ABSTRACTS

ISCFR 2012

July 26-29, Whistler, Canada

7th International Symposium on Canine and Feline Reproduction

In a joint meeting with

EVSSAR 2012

15th Congress of the

European Veterinary Society for Small Animal Reproduction

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Endocrine aspects of ovarian cycles, ovarian cyclicity and endogenous circannual cycles in dogs.

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Endocrine profiles during canine ovarian cycles have been extensively reported elsewhere for of estrogen (E), progesterone (P), 17a-OH P, testosterone (T), a'dione, immunoreactive LH and FSH, and prolactin, (PRL), as well as relaxin (RLX) in pregnancy. (1).

LH Surge and Onset of Estrus. The preovulatory (PO) LH surge and onset of estrus in dogs is initiated by the decline in the estrogen: progestogen ratio that follows the proestrus estrogen surge, based on observed temporal changes in endocrine profiles, experimental manipulation of estradiol and progesterone in ovariectomized animals, and computational models. What are unclear are (1) why does E peak at the end of proestrus and what factors contribute other than spontaneous follicle maturation; (2) whether factors controlling estrogen availability of the free E fraction at the time of the peak, including hepatic clearance and SHBG levels, change dynamically in late pro estrus; (3) whether as a consequence of follicle maturation the E peak and decline correspond to the rise and fall of E seen in induced ovulators like the cat in the absence of an LH surge and the onset of progressive atresia; (4) if the correspondence of LH surge and estrus-onset with the rapid fall in the E:P differs in cause or function from the equivalent correspondence in other species. The phrase "estrogen: progestogen" ratio is used preferentially in that catechol estrogens may play a role centrally and 17-OH-P, a ligand of neural membrane P receptors, undergoes a more rapid rise than Pat or preceding the LH surge (Concannon, 2011).

Follicular Phase and Proestrus Estrogen Peak. The common endocrine element of carnivore cycles is the estrogen peak. LH surge spontaneously in a few species. The E surge is the endocrine hallmark of ovarian cycles reflecting waxing-waning secretory capacity of ovulation-capable pro-estrus follicles, modified by availability of precursor androgen and metabolism and clearance by uterus, liver, and kidneys. Rising E elicits proestrus, and the declining phase is concomitant with, and likely the neuroendocrine effector of, estrus receptivity and, in spontaneous ovulators like the dog, surge release of GnRH, LH and FSH. The E:P is important at least in that in that P is an "anti-estrogenic". The transiency of the E surge presumably represents terminal events of normal follicle development, growth, selection and maturation to ovulatory-capacity, with an associated shift in steroidogenic type and capacity. The path to atresia is interrupted only by a gonadotrophic hormone insult. Observations on cats suggest that absent a spontaneous LH surge follicles are still capable of induced ovulation for 2-3 days of more of declining E. Associated morphologic and apoptotic changes are not well described for dogs or other carnivores.

Estrogen Secretion vs. Availability. The role of peripheral metabolism is also significant in producing the peak in E. Administration of increasing doses of E fail to result in continued proportional increases in plasma E because of increased clearance. The liver likely predominates in this phenomenon with liver microsomes and hydroxylation enzymes induced by increased E. Thus, plasma E likely increases more slowly and reaches a peak earlier and declines more rapidly than the actual rates of estradiol secretion. Peripheral and hepatic clearance of P is also likely increased in late proestrus .The initially slow and then rapid increases in P concentrations, before and during the LH surge respectively, may only partially reflect a more rapid increase in secretion.

E and P Profiles. Reported profiles presumably reflect a large part of the endocrine signalling during the cycle. Direct vs. indirect assays measure total vs. free E, or free plus some albumen-bound E, respectively. Complexities and implications of measuring sex steroids by kits and automated RIA or CIA assay have been reviewed. Results often do not translate well among labs. Most assays measure total E, although only a small fraction is bioactive. Factors that affect hepatic SHBG production may play a role in availability bioactive E available at different times during the cycle, especially the periovulatory period. (Matsumoto and Bremner 2004 JCEM 89, 520. Couke et al, 2007, Hum Reprod 22, 3204). In the absence of rapid and cheap mass spectrometry analyses, hormone profiles of total steroid by RIA or CIA still remain our best window into likely changes in availability of E and P to target tissues. Our assumption that free hormone is always available in excess , as in the case of P throughout most of pregnancy, may not be a valid assumption with the picogram levels of E, P and 17-hydroxy-P in the peri-ovulatory period.

