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30th Annual Canine Symposium

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Penn's 30th Canine Symposium on January 29, 2000 was sold out. The theme, Canine Reproduction, generated a great deal of interest and we had to turn a number of people away. Following are summaries of the presentations.

Reproductive Problems in Female Dogs

Apparent infertility in the bitch may actually be a manifestation of underlying problems. Dr. Margret Casal, assistant professor of medical genetics at the School, discussed reproductive problems in the bitch that impair fertility or otherwise hamper successful matings.

The number one reason for matings to be unsuccessful is that the bitch was bred on the wrong day of her cycle. On the other hand, some bitches fail to get pregnant because they are altogether acyclic. Acyclic bitches either have not experienced their first heat for reasons like delayed puberty or breed idiosyncracies (large-breed dogs, for example, tend to begin cycling at a later age than do small-breed dogs), or they have ceased their heat cycles due to disease such as ovarian cysts or tumors.

Other disturbances in the heat cycle can occur, said Dr. Casal. These include prolonged proestrus, prolonged estrus and split estrus. Bitches in prolonged proestrus often must be "pushed into ovulating" using hormone therapy. Prolonged estrus phases—which last more than 12 days—are sometimes anovulatory. Hormone therapy to treat prolonged estrus may result in marked side effects. Split estrus is characterized by a normal proestrus and the beginning of an apparently normal estrus in which the female initially allows mounting, then refuses, and then allows it again. Vaginal cytology often reveals that the bitch was not in true estrus until the second standing heat. These cycle anomalies cause difficulty in timing breeding.

Infections can also lead to the *appearance* of infertility in the bitch or the stud dog. Infections with *Brucella canis* or canine herpesvirus can cause early abortions. These aborted pregnancies, which often go unnoticed, may be mistaken for barren cycles. *Mycoplasma* has also been implicated as a cause for infertility. *E. coli*, *Staphylococcus* and *Streptococcus* are often associated with pyometra, another cause for infertility. Most bacterial infections are diagnosed by culturing vaginal secretions; a diagnosis of brucellosis or herpes infection is made by blood culture and/or by lymph node biopsies.

Other frequent medical barriers to reproduction in dogs include hypothyroidism, obesity, malnutrition and congenital defects. Vaginal hyperplasia and malformations may lead to reluctance to be bred, which gives the semblance of infertility. Abnormalities of sexual differentiation—in either chromosomal sex, gonadal sex and phenotypic sex—may also impair fertility. Many of these are breed related, such as pseudohermaphrodism in the miniature schnauzer.

Reproductive Problems in Male Dogs

Male infertility typically stems from primary defects in the sperm cells, which can result from a number of disease processes. Dr. Cynthia Ward, assistant professor of medicine at the School, reviewed semen evaluation procedures in dogs and discussed diseases of the prostate that can impair fertility.

"All infertility exams should involve semen collection and evaluation," said Dr. Ward.

Semen samples can readily be obtained from experienced stud dogs. Three semen fractions are present: clear pre-sperm fluid, sperm-rich fluid, and prostatic secretions. The sperm-rich fraction should be evaluated for sperm count, motility, morphology and cytology. Sperm motility should be examined promptly, as it decreases as the semen cools. The percentage of sperm swimming rapidly forward should be estimated from at least four different places on the slide, and the presence of sperm agglutination—which is associated with such causes of infertility as Brucella and anti-sperm antibody—noted. Normal dogs should have at least 200 million sperm per ejaculate. If consistently poor sperm motility is observed, infectious and/or inflammatory causes should be considered.

Sperm morphology is evaluated under staining. Generally, at least 75 percent of the sperm cells should be morphologically normal. The presence of white blood cells—especially degenerated ones, or red blood cells, may signal infection and/or inflammation. If infection is suspected, a semen sample should be cultured for aerobic bacteria.

The third semen fraction, which consists mainly of prostatic secretions, should be examined cytologically, said Dr. Ward. The presence of large numbers of bacteria , or red/white blood cells, may signal infection and/or inflammation.

