Symposium on Reproduction, September 6-7, 1973, School of Veterinary Medicine, University of California, Davis : sponsored by American Veterinary Society for the Study of Breeding Soundness, American College of Theriogenologists, School of Veterinary Medicine, University of California, Davis.

SYMPOSIUM ON REPRODUCTION

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CONTENTS

C O N T E N T S
(cont.)

TITLE: The Mare as an Oddity in Reproductive Endocrinology

SPEAKER: Dr. G. H. Stabenfeldt

In this discussion I shall emphasize the oddities of equine reproductive endocrinology. Not to discourage the veterinary practitioner, but a recognition of the oddities of the reproductive cycle of the mare should help the equine practitioner to develop a more rational approach to the management of the cycle of the mare. First, I shall discuss the basic events leading to ovulation from an endocrine viewpoint. The relationships of endocrine events have been quite well established during the past few years in a number of species. [First slide] I'll use the dog to emphasize the relationship of these events. FSH, and possibly some LH, are first of all necessary for follicular development. The growing follicles then begin to secrete larger and larger amounts of estrogen, as shown on the slide. At a certain point in time, estrogen levels have a positive feedback on the release of LH, and probably FSH, which results in a short ovulatory burst of these gonadotrophic hormones. Ovulation usually follows this surge of gonadotrophins within 24 hours. The shortness of this surge is emphasized in the next slide [Slide #2], which is from the work of Schalms and Karg in Germany. This slide shows that the LH surge in the cow is initiated and over with within a 12-hour period. The first oddity, then, of the mare's reproductive cycle concerns the breadth of the LH surge, which is seen in conjunction with ovulation.

The next slide [Slide #3] is courtesy of Irv Geschwind, in Animal Science, and detects the LH surge in the mare at the time of ovulation.

The LH rise begins at approximately the time regression of the corpus luteum is complete, or when progestin levels have declined to less than 1 ng/ml plasma. Another way of expressing this is, 3 days following the beginning of corpus luteum regression. Ovulation occurs approximately the third day of the LH rise, or on about the 4th or 5th day of estrus. Not only is there sustained rise prior to ovulation, but LH continues to rise several days postovulation. The reason for this sustained LH peak is not known. One main reason may be that it may have a long half-life. Parlow has found the half-life of most gonadotrophins in the rat to be a matter of minutes, whereas the half-life of equine LH and PMSG in the rat are a matter of days. The physiological reason for this broad LH peak is not apparent. Whether or not LH has anything to do with follicular growth remains to be proven. Pattison et al. have reported an estrogen surge that slightly precedes the LH surge. Considerable follicular development occurs prior to ovulation in regard to the number of follicles developed, which leads us to the second point:

The mare has a high percentage of multiple ovulations--approximately 25% in our experience--in lieu of the fact that only about 1% of births involve live twin foals. The mare seems incapable, as a rule, in developing two or more zygotes to term; yet she is often involved in multiple ovulations.

The next slide [Slide #4] shows the time intervals observed for multiple ovulations. As can be seen in the slide, most ovulations occur within 24 hours of each other.

The formation of two corpora lutea during the estrous cycle of the mare apparently does not result in higher levels of progesterone in

peripheral blood of mares, as indicated in the next slide [Slide #5]. The next slide [Slide #6] emphasizes that there is no difference as to cycle length, length of estrus, length of diestrus, or corpus luteum lifespan in mares that ovulate one or more follicles.

The mare is also somewhat unusual compared to other large domestic species, [Slide #7] in that she is receptive for a number of days prior to ovulation, whereas the sow and ewe ovulate shortly before they go out of heat, on about the 2nd day of heat, and the cow ovulates on the 2nd day following the onset of heat, often after she is out. The average ovulation time in our studies has been somewhere between the 4th and 5th day of estrus. [Slide #8]. The sequence of follicle development preceding ovulation during estrus can be seen as being linear in fashion with an ovulatory size of 45 mm reached slightly prior to ovulation.

The next slide [Slide #9] emphasizes the relationship of ovulation to the end of estrus. Our data confirms the data of many others in the literature, in that most ovulations in the mare occur one or two days prior to the end of estrus. The fact that a mare goes out of heat is strong retrospective evidence that she has ovulated.

The next point of interest in the mare is the fact that follicular development often continues to occur following ovulation. [Slide # 10] This follicular development at times results in ovulation during the luteal phase of the cycle anywhere from 2-12 days following the first ovulation. The veterinary practitioner who is only able to occasionally examine mares at weekly intervals, or greater, could be confused by the occurrence of ovulation during the luteal phase of the cycle. These luteal phase ovulations apparently result in the formation of normal corpora

lutea. These corpora lutea, however, do not in most cases prolong the diestrous phase of the cycle. The mare thus is somewhat unique as compared to other large domestic species of animals, in that follicular development is not strictly inhibited by luteal phase levels of progestins--in fact, neither at times, is ovulation.

We have utilized the measurement of progesterone in the peripheral blood of mares as an indicator of formation of corpora lutea, maintenance of corpora lutea and regression of corpora lutea during the estrous cycle in the mare.

The next slide [Slide #11] detects a composite, almost idealized profile of the corpus luteum of the mare during the estrous cycle. As can be seen in this slide, maximal corpus function is obtained by 6th or 7th day postovulation. Regression is very precipitous in the mare as in our other large species, in that it is complete in about 48 hours.

It is not generally recognized that corpora lutea can be palpated for some period of time following ovulation. [Slide # 12] John Hughes has found that corpora lutea can be palpated on the average of 9 days postovulation. In some mares they can be palpated the entire lifespan, particularly if they are located on the poles of the ovary or if the ovary or the corpora lutea have a particularly resilient nature to them.

The mare is also unusual compared to other large domestic species in that spontaneous prolongation of the corpus luteum occassionally occurs. This is shown by the next slide. [Slide #13]. This prolongation can last from 30-90 days. Estrus is suppressed during this time, but not necessarily follicular growth and, at times, ovulation.

The next slide [Slide #14] shows an ovary obtained from one of these

animals. The corpus luteum has a distinctive, connective tissue core surrounded by bands of luteal tissue. We have produced experimental prolongation of the corpus luteum by removing the uterus during the luteal phase of the cycle, as shown in the next slide. [Slide #15].

The next slide [Slide #16] shows a corpus luteum of nine days' duration in the mare, and the next slide [Slide #17] shows a corpus luteum obtained from one of our hysterectomized mares, which is very similar to the corpus luteum obtained from a mare with a spontaneous, prolonged corpus.

[Slide #18] Progestin data taken from mares with spontaneous prolongation of the corpus luteum and compared to those of hysterectomized mares show little or no difference. From this we have concluded that prolongation of the corpus luteum in the mare is due to a failure of the uterus to release a luteolytic factor during the latter part of luteal phase of the cycle, which normally causes regression of the corpus. It should be pointed out that our intact mares that have spontaneously prolonged corpora do recover their ability to control the corpus, as mentioned, with resumption of cyclic ovarian activity following this. The mare is one of a relatively small number of animal species--cow, sow, ewe and guinea pig--in which the uterus appears to play a role in the regression of the corpus luteum.

Another unusual aspect of the estrous cycle of the mare concerns sexual receptivity. We have observed some mares with regular ovarian activity, including ovulation, exhibit no signs of sexual receptivity. The next slide [Slide #19] shows progestin values from a mare that has a

number of unusual aspects about her, including failure to show estrus during the period of follicular development. She has a rather prophetic name, "Bashful Boots". This mare cycled normally for awhile with ovulation and formation of corpus luteum occurring. She did show estrus once during the period of time shown. She also had three episodes of prolonged corporus function during this time span, and finally she went into the classic winter anestrous period. Thus, mares may evidence anestrus or lack of sexual receptivity for a number of reasons--the most important, of course, being pregnancy. Secondly, the occurrence of spontaneous prolongation of the corpus luteum. Third, lack of sexual receptivity even though regular ovarian activity is going on, and--lastly, they can show no sexual receptivity because of the classic winter anestrum period. Light, as will be emphasized later in the day, seems to play a very important role in controlling the onset of the winter anestrum period. It should be noted that the response to light in the natural environment is a very slow response. This is typified by the fact that mares stop cycling in December or January, although light has been decreasing since June 21st. It is also typified by the fact that many mares that go into winter anestrum do not start cycling until March or April, even though light has begun increasing on December 21st. There are a number of other ways that the mare appears to be unusual, as compared to other domestic species of animals. I will mention them only briefly:

She is unusual in that she appears to differentiate the fertilized ova from nonfertilized ova very early in the estrous cycle. There have been reports that up to 15 ova have been found in the oviducts of a nonpregnant mare. Thus, if not fertilized, ova appear to be retained.

However, fertilized ova pass through the oviducts apparently bypassing nonfertilized ones.

The mare is also unusual in the fact that she produces endometrial cups in the uterus that produce a hormone, pregnant mare serum gonadotrophin, during pregnancy. This is somewhat unusual in that the cells that form the endometrial cups come from the placenta originally, and, also somewhat unusual in that we have no known function for pregnant mare serum gonadotrophin in the pregnant mare.

The mare is also somewhat unusual in that secondary corpora lutea are formed during pregnancy from about days 40-70. Until recently it had been thought that the primary corpus of pregnancy regressed at about the time the secondary crop came on. It appears this is not so; in fact, the primary corpus appears to last and regress at about the same time that the secondary corpora do--namely, days 150-180 of gestation. The placenta takes over support of pregnancy at this time.

The mare also seems to be somewhat unique as far as the termination of gestation, in that she is able to confine the time of delivery to night--and oftentimes during very few hours of evening--namely, 8 or 9 to midnight.

TITLE: Hormone Therapy in Equine Reproduction

SPEAKER: Dr. John P. Hughes

Hormones, chemical substances produced by the endocrine glands that serve as regulators of physiological processes within the body, are used frequently by veterinary clinicians in equine reproduction, and often with little scientific basis to the expected result. Research on the pharmacology and therapeutics of hormones in horses has been limited in the past, but the development of sensitive assay procedures and the revived importance of the horse is stimulating a considerable amount of vitally needed basic research effort throughout the world.

I would like to comment, in a limited way, on the present status of hormone therapy in the mare as viewed by a clinician.

Prostaglandins:

These substances are C_{20} unsaturated hydroxy fatty acids with a wide variety of pharmacologic effects.

Prostaglandin $F_{2\alpha}$ has recently been demonstrated to be luteolytic and there is ample evidence that it is the uterine hormone responsible for regression of the corpus luteum. While still in the experimental stage, it offers a means of controlling the estrous cycle of the mare similar to saline infusion into the uterus.

Mares injected on the 4th to 12th day of diestrus with $PGF_{2\alpha}$ (1.25 mg $PGF_{2\alpha}$ or 100-600 µg synthetic analogue) return to estrus within 3-4 days

of treatment and ovulate normally. The CL through 3 days of age is resistant to the luteolytic effect of $PGF_{2\alpha}$ or saline infusion. Interval from treatment to ovulation ranges from 7-12 days.

High doses result in signs of toxicity - sweating, hypermotility of the gastro-intestinal tract leading to diarrhea, mild colic, and increased rates of pulse and respiration. These substances should prove to be valuable in the treatment of persistent CL, which occur spontaneously in normally cycling or lactating mares and in mares that fail to resume cycling after early embryonic loss or early abortion. Saline infusion in these latter mares has proven to be inconsistent in bringing about the resumption of cyclic activity. Only future studies will tell us whether prostaglandins are more reliable.

Prostaglandins should be useful where AI is practiced or to give some control in preventing several mares from coming into estrus simultaneously when booked to a single stallion.

Anterior Pituitary Hormones:

Little experimental study of the physiology and pharmacology of anterior pituitary hormones in horses has been done; however, recent work shows LH starts to rise in the mare as progesterone drops. The rise is gradual and reaches a peak 1 to 3 days postovulation. When progesterone reaches peak levels, LH levels are back down to baseline. There is a surge of estradiol-17β that coincides with or just precedes the LH surge. It appears, therefore, that a surge of estrogen of follicular origin initiates the LH release which leads to ovulation. While FSH has been

used in the treatment of many mares to stimulate the growth of a follicle, there is no evidence that it will do so. Mares aborting after the endometrial cups become active in producing PMSG (levels as high as 3,000,000 IU) fail to return to estrus until after the normal lifespan of these structures. Allen injected 1,000,000 IU of FSH in an attempt to superovulate a mare without success.

PMSG, whose activity is primarily follicle stimulating with some luteinizing activity present, has been commonly used in broodmare practice to treat anestrus, stimulate follicle growth and induce ovulation, but no controlled evaluation has been made and favorable results reported are highly questionable.

Luteinizing hormone in broodmare practice is usually administered in the form of Human Chorionic Gonadotrophin (HCG). It is of proven value in inducing ovulation of existing follicles. An injection of 2,000 IU intravenously on the second day of standing heat resulted in almost 90% of mares ovulating within 48 hours. This gives us a valuable tool to predict optimum time of breeding. There is no evidence for the use of HCG to stimulate follicle growth.

Posterior Pituitary Hormones:

Oxytocin is used extensively by veterinarians. Retained placenta in the mare is one of the more common indications for its use. The uterine contractions elicited by oxytocin are often associated with expulsion of membranes. More favorable results have recently been reported by using an intravenous drip-infusion of 30-50 IU of oxytocin over a

period of an hour rather than a single, rapid injection. Oxytocin is also used to aid involution and expulsion of lochia from the uterus and in cases of tears or trauma to the uterus post-foaling.

Induced Parturition:

Parturition has been induced in mares by the use of oxytocin. Before induction of parturition in a mare is done, she should be within 2 weeks of term, should have milk in the udder, and the cervix should be softening and preferably showing a degree of relaxation. Two weeks prior to normal parturition, the cervix should be under the influence of estrogens and dilate when the uterus contracts under the influence of oxytocin.

One successful procedure has been 20 units of oxytocin IV followed by 10 units in 1 hour if parturition has not occurred.

Purvis at the AAEP meeting suggested 120 units/1000# IM. He injected estradiol (3 mg) or stilbesterol (30 mg) prior to the oxytocin if the cervix was not relaxing. His maximum dose of oxytocin was 140 units. Delivery of the fetus usually occurs in half an hour.

We have noted a few cases of premature separation of the chorioallantoris following this procedure.

Steroid Hormones:

Estrogens:

The behavioral response to injected estrogens varies with the reproductive state of the animal. Ovarectomized mares or mares in deep anestrus will respond to 0.5 to 1 mg of estradiol-17β or stilbesterol

and show estrus with correlated changes in the cervix and vagina. The response in these mares to estrogen occurs rapidly. Changes in the cervix and vagina are marked by 3 hours--maximum in 6 hours--and starting to subside by 12 hours. Initial behavioral changes are seen in 3 hours with estrus within 6 hours. Estrus may last from 12 hours to several days. Mares with active ovaries may not respond to doses up to 20 mg.

