used for analyses to define the protein, lipid, carbohydrate, enzyme and electrolyte compositions of the secretions during the estrous cycle or in response to hormone treatment. Oviduct secretions or components purified there from have also been used in in vitro assays to assess their ability to bind to sperm and/or influence on sperm viability, motility, sperm capacitation, the acrosome reaction, sperm-egg binding, egg penetration as well as subsequent embryo development. Compelling data have emerged which show that the composition of secretions differs during the estrous cycle and that their composition differs whether they originate from the ampullar or isthmic regions of the oviduct. These differences in composition are functionally relevant and associated with different responses by sperm. Evidence suggests that oviduct-specific glycoproteins, glycosaminoglycans, carbohydrates, norepinephrine, catecholamines, heat-shock protein and osteopontin are components of the oviductal milieu which have the capacity to modulate sperm function. Continued research on the livestock oviduct will likely unravel the role that specific oviduct secretions have in modulating sperm function and how these modifications ultimately affect fertilization and embryo development.

Key Words: sperm, oviduct, secretions

532 Role of the oviduct in maintaining sustained fertility in hens. M. R. Bakst*¹ and J. P. Brillard², ¹ARS, USDA, Beltsville, MD, ²INRA, Tours, France.

In poultry, sperm transferred by natural mating or artificial insemination (AI) into the distal end of the vagina immediately begin their ascent to the utero-vaginal junction (UVJ) located at the anterior end of the vagina. During their transport there is an intense sperm selection process that may reduce the number of sperm initially transferred by as much as 99.5%. Those "select" sperm reaching the UVJ enter the thousands of tubular invaginations of the vagina's surface epithelium located in the UVJ mucosa, collectively referred to as the sperm-storage tubules (SST). Sperm residing in the SST lumen are capable of surviving for several weeks while retaining their fertilizing capacity. Resident sperm are released gradually from the SSTs while the hen is in egg production, ascend to the site of fertilization, and interact with the next ovulated ovum. In this manner, given the absence of an estrus to synchronize ovulation with copulation, poultry, and birds in general, are assured a population of sperm at the site of fertilization during a daily succession of ovulated ova. Over the past decade several new and diverse observations have been published addressing the microanatomy of the

UVJ and SST, and the cellular and molecular mechanisms orchestrating oviductal sperm selection and storage. These include: the SST numbers in different poultry species and lines of high and low fertility; roles of the immune system and possibly neuroendocrine-like cells in the vagina in sperm selection and storage; the roles of aquaporins and a fluid exchange mechanisms contributing sperm release from the SSTs; and, gene expression of the SST epithelial cells with or without resident sperm. The objective of this presentation is to integrate these observations into a comprehensive understanding of the cellular and molecular events influencing the fate of sperm in the hen's oviduct, particularly in the area of oviductal sperm selection and storage.

Key Words: poultry, reproduction, oviduct

533 Effect of sperm mobility phenotype on fertility, sperm competition, and in vivo sperm storage in the domestic fowl. D. P. Froman*, *Oregon State University, Corvallis.*

Sperm mobility is a quantitative trait. Phenotype is determined by the extent to which sperm move against resistance at body temperature. This ability is measured in vitro by sperm penetration of 6% (wt/vol) Accudenz from an overlaid sperm suspension. The study of sperm mobility has provided: 1) an estimate of heritability, 2) an explanation for phenotypic variation based upon properties of individual motile sperm, 3) a plausible model that explains why a portion of sperm ejaculated by any given male are immobile in addition to why this proportion varies among males, and 4) loci of interest within the fowl genome. The distinction between sperm motility and mobility is critical. Whereas all mobile sperm are motile, not all motile sperm are mobile; for a motile cell must have a velocity >30 μm per second to be mobile in vitro. This distinction is biologically significant because sperm mobility phenotype predicts male fertility when hens are inseminated with a fixed number of viable sperm. Immobile sperm contain dysfunctional mitochondria, and the time course for mitochondrial failure begins before ejaculation. Percentages of affected sperm appear to range between 10 and nearly 100%. This variation is attributed to a genetic predisposition that puts sperm cells at risk as they pass through the deferent ducts of the testis. Sperm mobility is heritable ($h^2 = 0.30$), and phenotype is influenced by a maternal additive genetic effect, attributed to the Z chromosome based upon genome-wide SNP/typo analysis. To date, experiments have been reductionist in nature. Nonetheless, synthesis of experimental outcomes has afforded 4 new insights. These include: 1) how fitness varies among normal, fertile males within a population, 2) a likely mechanism enabling in vivo sperm storage, 3) a quantitative, gene-based definition of semen quality, and 4) a new approach to semen preservation based upon bioenergetic theory. In essence, sperm are self-propelled DNA delivery vehicles. This presentation will explain how the self-propulsive nature of sperm varies among males and how such variation affects male fitness.