Absolute Hormone Concentrations. Concentrations of estradiol measured in plasma presumably represent total with direct (no extraction) or free when measured in indirect (sample extraction) assays. Extraction efficiency can differ with method, animal status and binding protein levels. With E assay, as with P, results between laboratories are typically nor comparable, and results within animals may not accurately reflect changes in bioavailability. Interestingly, high levels of E increase SHBG and thus self-regulate the amount of bioavailable E exposure to neuroendocrine sites sensitive to rapid changes in E including GnRH and LH secreting cells and centers regulating behavior. While total T along with a fee-androgen index is often used in males, concern for distinction among free and total E other than the use of direct versus indirect assays seems to be minimal in most animal and human studies. Explicably, then, the precise concentrations of P at specific times relative to ovulation, perhaps important clinically, have varied significantly among studies, possibly due to differences in assays, dog breeds, blood sample collection, timing and handling protocols, and perhaps even in the accuracy of hormone concentrations in assay standard curves. The most reliable and repeatable method of timing and comparing results between studies in the absence of frequent sample LH determinations remains the detection of the day of the rapid increase in P from

baseline (0.2 -0.8 ng/ml) to substantially higher concentrations (0.9- 4.0 ng/ml) as the estimated day of the PO LH surge with ovulation assumed to occur 48-60 hr. later.

Luteal Phase. Theca versus granulosa cell contributions during CL formation varies among species; and even observations within species have yielded different conclusions on CL cellular origin. Preliminary histological study in dogs suggested a primarily theca origin for luteal cells all perhaps of a single type not unlike in some rodents (Concannon, unpublished). A rigorous examination of the question has not been reported for any carnivore. Canine luteal autonomy is negligible. A rapidly growing day 10 dog CL loses 90 % of its P output capacity within days of hypophysectomy. LH and prolactin are both luteotrophic in dogs and most likely all carnivores. LH, and/or an LH/FSH complex, is almost universally found to be a major if not only luteotropic requirement in mammals. The effects of LH on luteal cells are myriad and vary somewhat among species. LH is a required luteotrophin in rodents, primates Artiodactyls, and dogs and likely other carnivores by extension, regardless of when evolution divergence led to carnivores. In dogs, P declined in response to anti-LH serum; suppression of LH and FSH by GnRH-agonist reduces luteal function; and administration of GnRH-antagonist can terminate pregnancy. Prolactin functioning as a luteotrotphic as well as a mammotrophic hormone appears nearly universal in rodents and carnivores but not artiodactyla and primates, suggesting a genetic adaptation occurring at the time of the divergence of other groups from the rodent-carnivore groups. PRL appears a critical luteotropin as regards ensuring maintenance of luteal function throughout gestation. In dogs sensitivity to luteolytic effects of prolactin suppression or PGF insult increased from day 25 onward. Absent a uterine lutrolysis, luteal phases in carnivores are more like those of hysterectomized artiodactyls, i.e., with a natural lifespan being a significant proportion of a normal pregnancy.

Ovarian Cycle Length and Timing. Factors controlling both these aspects of ovarian cyclicity in domestic dogs are elusive. Short luteal phases do not always result in short cycles. Some litter mates can cycle synchronously within days of each other for years, others almost never. Length ranges from 100 to 380 days albeit average ca. 220 days in most breeds. Intervals in autumnal-breeding Basenji (and perhaps dingo dogs) are annual and apparently under the control of photoperiod. Great variation in cycle length in dogs is often stated as universally applicable, making reproduction in dogs perhaps different in character than in most. However, for some bitches housed together, the inter-estrus interval can be not only regular within bitches but synchronized among bitches including unrelated bitches within a kennel of colony. Our conclusion from this observation is that the timing of ovarian cycles in most breeds of domestic dogs represents an aspect of an endogenous circannual cycle unresponsive to photoperiod in all but the most ancestral breeds. And thus, not unlike reproductive cyclicity in sheep, horses and many rodents, is part of endogenous circannual cycles that free run at intervals of 6-10 months in the absence photoperiod control.