Estrogen treatment will not result in the initiation of a sequence of physiological events in ovaries of mares having no ovarian activity. There is some evidence that a surge of estrogen initiates the LH release which leads to ovulation. Thus follicles that are competent to ovulate may do so if estrogen is administered. It is well to remember that doses of 50 mg have been shown to inhibit follicle growth in the mare. High doses of estrogens may cause long-lasting changes in steroid sensitivity of the brain, with possible deleterious effects on reproductive function (gonadotrophin synthesis and/or release). (Stabenfeldt).

Estrogens can be used to induce behavioral estrus in ovarectomized or deeply anestrous mares if a jump mare is needed to collect semen from a stallion with the artificial vagina.

Combinations of estrogens and antibiotics for treatment of bacterial infections of the uterus have not proven more effective than the antibiotic alone.

Progestogens:

The primary use of progesterone in equine reproduction has been to prevent early fetal loss due to a deficiency of the hormone. There is

little evidence to support this concept but even less argument against its use in animals with histories of repeated fetal loss. In a study of 61 mares known to have recently undergone early fetal death or considered "pseudopregnant", 41 had normal to high progesterone levels and 20 had low progesterone levels. Thus, while progesterone deficiency may be a factor in early pregnancy loss, it is not a major one. Dosage of progesterone needed is another problem. Loy's work indicates a minimum of 100 mg/day is necessary in the mare. Another study has shown that mares with low progesterone levels between 25 and 50 days gestation required only 2 or 3 weekly injections before their blood progesterone levels rose to normal and stayed there.

Progestins are used to suppress estrus in the normal, cyclic mare that is being used for racing, rodeo or showing performance, as well as mares with disturbed psychic manifestations of estrus (nymphomaniac tendencies). At the present time we try to achieve a dosage of 100 mgs progesterone in oil/day. Silastic implants of progesterone offer one method of achieving uniform dosage over a prolonged period of time, but they are not available at this time and much work needs to be done on their efficiency.

Chlormadinone Acetate (CAP) has been used successfully in Europe to prevent signs of estrus. At least 3 days before the onset of estrus, 5 or 10 mg CAP (oral) was given daily for 8 to 12 days. Estrus was noted 4 to 8 days after medication was stopped.

Corticoids:

Dexamethazone administered during the latter part of gestation in cattle and sheep will induce parturition. Attempts to induce parturition

during the last two months of gestation was unsuccessful in pony mares using 20 mg dexamethazone, or in mares using 10 to 80 mg 2 to 18 days prepartum. Doses of 10 to 40 mg dexamethazone intravenously daily for 5 days had no effect on the pregnancy of mares 40 to 60 days pregnant. In one study 70 mgms of dexamethazone was reported to induce parturition in ponies.

Thyroid Hormones:

No relationship with any real degree of certainty has been established between thyroid function tests and a specific abnormality in reproductive function. Thyroidectomized mares will cycle, conceive and carry foals to term.

TITLE: Intrauterine Saline Infusion - Equine

SPEAKER: Dr. Dean P. Neely

The purpose of the project was to correlate the levels of plasma progestins associated with the shortened cyclic intervals produced by IU saline infusions - we wish to show that in order to bring a mare into estrus with the use of IU saline infusion, she must first have an active CL.

The basic theory on the mechanism involved on how saline infusions function to bring mares into estrus is by shortening the CL lifespan. The saline infusions supposedly stimulate the uterus to produce and/or release a luteolytic factor. This luteolytic factor is picked up by the venous drainage of the uterus. The exchange of this factor to the ovarian artery most likely occurs where the uterine vein and ovarian artery come into close apposition. The luteolytic factor which has been shown to be $PGF_{2\alpha}$ in sheep, may then cause vasoconstrictor activity on the ovarian arterial blood supply. The diminished circulation to the ovary then causes destruction of the CL which appears to need a rich supply of blood.

This study was run from January - August of the year. The mares were allowed to begin normal cycling and were then infused on either the 4th or 5th day postovulation. One to 2 recovery cycles were allowed to occur and the mares were then treated on the 6th or 7th day postovulation. The final recovery cycle was then followed. The mares had daily jugular blood samples taken to measure plasma progestins. Each mare was teased daily by 1 of 3 adult breeding stallions and recorded as being either in estrus or diestrus. Estrus indicating acceptance of the stallion; diestrus indicating non-receptivity to the stallion. Rectal palpations of the internal

reproductive tract were performed daily when follicular structures were present on the ovary and every other day when no follicular structures were present.

This slide (Slide 1) illustrates the progestin profile obtained from one of these mares throughout the period under study. Plasma progestin levels in ng/ml are indicated on the vertical scale. The period of estrus indicated by the darkened bars. The day of ovulation indicated by the arrows below the estrous bars. The time interval is on the bottom of the graph.

The initial levels of progestins where <1.0 ng/ml while this mare was in anestrus during the beginning of the year. Coming out of anestrus this mare had a rather long period of estrus followed by an ovulation. Plasma progestins then began to rise rapidly; the mare went out of heat approximately one day after ovulation. During the period of increased levels of plasma progestin, the mare remained in diestrus. On approximately the 14th day post ovulation, the plasma began to decrease rapidly, indicating the end of the CL lifespan. Approximately one day after the plasma progestin levels went below 1.0 ng/ml the mare returned to estrus and ovulated with a normal ovulatory interval of 21 days.

During the next cycle the mare was infused on day 4 postovulation. The plasma progestins continued at a normal level indicating this infusion had no effect on the cycle which resulted in a 20-day cycle length. A 20-day recovery cycle followed and the mare was then infused on day 7 postovulation of the next cycle. This resulted in a rapid decline in plasma progestins occurring the day following treatment. The cycle interval was shortened to 17 days. A recovery cycle of 23 days followed.

This slide (Slide 2) shows a mare with a similar plasma progestin profile during the period under study. This mare also came out of anestrus with variable days of estrus occurring and a double ovulation. A 24-day cycle occurred and the mare was infused on day 4 postovulation with a resulting rapid decline in plasma progestins beginning the next day and shortening the cyclic interval to 17 days occurring. Two recovery cycles followed. It should be noted this mare had a diestrous ovulation on the 7th day postovulation of the 24-day recovery cycle. The mare was then infused on the 7th day postovulation with plasma progestins again beginning to drop rapidly the following day with a 14-day cycle length resulting.

This is a composite of progestin profiles (Slide 3) for the 4th or 5th day infusions. A group of ten mares received infusion on either of these two days. A normal cyclic progestin profile is indicated by both our pretreatment and post-treatment cycle composites. It is noticed here again that the CL appears to have a lifespan of approximately 14 days from ovulation until the progestins begin to decrease rapidly.

During the treatment cycles six mares responded with shortened cyclic intervals and four did not respond and maintained a normal corpus lifespan of approximately 14 days and ovulatory interval of approximately 21 days.

Of the six responding, the progestins were declining rapidly by the 6th day and resulted in a shortened cycle length.

This is a composite of the progestin profile (Slide 4) of the 6th or 7th day treatment group. Ten mares were infused on either of these two days. Data from two of the mares could not be included for reasons I will soon discuss. Of the eight included, normal pretreatment and post-treatment plasma progestin profiles occurred. Seven of the mares responded during the treatment cycle with progestin rapidly declining the following day and

a shortened cycle resulting. One mare included here did not respond to a 7th day postovulation infusion. This mare had a diestrous ovulation occurring on this day. The plasma progestin did begin to decline following treatment but rebounded when this diestrous ovulation corpus luteum became fully active and carried this mare's cycle to normal length. The two mares which also received infusions on this date but were not included, also had diestrous ovulations occurring near the time of treatment.

One mare (Slide 5) showed a continuing increase in the level of plasma progestins and was ovarectomized 5 days later to examine the corpora lutea.

The other mare (Slide 6) developed a persistent corpus luteum which maintained elevated plasma progestins for approximately two months before decreasing again and she then ovulated.

This is a composite of the cycle lengths (Slide 7) of those treated 4th or 5th day. Examination of the composite cyclic ovulatory interval shows that both our pretreatment and post-treatment cycles were of a normal lnegth of 21-22 days. Those responding to treatment on days 4 or 5 had a shortened cyclic interval average of 14.8 days. Those not responding to treatment here had a normal interval of 21 days.

This is a composite of cycle lengths (Slide 8) of those treated on 6th or 7th day. Again, we see a normal ovulatory interval resulted for both the pretreatment and post-treatment cycle. Those responding to the treatment during these 6th or 7th day postovulation infusions had a shortened cycle length of 16 days. Thus it appears IU saline infusions can shorten the cycle length by approximately 5-7 days.

The length of estrus (Slide 9) remained similar for the pretreatment, treatment and post-treatment cycles without any significant differences. Estrus averaged approximately 5.3 days.

This illustrates (Slide 10) the length of diestrus for both groups. The normal diestrus length of approximately 16 days holds true for both our pretreatment and post-treatment cycles of both groups. Those responding to treatment on the 4th or 5th day postovulation had a shortened diestrous interval of approximately 9 days. Those not responding had a near normal 16-day diestrous period. In the 6th or 7th day postovulation treatments those responding had an 11-day diestrous interval.

Let us look more directly at the lifespan of the corpus luteum (Slide 11) which is that period from ovulation until progestins begin their rapid decline, or in terms of calculating it, it is that interval from ovulation until the last day before progestin levels decreased by at least 50% of the average of the three preceeding days.

The normal CL lifespan for the pretreatment and post-treatment cycles is approximately 14 days. Of those responding during the 4th or 5th day treatment, a 5.5-day CL lifespan occurred. Those not responding had approximately the normal 14-day CL lifespan. Those responding to the 6th or 7th day infusions had a CL lifespan average of 8.4 days. Thus, this shows that progestins begin to decline rapidly approximately one day after the IU saline infusions in those responding to the treatment.

Here we looked at (Slide 12) the interval from treatment to when plasma progestins decreased to <1.0 ng/ml, since we would normally expect estrus to occur approximately one day after this decrease occurred. An interval of approximately four days was obtained for both treatment groups.

Next (Slide 13) we looked at the interval from treatment to the first day of estrus and obtained a value of 6.2 days for those responding to treatment on the 4th or 5th day and a value of 5.3 days for those responding to the 6th or 7th day infusion.

Interval from infusion to ovulation. Finally (Slide 14) we looked at the interval from infusion to ovulation and obtained values of approximately 9-10 days in those which responded to treatment.

Summary:

We have shown that the intrauterine saline infusion appears to bring the mare into estrus by shortening the corpus luteum lifespan.

Our data suggests the corpus luteum has a variable response of regression to the effects of treatment if it is only 4 to 5 days postovulation. This is in agreement with Arthurs' data from England, in which he got no response to saline infusion during the first 5 days postovulation. Also a recent report by Allen and Rowson with the use of an analogue of $PGF_{2}\alpha$ indicates only 3 of 5 mares responded to treatment given during the 4th and 5th days postovulation.

The 6th or 7th day postovulation corpora lutea appear to respond well to IU saline infusion with the corpus luteum regression beginning approximately the following day. But, the occurrence of diestrous ovulations can interfere with this decline of plasma progestins and earlier than expected return to estrus.

Following the infusion, estrus can be expected to occur on the average in 5-6 days, with ovulation occurring approximately 9-10 days after the treatment.

TITLE: Relation of Postpartum Breeding Management to Efficiency of Reproduction

SPEAKER: Dr. Robert G. Loy

Traditionally horsemen have regarded foaling mares, as a class, to be one of the best breeding bets in the business. If only the overall seasonal conception rate is the criterion, this may appear to be true. If other measures of breeding efficiency are considered, however, the picture takes on a slightly less rosy hue. Consider the fact that for many breeds of horses such as the Thoroughbred, Quarter Horse, Standardbred, etc., the average gestation length is 345 days, give or take a few days,so that in order for a mare to foal as early next year as she did this year, we have just 20 days, on the average, to get her back in foal. The percentages show us that over a large number of mares and over the long haul, we will be successful in doing this in about 25% of our foaling mares as an upper limit. In other words, at least the remaining 75% of foaling mares will lose at least 10 days each year on the average. Actually, at least 25% will lose at least 30 days per year on the average. These time loss figures are for practical purposes those for optimum conditions. Any problem such as a transient uterine infection, a skipped heat, slower than normal uterine involution, even systemic illness or a case of founder or colic, may increase time. A difficult foaling is usually deadly, as is use of a stallion of low fertility. Within the limits of my own recent experience, the actual time loss in comparing foaling dates in succeeding years averages 25-30 days for foaling mares. Simply stated, this time loss gives rise to what I look at as the most serious single inefficiency in horse breeding.

Before going on to problems of breeding management of foaling mares, it should be pointed out that this problem can be substantially alleviated by getting barren and maiden mares in foal as early as possible in the breeding season. By doing so, you may delay the time when the mare goes empty a year simply because she foals too late to be rebred successfully. The simplest means of doing this consistently is by the utilization of artificial lighting regimes to initiate early onset of stable breeding patterns in these classes of mares on a fairly uniform basis. This subject of "lights" has been hashed and rehashed so that I will not belabor it further now.

Decreased efficiency of breeding performance during the postpartum period in mares undoubtedly results from a number of factors. To my present knowledge, no one has presented specific and pertinent data to substantiate a clear charge against any particular factor. However, the increased risk of unfavorable breeding result at foal heat has been attributed to reduced effectiveness of uterine defense mechanisms at this time and to the fact that the uterine epithelium is often incompletely repaired at the usual time of postpartum estrus. Andrews and McKenzie, for example, had reported in 1941 that the uterine epithelium returns to normal by 13 to 25 days postpartum but rarely by 10 days. They also found that repair of the endometrium is usually completed during the latter part of the postpartum estrus.

Most veterinarians will also have observed rather marked variation in the rate of gross uterine involution as indicated by return of the uterus to pre-gravid size, form and consistency. While most would probably agree that markedly reduced rates of gross uterine

involution are associated with poor breeding performance, the exact nature of the association is certainly not known. It might also be pointed out that the reproductive neuro-endocrine system has been rather massively blanked out for a relatively large part of later pregnancy by the endocrine activity of the fetoplacental unit. It should be reasonable to wonder if some time is not required for the system to reestablish a stable state with all controls, etc., functioning effectively. All may be important.

In contemplating these various areas, among others, some years ago, especially uterine involution and endometrial repair, we felt it to be possible that a relatively short delay of postpartum estrus and ovulation by means of progesterone inhibition might have beneficial results by providing additional time for involution and repair. In addition, we thought that if progesterone inhibition of gonadotropin secretion is quantitatively related to dosage, then a minimal dose that inhibits estrus and ovulation might permit secretion of sufficient gonadotropin to maintain a relatively high estrogen secretion rate in association with follicular development occurring during treatment. Alternatively, progesterone might inhibit a central nervous system center regulating a cyclic ovulatory discharge of gonadotropin while an area governing a basal or tonic gonadotropic release remains unaffected, the tonic release of gonadotropin stimulating follicular growth and maintaining an elevated estrogen secretion rate. In either case, the combined effects of the steroids might exert a beneficial effect on the uterus.