"I and others believe there's a lot of subclinical infertility caused by chronic prostatic disease," she explained. "Maybe we should be looking at the prostate more and more carefully when we're trying to diagnose some types of infertility in male dogs."

Prostatitis and prostatic abscesses can cause infertility in dogs. Both conditions are infectious in etiology. *E. coli* is the most common organism isolated, although others, such as *Brucella canis*, have also been found. Approximately ten percent of affected dogs have concurrent urinary tract infections. A dog with acute prostatitis is often systemically ill. A stilted gait, caudal abdominal pain and urethral discharge may be present. Ejaculation is painful, and these dogs may be reluctant to breed. Prostatic infection may spread hematogenously to other organs and, in chronic cases, may also cause infertility because sperm do not thrive in the presence of inflammatory mediators. Diagnosis is made by hematology, urinalysis, urine culture, or culture and cytology of semen or prostatic fluid. Prostatic infections are treated with antibiotics for four to six weeks, and cas-

tration is indicated in refractory cases.

Another common prostatic disease is benign prostatic hyperplasia (BPH), an age-related change occurring in people and dogs. BPH is a benign increase in the number and size of prostatic epithelial cells. Although BPH occurs in every aging dog, they don't all manifest the clinical signs of urethral discharge, hematuria, and tenesmus. BPH is diagnosed by clinical signs, physical exam, radiographs or ultrasound, hematology, urinalysis and prostatic fluid analysis. Castration is the treatment of choice for BPH, although the drug finasteride, an androgen antagonist, can be used to help decrease the proliferation of prostatic cells.

A far more serious affliction than BPH, prostatic neoplasia accounts for about five percent of all prostatic diseases. Occurring with equal frequency in intact and neutered males, prostatic neoplasia most commonly takes the form of adenocarcinoma, followed by transitional cell carcinoma. Prostatic neoplasia carry grave prognoses.

Advantages and Disadvantages of Using Fresh-Chilled or Frozen Semen

Creating the "choice" pup today is possible without ever having the "choice" parents in each other's presence. Dr. Melissa Goodman, a reproduction specialist who maintains a specialty veterinary practice at the Veterinary Referral Center in Frazer, Pennsylvania, presented the pros and cons of remote reproduction via frozen or chilled semen shipments.

By employing sophisticated technology available for obtaining, processing and using chilled or frozen semen, one can greatly increase the gene pool available for matings. These reproductive methods also enable breeders and owners to achieve pregnancies without shipping the bitch or stud dog or hosting the bitch. Yet the added convenience and versatility of these technologies comes with tradeoffs, explained Dr. Goodman.

While the use of chilled semen eliminate limitations of geography, it introduces limitations of time: Although semen extenders provide the cells with a nutrient source and protection during transport, the life span of the sperm is greatly reduced, necessitating that the stud be available at the time the bitch is ovulating.

Furthermore, said Dr. Goodman, "Whenever we process sperm—chilling, shipping, freezing and thawing—we are damaging it. So, by definition, we will always decrease fertility whenever we use these procedures."

To obtain a high-quality ejaculate, the semen collection process must be performed with care and foresight. Since, at best, ten percent of the ejaculate is lost during collection, processing and shipping, Dr. Goodman recommends that only dogs with normal ejaculates (normal sperm count and motility) be used. The quantity and quality of semen produced in an ejaculate are affected by the dog's age, size, breed and general health status. Some medications and recent ejaculation may also affect semen quality. A period of sexual rest of 10-14 days is suggested before ejaculation to maximize the sperm count. Libido, which also affects the quality of the ejaculate, can be heightened by an experienced collector and the presence of a teaser bitch.

Following collection, the ejaculate—which contains three fractions—should be separated, and only the sperm-rich fraction used.

The use of *chilled* semen requires skillful choreography. Because the sperm begins to degrade immediately after ejaculation, insemination should be performed within 24 hours of collection. Therefore, the schedules of the stud owner, bitch owner, collecting veterinarian and inseminating veterinarian must be coordinated, and shipping arrangements must be made in advance.