Key Words: domestic fowl, fertility, sperm

534 Bovine oviduct-sperm interactions preceding fertilization. S. S. Suarez*, *Cornell University, Ithaca, NY.*

The bovine oviduct is not a simple conduit for sperm. The epithelium and luminal fluids of the oviduct affect the physiological state of sperm and movement of sperm into and through the oviduct. There is evidence that the oviduct is open to sperm for only a limited time after insemina-

tion; furthermore, sperm may require certain cell surface proteins to gain access. After entering the oviduct, bull sperm bind to the oviductal epithelium using 3 proteins in the BSP family (PDC109 or BSPA1/A2, BSPA3, and BSP30K), which are the major secreted proteins of the seminal vesicles. The BSP proteins coat the heads of sperm when they come into contact with vesicular secretions at ejaculation. Putative oviductal receptors for the BSP proteins are 4 proteins in the annexin family (ANXA1,2,4,5). Sperm binding to oviductal epithelium results in the development of a storage reservoir of sperm in the lower oviduct. There is evidence that binding prolongs the fertility of sperm; that is, when sperm coated with BSP proteins are incubated with apical plasma membranes of oviductal epithelium, their motile lives are extended. Release of sperm is likely a gradual process, with sperm breaking loose and then reattaching several times. The gradual release of sperm would reduce the numbers that arrive at the oocyte at any one time, thereby preventing polyspermy, and would also prolong the arrival of sperm in the upper oviduct to ensure that fertilization takes place. Capacitation may play a role in sperm release, because the process involves shedding of BSP protein and reduces bull sperm binding to oviductal epithelium. Capacitation of bull sperm is enhanced in vitro by heparin and heparin-like glycosaminoglycans have been detected in bovine oviduct fluid. Considering the role of heparin in capacitation, it is interesting that both BSP and annexin proteins bind heparin. Differential shedding of the BSP proteins and their differing affinities for the 4 annexin proteins may result in a gradual and directed release of sperm toward the site of fertilization.

USDA CSREES NRICGP 2008–35203–19031.

Key Words: sperm, oviduct, seminal vesicles

535 In vivo imaging of in situ motility of fresh and liquid-stored ram spermatozoa in the ewe genital tract. X. Druart^{*1}, J. Cognié¹, G. Baril¹, F. Clément², J.-L. Dacheux¹, and J.-L. Gatti¹, ¹UMR 6175 INRA, CNRS-Université de Tours-Haras Nationaux, Nouzilly, France, ²INRIA Paris-Rocquencourt, Le Chesnay Cedex, France.

The fertility of ram semen after cervical insemination is substantially reduced by 24 h of storage in liquid form. The effects of liquid storage on the transit of ram spermatozoa in the ewe genital tract was investigated using a new procedure allowing direct observation of the spermatozoa in the genital tract. Ejaculated ram spermatozoa were fluorescently labeled and used to inseminate ewes in estrus either cervically through the vagina or laparoscopically into the base of the uterine horns. Four hours after insemination, the spermatozoa were directly observed in situ using fibered confocal fluorescence microscopy. The high resolution video images obtained with this technique allowed determination of the distribution of spermatozoa and individual motility in the lumen of the ewe's genital tract. The results showed a gradient of increasing concentration of spermatozoa from the base of the uterus to the UTJ 4 h after intrauterine insemination into the base of the horns. The in vitro storage of spermatozoa in liquid form decreased their migration through the cervix and reduced the proportion of motile spermatozoa and their straight line velocity at the UTJ and their transit into the oviduct.

536 Comparison of timed AI pregnancy rates in Santa Gertrudis (SG) and SG crossbred heifers following the 7-d or 5-d CO-Synch+CIDR protocol. R. L. Stanko^{*1,3}, K. D. Arnold¹, J. R. Ramirez², S. Moore², and R. Silguero², ¹Texas A&M University-Kingsville, Kingsville, ²King Ranch, Inc., Kingsville, TX, ³Texas AgriLife Research, Beeville.