Eighteen mares were used in an initial trial intended to evaluate these hypotheses. Seven were not treated after foaling. Nine mares

were given intramuscular injections of 100mg of progesterone in oil solution daily from postpartum day 5 through 14. Two mares received 200mg of progesterone per day for the same period. Beginning the day after foaling, mares were tested for estrus daily, using a teaser stallion. Manual examination of ovaries and reproductive tracts per rectum was done every other day between day 5 postpartum and estrus, and daily during estrus. Urine was collected for estrogen assay on the same schedule. Uterine biopsies were taken on days 5, 10 and 15 postpartum.

Six progesterone-treated mares were bred at first estrus following treatment.

Trial II. A separate trial was done to determine the effects of a different period of progesterone treatment on the degree of control of ovulation time that could be obtained following delay of postpartum estrus. Six mares received 100mg of progesterone IM per day from the day after foaling (day 1) through day 10 postpartum. Teasing was done as in Trial I as were rectal examinations except that they were begun on day 1 postpartum. These six mares were bred at the first estrus following treatment.

RESULTS

Trial I. Three of seven untreated mares failed to show estrus or ovulate within three weeks after foaling. Two of the remaining four mares first showed estrus at seven days and two at eight days postpartum. The length of estrus ranged from two to six days (average 3.8). Ovulations occurred on days 9, 10, 11, and 12, averaging 10.5 days postpartum.

The mares failing to show postpartum estrus did not show peak levels of excreted estrogens between five and fifteen days after

foaling. A moderate level of excreted estrogen observed in one of these mares at day 5 declined to day 11 and then rose slightly in association with anovulatory follicular development.

Levels of excreted estrogen in four control mares showing postpartum estrus rose from day 5 to peaks that occurred between days 7 and 11 postpartum. Peak levels ranged from 8.8 to 23.0 Mcg percent, averaging 18.4 Mcg percent. A marked drop in estrogen excretion rates occurred in association with ovulation.

Although none showed estrus, ovulation occurred during treatment in three of nine mares receiving 100mg of progesterone daily (ovulation on days 11, 13 and 15 postpartum). In two mares, large follicles present at the end of treatment progressed to ovulation at 17 and 18 days postpartum. Only the mare ovulating at the later time showed estrus from day 16 through 19 postpartum. Follicles present at the end of treatment did not ovulate in three other mares receiving 100mg of progesterone per day. These follicles regressed slowly as new follicles appeared and progressed to ovulation at 20, 26 and 28 days postpartum. Ovulation was accompanied by estrus in all three mares (duration of estrus 2, 4, and 11 days). A ninth mare on this dose failed to show postpartum estrus or to ovulate through day 24 postpartum and at that time had no significant ovarian activity.

In two mares treated with 200mg of progesterone per day, follicles developing during the latter part of treatment failed to ovulate after withdrawal of progesterone. As in the cases of three mares treated with 100mg, these follicles regressed and were replaced by new follicles that progressed to ovulation 24 and 25 days postpartum. In both mares ovulation was accompanied by estrus (duration 7 and 9 days).

In all mares treated with progesterone, a maximum in urinary estrogen excretion rates occurred between days 9 and 15 postpartum. These excretion maxima were associated with attainment of maximum size of follicles present whether ovulation or regression of those follicles occurred. Peak estrogen excretion levels occurring in treated mares at this time ranged from 4.2 to 9.7 Mcg percent, averaging 7.4 Mcg percent. Progesterone injected at 200mg per day did not result in lowered peak estrogen excretion rates (8.9 and 9.7 Mcg percent) as compared to 100mg per day (range 4.2-8.9 Mcg percent, average 7.1 Mcg percent).

Evaluation of the characteristics of gross uterine involution by manual palpation was not sufficiently sensitive to distinguish whether or not the rates of involution differed between control and treated groups. In general, uterine involution, as indicated by the return of the uterus to prepregnant size, form and consistency, took place quite rapidly in both groups. The uterus was more completely involuted at the time of first estrus postpartum in treated mares due to the additional time provided by the delay.

Generally, a comparison of uterine structures at the different biopsy stages showed that at 15 days postpartum the mucosa had a more regular appearance with glands and epithelial cells, both superficial and glandular, being more numerous. In addition, the stroma was looser and there were fewer inflammatory cells at 15 days postpartum. Subjectively, it appeared that biopsies taken from progesterone-treated mares at ten days postpartum tended to be more similar to those from mares at 15 days postpartum than did those from control mares at ten days postpartum. Differences were small, however, and a more critical

study would be required to substantiate such an impression.

Four mares treated with 100mg progesterone per day were bred at first estrus following treatment as were the two mares receiving 200mg per day. Ovulation days for mares on the 100mg dose were 18, 20, 26 and 28 postpartum and for those on the 200mg dose, 24 and 25 days postpartum. Three of the six mares including both mares on the 200mg dose, conceived at the estrus and all three delivered live, healthy foals.

Trial II. Of six mares given 100mg of progesterone from day 1 through day 10 postpartum, four ovulated on days 15 through 17 postpartum. In all four, the follicles ovulating were the first that developed after parturition. Two other mares ovulated on days 22 and 23 postpartum. In both instances, significant follicular development occurred during treatment. In one mare, a single large follicle first became palpable two days postpartum, growing to a maximum diameter of more than 45mm on day 10 postpartum. This follicle then slowly regressed and was replaced by a second follicle which progressed to ovulation on day 23. The second mare produced a number of follicles up to 25 mm in diameter in both ovaries at about ten dyas postpartum. These regressed and were replaced by a single large follicle which ovulated on day 22 postpartum.

Three mares showed faint estrus but of sufficient intensity to permit breeding under mild restraint at the time of first ovulation after treatment. Three showed normal estrus ranging from four to ten days in length. Five of the six mares conceived to breeding at the delayed estrus and delivered live, healthy foals.

DISCUSSION

It was not possible to effect a consistent delay in ovulation time with the treatments imposed in these trials. In mares in Trial I, considerable follicular development had already occurred by day 5 postpartum. Under these circumstances a dosage of 100mg of progesterone per day was not sufficient to block ovulation in all instances. When ovulation of existing follicles was blocked by administration of either 100 or 200mg per day till as late as day 14 postpartum, these follicles apparently lost the ability to ovulate when progesterone treatment ceased. In an earlier study follicles present when treatment was terminated at 12 days postpartum ovulated from three to six days after the end of treatment in five of six mares.

In trial II progesterone treatment from day 1 through day 10 postpartum did not prevent early development of follicles in two mares. Again it appeared that when ovulation (or maturation) of such follicles was blocked for a sufficient period their competence to ovulate after withdrawal of the block was lost.

The fact that ovulation of the first follicles that developed after parturition occurred on days 15 to 17 postpartum in four mares on this treatment and on days 17 and 18 in Trial I suggests that a slight delay of follicular development may be possible without impairing ovulatory competence.

The results of Trial I indicate a definite elevation of estrogen excretion rates at the expected time of postpartum estrus in progesterone treated mares as compared to untreated mares showing no estrus. The elevated levels showed a peak-like character which was, however, less than half the peak value seen in untreated mares that showed postpartum estrus. The fact that ovulation occurred in association with the

estrogen excretion peak in three treated mares as well as in untreated mares might suggest a quantitative inhibition of an ovulatory "surge" of gonadotropin by progesterone so that estrogen secretion was depressed, as compared to mares showing normal postpartum estrus, but that in a few instances sufficient gonadotropin was released to cause ovulation.

It is interesting to note, in mares in which ovulation was blocked, that when progesterone was withdrawn after the peak of estrogen excretion, ovulation did not occur even though large follicles may have been present, persisting well into the developmental period of a new follicle. It is likewise of interest that there apparently was no further increase or resurgence in estrogen secretion by these follicles following progesterone withdrawal after the initial peak excretion. Under these circumstances levels of excreted estrogens remained low until well into the growth phase of a new follicle. If these relationships are interpreted to suggest a limited time during the life of a follicle when it is capable of secreting high levels of estrogen and/or ovulating, then a constant level of gonadotropin might be postulated to exist under conditions of these treatments. The observation that the 200mg per day progesterone dosage did not depress estrogen excretion to a greater extent than 100mg also suggests a plateau type of inhibition. Higher doses of progesterone alone will not do it.

Observations concerning gross uterine involution provided little or no indication of effects of progesterone treatment on the rates of the processes involved. There was little objective evidence that treatment effected any increase in the rate of endometrial repair. The subjective observations that the endometrium of treated mares at day 10 postpartum appeared to have fewer inflammatory cells, more regular appearance of mucosa and glands, and looser, more edematous stroma than control mares at that stage require further examination. The finding

that the differences in repair of the uterine epithelium from day 5 to day 15 postpartum are relatively minor is not in agreement with the observations of Andrews and McKenzie. The present study showed that major repair already had occurred by day 5.

Fertility of mares bred at the delayed postpartum estrus was quite satisfactory. A conception rate of 67% (eight of twelve mares) for Trial I and II combined suggests that if delay of postpartum estrus by progesterone did not improve fertility, it did not depress it as compared to that previously reported for breeding at the normal first estrus postpartum. Comparison of this conception rate with those regularly achieved in practical situations also is favorable. All four mares in an earlier study conceived when bred following a short progesterone delay of postpartum estrus. Thus for all mares that were bred in these two studies following a progesterone delay of postpartum estrus, 75% conceived (twelve of sixteen) and all delivered live foals except one mare that died at 150 days postbreeding while carrying a normal fetus.

If we include all mares that have been utilized in postpartum delay treatments, a total of 45 mares, 34 conceived for 75.6% conception to one breeding. Through 1970, 31 mares were treated, 24 conceived for 77.4%, 23 foaled for a 74.2% live foal rate from breeding in one heat period.

The results of breeding mares at progesterone delayed postpartum estrus are interpreted as suggesting an improvement of fertility compared to breeding at normal postpartum estrus. Reasons for the improvement are not clearly indicated in the present study. Grossly, the

uterus was more involuted and the endometrium appeared to have undergone a greater degree of repair in treated mares but neither of these differences was great and might well be accounted for by the additional time allowed before breeding in delayed mares rather than by more direct effects of treatment.

TITLE: Equine Cushing's Disease

SPEAKER: Dr. David Gribble

Horses with Cushing's disease have consistantly had pituitary tumors arising in the pars intermedia. In addition, the adrenal glands are enlarged due to hyperplasia of the adrenal cortex. The disease occurs more frequently in aged animals (mean age 20 years) and in females more often than males (about 2:1). Typically affected animals have persistent bacterial infections, long hair throughout the year, polyuria and polydipsia, muscle wasting and a more docile than normal nature. Neutrophilia, lymphopenia, hyperglycemia and glucosuria are frequently observed. Indeed, cases may be incorrectly diagnosed as having diabetes mellitus. Plasma insulin levels, however, are usually normal or elevated. In affected horses plasma levels of ACTH are elevated in comparison to normal horses while plasma cortisol levels are often similar to those of normal horses or only slightly elevated. Because resting plasma cortisol level determinations often do not aid in the diagnosis of this disease, we have relied on the abnormal adrenal response by affected animals to either exogenous ACTH or dexamethasone. One unit of ACTH per kilogram of body weight given I.M. causes a rapid maximal rise (2 to 4 hours) in plasma cortisol levels (20-30 µg/100 ml) in horses with Cushing's disease. Normal horses respond more slowly with peak levels being at 6 to 8 hours post-injection and the levels usually being 15-20 µg/100 ml. Forty micrograms of dexamethasone per kilogram of body weight I.M. will cause a percipitous drop in the plasma levels of corticosteroids in normal horses. The suppression being most consistent if the dexamethasone is given at the time ACTH levels are normally peaking, late evening or early morning.

We collect our zero hour blood sample and inject at midnight. The maximal effect being at 12 to 16 hours and often persisting for more than 24 hours. Plasma cortisol levels are usually less than 1 µg/100 ml at the time of maximal inhibition. Plasma cortisol levels in horses with Cushing's Disease do not have a decrease to the same degree. Indeed, we have found only one horse in which at 12 hours post dexamethasone injection the plasma cortisol levels were less than 3 µg/100 ml. When this horse was suppressed with a smaller dose of dexamethasone (10 µg/Kg body wt.) the plasma cortisol did not decrease to the same degree as normal horses.

TITLE: Physiology of the Normal Estrous Cycle

SPEAKER: Dr. Keith J. Betteridge

Lecture summary prepared by Michael Van Noy

Riding is not necessarily a sign of estrus - standing to be ridden is a more reliable sign. Many beef breeds do not exhibit riding as often as do many dairy breeds. The period of receptivity is timed to coincide with ovulation. The actual mechanism which controls estrous behavior is the brain. The thalamus and hypothalamus are involved in integration of internal and external stimuli that control the cycle.

External Stimuli.

1. Sensory - as perceived by the 5 senses:

   (a) Light

   (b) The presence of a male. Russian workers have found that running a vasectomized male with freshly calved cows causes these cows to have stronger heats, shorter periods of estrus, and conceive 4 weeks earlier than control cattle.

   (c) Sense of smell detects pheromones. Example: Sows will show strong estrus if exposed only to odor of a boar which is sprayed on her snout. In the bull the sense of smell alerts him, too, to when a female is in estrus. This may involve a pheromone which is perhaps produced by the vestibular glands in the vagina.

Internal Stimuli. (Or hormonal control of the estrous cycle)

Ovarian steroids have a direct effect on the brain, and hence

on behavior.

Estrogens are a family of compounds which are changed during metabolism and excretion.

Progesterone is the principal progestin. Estrogens and progesterone are produced throughout the cycle, and therefore changes in behavior are probably brought about by changes in their ratio.

Estrus results from a high estrogen/progesterone ratio, not from the presence of estrogen and complete absence of progesterone. Under Normal Circumstances luteal phases and follicular make up the ideal estrous cycle:

Estrus 18 hrs. on day 0

Ovulation occurs approximately 12 hours after the end of estrus. This begins the luteal phase which lasts from ovulation, with CL formation, to about day 17, at which time the CL regresses. The follicular phase then begins and lasts through estrus until ovulation occurs again.

There is a large pre-ovulatory peak of estrogen with lesser peaks throughout the cycle. Progesterone levels rise from day 2 or 3 as the CL becomes established, maintain a plateau until approximately day 17, then drop precipitously.

An ovariectomized heifer may be brought into heat by using 0.1 mg, estradiol benzoate. Large doses, such as 10 mg., may actually make a heifer refractory to further estrogen treatment. Hence, there are dangers in estrogen therapy.