Accurate ovulation timing is crucial to the success of chilled semen breeding. The stress of chilling and shipping reduces the life span of the sperm cells from about 5-7 days to just 2-4 days. Inseminations must be performed during the bitch's 72-hour fertile period. The most accurate indicator of ovulation and the fertile period is the luteinizing hormone (LH) surge, which is best identified by a combination of blood assays for LH and progesterone, as well as vaginal cytology and vaginoscopy. Dr. Goodman advocates two inseminations, usually on days four and six post-LH surge. The average conception rate for chilled, extended semen is approximately 80 percent.

Unlike chilled semen, frozen semen allows the genetic potential of a stud dog to be preserved for future use, in the event of disease, death, unexpected sterility or sale of the dog. Freezing the semen also facilitates long-distance and international breedings, and is useful when the stud is not available during the bitch's fertile periods. When properly processed, frozen, and stored, canine semen will last an estimated 10,000+ years. The sample is extended with a buffer solution that protects the sperm cells during freezing and thawing. The sample is then frozen either in straws over liquid nitrogen or in pellets in dry ice. Storage is done in liquid nitrogen.

For an insemination using frozen semen, only a bitch with good fertility should be used, since the sperm quality will be compromised. Approximately 100 million progressively motile sperm are needed in order to achieve a normal pregnancy. Therefore, post-thaw sperm count and quality should be assessed in order to determine the number of straws to use.

Precise ovulation timing is essential, since the stress of freezing and thawing damages the sperm membranes, shortening the life span of the thawed sperm cells to 12-24 hours. Daily blood testing is recommended to identify the LH surge, enabling insemination during the bitch's short fertile period. If a frozen insemination is performed even a few hours too early, said Dr. Goodman, "you can have high-quality frozen semen and a very fertile bitch, but the two will pass like ships in the night."

Once thawed, the semen can be deposited into the uterus via a catheter passed through the cervix. The most common, and perhaps most successful, method, is a quick surgical technique that allows direct deposition of the

semen at the ends of the uterine horns, just adjacent to the oviducts.

Managing the Breeding

Most dogs that fail to conceive have normal fertility status. Dr. Matthew Ellinwood, resident in medical genetics at the School, revealed the real reason behind unsuccessful matings and outlined steps for enhancing breeding success.

In cases of apparent infertility, the matings have not been accurately synchronized with ovulation. "The biggest problem is not the dogs having a primary infertility," said Dr. Ellinwood. "It's that they haven't been managed properly."

Planning successful breedings requires a good understanding of the estrus cycle. The bitch experiences her first estrus between six and 18 months of age, with size and breed variations. The estrus cycle is divided into four phases that are differentiated by cytologic, hormonal and behavioral features.

The first phase, proestrus, is characterized by bloody vaginal discharge. During proestrus—which lasts one week, on average—estrogen becomes the dominant hormone, the uterine lining begins to proliferate, and the vaginal epithelium starts to cornify. The next phase, estrus, spans from the point at which the bitch willingly stands for mating to the point at which she refuses to be bred. Estrogen levels fall and progesterone rises, the vaginal epithelium further cornifies, and ovulation occurs. During estrus, which lasts about a week, the bitch experiences behavioral changes that culminate in "standing heat," or submission to breeding. Diestrus follows, lasting 57 days in the pregnant bitch and slightly longer in the nonpregnant bitch. During diestrus, the bitch refuses to be bred. Progesterone dominates and the vaginal cytology changes to less than 50 percent cornified cells. Ensuing anestrus—which lasts five months, on average—is the period from the end of the progesterone elevation to the beginning of the next cycle.

In preparing for breeding, one should have physical exams—including reproductive exams—performed on both the bitch and the stud dog. Routine lab work, including a complete blood count, a serum biochemistry panel and a urinalysis, should be done on the breeding pair, particularly the bitch. A *Brucella canis* titer should be obtained on both dogs. And if either dog has a history of infertility, vaginal and preputial cultures should be performed.

