# Physiology and Endocrinology Symposium: Progesterone as an endocrine regulator of fertility in cattle

**323 The role of progesterone in uterine biology of ruminants.** Thomas E. Spencer*, *University of Missouri, Columbia, MO.*

This review integrates established and new information on the role of progesterone in uterine biology of ruminants. Establishment of pregnancy in ruminants occurs during the peri-implantation period and involves growth of the blastocyst, elongation of the conceptus (embryo and extraembryonic membranes), and suppression of the endometrial luteolytic mechanism to maintain progesterone production by the ovary. Conceptus elongation involves exponential increases in length and weight of the trophectoderm and onset of extraembryonic membrane differentiation, including gastrulation of the embryo and formation of the yolk sac and allantois that are vital for embryonic survival and formation of a functional placenta. Antiluteolytic effects of the elongating conceptus are due to production of interferon tau (IFNT) by the trophoblast that has a paracrine effect to inhibit upregulation of oxytocin receptors in the endometrial epithelia, thereby disrupting uterine release of luteolytic prostaglandin $F_{2\alpha}$ (PGF) pulses. Survival of the blastocyst and elongation of the conceptus requires embryotrophic factors (ions, amino acids, carbohydrates, proteins, lipids, and other substances) from the epithelia of the uterus, and those embryotrophic factors are primarily regulated by progesterone. Available results from studies in sheep support the idea that the individual, interactive, and coordinated actions of progesterone, IFNT, prostaglandins, and cortisol regulate expression of elongation- and implantation-related genes in the endometrial epithelia and are essential regulators of conceptus elongation. The outcome of these gene expression changes is alterations in endometrial epithelial secretions that govern conceptus elongation. Elevated concentrations of circulating progesterone immediately after conception have been associated with an advancement of conceptus elongation, an increase in IFNT production and, in some studies, higher pregnancy rates in cattle. An increased knowledge of progesterone biology and conceptus-endometrial interactions is necessary to understand and elucidate the causes of recurrent pregnancy loss and to provide a basis for new strategies to improve pregnancy outcome and reproductive efficiency in ruminants.

**Key Words:** uterus, conceptus, progesterone

**324 Cellular and molecular mechanisms controlling corpus luteum function and progesterone concentrations in cattle.** Milo Wiltbank*[1], Giovanni Baez[1], Julian Ochoa[1,2], Joao Ferreira[2], Eduardo Trevisol[2], Wenxiang Luo[1], and Roberto Sartori[3], *[1]University of Wisconsin-Madison, Madison, WI, [2]São Paulo State University, Botucatu, SP, Brazil, [3]University of São Paulo, Piracicaba, SP, Brazil.*

Steady-state, circulating progesterone (P4) concentrations are determined primarily by 2 factors: the rate of P4 production, principally by the corpus luteum (CL), and the rate of P4 metabolism, mainly by the liver. Rate of P4 production is primarily related to number of large luteal cells (LLC), differentiation state of LLC, and provision of substrate for P4 production. Steroid production increases 1000-fold from estrus to d 7 with growth of the follicular granulosa cells from 10 mm diameter to 38 mm LLC, a 50-fold increase in cellular volume. The LLC of ruminants are distinguished by a large number of mitochondria and high constitutive P4 production meaning that luteotrophic hormones are not required for P4 production. Nevertheless, cholesterol substrate, primarily in the form of high-density lipoprotein (HDL) is essential for high P4 production by ruminant LLC. Circulating HDL is not limiting in well-fed cattle but may be manipulated by diet. One limiting factor is number of granulosa cells in the follicle and therefore number of LLC. Ovulation of larger follicles or ovulation of multiple follicles can produce greater quantity of luteal tissue and therefore increased luteal P4 production. Regression of the CL is marked by decrease P4 production due to decreased transport of cholesterol to the inner mitochondrial membrane of the LLC. The rate-limiting step in cholesterol transport is StAR protein. Other steroidogenic enzymes remain active as P4 production by the CL plummets. Thus, luteal P4 production can be increased or decreased by hormonal, dietary, and management manipulations. Metabolic clearance rate (MCR) of P4 has been found to vary substantially between cows in different physiological conditions. For example, P4 MCR more than doubles in cows during high milk production as compared with similar size and age nonlactating cows. The P4 metabolism enzymes are concentrated in the liver. Thus, MCR of P4 and other steroids is primarily related to the rate of liver blood flow, which has been related to dry matter intake in dairy cattle. In conclusion, manipulation of P4 production and metabolism appear to be fertile areas for future research aimed at improving fertility in cattle.