Glucocorticoids also tend to peak near the time of estrus. The

Pharmaceutical Arithmetic Quiz III

February 22, 1974 Name _____

1. 5 ml = _____ minims
2. 1 cup = _____ tablespoons
3. 3 Gm. = _____ gr.
4. 2 pints = _____ ml.
5. 1 gallon = _____ fld. oz.

Problem No. 1: If you administer IV, 10,000 units/lb. potassium penicillin G to a dog, how many micrograms of K+ is the dog receiving at this dosage level?

1 unit = 0.6 µg. potassium penicillin G ($C_{16}H_{17}KN_2O_4S$)
Molecular weight of potassium penicillin G = 372
Atomic weight of K+ = 39

Problem No. 2: In how many ml. of water should a 1/100 gr. atropine sulfate tablet be dissolved to provide a 1:10,000 solution?

major follicular phase peak of estrogen is from the major follicle under development. The lesser peaks which occur throughout the cycle are probably produced by waves of follicles which are being produced continually. Determination of the stage of the cycle by rectal palpation of follicles can be misleading, and palpation of the CL is a more accurate index of cycle stage.

Effects of Ovarian Steroids.

A high estrogen level is required to bring the animal into behavioral estrus. A high estrogen/progesterone ratio leads to a flow of cervical mucus and the characteristic increase in uterine tone at estrus - hence peripheral effects. During the luteal phase or a low estrogen/progesterone ratio, the uterus becomes prepared to receive the fertilized egg and implantation.

The Effects of Ovarian Steroids on the Brain.

The pineal gland is light sensitive in lower animals, and probably still has a function in controlling the onset of estrous cycles of seasonal breeders, such as the mare and ewe. Its significance in cattle is still unknown but in laboratory species it produces anti-gonadotropins.

Releasing factors are produced by the hypothalamus and affect the gonadotropins produced by the pituitary. In one case, the hormone produced by the hypothalamus is an inhibiting factor involved with prolactin release.

Circulating ovarian steroid levels, estrogen/progesterone ratio, determine which hypothalamic hormones will be produced and passed to

the pituitary.

Pituitary gonadotropins of concern in normal cycle regulation are FSH and LH - and possibly prolactin.

Placental gonadotropins, such as PMSG and HCG do not have exactly the same effect as do their pituitary counterparts. PMSG has "mostly" FSH-like activity and HCG has "mostly" LH-like activity but they are best regarded as distinct hormones.

Most strinking feature when measuring blood levels of pituitary gonadotropins is the sharp peak of LH at the onset of estrus. FSH peak is seen to occur at the same time as the LH peak.

Ovulation occurs as a result of a change in form of gonadotropin release, rather than proportion. It may be that follicular development occurs under a slow and steady release of the gonadotropins, whereas rupture occurs from a surge of gonadotropins, which could be LH, FSH, or both.

Gonadotropin surge is responsible for shutting off estrogen production by the follicle and initiating progesterone production. Gonadotropins have other effects on the ovary than just rupture of the single follicle and maturation of the egg it contains in preparation for fertilization.

Other immature follicles must be stimulated and brought to maturity. This recruitment of nonproliferative primary follicles to development of proliferative secondary follicles which, in turn, continue to mature or become atretic is a major function of the gonadotropins. After ovulation, continued gonadotropin support is required

for the newly-formed CL. LH is the principal luteotrophic hormone in cattle.

The uterus is involved in controlling the lifespan of the CL. The CL does not regress in hysterectomized cattle. It has been suggested that prostaglandin F2α is the uterine luteolysin responsible for regression of the CL.

It is postulated (but disputed) that prostaglandin F2α is transported to the ovary by a countercurrent exchange mechanism involving the uterine vein and the ovarian artery.

There are doubtlessly other endocrine influences on the estrous cycle which remain to be discovered e.g., the roles of thyroid, adrenal and pineal hormones.

It is important to remember that internal and external influences on the estrous cycle need to dove-tail together if normal function is to be maintained. More understanding of the normal cycle is essential if artificial control or correction of abnormalities is to be undertaken.

TITLE: The Use of Prostaglandins in Large Animals

SPEAKER: Dr. James Sokolowski

Lecture summary prepared by Michael Van Noy

Von Uller in 1934 recognized activity of prostaglandins and gave them their name. There are a number of different natural prostaglandins with different activities and these vary from species to species.

The dominant structure of the estrous cycle of cattle is the corpus luteum. The CL being present for about 80% of the cycle. The CL plays a major role in determining the length of the estrous cycle in domestic species. The CL is also involved in controlling the time of ovulation--due to the inhibition of the preovulatory surge of LH by progesterone.

One approach to cycle control is to prevent the preovulatory surge of LH by administering progestogens orally or parenterally.

Alternate method of control involves removal of the CL or by CL regression--until recently removal of the CL required use of the potentially dangerous enucleation technique or daily administration of oxytocin.

In 1965 it was first suggested that a prostaglandin might be the luteolytic factor from the uterus. This fact has since been demonstrated in most laboratory rodents, cattle and sheep.

Prostaglandin F-2α luteolytic effect is probably due to a reduction in progesterone secretion, rather than a decrease in ovarian blood flow as was previously believed.

Clinical fields of use of prostaglandins include:

1) grouping estrus in cows, ewes, and mares;
2) to terminate pregnancy as an abortifacient, or to induce term labor.

The use of 30mg prostaglandin F-2α Tham salt, water soluble SQ, produced estrous activity in cattle 2-4 days later, when injected between days 6-16 of the cycle.

Prostaglandins administered subcutaneously, intravenously, intrauterine or intravaginally, have been effective in causing luteolysis. Studies have shown that they are effective only when administered after day 5 and before day 16 of the cycle, the period when the CL exerts its effects.

The size of the CL decreases significantly within 2-4 days following injection of prostaglandin F-2α.

These results indicate that a potentially practical method for estrous grouping may exist.

The need to abort feedlot heifers and to induce timed parturition in cattle and sheep are real needs in the livestock industry.

Prostaglandins at the level of 45mg and above, terminated pregnancy in cows until about day 120 of pregnancy.

Post-abortion fertility was not affected.

Studies on parturition induction indicate that 15mg or more of prostaglandin F-2α, given subcutaneously, resulted in a mean calving interval of 3 days. An unfortunate sequela to prostaglandin-induced parturition is retained placenta. This same problem has been observed when corticoids were used to induce parturition.

TITLE: The Pharmacology of Endocrine Products Used for Treating Cattle

SPEAKER: Dr. D. R. Lamond

INDRODUCTION:

The purpose of my presentation is to:

1) Draw attention to the relatively high level of sophistication or knowledge in pharmacology and the relative naivety, old-fashionedness and even inaccuracy of the labels on the products available.
2) To emphasize some of the pharmacological aspects of some drugs currently available.

The compounds I should like to discuss fall into the following classes:

Gonadotropins

Estrogens

Progestins

Androgens

Corticosteroids

Oxytocin

Time will not permit a thorough discussion of all these hormones. I propose, therefore, to concentrate on those gonadotropin and the estrogen preparations which are available to the clinician. The discussion will include the use of hormones both to treat disorders and to enhance reproductive function beyond normal limits, that is to say, by control of reproductive function.

TWO TYPES OF GONADOTROPINS - PITUITARY and PLACENTAL

Preparations available: Available gonadotropic preparations comprise the following:

a. Pituitary preparations: FSH-P (porcine-Armour-Baldwin Labs); PLH-(pituitary LH-Armour-Baldwin Labs); Vetrophin-(Abbott, Diamond), 5 mg LH and FSH for cystic ovaries and anestrus (from sheep pituitaries).

b. Chorionic Gonadotropin: Chlorisol (Burns) 5,000 or 10,000 i.u. for nymphomania. Chorionic gonadotropin (Haver-Lockhart) for i.v., i.m. or intrafollicular injection for cystic ovaries. Also Jen-Sal and Ft. Dodge preparation.

c. Serum Gonadotropin: Freezed dried whole serum (Gonadin, Haver-Lockhart); purified preparations not easily obtained. Gonadin is for functional impotency in male and female, especially delayed sexual development.

General Characteristics:

The pituitary preparations are generally crude preparations obtained from the pituitary glands of pigs, sheep or horses. These materials have low potency on a weight basis and there are some difficulties in standardization (no pure standard). They tend to be expensive, but have a short half life, which appears to be an advantage, at least from a physiological point of view. They do not produce high titres of antibodies and hence repeated treatments may be tolerated.

The gonadotropins differ between species, especially in the βchain. They have molecular weights of about 30,000 and most consist of 2 sub-units, α and β. Pituitary hormones, FSH and LH, have many chemical similarities,

though there are also differences by chemical and biological methods of evaluation.

The pituitary preparations are extracted in such a way as to tend to be rich in either FSH or LH like activity. It has been well established that FSH and LH do not act alone. Hence, so-called crude preparations are better than apparently pure materials. By grading the doses of pituitary preparations it is possible to grow more than one follicle and/or to cause ovulation. As mentioned earlier, however, preparations available to the practitioner contain both FSH and LH and therefore it is mainly a question of determining the correct doses and intervals between doses to produce the response desired. Frequent injections can be given and the growth of follicles can be monitored by palpation or by other means. In general, there are not many situations in veterinary practice where an expensive method of growing follicles is required. However, with the present interest in egg transfer and the great value of some cows one can expect that costs will no longer be a factor and that pituitary preparations will be utilized to develop follicles in anestrous individuals, or under controlled conditions in cyclic animals. The pituitary preparations are mainly of value in anestrus although some claims have been made for their administration in cases of ovarian dysfunction such as persistent follicles. However, there seems no real need to use them under these conditions. The pituitary preparations have been used in association with progestins to cause multiple ovulation. Doses of 1 to 3 mg per day for 3 to 5 days beginning on Day 14 of an estrous cycle have caused multiple ovulations.

Labels on Pituitary Gonadotropins - references to current labeling practices in hormone preparations.

Vetrophin - indications as a pituitary extract are:

1) to correct nymphomania and infertility as a result of cystic ovaries.

[A single injection of a pituitary preparation such as Vetrophin will produce the desired results; however, it is much cheaper to use HCG.]

2) to induce heat and aid in restoring the estrous cycle to normal.

[This is a "naive and quite inaccurate description of what pituitary preparations do". It is not likely that this label would be acceptable to the FDA if presented at this time.]

3) to treat non-functioning ovaries (where ova are not produced, or ovulation is infrequent or production of estrogen or progesterone is low.

[These are difficult objectives to monitor; very rare cases or true "non-functional ovaries"; thus label is not very scientific.]

At present, more reasonable indications for pituitary preparations would be:

1) to stimulate follicle growth and maturation, particularly in anestrus or immature animals

2) to stimulate multiple ovulations

It is the responsibility of the veterinarian to understand what pituitary preparations can do and to use them accordingly.

The placental preparations, particularly chorionic obtained from pregnant womens' urine, usually assay better than 2000 international units per mg.

Commercial preparations are still relatively impure since concentrations of up to 20,000 units per mg have been obtained. The HCG preparations are relatively cheap. Because the half-life is about half a day, HCG does not exactly mimic ovulating hormones whose function it resembles, though HCG is follicle stimulating, especially if given when follicles not quite ready to ovulate. Antibody production after i.v. injection in water, is negligible although repeated treatments will result in diminished response.

Serum gonadotropin, PMSG, is generally available as the whole dried serum which is difficult material to standardize and will cause anaphylactic responses if used too often. The purified preparations of PMSG also will produce antibodies because the half-life of PMSG is 30 to 40 hr.

PMSG is produced by the endometrial cups in the mare and reaches its maximum between 50 and 80 days of pregnancy. Like HCG, which is produced by the placenta by the pregnant woman from as early as the 10th day after fertilization, its function is to support pregnancy by maintaining progesterone secretion by the corpus luteum. PMSG is believed to be responsible for the production of accessory corpora lutea in the mare.

PMSG, because of its longer half-life, 30-40 hrs, requires only single s.c. injections for its action. However, because of this, it is extremely difficult to control the numbers of follicles which are sitmulated by the compound. The PMSG molecule seems to be quite different from HCG and the pituitary preparations. It has FSH to LH activity in ratio of about 5 to 1 based on uterine weight and ovulation studies. In studies in rodents,for example, PMSG can be used both to grow follicles and to cause ovulation. In farm animals,particularly the sheep,a single injection of PMSG will cause ovulation during anestrus. It has limited use in the mare except possibly to initiate the growth of a follicle in the breeding season.

HCG can be given by any route and is often used i.v. because its primary use is as an ovulating hormone. Nevertheless, HCG will also grow follicles if used in the proper manner. Considerable skill is involved in the use of PMSG or HCG because selection of dose and the precise timing of injections relative to steroid treatment and/or endogenous steroids will markedly influence the ovarian response.

Gonadotropins are used primarily in anestrous animals, including immature animals, lactation anestrus, low body condition, or the winter and fall. Also, gonadotropins have a role in solving problems of anestrus due to such factors as cystic follicles. Doses for above indications range from 1500 to 3000 for PMSG and 2000 to 4000 for HCG. If PMSG is used too late during follicle growth, rapid growth and partial luteinization occur - ovulation does not occur.

Labels on Placental Gonadotropins.

Labeling on PMSG preparations are rather non-specific. Many of the indications for PMSG have their basis in poor husbandry, such as anestrus, or delayed sexual maturity due to poor nutrition. PMSG will cause follicle growth and ovulation in these animals but in most cases fertility will remain low.

Labeling of HCG is actually rather good. HCG being used specifically for inducing ovulation. Again, timing is critical. HCG should not be given to a growing follicle as rapid growth and failure to ovulate will result.

ESTROGENS

Estrogenic activity is a property which is shared by a great many chemical compounds including the natural estrogens, estradiol 17β, estriol and estrone. The mare urinary estrogen, equilenin, is a potent estrogen. The natural hormones are generally very active but have to be given by injection in oily solutions because they are not water soluble. The esters of the natural hormones are also insoluble in water but are more slowly hydrolysed in the animal since esterification seem to protect the estrogens from degradation by the liver. The best known esters are estradiol benzoate, estradiol propionate, estradiol valerate, and estradiol cyclopentapropionate. Numerous non-steroidal estrogens (phytoestrogens) occur in plants of the legume family. Synthetic estrogens such as diethyl stilbestrol (DES) are well known. Many of the synthetic compounds (ethinyl estradiol and stilbestrol) are active orally.

The preparations available to the veterinary profession include the natural estrogens which can be purchased from chemical firms, and dissolved in an appropriate medium such as corn oil or propylene glycol for injection. The natural estrogens are short-acting and very potent and hence are the estrogens of choice for most circumstances.

Various types of suspensions of estradiol and DES are available. Repository estrogens comprise the compound dissolved in alcohol and then emulsified in water. When the material is injected, the crystals come out of solution and form a depot from which absorption occurs very slowly. This method maintains absorption for long periods of time and is useful in those

situations which warrent a long acting estrogen.

