

Hormone Therapy and Estrus Cycle Control

Hormones used for estrus control:

A) Progesterone

Progesterone is a steroidal hormone produced by the ovary (corpus luteum) and is responsible for maintaining pregnancy. Progesterone is also produced by the corpus luteum (CL) during diestrus (the period between estrus) in the non-pregnant animal. During diestrus or pregnancy progesterone suppresses ovulation and expression of estrus when a dominant follicle is present on the ovary.

Progesterone compounds are used pharmacologically to suppress the pituitary release of gonadotropins (FSH and LH), which effectively suppresses estrus. While the animal is being supplemented with the progesterone compound estrus will not occur. This can be of benefit when synchronizing estrus. Soon after withdrawing the progestogen, the animal will come into heat in 2 to 5 days. In addition to natural progesterone there are several other "progestogen" compounds that have the same physiological effect as progesterone. These synthetic progestogens are available commercially for estrus control.

Progestogens can be administered by injection, orally, as a removable sub-cutaneous implant or in an intravaginal drug releasing devices. Animals can be kept on progestogens for a short (7 to 16 days) period for estrus synchronization. It is also possible to achieve absolute estrus suppression or birth control by administering a progestogen for an extended period of time, as with the use of oral MGA in feeder heifers or oral Regu-Mate for mares in training or competition)

- 1) Megestrol, Ovaban tablets 5 and 20 mg tablets, are typically used for estrus suppression or birth control in companion animals.
- 2) Norgestomet, Syncro-Mate-B implants 6.0 mg/SQ implants. Syncro-Mate-B has been used for estrus synchronization in cattle, but is not currently available in US, although it is still an approved drug.
- 3) Melengestrol, MGA feed additive. MGA is typically supplied as a feed supplement and is fed at a rate of .5mg MGA per head per day. Usually MGA supplement is formulated to contain .5mg MGA per pound of feed, and 1 pound of MGA supplement is fed per head per day. MGA is typically used for long-term estrus suppression in feeder heifers. It has also been used to synchronize estrus in various short-term protocols.
- 4) Altrenogest, Regu-Mate, oral solution. 2.2mg/ml. Used in horses for estrus suppression or can be used to induce fertile estrus in transitional mares. Regu-Mate is also used in swine and experimentally in cattle.
- 5) Progesterone releasing intravaginal device, The CIDR-B Vaginal Insert, with 1.38gm natural progesterone, is now available in the U.S. The CIDR-B Vaginal Insert, with 1.9gm natural progesterone, is available in the rest of the world. Either CIDR appears to work fine. CIDRs are used in cattle for estrus synchronization. They are placed in the vagina for 7 to 8 days, where the progesterone that they release is readily absorbed. When removed a prostaglandin injection is given and the rapid drop in progesterone levels in the circulatory system allows the animal to come into heat in 2 to 4 days.

The following progestogen releasing devices are not currently available in the United States but may eventually be approved for use by the FDA

- 6) Medroxyprogesterone acetate, MAP, Repromap sponge
- 7) Fluorogestone acetate, FGA. Cronogest sponge

B) Estradiol

Estradiol is the hormone produced by dominant follicles prior to and at estrus. In addition to causing a female to be receptive to breeding, estradiol also prepares the reproductive tract for breeding and sperm transport. Estradiol is also responsible for positive feedback on the hypothalamus during proestrus, stimulating the release of GnRH, and thus the LH surge, that is responsible for ovulation. Many of the effects of estradiol on the reproductive tract, endocrine system and on the brain (receptivity) are only evident when progesterone levels are less than 1 ng/ml, or in other words, when there is not an active CL present on the ovaries. In the presence of elevated progesterone, (an active CL is present, or the animal is supplemented with a progestogen) estradiol exerts negative feedback on the hypothalamus and the pituitary gland, suppressing the release of GnRH and the gonadotropins FSH and LH. Without gonadotropin support of the growing follicles, atresia (degeneration) of all growing follicles present on the ovary, including the dominant follicle that is producing the estradiol, will occur.

Estradiol is used in estrus synchronization in two ways.