Our objective was to evaluate the effectiveness of CO-Synch + CIDR protocols and timed AI (TAI) in beef heifers typical to the gulf coast states. Heifers (n = 239) of 2 breed types were used: Santa Gertrudis (SG, n = 145) and SG x Red Angus, F1 (SGX, n = 94). Heifers (n = 6, 2.5%) were removed from data set due to lost CIDR or absence at TAI or pregnancy determination. Age was determined from recorded birth date, and BCS and BW was recorded at CIDR insertion. All heifers were administered GnRH (100 µg, i.m.) and either a new (n = 121) or an autoclaved, once-used (n = 118) CIDR insert (1.38 g progesterone) at random on d 0. CIDR inserts were removed and prostaglandin F2α (PG, 25 mg, i.m.) was administered on d 7 (n = 160) with TAI performed 55 to 58 h after PG or CIDR inserts were removed and PG administered twice on d 5 (n = 73) with TAI at 72 to 75 h after PG. All heifers were administered GnRH (100 mg, i.m.) following TAI. Pregnancy was diagnosed by transrectal ultrasonography at 34, 35 or 36 d after TAI. Heifer age, BW, BCS, and TAI pregnancy rate (PR) was 372.0 ± 2.0 d, 331.0 ± 3.1 kg, 5.6 ± 0.04, and 43.8%, respectively. Chi-squared analysis was used to determine differences in PR. Overall, PR was greater (P < 0.01) in SGX (51/92, 55.4%) than in SG (51/141, 36.2%) heifers. Similar (P > 0.1) PR was observed between heifers receiving the once-used or new CIDR (56/115, 48.7% vs. 46/118, 39%), and between heifers on the 7-d or 5-d CIDR insert (68/160, 42.5% vs. 34/73, 46.6%). SG heifers receiving the 7-d CO-Synch + CIDR protocol had lower (P < 0.01) pregnancy rate (30/91, 33.0%) than SGX (38/69, 55.0%) receiving the same protocol. However, SG and SGX heifers receiving the 5-d CO-Synch + CIDR protocol had similar (P > 0.1) PR (21/50, 42.0% vs. 13/23, 56.5%, respectively). Heifers with less than 1/4 Brahman influence had acceptable PR with the CO-Synch + CIDR protocol, regardless of duration or CIDR type. The 5-d CO-Synch + CIDR protocol may prove to be an acceptable TAI protocol for SG heifers. Other synchronization of estrus and ovulation protocols are needed to enhance success rate of TAI in cattle adapted to the gulf coast region.

Key Words: heifer, CIDR, synchronization

537 Neither temperament or residual feed intake affect sexual maturity in Brahman heifers. A. N. Loyd^{*1}, D. A. Neuendorff¹, A. W. Lewis¹, T. D. A. Forbes², and R. D. Randel¹, ¹Texas AgriLife Research, Overton, ²Texas AgriLife Research, Uvalde.

Selection of calm and feed efficient cattle based on temperament and residual feed intake (RFI), respectively may improve the overall profitability of beef cattle operations. While studies have investigated the relationships of temperament and RFI with growth parameters, only limited data are available concerning reproductive traits. The objective of this study was to evaluate the effects of temperament and RFI on sexual maturity of Brahman heifers. Brahman heifers born in 2005 (n = 48) and 2006 (n = 54) were evaluated at weaning for temperament using pen score (PS) and exit velocity (EV). Temperament score was calculated for each heifer as the average of PS and EV. Heifers (n = 38 in 2005; n = 41 in 2006) were fed a balanced ration at 2.5% BW twice daily at 0800 and 1600 h in Calan gates. Residual feed intake was calculated from weekly feed intake and BW data collected for 70 d. Following the feeding trial, heifers were allowed to graze coastal bermudagrass pasture and were exposed to a mature Brahman bull for natural breeding. Age at calving was recorded and age at sexual maturity was defined as 292 d before calving. Weight at sexual maturity was determined from BW and ADG data collected every 28 d. For statistical analysis, heifers were categorized as calm, intermediate or temperamental based on ± 0.5 standard deviation (SD) of both the mean EV and mean temperament score. RFI sign was used to categorize heifers as efficient (negative RFI) or inefficient (positive RFI). Heifers were also classified as efficient,

intermediate or inefficient based on ± 0.5 SD of the mean RFI. Using EV, temperament score, RFI sign, and RFI category as class variables, age and weight at sexual maturity were analyzed by PROC GLM. No differences ($P > 0.05$) were detected among these parameters comparing

all temperament and RFI classifications. These data suggest that selection for RFI or temperament should not affect age or weight at sexual maturity in Brahman heifers.

Key Words: heifer, residual feed intake, temperament