It is worth mentioning that the term pharmacology relates to the actions of hormones above and beyond the normal. Veterinary use of estrogens is usually pharmacological and not physiological. To cause changes in the uterus of an immature lamb, for example, microgram quantities of estrogen are required. Estrus in a suitably primed ovariectomized ewe can be caused by about 15 micrograms of estradiol benzoate and estrus in a suitably ovariectomized cow can be brought about by as little as 200 microgram of estradiol benzoate.

Stilbestrol in oil is believed to be effective up to 36 hours, estradiol and benzoate dipropionate will be effective for about 48 hours or longer and E.C.P. and estradiol valerate are effective for periods of days. Estrogens are anabolic and the well-known effects of DES and Synovex (estradiol plus progesterone for heifers, and estradiol plus testosterone for steers) are based on this action. In terms of potency, estradiol is about 10 times more potent than stilbestrol on a weight basis.

Estrogens are widely used in veterinary medicine, especially to evacuate the uterus, e.g. mummified fetus or pyrometra and when used early in the pregnancy, abortion. This latter effect is probably due to regression of the corpus luteum since estrogens particularly when given in the cycle, will cause CL regression. However, two or three injections of 2-5 mg of estradiol valerate for example just a few days apart will generally cause abortion up to about 5 months. Estrogens can be used in immature or anestrous animals to cause estrus although continued use may lead to

refractory conditions. Intermittent use of progesterone will tend to maintain the responsiveness to the estrogens. DES is not a satisfactory hormone for producing estrus because the refractory condition tends to set in earlier than with estradiol and is more prolonged.

The use of estrogens mentioned above in anestrous animals to produce estrus is unphysiological because the amounts which are required under suitable conditions are extremely low and very few veterinarians give physiological doses. Pharmacological doses will interfere with gamete transport and with genital tract function. Estrogens can be used as implants especially with progesterone to initiate milk secretion. Very large doses may inhibit lactation. Excessive or prolonged dosage with estrogens, particularly DES, will produce cystic ovaries.

Estradiol under appropriate conditions (anestrus + follicle) may cause release of LH and ovulation. The problem is one of timing, but this does explain why ECP, for example, will initiate luteal function in anestrous heifers.

PROGESTINS

There are no synthetic progestins available to the veterinarian at the present time. The synthetic progestins cover a wide variety of very active and useful products but since they are at present under a cloud with FDA they are not available. The only progestin available is the natural product progesterone which can be purchased as a powder from the chemical supply houses and which is soluble in oil. A standard preparation is progesterone

in arachis or sesame or corn oil 10 mg/ml. Elanco put up a progesterone solution of 25 mg/ml. There are repositol progesterones on the market. These are progesterone in alcohol and when the alcohol is absorbed the progesterone crystals come out of solution and are absorbed from the site. Progesterone is also put up in pellets for use in horses. In general, the amounts of progesterone which are absorbed from repositol preparations or from implants are so slight as to be of negligible significance. It has beeen found that to simulate the blood levels which occur during the estrous cycle of a cow it is necessary to give about 100 mg of progesterone i.m. daily. Even from the most efficient implant it would be unlikely that these amounts would be absorbed.

Progesterone has a half-life in the blood of 3 to 5 minutes; it is normally transported by proteins. There is some evidence that when it is given in oil i.m. or s.c. its' half-life is prolonged. This may be explained by different type of binding to different serum proteins and possible also protection from degradation. **Inactivation** of progesterone takes place largely in the liver. Progesterone, like estrogen, is stored in body fat in varying quantities, depending on the concentrations in the blood. Progesterone by injection is useful in priming the genital tract and is particularly valuable in antagonizing the action of estrogen from cystic follicles. Since it has a direct action on the endometrium it is very valuable in increasing tone and activity of the atonic endometrium.

HORMONES	ACTION	DOSE	CURRENT STATUS
Progestins			
MAP (Repromix)	Suppress ovulation	180 mg/day/oral	Not available
MGA ®	Suppress ovulation	0.5 mg/day/oral	Feed-lot heifers only
CAP	Suppress ovulation	10 mg/day/oral	Not available
Progesterone	Suppress ovulation	50 mg/day/i.m.	Available
Numerous others	Suppress ovulation	silastic implants injectable combinations	None approved
Estrogens			
Estradiol valerate	Regress Corpus	5 mg i.m.	Available(medical)
Estradiol 17β	Stimulate ovulation	1 mg i.m.	Available(chemical)
Gonadotropins			
PMS	Stimulate follicles	1500-2500 i.u. s.c.	Available
HCG	Ovulation	1000-3000 i.u. i.v.	Available
Pituitary preps	Stimulate follicles and ovulation	5-10 mg i.u.	Available(limited)

TITLE: Factors Affecting the Utilization of Frozen Bovine Semen for Maximum Reproductive Efficiency

SPEAKER: Dr. B. W. Pickett

Lecture summary prepared by Michael Van Noy

Until recently most cows were bred by full-time technicians who were closely supervised by an Artificial Insemination Association. The current trend is toward the sale of semen directly to the herd owner. The herd owner,or his employee, breeds the cows, generally on a single farm. Under these circumstances,a new set of problems has been imposed upon the industry.

There is little doubt that most of the fertility problems associated with the use of frozen semen are due to improper handling or deposition, of the semen by the technician in the field or on the farm.

The comments and recommendations which follow, apply only to semen in glass ampules stored in liquid nitrogen at approximately -196°C.

Transfer precautions.

1. When the semen is delivered by common carrier, the unit in which the semen arrives must contain liquid nitrogen. Unless there is a measurable amount of liquid nitrogen in the unit,the shipment should not be accepted. The amount of liquid nitrogen should be determined, or the temperature, before adding more liquid nitrogen. If the temperature has risen above -80°C, fertility of the semen could be lowered.

2. The unit into which the semen is transferred should be full of liquid nitrogen. Frozen semen is exposed to elevated temperature:

1st when it is transferred to the technician's tank;

2nd when ampules are removed - primarily those ampules which are repeatedly exposed while others around them are being removed - when inventory is being taken.

The magnitude of the temperature rise is determined by:

1. length of exposure
2. ambient temperature
3. air circulation
4. intensity of solar radiation
5. level on nitrogen in the tank
6. height to which the canister is raised.

As ampules are exposed to room temperature, the motility decreases. Decrease in motility correlates well with a decrease in fertility. When working with dry ice, motility decreases more rapidly during the same length of exposure than when working with liquid nitrogen.

It is important to keep the number and length of exposures to an absolute minimum.

Ampules should be moved from the field unit to the thawing container in as little time as possible. With practice and the proper arrangement, this may be done in 3-5 sec. If an ampule is not removed in 10-11 secs., the canister should be lowered into the tank until the temperature of the uppermost ampules has returned to the temperature of the tank. The time required for this to occur depends on the amount of liquid nitrogen in the tank. A correct up-to-date inventory and a knowledge of the precise location of the ampules will reduce the number of exposures required to retrieve a particular ampule. Ampules should not be elevated above the frost line.

Thawing.

There is considerable difference of opinion in regard to the proper thawing technique for maximum fertility. In a series of laboratory studies, it was found that there was no difference in motility between

thawing in ice water at 1°C and water held at 40°C. All other methods resulted in lower survival of the spermatozoa. The difficulty of maintaining a water bath at 40°C usually eliminates this method of thawing frozen semen. Thus thawing in ice water becomes the method of choice.

Steps which should be taken in preparation for a days breeding operation.

1. Prepare an ice bath at least 30 min. in advance. Use a minimum of 1 pint water.
2. Have an arrangement which prevents the ice cubes from coming in contact with the ampules which might freeze to the ice cubes.
3. Remove ampules and place them in the thawing container as quickly as possible and in such a manner as to prevent them from freezing together, causing the rate of thaw to decrease
4. Allow 8-10 minutes for the ampule to thaw before removing it from the thawing container.
5. Do not disturb the ampule while it is thawing.
6. Do not use the ampule after it has been thawed for more than one hour.
7. Ampules should be dried and wiped clean before cutting and breaking the neck.
8. The ampules should always be removed and held in the upright position to avoid loss of semen in the top of the ampule.
9. The catheter should be inserted slowly and withdrawing semen as it is lowered. The catheter and the ampule should be held in a position that allows all the semen possible to be withdrawn without breaking the liquid column.

The rate at which the semen was expelled from the catheter had a significant effect upon the amount of semen removed from the catheter; more semen was removed using a slow squeeze. A steady 5-second squeeze was the best method, resulting in delivery of about 95% of the semen.

TITLE: Response of the Bovine Fetus to Infectious Agents

SPEAKER: Dr. B. I. Osburn

Lecture summary prepared by Michael Van Noy

Most veterinary pathology laboratories report that they can make a successful diagnosis in cases involving abortion in 1 out of 5 cases. One lab using all available methods to arrive at a diagnosis, reported the average cost per fetus examined to be in excess of $120.00.

It is not known why the bovine is so susceptible to congenital infection. Factors which influence fetal susceptibility:

1) The fetus is agammaglobinemic;
2) No antibodies are present within the fetal calf;
3) In the bovine species maternal antibodies do not cross the placenta;
4) No passive immunity until after birth when calf consumes colostrum.

Comparison of maternal vs fetal serum by means of electrophoresis again demonstrated the absence of gamma globulins in the serum of the bovine fetus.

Investigation with the fetal lamb has shown these organs to be involved in producing an immune response: thymus, spleen, lymph nodes, Pyers patches in the intestines.

Lymphocytes which are involved in producing an immune response are first seen in the thymus in fetal lambs at 42 days of gestation. The fetus develops in a sequential manner the ability to produce antibodies to various substances. If infection of the fetus occurs prior to its ability to mount an antibody response to the particular infecting agent then the

fetus will probably succumb to the infection.

Innoculation of fetal calf with vibrio fetus prior to 6 months gestation, the organism will kill the fetus within 3-4 days. It is expelled 2-3 days after the fetus dies. However, injecting the agent vibrio fetus after the 7th month of gestation, the fetus generally survives the infection and calves are born alive although they may die shortly after birth, but antibodies are now present in their circulation.

Studies involving innoculation of Blue Tongue virus into the fetal lamb at about 50 to 55 days result in severe necrosis of the brain. Process seems to be similar in calves when innoculated at 90 days of gestation, resulting in so-called dummy calves.

Again,in studies with lambs, it was found that innoculation during the first half of gestation resulted in lambs born with severe hydranencephaly. As a result of necrotizing lesion, virus may easily be recovered from the brain until day 150. And no evidence of antibody production can be detected. Once antibodies are produced in the fetus then the virus can no longer be isolated.

In the bovine fetus, the absence of lymphocytes or active germinal centers indicates a lack of immunological competence. First evidence of immunoglobulin Classes IgM and IgG are found at approximately 120 days of gestation. The levels of these globulin continue to increase to reach minute levels at birth in noninfected animals.

In animals which have been infected, examination of lymph nodes indicate an increase in lymphocytes and plasma cells. The plasma cells are responsible for the production of specific serum antibodies.

It is now felt that if serum samples or possibly peritoneal fluid samples were collected from aborted fetuses and examined for elevated immunoglobulin levels that a more accurate diagnosis as to the cause of the abortion could be made.

TITLE: Estrous Cycle of the Dog - Physiological

SPEAKER: Dr. George H. Stabenfeldt

Knowledge of the estrous cycle of the bitch has been slow in developing in comparison to our so-called large domestic species of animals. This is undoubtedly a result of the availability of monies in Agricultural Experiment Stations for reproductive work in economically important species. One of the first reports on the reproductive cycle of the dog was by one of our long-standing faculty members at Davis - Dr. Harold Cole - who, together with H. M. Evans, published on the estrous cycle of the dog in 1931 (Memoirs of the University of California, Vol. 9: 65-101). It was not until the latter part of the 1960's that knowledge of the basic physiology of the bitch's estrous cycle began to unfold. The impetus for this work, at least in part, arose from a concern over control of our dog populations. The last year or two has shown a tremendous interest by the public in ways to inhibit fertility in dogs.

The bitch has a number of unusual aspects of the reproductive cycle in comparison to the cow, sow, ewe and mare. These differences will be emphasized in this discussion - as well as points of similarity.

First of all, the estrous cycle of the bitch is prolonged with an average interval of 7+ months (range 6.5-11.5 months). A seasonal aspect as to the occurrence of estrus has been suggested by a number of investigators. Data from recent investigators, Christie & Bell & Sokolowski, however, have emphasized the rather uniform occurrence of estrus during the year.

Sokolowski has made the point that with a 7+ month interval between cycles, most dogs will eventually exhibit heat during all 4 seasons - not in the same year, of course. Proestrus averages approximately 9 days, estrus 10 days, metestrus 80 days and anestrus 115 days. The first slide [Slide 1] taken from McDonald emphasizes these relationships. One of the first discrepancies in nomenclature is apparent through the lack of use of the term "diestrus" - normally used to indicate the period of full luteal activity, with metestrus used to indicate the period of transition from follicle to CL - usually of few days' duration. In the dog, metestrus has been used to indicate the time corresponding to both metestrus and diestrus in our large animal species. Several people have used the term "metestrus" to indicate the entire time from ovulation to involution of the uterus (150 days). I don't believe this is proper - the term in the dog has been used to indicate only the time of luteal function, and this is even not proper in terms of prior usage in other animals.

In the last few years, the endocrine events that resulted in ovulation have been well delineated in a number of species. In this instance, the bitch is no different. The next slide [Slide 2] Jones et al. shows the endocrine events that result in ovulation. Increasing levels of estrogen during proestrus, presumably from follicles developing under the influence of FSH and some LH, finally cause a positive feedback release of LH, and possibly FSH, which results in ovulation usually within 24 hours. The question is still not clearly answered as to when the LH release occurs. In one report by Smith and McDonald, who studied young females, the LH release occurred on the last day of proestrus with ovulation occurring on

the first day of estrus, probably within a matter of a few hours. Phemister et al. have described an LH surge occurring at the onset of estrus, with ovulation occurring approximately 2 days later, i.e., on days 2 and 3 of estrus. Dr. Bud Andersen, Radiobiology Laboratory, UCD, has indicated that he has collected data in young bitches which indicates they tend to ovulate within the first 24 hours of acceptance, while older bitches may ovulate slightly later. We have discussed the possibility that older bitches may become sexually receptive sooner in relationship to the changing endocrine events which would tend to place ovulation deeper into estrus. In any case, Dr. Andersen feels strongly that ovulation time is a function of age.

The ova of the dog are shed prior to the extrusion of the first polar body. This probably means the ova are not fertilizable until about the second day of estrus, if ovulated on day 1 of estrus. It may also mean that these ova may be more resistant to aging changes as compared to our domestic species. Gier has reported inseminations resulting in pregnancy as early as 3 days before ovulation and 2 1/2 days after. However, one has to wonder at the precision of his knowledge of the time of ovulation.