Endogenous Circannual Cycles. Evidence of a sort supporting the phenomenon of non-photo-responsive endogenous circannual cycles as underlying ovarian cycles in dogs is provided by the occurrence of photoperiod sensitive annual ovarian cycles in Basenji dogs, one of the breeds of dogs genetically closest to the wolf ancestors of domestic dogs, as well as in wolves and other wild canids. Annual cycles in wild canids and apparently Basenji, where studied, as in most seasonal breeders, adapt to the photoperiod of the non-native temperate zone when translocated. The basenji might therefore, in comparison to other breeds of dogs, provide clues as to the genes involved in photo-responsiveness of circannual cycles, presumably present in that breed but not the majority of dog breeds.

The sometimes observed lack of variability in ovarian cycles among bitches is likely a phenomenon dependent on factors such as common genetics, perhaps especially when observed in litter mates, and on pheromones. Such a pheromone influence has been anecdotally documented by the practice in large breeding colonies of relocating mid anestrus bitches to the proximity of females in proestrus or estrus and finding the subsequent proestrus to be clearly advance from the predicted onset based on prior cycles or colony averages. Prospective study of the cycle synchronization due to pheromonal influence appears not to be reported.

Endogenous circannual cycles in many species are best if not only reflected in reproductive activity and reproductive hormone secretion patterns. In some species, concomitant endocrinologic or biologic changes are clearly expressed, including often dramatic cycles in one or more of the following; PRL, hair coat, molting leptin, food intake, fT4, TT4, TBG, liver function, (e.g., Concannon et al 2001, AJP- Regu Physiol 281, R951-R959). It would be interesting to see if such cycles occur annually in Basenji and wolves and whether or not they can be documented in cycles of 6-11 month durations within individualdogs of breeds that are not photo-responsive. They may interact with other factors affects timing of canine cycles including length of luteal phase P section, intensity and duration of lactation, and pheromone exposure. One study in male dogs (2) found circannual patterns of PRL unrelated to the calendar yr and varying in length from 6 to 11 months. Endogenous PRL rhythms in dogs would likely reflects endogenous rhythms in dopamine or serotonin availability or sensitivity that even independent of PRL might affect the timing of transitions from anestrus to proestrus in individual cycles . Interestingly, multiyear reproductive hormone patterns in groups of dogs are remarkably similar to reproductive hormone patterns in free-running sheep and other annual breeders if deprived of normal photoperiod cues.

(1) Concannon, P. 2011, Reproductive cycles of the domestic bitch... Anim. Reprod Sci. 124, 200-10.
(2) Verstegen, J., Onclin, K., Lauwers, F., Concannon, P., 2008. Potential role of prolactin in patterns of reproductive activity in dogs: a male model. In: Abstracts, International Symposium Canine Feline Reproduction VI, July 2008, Vienna, Austria.

Additional References

Concannon, PW, 2013. Research challenges in endocrine aspects of canine ovarian cycles. Reprod. Domest. Anim. (In Press)

Arreguin-Arevalo JA, Ashley RL, Wagenmaker ER, Oakley AE, Karsch FJ, 3 and Nett, TM, 2012: Membrane-initiated actions of estradiol (E2) in the regulation of LH secretion in ovariectomized (OVX) ewes. Reproductive Biology and Endocrinology 8, 40 (doi:10.1186/1477-7827-8-40 url: http://www.rbej.com/content/8/1/40)

Christian, CA and Moenter, SM, 2010 The Neurobiology of Preovulatory and Estradiol-Induced Gonadotropin-Releasing Hormone Surges Endocrine Reviews 31, 544–577

Concannon, P.W , Cowan, R.G and Hansel, W, 1979: LH release in ovariectomized dogs in response to estrogen withdrawal and its facilitation by progesterone. Biol Reprod 20, 523 531.

Concannon, PW, 1993: Biology of gonadotropin secretion in adult and prepubertal female dogs. J Reprod Fert Suppl 47, 3-27

Concannon, PW, 2009: Endocrinologic control of normal canine ovarian function. Reprod Domest Anim 44 Suppl 2, 3-15.

Concannon PW, 2011: Reproductive cycles of the domestic bitch.. Anim Reprod Sci 124 , 200-10

Concannon, PW, Castracane, VD, Temple, M, Montanez, A, 2009: Endocrine control of ovarian function in dogs and other carnivores. Anim Reprod 6 , 172-193.

Concannon P, Levac K, Rawson R, Tennant B, Bensadoun A, 2001: Seasonal changes in serum leptin, food intake, and body weight in photoentrained woodchucks. Am J Physiol Regul Integr Comp Physiol. 281, R951-9.