Accurate timing of breeding is perhaps the greatest breeding success factor. In order to detect proestrus, the bitch's vulva should be examined once weekly beginning four-and-a-half months after the beginning of her last cycle. After the bitch has begun to show vaginal discharge, signaling proestrus, vaginal cytology should be done every three to four days until she has 90 percent cornified cells (estrus). Once the bitch has reached standing estrus, breeding should be allowed every two days for two to three breedings. After this, breeding frequency can be decreased to once every three or four days until the bitch refuses to be mounted.

While the behavior of the bitch and, in some cases, the stud are reliable indicators of estrus, a bitch will occasionally refuse to stand for breeding during estrus under any circumstances. In such cases, artificial insemination should be considered.

In conclusion, Dr. Ellinwood recommended that owners and breeders keep thorough records of previous cycles in order to better predict breeding variables and plan matings. In timing breeding, these records should be considered in tandem with vaginal cytology and behavioral cues in the bitch and the stud.

Whelping the Litter

The complexity of the birthing process is manifest in the post-whelping period, during which a variety of complications may arise in the dam. Dr. Margret Casal, assistant professor of medical genetics at the School, discussed these disorders and their clinical signs, diagnosis and treatment.

Following whelping, uterine involution is complete four weeks postpartum, but the uterus does not return to its anestrus histologic state until about 150 days postpartum. A bloody discharge is passed for up to 10 days to two weeks after whelping.

Persistent heavy bleeding after whelping may indicate rupture of the blood vessels within the uterus or the birth canal. The most common reasons for uterine bleeding are overdoses of oxytocin and fetal extraction using surgical instruments. Purely vaginal bloody discharge is typically caused by trauma to the birth canal. Hemorrhage, which usually begins several days after birth, usually occurs in the morning when the bitch arises. Bleeding from damaged vessels in the birth canal can be controlled with tamponage or, in the case of larger vessels, surgical repair. Uterine hemorrhage is usually controlled with pregnancy hormones. If blood loss is extensive, transfusion may be necessary.

Metritis—inflammation of the uterus—may also occur postpartum. Metritis is the result of bacterial invasion into the uterus secondary to abortion, fetal infection, manipulation during whelping, placental retention or ascending infection. Bitches with metritis typically neglect their pups. Diagnosis is made by ultrasound and vaginal smears. If not treated promptly, metritis can lead to more serious conditions, such as pyometra and sepsis. Treatment includes antibiotics, oxytocin and prostaglandins.

In a bitch with retained placenta, the discharge may also contain necrotic particles. Clinical signs are fever and persistent greenish-black, watery, foul-smelling discharge. Diagnosis is made by digital palpation, vaginoscopy or ultrasound. Placental retention is treated with oxytocin and prostaglandins.

Uterine prolapse—protrusion of parts or all of the uterus through the vulva, occurs typically after the birth of the last pup. Rapid repositioning of the uterus is critical to preventing tissue necrosis, an indication for immediate spaying.

Subinvolution of placental sites (SIPS), a condition wherein the sites of placental attachment do not revert to their normal, nonpregnant state, occurs occasionally in bitches younger than 2.5 years of age. Affected bitches present

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with persistent, serosanguinous, odorless vaginal discharge. Diagnosis is made by ultrasound. Subinvolution sites normally heal on their own; however, if necrosis becomes severe, the uterus can rupture, requiring immediate surgery.

A common finding in bitches producing copious amounts of milk, eclampsia ensues when heavy lactation depletes the bitch's calcium reserve. Eclampsia is seen at the height of lactation (2-3 weeks after whelping), and occurs more commonly in small- than large-breed dogs. Clinical signs include restlessness, muscle tremors, increased respiratory rate and, eventually, stiff gait, high fever (>104F) and convulsions. Therapy consists of intravenous calcium and if needed sodium bicarbonate and glucose. Following initial treatment, the bitch is sent home with calcium and vitamin D3 to prevent further occurrences.