It is of practical interest that very little success has been reported by investigators as to the initiation of proestrus, estrus and ovulation by hormonal means. Several investigators have been able to induce estrus and some follicle growth by the use of FSH or PMSG, but not much success has been reported on the actual occurrence of ovulation.

[Slide 3] The dog is unusual as concerns the association of sexual receptivity and ovulation. The sow, ewe and mare ovulate shortly before the

end of heat, the cow after heat is ended. The dog, on the other hand, is often in heat for 7 or 8 days following ovulation, even in the face of active CL. The reason for this prolonged receptivity in the presence of active CL is not apparent, although the presence of large, unovulated follicles has been reported during the entire time of estrus in the dog, which could result in significant production of estrogen. Estrogen seems to be declining several days prior to first refusal in that vaginal cytology is beginning to change at this time.

[Slide 4] The dog is also unusual in that the CL function is essentially the same length of time regardless of whether pregnancy ensues or not. The typical pattern of CL function, as indicated by progesterone secretion into the blood in the nonpregnant animal, is as follows: Progesterone levels peak about 20-25 days postovulation with the establishment of a rather transient plateau, followed by a prolonged decline with progesterone becoming non-detectable at about 75-80 days postovulation. This is almost identical to the progesterone pattern in the pregnant animal, with some slight modifications. Smith and McDonald have reported an enhancement of luteal function at about day 15 postovulation in the pregnant animal, with resultant higher progesterone levels as compared to the nonpregnant animal. This is suggestive of the presence of a placental luteotrophin. Other workers, however, have not observed this. Also, it is of interest that CL function appears to cease about 24 hours prior to delivery in the bitch, i.e., about day 62 of gestation, as per the work of Concannon & Hansel. These differences, however, should not detract from the striking similarity between CL function in the nonpregnant and pregnant animals.

Another point of interest in the dog involves the length of time required for uterine involution. The studies of Dr. Bud Andersen, Radiobiology, UC-Davis, have been particularly enlightening. He has found that uterine involution is not complete in the dog until about day 150 postovulation in either the animal undergoing a pregnancy or even the nonpregnant animal. This prolonged involutionary period probably comes about because of the prolonged luteal phase of the cycle, coupled with an apparent genetic sensitivity to progesterone. These prolonged periods of endometrial stimulation certainly predispose to glandular hyperplasia and cyst formation which, in turn, establish an environment conducive to the development of the endometritis and pyometra so commonly observed in the dog. Knowledge of the prolonged involutionary period could be valuable from the view of safe hormonal control of the estrous cycle. It would appear that administration of a progesterone-type compound during any period in which involution of the uterus is still underway could prolong the involutionary period--thus further predisposing the animal to the onset of the cystic hyperplasia-endometritis syndrome. It would appear that the safest time for drug administration would be approximately 5 months after the onset of estrus.

Studies involving hysterectomy during the luteal phase of the cycle in the cow, sow, ewe and mare have indicated CL lifespan is prolonged in this situation in these species. From this has arisen the concept that the uterus produces a substance - luteolysin - during the late luteal phase of the cycle which is eventually transported to the ovary causing regression of the CL. There seems to be no such uterine control over the ovary in the

dog. While the effect of hysterectomy on ovarian activity in the dog is not particularly well documented in the scientific literature; the fact is ruefully attested to by veterinary surgeons who have failed to completely remove the ovaries with an eventual return to cyclic ovarian activity, including heat. From an academic view, the very prolonged decline in progesterone in the bitch suggests that the regression is gradual - a "wearing out" process - and not caused by an active substance which would result in a precipitous decline in CL activity.

[Slide 1] A syndrome is observed in the nonpregnant bitch in which clinical signs of pregnancy occur, namely, gradual deposition of abdominal fat, mammary gland development, mothering instincts at approximately 63 days postovulation, and even lactation. This syndrome is undoubtedly brought on by the endocrine environment of the animal, i.e., the presistence of CL over a relatively long period of time. There is no evidence to date, however, that progesterone levels are different in pseudopregnant females. Thus the specific endocrine basis for pseudopregnancy is not understood. One comment about terminology: Some individuals have called all nonpregnant bitches that are in metestrus as being "pseudopregnant". I think this is an improper usage of the term. A prolonged interval between heats is normal in the dog - it certainly is not reason to say a dog is pseudopregnant - thus, I think only those animals that have actual clinical signs suggestive of pregnancy, but who are not, should be called "pseudopregnant".

In the realm of behavioral changes, it is of interest that bitches show, at times, unusual patterns prior to proestrus. Improvement of appetite and appearance, preferred association with males, and resentment of

females have been observed several weeks before the onset of proestrus. Immediately before proestrus, changes such as listlessness, inappetence and nervous signs, including convulsions (admittedly rare) have been seen. These signs disappear at the start of proestrus. Homosexual mounting behavior has also been observed most commonly during metestrus.

TITLE: Normal Canine Estrous Cycle - Structural

SPEAKER: Dr. Victor M. Shille

Gross and microscopic anatomical changes accompany the rise and fall of hormones occurring during the estrual cycle.

Clinical signs consist of changes in the appearance of the vulva and in the vaginal discharges. In the light of much of the present knowledge it seems that these signs are still most reliable indicators of the sexual state of the bitch, furnishing us with a valuable diagnostic framework useful for arranging laboratory data in a meaningful way.

In proestrus the influence of rising estrogens causes edema of the vulva. The swelling is very firm and can be surprisingly large in certain breeds. The vaginal discharge consists of pure blood, but it should not clot. Appearance of clots should make one suspicious of persistent proestrual bleeding caused by follicular cysts or of other abnormalities.

Shortly before ovulation occurs, plasma estrogens are seen to drop and after the ovum is released their influence diminishes and is replaced by the rising progestins. Vulvar edema decreases resulting in a softer, more pliable texture. The vaginal discharge loses its intense redness and becomes more serous or occasionally brownish tinged. This is estrus or the period of acceptance. Behaviorally estrus is signified by willingness to stand for the male in a characteristic stance with rear legs firmly planted and tail skewed to the side. I might add here that the bitch will not always stand for every male, and may be quite choosy in this respect.

Concurrently with the rising progestin levels the bitch begins to refuse advances from males and soon becomes not sexually attractive herself.

Her vulva loses its turgidity and the discharge pales and gradually disappears. She enters thus the state of metestrus dominated by the corpur luteum and its progestin output. Unless false pregnancy occurs, the bitch will be clinically and behaviorally indistinguishable from an animal in anestrus or the quiescent period.

The most commonly employed practical laboratory aid for determining the sexual status of the bitch is the vaginal smear, stained and examined for changes in the exfoliated cells. Collection of the cells can be accomplished by means of aspirating with a pipette, by rolling a sterile cotton swab around the vaginal tract, or by gently scraping with a spatula made of wood or metal. In our experience we have found that the cotton swab or the disposable wooden spatula (coffee stirring sticks) that has been soaked in Zephiran have been the most satisfactory. The use of the spatula results in slightly better looking, more distinct epithelial cells. When rolling the swab or wiping the spatula across the slide one should make the motion in one direction only, to prevent scrambling the cells by a back and forth movement. Staining can be accomplished by Wrights stain, by Giemsa, by rapid methylene blue or by Shorr's trichrome stain which will give a clear indication of the cornification index of the cells. Christie and coworkers have recently made a concentrated effort to organize the vaginal smear in a quantitative manner. Using Schuttes classification of exfoliated cells they name four classes of epithelial cells that vary with the stage of the cycle. (Slide 1): This is a high dry power view of Giemsa stained vaginal smear of a bitch in proestrus. Note the presence of erythrocytes and of the noncornified cells. Here is a good example of what Christie terms the parabasal cell. Note the rounded cytoplasm and large healthy

nucleus. (Slide 2): This is the next stage of cornification; the cytoplasm is squarish and more brittle, the nucleus is still well. (Slide 3): This is an intermediate cell. Here is a square-shaped cytoplasm but the nucleus is fading. In some the nucleus will become pyknotic, very dark and small. (Slide 4): This is the next stage of cornification, called the superficial cell. As the influence of estrogen progresses, cornification of the epithelial cells will proceed also, so that the more cornified stages will appear in relatively larger numbers. (Slide 5): Finally, when estrogens peak just before ovulation, the most cornified stage will predominate. This, for obvious reasons, is called the anuclear cell, and it appears in early estrus. As estrogens drop in peripheral blood and their influence on the vaginal epithelium decreases, cornification disappears, and in late estrus, just before refusal, (and of course in metestrus) the noncornified cells appear again, accompanied, however, not by erythrocytes but by neutrophils. As all of you know, and as Christie has shown by counting the cells in vaginal smears, the situation is far from simple, because each individual bitch will vary considerably from the presented average. This makes repeated smears necessary to arrive at an intelligent conclusion. Changes surrounding ovulation appear so gradually in the vaginal smear that it is virtually impossible to pinpoint ovulation to a day.

Fluctuations of body temperature have been measured by Spano at Colorado and Christie and coworkers. It appears not to be a reliable way to determine ovulation time in the bitch.

Abdominal palpation, however, does offer an additional means of estimating the cyclic state of the bitch. It has been found to be successful in

bitches that are calm and not obese. Christie and coworkers, as well as Sokolowski in this country, have performed measurements on the uterus of the cycling bitch. The outline of the quiescent uterus is rather flattened but as proestrus approaches it becomes rounded and enlarges gradually to reach its largest diameter in late estrus. This enlargement persists through metestrus after the peripheral progestins have dropped to base levels. Sokolowski has found that the uterine index, that is, diameter times length expressed in square millimeters, returns to pre-proestrual levels about 120 days after initial bleeding commences.

Qualitatively, the histological changes induced by the variations in the relative amounts of estrogen and progestins, have been studied by Sokolowski, and earlier by R. M. Mulligan. Bud Andersen, from UC Davis Radiobiology Laboratory, has kindly provided us with examples of these changes as they occur in the cycling bitch. Incidentally, some of this material will be included in Dr. Andersen's soon-to-be-published book on the ovary of the dog.

(Slide 6): This depicts an ovary with a ripe Graafian follicle. This bitch has accepted a male for the first time about one hour before this was obtained. Notice the ovum still present in the follicle. (Slide 7): This shows ovulation occurring - the follicle has ruptured and the ovum is gone. Liquor folliculi are streaming out and Theca and Granulosa cells are in the antrum, also free blood from the ruptured capillaries. This was taken from a bitch that accepted a male three hours before the tissue was harvested. (Slide 8): Twenty six days after ovulation, we see the corpora lutea that have formed from the ovulated follicles. Remember that the peripheral progestins are just past their peak at this time. These corpora are the source

of the progestins we measure in the blood. (Slide 9): At 90 days after ovulation you will note that the corpus luteum has degenerated. At this time you will remember the plasma progestins have reached their basal level. However, look what is happening in the uterus. (Slide 10): A section taken 98 days after ovulation reveals fluid in the lumen, a well-developed endometrium with active glands and a thickened myometrium. Certainly not a completely involuted uterus as shown in this section (Slide 11) taken by Dr. Andersen 150 days after ovulation.

As we look at these rather startling facts, we should realize that the bitch has taught us a rather valuable lesson.

Having heretofore concentrated our research in reproductive physiology on a few domesticated species on the farm and in the laboratory, we have had a tendency to measure all females with the same yardstick, as it were.

The bitch's cycle is unique in its apparent domination by the corpus luteum that gradually wears down rather than being subject to luteolyzing influences. Its effects dominate the endocrine profile for a very long time, subjecting the body of the bitch to prolonged progestin action. It almost seems as if pregnancy would be a normal state for the bitch, and we can't help but agree with Conway when he states that in natural populations a nonpregnant cycle is a pathological luxury that is to be avoided.

We need to keep these thoughts in mind as we work on solutions for canine uterine disease and methods of canine contraception.

TITLE: Pharmacological Control of Canine Fertility

SPEAKER: Dr. James Sokolowski

Lecture summary prepared by Michael Van Noy

Assumptions involving the pet population explosion: 210 million people in U.S.; 33% of canine population are fertile females, capable of producing an average of 4 puppies per litter; 1 owned dog per 7 people = approximately 30 million owned dogs.

Several potential ways of controlling the estrous cycle of the bitch:

1. Block the releasing factors for the gonadotropin
2. Block FSH release
3. Block LH Release
4. Block the receptors at the target tissues
5. Block the receptor sites for estrogen
6. Block pheromone production.

As early as 1948, progestational steroids have received major attention as a method of altering cyclic activity.

Murray & Eden 1952 first successful attempt to control estrus in canine. Repository progesterone 1.0mg - 1.5mg/lb. bodyweight. Repeated 14 - 21 day interval delayed estrus. Necessity for repeated injections has limited use.

Evaluation of orally active progestational steroids 1960 & 1961. Hydroxyprogesterone Acetate (Brian) 2.5mg/kg orally/day - resulted in

estrus delay when given in suspension, but this dose was not effective when given mixed with dog food.

1962 (Brian) reported on use of medroxyprogesterone acetate. (Promone) 50mg SQ at 6 month intervals delayed estrus - prolonged delay without repeated injections.

Harris & Woolchuck 1963 oral megestrol acetate for estrus inhibition in the dog. .01mg/kg daily. Minimal effective dose.

Evans 1969 reported that one problem with megesterol is timing of administration. If started too late then estrus will not be inhibited and subsequent estrus will occur earlier.

Melengesterol acetate (MGA) orally,at 80 micrograms/10kg daily inhibits estrus for 365 days. Ethamoxytriphetol, a nonsteroidal estrogen antagonist in bitches, given in proestrus and estrus orally, prevented mating in some cases and pregnancy in all cases.

Nonprogestational steroid, generic name mibolerone, now undergoing clinical testing. Given orally on continuous basis will prevent estrus in majority of bitches.

Progestagens can and do have deleterious effects in the bitch. It is difficult to make similar statements concerning the queen due to lack of carefully controlled studies in the feline. Progesterones have been demonstrated to cause endometrial gland growth and endometritus can be caused by this hormone alone.

Dow reported that daily injections of progesterone for 60 days resulted in cystic changes in the endometrium.

Cystic endometrial hyperplasia occurred following use of long-acting progesterone, medroxyprogesterone acetate, promone.

Other side effects associated with the use of various progesterones include stimulation of mammary nodules. Under experimental conditions, progesterone has been shown to cause a mild transitory increase in blood glucose and cholesterol.

Mibolerone, being anabolic and mildly androgenic, does result in some enlargement of the clitoris and reversible ossification of the os clitoris. Studies have not revealed estrogenic or progestational activity in the bitch and therefore have been devoid of side effects associated with these compounds.

Therophalyne is a compound which is supposed to eliminate the mating odor, reduces chances of breeding.