**Key Words:** P4, corpus luteum, fertility

**325 Novel concepts on the role of prostaglandins on luteal maintenance and maternal recognition of pregnancy in ruminants.** Joe A. Arosh*[1], JeHoon Lee[1], Jone A. Stanley[1], John A. McCracken[2], and Sakhila K. Banu[1], *[1]Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, [2]Department of Animal Science, University of Connecticut, Storrs, CT.*

In ruminants, the corpus luteum (CL) of early pregnancy is resistant to luteolysis. $PGE_2$ is considered as a luteoprotective mediator. Early studies indicate that during the establishment of pregnancy in sheep, a factor(s) from the conceptus or gravid uterus reaches the ovary locally through the utero-ovarian plexus (UOP) and protects the CL from luteolysis. The local nature of the embryonic antiluteolytic or luteoprotective effect precludes any direct effect of a protein transported or acting between the gravid uterus and CL in ruminants. At the time of establishment of pregnancy, interferon tau (IFNT) secreted by the trophoblast of the conceptus inhibits endometrial pulsatile release of PGF2a and increases endometrial PGE2. Our recent studies indicate that (1) luteal PG biosynthesis is selectively directed toward PGF2a at the time of luteolysis and toward $PGE_2$ during establishment of pregnancy; (2) the ability of CL of early pregnancy to resist luteolysis is likely due to increased intraluteal biosynthesis of $PGE_2$ and signaling; (3) IFNT protein is not transported from the uterus to the CL through the UOP vascular route; and (4) a large proportion of endometrial $PGE_2$ is transported from the uterus to the CL through the UOP vascular route during the establishment of pregnancy in sheep. Our ongoing studies indicate that intrauterine co-administration of IFNT and PGES-1 inhibitor inhibits IFNT action and IFNT fails to rescue the CL in cyclic sheep. By contrast, intrauterine co-administration of IFNT and PGES-1 inhibitor along with intraovarian administration of PGE2 rescues the CL in cyclic sheep. These results suggest that IFNT may prolong the lifespan of the CL by increasing endometrial secretion of $PGE_2$ which in turn increases luteal $PGE_2$ biosynthesis and signaling and promotes luteal resistance. Together, our studies provide compelling evidence

that PGE2 produced by the CL in response to endometrial PGE2 may protect the CL, as a component of luteoprotective mechanisms, at the time of maternal recognition of pregnancy in ruminants. These novel findings may offer therapeutic strategies to target PGE2 biosynthesis and signaling selectively in the endometrium and/or CL to improve reproductive efficiency in ruminants.

**326 The role of progesterone receptor on control of ovulation and oviductal transport in mammals.** Rebecca Robker*, Darryl Russell, and Lisa Akison, *University of Adelaide, Adelaide, South Australia, Australia.*

Progesterone is critical for many aspects of female reproduction such as ovulation, oviductal transport, implantation, and maintenance of pregnancy. Its effects are mediated by the progesterone receptor (PGR), which occurs in both PGR-A and PGR-B isoforms, acting as nuclear hormone transcription factors. In the ovary, PGR is transiently expressed, specifically in granulosa cells of preovulatory follicles in response to the luteinizing hormone (LH) surge that triggers ovulation. To identify PGR regulated genes that are responsible for these key reproductive events we conducted microarray analyses of mRNA from granulosa cells and oviducts comparing progesterone receptor null mice (PRKOs) and heterozygous littermates. Microarray results confirmed that proteases Adamts1 and Cathepsin L are expressed at greatly reduced levels in PRKO granulosa cells, and identified novel gene products involved in cellular migration and invasion processes that are likely to mediate the dynamic tissue remodeling that facilitates ovulation. In the oviduct, PGR is expressed predominantly in luminal epithelial cells but also muscle cells. A large number of oviductal genes were also dysregulated in PRKO mouse oviducts during the peri-ovulatory period and we identified and subsequently validated several that have potential roles in cumulus-oocyte capture and transport following ovulation. The majority were genes associated with adhesion and muscular contractility including integrin α 8, endothelin 3, myocardin and angiotensin II receptor. Thus, the actions of PGR play key roles in coordinating the functions of multiple tissues, including the important peri-ovulatory events of oocyte release and acquisition of oocyte developmental competence, as well as subsequent oviductal transport of the newly formed embryo and immunological events at implantation.