1. If given during diestrus, when there is an active CL or during progesterone supplementation, it will cause atresia of growing follicles and result in a new follicular wave in 3 to 5 days. This effect can be utilized to synchronize follicular development in groups of cattle undergoing estrus synchronization.
2. If given 18 hours prior to the scheduled estrus in synchronized cattle, after progesterone levels have decreased to a baseline level (progesterone supplementation is removed and prostaglandin F2 alpha have been administered at least 24 hours previously to lyse the CL), it will effectively synchronize ovulation by stimulating a LH surge. This also assumes of course that the follicular wave was synchronized 7 to 8 days earlier so that all cattle have a dominant follicle that is capable of responding to the LH surge.

Unfortunately there are no FDA approved estradiol preparations available in the United States that can be used to synchronize follicular waves or synchronize ovulation. Estradiol Cypionate (ECP, Upjohn) is FDA approved but not currently available. ECP is not an ideal estradiol compound for follicular synchronization though, since it is a long acting compound. Estradiol Benzoate (E2-B) is a short acting estradiol compound that is available throughout the rest of the world. E2-B has been used for many years to safely synchronize estrus and ovulation in ruminants. Hopefully we will have a short acting estradiol compound available in the U.S. some time, but I'm not holding my breath. Until E2-B or another short acting estradiol is available, we must rely on GnRH to synchronize the follicular cycle and ovulation (to be legal).

C) GnRH

GnRH is "gonadotropin releasing hormone". GnRH is produced by the brain (hypothalamus) and is solely responsible for causing the release of luteinizing hormone (LH) from the anterior pituitary gland. GnRH also stimulates increases in FSH release from the anterior pituitary. LH and FSH are gonadotropin hormones, which are responsible for follicular development (FSH) and ovulation (LH). Without GnRH, LH would not be released and ovulation would not occur.

Pharmacologically GnRH is approved for treating cystic ovarian disease (LH deficiency) in cattle by causing luteinization of follicular cysts. There have been several other uses for GnRH in cattle and horses. These include; use cows at 15 to 25 days postpartum to increase fertility and accelerate the return to cyclicity, synchronize follicular waves in cattle prior to estrus synchronization or superovulation, induction of ovulation at estrus in cattle, and induction of ovulation in mares when the dominant follicle is larger than 30mm (Ovuplant SQ implants).

All available GnRH products are synthetic analogs of natural GnRH.

- 1) Injectable GnRH preparations include: Cystorelin, Factrel and Fertagyl. All three preparations contain Gonadorelin 50 µg/ml. A typical dose is 2ml or 100µg for cattle.
- 2) Sub-cutaneous GnRH implants are used in mares: Ovuplant. Contains 2.1mg Deslorelin

D) Prostaglandin F2 alpha

Natural prostaglandin F2 alpha (PGF) is produced by the uterus lining and targets luteal tissue in the corpus luteum (CL) on the ovary. Starting about 15 to 17 days after estrus, if the animal is not pregnant, the uterus will start to increase production of PGF. Increased PGF will cause lysis (luteolysis), or destruction, of the CL. This results in a rapid decrease in progesterone. As a result, if, or as soon as, a mature dominant follicle is present on one of the ovaries, the cow or mare will come into heat and ovulation can occur. In the case of a pregnant animal, the embryo will secrete a substance that suppresses the release of PGF from the uterine lining. This is called "maternal recognition of pregnancy", and prevents the cow or mare from coming back into heat.

Prostaglandin F2 alpha and its synthetic analogs are 20 carbon cyclic fatty acids that have the same action on the ovary as naturally released uterine prostaglandins. If an intramuscular injection of PGF or one of its analogs is given to a cow or mare with a mature CL, it will cause regression of that CL, allowing the cow or mare to come into heat. The available preparation of prostaglandin include:

- 1) Dinoprost tromethamine, Lutalyse 5000 mcg/ml, 5 mg/ml, (5ml cattle, 2ml equine, 1 ml / 20 Kg canine) approved for use in cattle and horses.
- 2) Cloprostenol, Estrumate 250 mcg/ml (2ml) for use in cattle

D) Gonadotropins

FSH and LH are the two natural gonadotropins produced by the pituitary gland. These are available commercially. In addition two other gonadotropins of embryonic or fetal placenta origin are also available. These are equine chorionic gonadotropin (eCG has FSH and LH activity) and human chorionic gonadotropin (hCG has LH activity). FSH and eCG are used to stimulate follicular development while LH and hCG are used to induce ovulation or treat cystic ovarian disease.