Estrogens such as DES and ECP have been used within 72 hours of mating to terminate a pregnancy. ECP 1mg/10kg bodyweight IM. Estrogens also have side effects, including bone marrow depression and thrombocytopenia leucocytosis followed by leucopenia and even pyometra. These effects may be produced by the same dose as used for therapy.

Use of prostaglandins as luteolytic agents in terminating pregnancy, F2α Tham salts. etc., sometimes required multiple injections to obtain the desired results.

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Mibolerone, being anabolic and mildly androgenic, does result in some enlargement of the clitoris and reversible ossification of the os clitoris. Studies have not revealed estrogenic or progestational activity in the bitch and therefore have been devoid of side effects associated with these compounds.

Therophalyne is a compound which is supposed to eliminate the mating odor, reduces chances of breeding.

Estrogens such as DES and ECP have been used within 72 hours of mating to terminate a pregnancy. ECP 1mg/10kg bodyweight IM. Estrogens also have side effects, including bone marrow depression and thrombocytopenia leucocytosis followed by leucopenia and even pyometra. These effects may be produced by the same dose as used for therapy.

Use of prostaglandins as luteolytic agents in terminating pregnancy, PGa Tham salts, etc., sometimes required multiple injections to obtain the desired results.

TITLE: Immunologic Control of Reproduction in Dogs

SPEAKER: Dr. Mauricio H. Pineda

The American Humane Association has stated (1) that it is an indisputable fact that the United States is faced with a tremendous surplus of unwanted dogs and cats. Handling millions of surplus dogs and cats is not prevention but a short-range attempt to meet an immediate need caused by a universal problem which has reached epidemic proportions. The problem and its increasing severity are apparent when one considers that while 415 human being are born each hour in the U.S.A., 2,000 - 3,400 dogs and cats are born during the same time (2).

Our interest in the immunologic control of reproduction stems from the discovery that active immunization against a purified preparation of bovine luteinizing hormone (NIH-LH-B3, BLH) was associated with severe atrophy of the primary and secondary organs of reproduction in male (3) and female (4) rabbits.

In further studies (5), we found that antibodies in rabbits immunized against BLH also cross-reacted with factors (presumably gonadotropins) in crude extracts of canine and feline pituitary glands.

These findings raised the possibility that if bovine LH would

stimulate the production of antibodies in dogs and cats, those antibodies might neutralize endogenous gonadotropin and cause atrophy of the reproductive organs in these species as well.

In further studies (6), male dogs were immunized against BLH. Reproductive function in these dogs was severely impaired for as long as a year after the first dose of the immunogen was given. We used 6 groups of 4 adult male dogs each. We used male dogs in this study since we could monitor the effects of immunization by studying sexual behavior and seminal quality. Bitches were not included because of the long interestrous interval; the bitch is a remarkably poor experimental model as compared to the male dog in which the reproductive function is continuous. Moreover, we had previously shown (3, 4) that the reproductive organ atrophy in rabbits immunized against BLH was present in males as well as females. In this experiment we studied the short-term (15 weeks) and long-term (52 weeks) effects of active immunization. In the short-term experiment, the immunized dogs at necropsy had considerable atrophy of testes and sex accessory organs, including degenerative changes in the germinal epithelium of the seminiferous tubules. The BLH-immunized dogs ceased to ejaculate by the 5th week post-immunization. The atrophic and degenerative changes of the reproductive organs were also present in the immunized dogs of the long-term experiment.

Antibody titers in the serum of the immunized dogs correlated negatively with androgens levels. While the antibody titer to BLH was high, the serum androgens in terms of testosterone levels were low.

We did a pilot study in which a single male dog was immunized against a commercial preparation of ovine gonadotropins. Severe testicular and sex

accessory glandular atrophy ensued.

These studies provided ample evidence that an immunologic approach to the control of reproduction in dogs was scientifically feasible. However, the immunogen (except for the dog which was immunized with the commercial preparation of ovine gonadotropins) was available only in experimental, limited quantities from the National Institutes of Health. Moreover, the immunization schedule in these early studies consisted of a series of 12 injections over a period of 94 days (3-6).

We were concerned that any of the commercially available gonadotropins would be so badly contaminated with other pituitary hormones - which could be as good as or more antigenic than the gonadotropic hormones- that side effects could be a serious problem in developing a practical immunological approach to the dog population control. We decided to study the effects of immunizing dogs against human chorionic gonadotropin (HCG), since this hormone is readily available and free of pituitary contaminants.

An investigation was designed (7) to study the possibility of stimulating immunity against gonadotropins with a single administration of the immunogen. If this was not possible, the impairment of the reproductive function, we have described, would be experimentally fascinating but not technologically feasible for practical application to the problem of controlling dog population.

Dogs immunized against a single dose of HCG had antibody titer from 4 to 24 weeks after treatment, and the antibodies were biologically active in mouse uterine weight neutralization assays; unfortunately, these antibodies to HCG failed to cross react with canine pituitary preparations in radioimmunoassay, microimmunodiffusion and in bioassay.

Even though the dogs immunized against a single dose of HCG showed no appreciable changes in reproductive function, this investigation suggested that it was possible to induce antibodies against a gonadotropin by the administration of a single dose of the immunogen.

In a preliminary study still in progress, we immunized 3 male dogs with a single dose of a commercially available ovine pituitary gonadotropic preparation, containing both LH and follicle-stimulating hormone (FSH) activities. Each dog had ceased to ejaculate by 21 days after immunization, failing to produce pre-sperm, sperm-rich or post-sperm fractions in spite of prolonged digital manipulation in the presence of a bitch in estrus. All 3 dogs remained with ejaculatory arrest for 140 days after the immunizing dose of ovine pituitary gonadotropins. One of the dogs (No. 8150) recovered ejaculatory capability at 147 days post-immunization, and reached pre-immunization values of total number of sperm cells per ejaculate at 168 days post-immunization. The second dog (No. 7538) recovered ejaculatory capability at 168 days, and reached pre-immunization values of total number of sperm cells per ejaculate at approximately 196 days post-immunization, and the third dog started ejaculating again at 210 days post-immunization but failed to reach pre-immunization levels of total number of sperm cells per ejaculate even at 49 days after this dog recovered ejaculatory capability.

When boostered with a single dose of the ovine pituitary gonadotropin 2 dogs (Nos. 6253 and 7538) ceased to ejaculate again at 21 days post-booster, while the third (No. 8150) ceased to ejaculate at 28 days post-booster.

Erection and libido were not apparently affected.

Testicular diameter, as measured through the scrotum with calipers, was significantly decreased between 35 and 126 days post-immunization,

reached pre-immunization size between 133 to 189 days post-immunization, and was significantly decreased again at 21 days post-booster injection of the ovine gonadotropic preparation.

Microimmunodiffusion studies with the antisera of dogs immunized against the ovine pituitary gonadotropin indicated the presence of precipitating antibodies against the immunogen. Radioimmunoassay studies using the antiserum from the immunized dogs and high purified ovine LH-^{125}I demonstrated the presence of antibodies to LH. High antibody levels, in general, were detected in the serum of immunized dogs when they were in ejaculatory arrest.

Except for an inflammatory reaction and abcessation at the site of injection of the immunizing dose (when the immunogen was given in adjuvant) we have not observed clinical side effects in the immunized dogs. No inflammatory reaction at the site of injection was observed when the booster dose of ovine pituitary gonadotropin was given.

Our finding that immunization of male dogs against a commercially available ovine pituitary gonadotropic preparation induced the formation of antibodies which were and continue to be associated with impairment of reproductive function, constitutes a significant step forward toward a practical method for dog (and probably cat) population control. THIS FINDING CLEARLY INDICATES THAT IT IS FEASIBLE TO INDUCE PROLONGED BUT REVERSIBLE DYSFUNCTION OF THE REPRODUCTIVE SYSTEM WITH A SINGLE IMMUNIZING DOSE OF A COMMERCIALLY AVAILABLE PITUITARY GONADOTROPIN.

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Total Number of Sperm Cells/Ejaculate (X 10^6) in Dogs Immunized against Ovine Pituitary Gonadotropin

Days after immunization	Dog number		
	6253	7538	8150
- 5	672	500	432
0	680	546	714
14	0	450	15
21 - 140	0	0	0
147	0	0	8.3
154	0	0	54.4
161	0	0	180
168	0	21	320
175	0	176	392
182	0	135	910
189	0	270	1,206*
196	0	300	1,272
203	0	182	231
210	0.2	308*	3.2
217	0.1	360	0
224	0.8	50	0
231	1.5	0	0
238	1.2	0	0
245	3.2	0	0
252	2.0*	0	0
259	1.6	0	0
266 - 273	0	0	0

Dogs received a single dose of ovine pituitary gonadotropin in Freund's complete adjuvant at Day 0.

*Booster injection of ovine gonadotrpin in saline only.

Average Testicular Diameter of Dogs Immunized against Ovine Pituitary Gonadotropin

Days	Testicular diameter (cm.)
- 5	4.1 ± 0.3 [a]
Immunization*	4.0 ± 0.4 [a]
14 - 28	3.6 ± 0.3 [a]
35 - 126	2.5 ± 0.3 [b]
133 - 189	3.4 ± 0.3 [a]
Booster**	3.7 ± 0.2 [a]
7	3.8 ± 0.3 [a]
14 - 21	3.0 ± 0.2 [b]

* Ovine pituitary gonadotropin in Freund's complete adjuvant

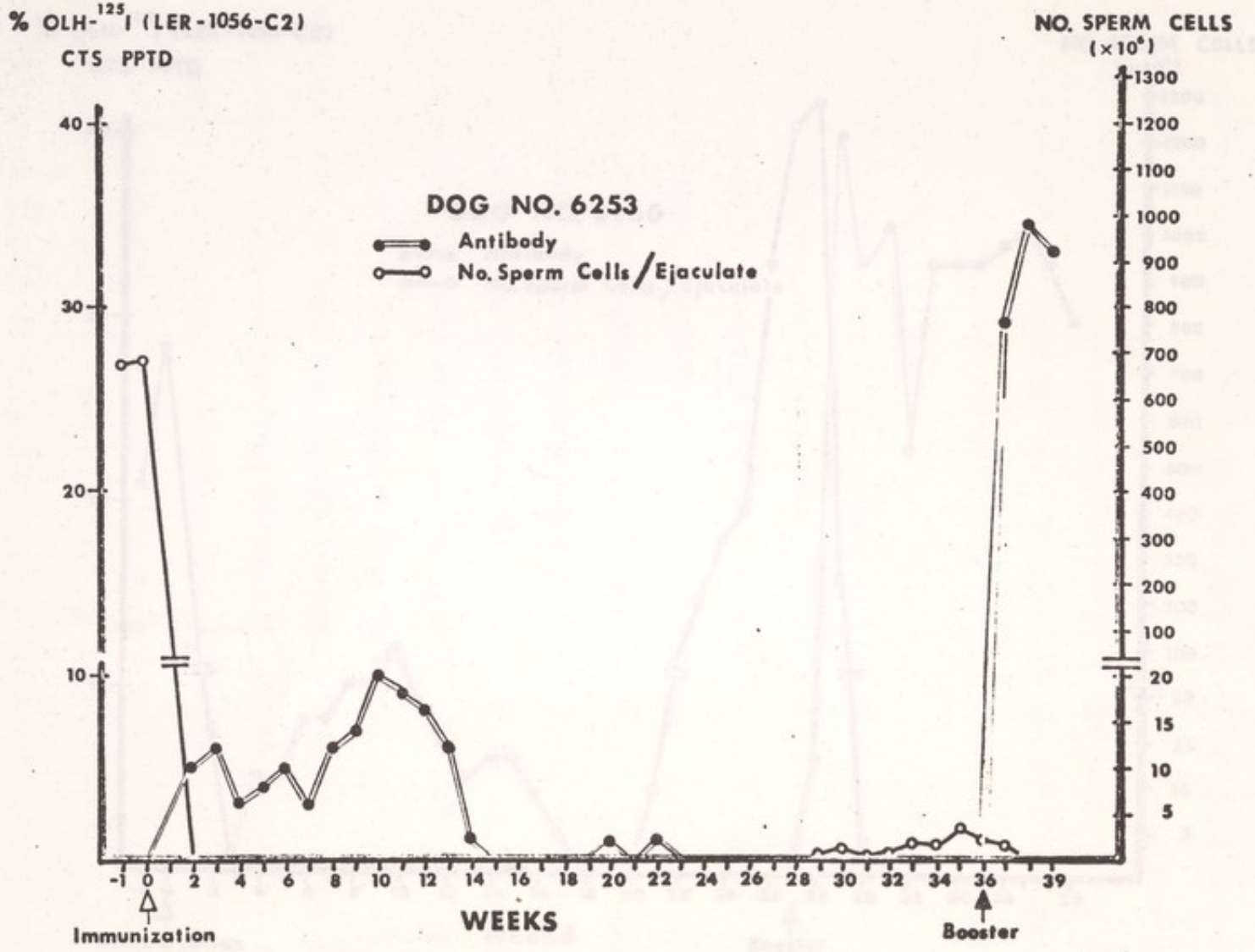
** Ovine pituitary gonadotropin in saline solution

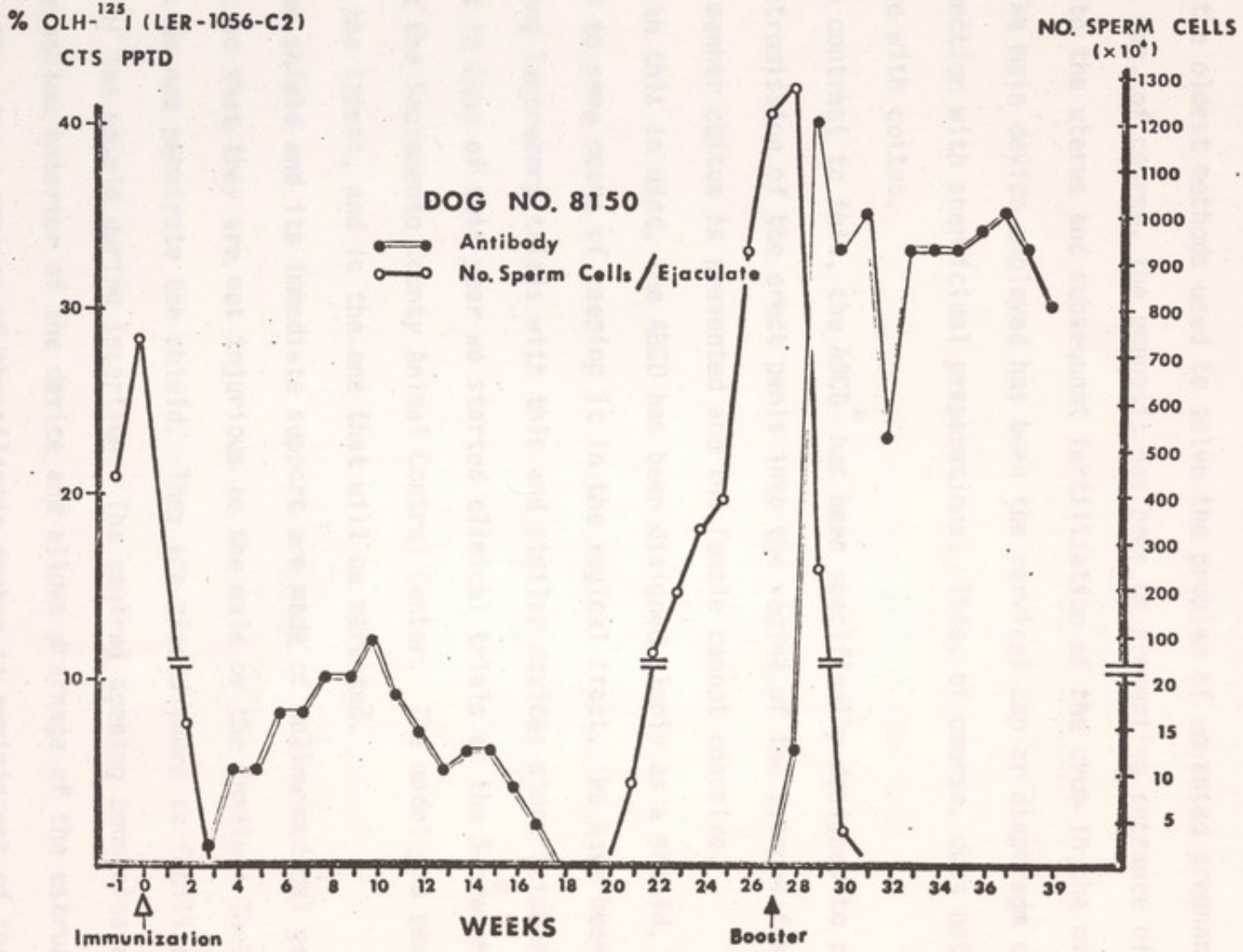
Values are given as mean $\pm$ standard deviation for 3 dogs. Means with a different superscript are significantly different ($P < 0.05$).