**Key Words:** progesterone receptor, ovulation, oviduct

**327 Contrasting effects of progesterone on fertility of dairy and beef cows.** Jeffrey S. Stevenson*[1] and G. Cliff Lamb[2], *[1]Kansas State University, Manhattan, KS, [2]University of Florida, Marianna, FL.*

Role of progesterone in maintaining pregnancy is well known in the bovine. Subtle differences exist between dairy (milked) and beef (suckled) cows because of differing concentrations of progesterone during recrudescence of postpartum estrous cycles, rate of follicular growth and maturation, proportions of 2- and 3-follicular wave cycles, and other effects on pregnancy outcomes per AI (P/AI). Because proportions of anovulatory cows at the onset of the AI period are greater and more variable in beef (usually ranging from 30 to 70%) than dairy (25%) cows, AI programs were developed to accommodate anovulatory and cycling beef cows enrolled therein. Incorporating a progestin as part of an AI program in beef cows improved P/AI by reducing the proportion of cows having premature luteal regression and short post-AI luteal phases. Two- vs. 3-follicle wave cycles are more common in beef cows (86 vs. 14%) than in dairy cows (68 vs. 32%), respectively, even though P/AI may not differ in 2- vs. 3-wave beef cows (82 vs. 100%), but differed in dairy cows (63 vs. 81%). When dominant follicles matured in subluteal-phase progesterone concentrations compared with those matured in luteal-phase concentrations, P/AI increased in beef cows, but were reduced in dairy cows when the first dominant follicle (matured in sub-luteal milieu) was induced to ovulate compared with cows ovulating a second-wave dominant follicle. Further, supplementing progesterone during growth of the first-wave dominant follicle improved fertility in dairy cows. Initiating timed AI programs in dairy cows in a greater progesterone environment and during cycle d 5 through 12 enhanced fertility. In contrast, progesterone status in beef cows at the onset of synchronization does not seem to be related to P/AI in multiparous cows, whereas P/AI were suppressed in primiparous cows that began a timed AI program in a low-progesterone environment. Pregnancy losses after AI between 35 and 60 to 70 d are less than 5% in beef cows and are not associated with pre-AI progesterone or cycling status, whereas losses in dairy cows (6 to 20%) are inversely related to progesterone and adversely affected in anovular dairy cows.

**Key Words:** beef and dairy cattle, progesterone, fertility

**328 The influence of progesterone (P4) during follicle development on endometrial and conceptus biology and fertility in dairy cows.** Rafael S. Bisinotto*, Eduardo S. Ribeiro, Leandro F. Greco, Natalia Martinez, Leticia D. P. Sinedino, Fabio S. Lima, William W. Thatcher, and Jose E. P. Santos, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

The effects of P4 concentrations during follicle growth on fertility responses in dairy cows were evaluated. In study 1, pregnancy per artificial insemination (P/AI) increased ($P < 0.01$) in cows initiating the timed AI program in diestrus compared with anovular and cyclic cows without a corpus luteum (CL; 36.9, 25.1, 28.1%); however, pregnancy loss was smaller ($P = 0.08$) in cyclic cows without CL, followed by cows in diestrus, and anovular cows (10.0, 13.5, 15.0%). In study 2, concepti on d 15 after AI were longer ($P < 0.05$) in anovular compared with cyclic cows (47.8 ± 8.8 vs. 9.4 ± 5.8 mm). Concepti transcriptome analysis depicted 417 genes differentially expressed in response to cyclic status, mostly involved with embryonic development. In study 3, cyclic cows were induced to ovulate the dominant follicle from the first wave (FW; P4 = 1.2 ± 0.3 ng/mL), first wave supplemented with P4 (FWP4; P4 = 4.3 ± 0.3 ng/mL), or second wave (SW; P4 = 5.4 ± 0.2 ng/mL). Cows in FW had ($P < 0.05$) larger ovulatory follicles (19.6 ± 0.6, 15.6 ± 0.6, 15.2 ± 0.5 mm), greater estradiol concentrations during proestrus (8.0 ± 0.6, 7.0 ± 0.7, 5.9 ± 0.6 pg/mL), and a faster P4 rise after AI. Concepti on d 17 after AI were longer ($P = 0.05$) for FW compared with FWP4 and SW (16.6 ± 2.3, 9.8 ± 2.2, 9.6 ± 2.0 cm); however, major changes on transcriptome were not observed. Treatment did not affect any biological process in the endometrium on d 17 of gestation. In studies 4 and 5, a single ultrasound evaluation identified a low-fertility cohort based on the absence of CL at the initiation of the timed AI program. Increasing P4 concentrations to at least 2.0 ng/mL improved ($P \leq 0.01$) P/AI in cows lacking CL similar to those in diestrus in the 5-d timed AI (No CL = 28.6, 2CIDR = 43.7, Diestrus = 47.3%) and the Ovsynch protocols (No CL = 28.9, 2CIDR = 37.2, Diestrus = 33.9%). Low concentrations of P4 during the development of the ovulatory follicle impair fertility and a minimum of 2.0 ng/mL seems to be needed to restore P/AI. Lack of differences on conceptus and endometrium on d 17 suggest that effects occur early in gestation.