Mastitis is a condition in which one or more mammary gland are enlarged, hot and red due to bacterial invasion by *E. coli, Staphylococcus* or *Streptococcus*. The affected bitch is typically febrile, anorexic and neglects her pups. The milk may be rancid. Pups should be prevented from suckling on affected teats. Abscesses are drained and adjunct treatment consists of antibiotics and warm compresses. Severe mastitis may warrant surgical removal of affected glands.

Occasionally, milk may not be expressed from the bitch's mammary gland. To test if she is not "letting down" the milk or if she is actually not producing any (agalactia) a single dose of oxytocin on the tongue will cause letdown if milk is being produced. Absence of milk production is an untreatable condition, and pups will need to be provided an alternate source of nutrition.

Chronism, or postpartum hysteria, is a breed-specific condition in which the bitch mutilates her normal pups. Occasionally, chronism is manifest as excessive grooming of the pups, abrading the skin off of them.

Stillborn and Fading Puppies: What Can They Tell Us?

For owner and breeder alike, a dead pup is a heartbreak. But it is also a medical opportunity. The information veterinarians can glean from a pup that has died in utero or neonatally is paramount to preventing illness and reducing the likelihood of congenital defects in future generations.

"If you have a large litter and several of the pups die, you want them treated as a litter. Therefore, the health of that litter is dependent upon knowing what went wrong with the animals that haven't survived," said Dr. Mark Haskins, professor of pathology at the School, who lectured on neonatal and pediatric pathology in dogs.

The importance of the autopsy in this age group is underscored by the statistics: among purebred dogs, ten percent are stillborn or die before their first veterinary examination and nearly 20 percent die within the first week of life. Overall pre-weaning mortality is 30 to 45 percent.

If a pup dies abruptly, the two major concerns are infection and genetic defects. The former puts the surviving litter mates at risk; the latter, future generations. Autopsy data can be used to protect these two groups of pups, although pathology is not a perfect science. The clinical signs of "fading puppy syndrome," a term that describes a variety of conditions in which pups that are apparently normal at birth gradually weaken and die, are very nonspecific. Many of these pups suffer inborn errors of metabolism, which are difficult to diagnose, explained Dr. Haskins.

"The majority of the time, they're going to come back to you and say, 'I don't know why the puppy died.'"

The autopsy of the pup differs in many ways from that of the adult dog. In the pup, the skeletal muscle is comparatively paler in color, and adipose tissue is light brown and relatively sparse. The thymus, which regresses with age, is still prominent. Ossification is limited, and the brain and kidneys are still in the process of maturation.

Such differences are taken into account when detecting for the presence of developmental malformations, which are a major concern in neonatal pathology. Malformations have been reported in seven to 20 percent of neonatal deaths in dogs and cats. Congenital malformations take many different forms, from duplications to arrested development (aplasia or hypoplasia), failure to regress (imperforation), and failure to close (persistent patency)—as in the case of cleft palate, diaphragmatic hernia and neural tube defects.

At the molecular level, developmental malformations are caused by alterations in DNA structure and nucleic acid function, altered energy states, changed membranes and enzyme inhibition. The most sensitive period for the occurrence of these anomalies is the first trimester (days 1-20)—the period of organ formation. The underlying causes for these molecular alterations are biological agents (i.e., viruses, bacteria and fungi), physical agents (i.e., ionizing radiation), chemical agents (i.e., certain drugs) and inherited genetic defects.

Less significant in dogs than developmental malformations, prematurity is manifest primarily as immaturity of the mature surfactant system that facilitates expansion of the lungs. Prematurity is a diagnostic challenge in dogs because of the great variation in size between breeds.

Mortality in neonatal pups can also be directly caused by infectious agents, such as herpesvirus, parvovirus, adenovirus (infectious canine hepatitis), paramyxovirus (canine distemper), bacterial infections (group B Streptococcus and *E. coli*) and trauma.

Neonatal and Pediatric Care

Neonates require special care during their precarious first weeks of life. Dr. John Melniczek, lecturer in medical genetics at the School, discussed early postnatal development and care in pups.