Summary of Results Obtained at C.S.U. on the Effects of Immunization against Pituitary Gonadotropins

Species	Hormone used as antigen	Immunization	No. of immunizing doses	Effects on sex organs	Days after first immunizing dose
Male rabbits	Bovine LH	Prolonged	12	Atrophy and degenerative changes	112
Female rabbits	Bovine LH	Prolonged	12	Atrophy and degenerative changes	112
Male dogs	Bovine LH	Prolonged	12	Atrophy and degenerative changes	105
Male dogs	Bovine LH	Prolonged	12	Ceased to ejaculate	35 to 364
Male dog	Ovine Pituitary Gonadotropins	Prolonged	12	Atrophy and degenerative changes	150
Male dogs	Ovine Pituitary Gonadotropins	Short	1	Ceased to ejaculate	21 to 140*
				Decreased testicular diameter	49 to 140

* Recovered ejaculatory capability at variable times after immunization (147, 168 and 210 days), but ceased to ejaculate again 21 days after a booster injection of the immunogen.


% OLH-^{125}I (LER-1056-C2)
CTS PPTD
NO. SPERM CELLS
(×10^6)
DOG NO. 6253
Antibody
No. Sperm Cells/Ejaculate
40
30
20
10
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
20
15
10
5
-1 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 39
WEEKS
Immunization
Booster


% OLH-^{125}I (LER-1056-C2)
CTS PPTD
NO. SPERM CELLS
(×10^6)
DOG NO. 8150
Antibody
No. Sperm Cells / Ejaculate
40
30
20
10
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
20
15
10
5
-1 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 39
WEEKS
Immunization
Booster

TITLE: Mechanical Control of Canine Fertility

SPEAKER: Dr. Victor Shille

Intravaginal mechanical means of preventing conception are certainly some of the oldest methods used to solve the problem of unwanted pregnancies. In man, of course, the emphasis has been on preventing entrance of sperm into the uterus and subsequent fertilization of the ovum in the oviduct. The main device employed has been the cervical cap or diaphragm used in conjunction with spermicidal preparations. This, of course, does not interfere with coitus.

In contrast to this, the ABCD* has been specifically designed to preclude intromission of the erect penis into the vagina of the estrous female. In this manner coitus is prevented and the female cannot conceive.

With this in mind, the ABCD has been disigned simply as a shield, attached to some means of keeping it in the vaginal tract. We have been conducting laboratory trials with this and similar devices since October 1972 and in June of this year we started clinical trials at the Sacramento SPCA and the Sacramento County Animal Control Center. The model you see here is the latest, and is the one that will be marketed.

The shield and its immediate support are made of hollow medical grade silastic so that they are not injurious to the male or the female. These openings do not penetrate the shield. They are placed there to facilitate bending of the shield during insertion. The central opening communicates with the hollow interior of the device and allows drainage of the estrual fluids. The curved portion of the silastic anchor is reminiscent of the erect bulbus glandis. This is not a coincidence, since it is designed for maximum retention efficiency by the vestibulovaginal sphincter. The polyethylene tybe extending from the silastic anchor is perforated at its

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*Agrophysics Breeding Control Device

origin and communicates with the hollow interior for estrual fluid drainage.

The diameter of the anchor and the length of the tube with the silastic end are determined for each individual animal by the measuring devices shown here. The tube serves two functions. First, it aids balancing the device inside the tract. Second, it prevents an overeager male from pushing the device forward.

Each measuring instrument serves a dual purpose: the silastic bulb measures diameter of the vaginal sphincter, and is made in three sizes: 19, 22, 25mm. The polyethylene tube is marked off in 25mm increments and is used to measure the length of the tract from the fornix to the vulvar lips.

We recommend that the vagina be examined visually before devicing, and this is the vaginoscope we use attached to a battery handle.

In our investigational work, we have been collecting vaginal cultures before and after devicing.

We do not recommend sedation.

The perigenital area of the patient is cleansed with aqueous zephiran and wiped dry. Bacteriological sampling is carried out. Digital examination is used to determine the character of the sphincter and the presence of any strictures, neoplasms or other abnormalities, in the vestibule. Then we visually inspect the entire tract, and if we find no cervical or vaginal disease we are ready for measuring. Length is measured first, then diameter. We find that there is a characteristic popping sensation when the correct size measuring instrument is extracted from the vagina. A shield of appropriate diameter is selected, and the tube is cut to size. We must subtract 37mm from the total length

measured to allow for the vestibule. After cutting, a silastic end plug is placed in the tube. The ABCD is now ready for insertion. We introduce it, well lubricated with KY Jelly, until the shield reaches the vulvar lips. At this point it has to be folded to pass into the vestibule. Steady pressure here causes the sphincter muscles to relax and the device pops into place. It may take 10-15 seconds at this point.

Once positioned, the device will remain in place for what appears to be an unlimited time. The position of the device seen in this radiograph shows rather well how the bitch's vaginal tract terminates well forward of the pelvic brim.

Other than the creation of some cornification, in dogs that have been deviced for a longer period of time, there seems to be no abnormality observable in histologic studies of deviced bitches. We have examined 21 tracts from bitches sacrificed during the course of the investigation. 6 of these had been deviced for 61 to 90 days. This is the appearance of the vaginal epithelium of these dogs. Here is a hi-power view of an area that developed a microscopic ulcer at the point of contact with a device that has since been replaced by the present model.

Sections of ovaries and endometrium from these animals appear to be characteristic of the anestrous bitch. Progestin levels agree with this.

The balance of the animals sacrificed had been deviced for 7 to 29 days, and their organs were typical of their respective state in the estrous cycle.

We have found that bitches will reject the device if they are suffering from vaginitis, or if they are pregnant or have been spayed prior to devicing. There are multiparous bitches with relaxed sphincters

that cannot retain the device. There are immature bitches with undeveloped vulvas that cannot be deviced with our present model. We are working on an approach for prepuberal bitches at the moment. Loss of the device when it occurs does so invariably within 30 days after devicing. 71.7% rejections occur within the first week, 95% within the first two weeks. We find that if loss of device has occurred, the bitch should be examined thoroughly to find the cause of the rejection.

We had problems testing the device effectiveness as a coitus preventative because we have had a lack of deviced bitches that have come in heat. One of the bitches that experienced estrus had been deviced 116 days, but the males could not breed her because of her rather small vulva. The others were either deviced shortly before their season or they had been induced by injections of estradiol cypionate. A total of 164 breeding trials were held in 36 bitches. 3 bitches were bred successfully and subsequently carried healthy litters to term. They rejected their devices 8 days after breeding. The model of device used in them has been changed since then.

There is no suggestion that the device induced any infectious process in the trial bitches. Our pre-devicing survey showed a surprising variety and number of organisms existing in clinically normal-appearing tracts. E. coli, alpha and beta streptococci comprise 67% of the types found.

In light of our present knowledge, we consider this device to be one of the possible answers to the canine population problem. Further studies must be undertaken to broaden its application in various sizes of genital tracts including the feline. Long-range effects of the device need to be studied, especially if we take into considera-

tion that the canine cycle moves slowly. The phenomenon of apparent lack of sexual receptivity in long-term subjects is of interest and will be investigated.

TITLE: Treatment of Selected Reproductive Problems in the Dog

SPEAKER: Dr. Gary Ewing

Lecture summary prepared by Michael Van Noy

Perhaps all reproductive problems should be treated by ovariohysterectomy. However, ovariohysterectomy is not the answer to all reproductive problems. Many animals are of such value that their owners wish to maintain their reproductive functions in spite of an apparent disease. Alternatives other than surgery should be available in these cases.

Infertility in the bitch may be divided into 2 categories: those bitches which are cycling and those which are not. The common factor in all cases is infertility.

Hypothyroidism is a significant cause of infertility. Bitches which are affected by hypothyroidism may be completely anestrus or may exhibit completely normal estrous cycles, depending on the severity. When anestrus is present, there are usually other clinical signs reverable to the thyroid deficiency:

1. lethargy
2. hypothermia
3. alopecia
4. obesity.

Correction of thyroid deficiency results in return to normal cycles and in many cases fertility will be restored.

In borderline hypothyroidism the bitch is clinically normal. The presenting complaint is one of normal cycles, being bred to proven sires, but no puppies, thyroid levels are run on all infertile bitches. In those

bitches exhibiting borderline hypothyroidism when thyroid supplement was administered, no external changes were noted, but on subsequent breeding the bitches became pregnant, and carried normal healthy litter.

Another cause of infertility in the bitch that is cycling, is chronic low grade endometritis. Those which have minimal exudate may go unnoticed by the owner, but are apparent to the veterinarian. Others are noticed only during careful vaginoscopic exam. Still others are detected only upon cervical or uterine culture.

Culture of infertile bitches is part of my routine approach to infertility. I make use of a sterile scope and Teigland swabs to obtain the culture.

Two choices of therapy in cases of low grade uterine infection are:

1) Direct intrauterine infusion, of appropriate antibiotics;

2) Systemic administration of antibiotics.

Due to the low levels of movement of antibiotics from blood to the uterine lining in the anestrus bitch, in most cases of parenteral injection or oral antibiotics, the desired results are not achieved with antibiotics alone.

When for some reasons, intrauterine infusion is not possible, low level stilbesterol therapy is used in combination with parenteral or oral antibiotics, to enhance blood flow to the uterine wall. 1-2mg/day for 5-7 days of stilbesterol.

In those cases treated by direct infusion of antibiotics, one should obtain a culture sensitivity, select appropriate antibiotics, suspend them in 5-10cc sterile saline, instill in the uterus by means of plastic artificial insemination pipette. (Bovine) This treatment is repeated every other day for 10 days - or 5 treatments. This may be difficult in dogs

weighing less than 15 lbs.

Puppy Vaginitis.

The exact cause and clinical significance are debatable. The most frequent case involves a modest purulent exudate which crust hairs of vulva: the owner reports no abnormality. Less frequently there is persistent licking, a noticable purulent exudate, and these dogs may attract the sexual attention of mature male dogs. When the owner is concerned and the dog appears to be showing discomfort, treatment is advisable. There are many methods of treatment with apparant remission as long as therapy is continued, but relapses are common when treatment is stopped. This is usually the case when antibiotics and antibiotic steroid ointments are used.

This condition usually resolves spontaneously at first estrus. Therefore low level stilbesterol - 1/2mg/day orally for 5 days in combination with antibiotic/steroid-mastitis ointment - is advisable.

Nymphomania.

Occurs in Dobermans and German Shepards. The history is one of a young bitch on her first estrus, who comes in heat, stays for a week, goes out and comes back in a week later, until she is in constant heat. Ovariohysterectomy is still probably the treatment of choice. Medical treatment consists of administration of LH hormone, 1mg/10lbs. bodyweight - single SQ injection. The vaginal epithelium undergoes normal changes as the bitch goes out of heat and loses interest in males.

Incomplete involution of the uterus appears clinically as a persistent postpartum vaginal discharge for 9-12 weeks, otherwise the bitch seems healthy. The discharge is usually not purulent. It may be colorless,

flecked with blood spots, and is odorless. Spontaneous recovery has been noted. Therapy consists of diethylstilbesterol 1-2 mg/day for 7-10 days.

Post Partum Endometritis.

The bitch is clinically normal with a persistent vaginal discharge. This condition must be differentiated between incomplete involution of the uterus. The discharge is slightly malodorous, cloudy and contains many white cells. This condition may later result in infertility. The therapy is similar to that of chronic endometritis.

Post partum metritis is a much more severe infection; with systemic signs, and involves all layers of uterus which may eventually produce peritonitis. This condition usually occurs 7-10 days post partum. The bitch is not nursing the pups. An emergency ovariohysterectomy is probably the best treatment. Because of the extensive involvement of the uterus it is doubtful that these bitches would again regain fertility.

The majority of cases of pyometra should be handled by ovariohysterectomy, especially older bitches and bitches which are extremely toxic with a closed cervix and retained secretions. In young dogs under 4 years with no prior history of reproductive problems, which are of value as breeding animals, alternative therapy should be attempted.

Treatment of open cervix pyometra consists of a combination of surgical and medical therapy:

1. surgical drainage of the uterus and surgical removal of the corpora lutea, if these are accessible;
2. use of appropriate antibiotics and ergonovine, an ergot alkaloid which stimulates uterine contractions to keep the uterus empty. Also, diethylstilbesterol in small doses enhances uterine blood

flow and transfer of antibiotics across the uterine wall and keeps the cervix open.

Diethylstilbesterol must be used in small doses to avoid toxicity. 1-4mg/day,according to size of dog, for 7 days is recommended.

The ergonovine dose is 0.2mg-0.4mg BID for 7 days.

The animal is monitored by several white blood cell counts and abdominal palpation or x-rays.

Persistent vaginal discharge is considered a good sign during therapy.