**Key Words:** anovulation, embryo, progesterone

**329 Effect of manipulating progesterone before timed artificial insemination on reproductive and endocrine parameters in Irish Holstein-Friesian dairy cows.** Paul M. Fricke*[1], Paulo D. Carvalho[1], Matthew C. Lucy[2], Francis Curran[3], Mary M. Herlihy[3], and Stephen T. Butler[3], *[1]University of Wisconsin - Madison, Madison, WI, [2]University of Missouri, Columbia, MO, [3]Teagasc Moorepark-Animal & Grassland Research and Innovation Centre, Fermoy, Ireland.*

Irish Holstein-Friesian dairy cows managed in a seasonal calving, grazing-based dairy system were randomly assigned to 2 treatments to manipulate progesterone (P4) before timed AI (TAI). Cows in the first treatment (High P4; n = 30) were submitted to a Double Ovsynch protocol [Pre-Ovsynch protocol (GnRH; 7 d, $PGF_{2\alpha}$; 3 d, GnRH) followed 7 d later by an Ovsynch-56 protocol (GnRH (G1); 7 d $PGF_{2\alpha}$ (PGF); 24 h, $PGF_{2\alpha}$; 32 h, GnRH (G2); 16 h, TAI)]. Cows in the second treatment (n = 30; Low P4) received the same protocol with an additional PGF injection 24 h after G1. Data were analyzed using PROC MIXED and PROC GLIMMIX of SAS. Overall, synchronization rate was 92% (55/60) and did not differ between treatments. As expected, High P4 cows had more ($P < 0.001$) CL at PGF (1.93 ± 0.09 vs. 1.04 ± 0.04), and P4 (ng/mL) did not differ between treatments at G1 (3.8 ± 0.3 vs. 4.1 ± 0.4). Circulating P4 was greater ($P < 0.001$) for High vs. Low P4 cows at PGF (8.5 ± 0.9 vs. 1.6 ± 0.4) and G2 (0.30 ± 0.04 vs. 0.08 ± 0.02). Although diameter (mm) of the preovulatory follicle was greater ($P < 0.001$) for Low vs. High P4 cows at G2 (17.1 ± 0.4 vs. 15.6 ± 0.4), neither CL diameter nor volume differed between treatments 15 d after TAI. The proportion of pregnant cows 39 and 60 d after TAI did not differ between treatments (63%; 17/27 vs. 61%; 17/28 for Low P4 vs. High P4 cows), and no pregnancy loss occurred. Both treatment ($P = 0.006$) and time ($P < 0.001$) affected P4 concentrations in pregnant cows from 1 to 39 d after TAI with Low P4 cows having greater P4 than High P4 cows. Treatment did not affect plasma pregnancy-associated glycoprotein (PAG) concentrations for cows diagnosed pregnant 39 d after TAI; however, pregnant primiparous cows had greater ($P = 0.02$) PAGs from 20 to 39 d after TAI than pregnant multiparous cows. We conclude that although low P4 before TAI increased follicle growth before TAI and P4 concentrations in pregnant cows after TAI compared with high P4, low P4 before TAI did not negatively affect fertility, pregnancy loss, or PAGs after TAI in Irish Holstein-Friesian dairy cows.

**Key Words:** progesterone, timed AI, dairy cow