The most stressful period in a pup's

life is the first week of age, which is the interval of highest mortality; weaning is the second most stressful time. The breeder can help a pup cope with stressors by examining it shortly after birth to detect for the presence of any obvious congenital defects, which may impair development and hinder survival. Some of the more common congenital abnormalities in pups are cleft palate, open anterior fontanel, hydrocephalus, heart disease, umbilical hernia and inborn errors of metabolism.

Within hours after birth, the pup should begin to consume colostrum, which is the source for over 90 percent of the maternal antibodies the pup receives. Prior to breeding, the bitch should have been brought up to date on her vaccinations, thereby maximizing the presence of maternal antibodies in her colostrum.

Because of their immature glucose storage systems and renal function, pups are prone to hypoglycemia and dehydration. Hence, adequate nutrition is critically important. The pup's growth rate is a sensitive indicator of nutritional status. Pups should be weighed daily for the first three weeks of life, during which time it gains about ten percent of its body weight daily. If weight gain is inadequate, the dam's mammary glands should be expressed to ensure adequate milk production, and her nursing behavior should be observed. Supplementation with milk replacers and bitches' milk should be considered in cases of poor weight gain. In nursing pups, weaning can begin at three to four weeks of age, but should not be completed until six weeks.

Neonates also have undeveloped thermoregulatory systems. Because of their large surface area-to-body mass ratio, sparse body fat, high water composition, poor blood flow to the extremities, and immature shivering and panting responses, pups have difficulty regulating their body temperatures. During the first weeks of life, the ambient temperature in the nesting area should be kept at 86-90F, with gradual reductions to 75F over the next three weeks. Maintaining normal body temperature—which is 96-97F during the first two weeks of life, and increases to 100F by four weeks of age—is important for normal function of the pup's metabolic pathways. Normal body temperature is also a deterrent to infectious diseases, many of which grow best at low body temperatures.

Neonates can fall prey to a variety of infectious agents. Canine herpesvirus, most common in pups under three weeks of age, can cause depression, diarrhea, respiratory disease and sudden death. If contracted during pregnancy, it can cause abortion. Affected pups should be kept warm and well hydrated, and any electrolyte imbalances should be corrected. Although no vaccine is available, an affected dam's subsequent litters are usually immune if they've received adequate colostrum.

Canine parvovirus type 1, seen primarily in pups aged 5-21 days, causes diarrhea, pneumonia and death, as well as abortion and infertility in infected bitches. Like canine herpesvirus, treatment is symptomatic and no vaccine is available.

Pups of all ages are subject to bacterial infections. "The young animal is prone to these because the immune system is not yet what it should be," said Dr. Melniczek. Pups can develop bacterial respiratory infections through aspiration secondary to cleft palate, vomiting or regurgitation. Kennel cough, caused by *Bordetella bronchiseptica*, is also frequently seen in neonates. Puppy pyoderma, usually caused by Staphylococcus, is a common skin affliction in pups.

Vomiting and diarrhea is often seen in pups aged three to five weeks. Usual etiologies in the pup are parasites (roundworms and hookworms), Coccidia, Campylobacter, Clostridia, Salmonella, distemper, metabolic disease (i.e., liver shunt) and dietary indiscretion.

Genetic diseases cause a variety of syndromes in pups, such as cystinuria in the Newfoundland, copper toxicosis in the Bedlington terrier and phosphofructokinase deficiency in the English springer spaniel. Sensitive metabolic screens and genetic tests for the identification of affected and carrier animals have been developed at the School. *J.C.*

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The Conference will be held at the Adam's Mark Hotel in Philadelphia on Wednesday, January 31 and Thursday, February 1, 2001. Please look for the Conference Brochure in your mail in November or take a sneak peek now by checking out our web site.

If you have any questions regarding the Conference, please call the Office of Continuing Education at (215) 898-1882 or e-mail us at: **penn-conference@vet.upenn.edu**