Concannon, P W , Parks, J E , Roberts, P J , and Tennant, B C, 1992: Persistent free-running circannual reproductive cycles during prolonged exposure to a constant 12L:12D photoperiod in laboratory woodchucks, Marmota monax. Lab Animal Sci 42, 382-391.

Concannon, P W and Temple, M, 1988: Periovulatory decline and late anestrus increase in naloxone-inducible LH release in adult domestic bitches. Biol Reprod 36 Suppl 1, 101.

Coucke W, Devleeschouwer N, Libeer JC, Schiettecatte J, Martin M, Smitz J, 2007: Accuracy and reproducibility of automated estradiol-17beta and progesterone assays using native serum samples: results obtained in the Belgian external assessment scheme. Hum Reprod 22, 3204-3209.

Fahiminiya S, Reynaud, K, Labas, V, Batard, S, Chastant-Maillard, S and Gérard, N, 2010: Steroid hormones content and proteomic analysis of canine follicular fluid during the preovulatory period. Reproductive Biology and Endocrinology 8, 132 (doi:10.1186/1477-7827-8-132; URL http://www.rbej.com/content/8/1/132)

Goodwin M, Gooding KM, Regnier F , 1979: Sex pheromone in the dog. Science 9, 203 (4380), 559-61.

Hale, PA, 1982: Periodic hair shedding by a normal bitch. Journal of Small Animal Practice 2, 345–350,

Hammes, SR, 2004: Steroids and Oocyte Maturation—A New Look at an Old Story. Molec Endocrin 18, 769-775

Inaba T, Namura T, Tani H, Matsuyama S, Torii R, Kawate N, Tamada H, Hatoya S, Kumagai D, Sugiura K, Sawada T, 2002: Enhancement of aromatase gene expression in the mediobasal hypothalamus during anestrus in the beagle bitch. Neurosci Lett 333, 107-110.

Kawashima I, Okazaki, T, Noma, N, Nishibori, M, Yamashita, Y, and Shimada M, 2008: Sequential exposure of porcine cumulus cells to FSH and/or LH is critical for appropriate expression of steroidogenic and ovulation-related genes that impact oocyte maturation in vivo and in vitro. Reproduction 136 9-21

Martinet L, Mondain-Monval M, Monnerie R, 1992: Endogenous circannual rhythms and photorefractoriness of testis activity, moult and prolactin concentrations in mink (Mustela vison). J Reprod Fertil 95, 325-338.

Matsumoto AM, Bremner WJ, 2004: Serum testosterone assays--accuracy matters. JCEM 89, 520-524.

McBride MW, Aughey E, O'Shaughnessy PJ, Jeffcoate IA, 2001: Ovarian function and FSH receptor characteristics during canine anoestrus. Reprod Fertil Suppl 57, 3-10.

Radovick, S, Levine J, Wolfe A, 2012: Estrogenic regulation of the GnRH neuron Frontiers in Endocrinology 3, 00052. URL = http://www.frontiersin.org/Journal/Abstract.aspx?s=473&name=genomic_endocrinology&ART_DOI=10.3389/fendo.2012.00052

Robker RL, Akison LK and Russell DL, 2009: Control of oocyte release by progesterone receptor-regulated gene expression. Nuclear Receptor Signaling 7, e012.

Rosner, W, Hryba, DJ, Kahn, SM, Nakhla, AM, and Romas, NA, 2010: Interactions of sex hormone-binding globulin with target cells. Molecular and Cellular Endocrinology 316, 79–85

Stormshak, F, Estill CT, Resko JA, and Roselli CE, 2008: Changes in LH secretion in response to an estradiol challenge in male- and female-oriented rams and in ewes. Reproduction 135, 733-738

Tortonese DJ, 1999: Interaction between hypothalamic dopaminergic and opioidergic systems in the photoperiodic regulation of pulsatile luteinizing hormone secretion in sheep. Endocrinology 140, 750-757.

van Haaften B, Bevers MM, van den Brom WE, Okkens AC, van Sluijs FJ, Willemse AH, Dieleman SJ, 1994: Increasing sensitivity of the pituitary to GnRH from early to late anoestrus in the beagle bitch. J Reprod Fertil 101:221-225.

Vickery BH, McRae GI, Goodpasture JC, Sanders LM, 1989: Use of potent LHRH analogues for chronic contraception and pregnancy termination in dogs. J Reprod Fertil Suppl 39, 175-187.