The following gonadotropin preparations are available in the United States

- 1) Porcine FSH: Folltropin-V, (Bioniche Canada Inc),
- 2) Porcine FSH: Pluset (Serono, Italy / Calier, Spain)
- 3) Ovine FSH: Ovagen (ICP Bio, ICP, Auckland, New Zealand)
- 4) hCG 10,000 IU: Chorulon, Follutein
- 5) PMSG + hCG: PG600, 400 IU eCG and 200 IU hCG

The following gonadotropin preparations are not currently available in the United States

- 6) Porcine LH: Lutropin-V, 25mg Armour standard (Bioniche Canada Inc)
- 7) PMSG: Equinex, Folligon, 5000 IU per 10ml vial

Common Reasons for female hormone therapy:

The goal of estrus control is usually to: allow breeding to occur when it is convenient, synchronize estrus in a group of animals for AI or ET, induce a fertile estrus for breeding in pre-pubertal heifers, induce a fertile estrus for breeding in post-partum cows, induce superovulation, induce estrus in superovulated animals, suppress estrus behavior in female athletes, show animals, feedlot heifers and pets, and also for birth control.

Initiation of estrus cyclicity

Frequently when estrus synchronization for breeding is scheduled, some of the animals to be bred are not cycling. This is particularly true with 15-month old heifers, post-partum cows and non-lactating mares early in the breeding season. Many protocols have been used on these animals to initiate cyclicity. The most effective

protocols utilize progestogens to mimic diestrus. The progestogen will suppress LH release and estrus. When the progestogen is removed, there is a rebound effect on FSH and LH release, which will stimulate follicular maturation and the animals are thus induced (some of the time) to come into an ovulatory estrus.

Induction of puberty

Inducing puberty requires that the animals are sufficiently old enough for natural puberty, have sufficient weight, uterine development, gonadal maturity and follicular development. Also important is that the animals are on an adequate plain of nutrition for maintenance and growth. Hormonal induction of puberty is most effective if the animals are at or near puberty

Induction of postpartum cyclicity

It is often desirable to synchronize dairy cattle or beef cows that are lactating heavy and have not yet resumed cycling. It is particularly beneficial for beef cows that have calved late in a calving season. Shortening the interval from calving to breeding will cause the cow to calve earlier the following year, with the rest of the herd. If a cow is not cycling special procedure must be used to synchronize estrus.

The requirements for successful initiation of cyclicity in postpartum cows include many management factors. First of all it is important for cows to calve with a good body condition score. If the animal has adequate fat reserves at the time of parturition she will be better equipped to cope with the stresses of parturition and the negative energy balance and weight loss that occurs in peak lactation. A cow should still be in moderate body condition at estrus synchronization for breeding to be successful. See: <http://ohioline.osu.edu/l292/> for body condition scoring of beef cattle and: <http://www.ianrpubs.unl.edu/epublic/live/g1583/build/g1583.pdf> for body condition scoring of dairy cattle).

Nutrition at the time of estrus synchronization and breeding must be adequate for maintenance of body condition and lactation. Adequate protein and mineral supplementation are also essential. It is important for cows that are in peak lactation to receive an adequate quantity of feed or have access to a high quality pasture so they are able to maintain their weight and body condition, or only lose weight slowly.

A sufficient postpartum interval, usually 45 days, is recommended before estrus synchronization is initiated. It is amazing though, how some cows may breed back as early as 30 days postpartum.

Induction of estrus during seasonal anestrus

Sheep, goats and deer are seasonal breeders with the normal breeding season occurring in the fall. It is possible with these species to breed out of the normal breeding season if progesterone based estrus synchronization methods are utilized. In sheep there are distinct economic benefits if lambs can be born in the late summer or fall so that they can be ready for slaughter in the spring for the "Easter market". This requires that the ewes be bred outside of the normal fall breeding season. In some breeds of sheep it is possible to achieve 2 lamb crops per year with top management, excellent nutrition and hormonal induction of estrus in the spring when ewes are still nursing their lambs.

Initiation of cyclicity in "Transitional" mares

Mares that are not pregnant will normally not start cycling until after the 1st of March in the northern hemisphere. The time from February 1st, until a mare ovulates and resumes cycling, is called the "transitional period". This is the transition from winter anestrus to the normal cyclicity that occurs during the spring and summer breeding season. With the use of artificial lighting and progestogen therapy many mares will commence normal cycling as early as January 1st.

Detection of silent heat or breeding with no estrus detection

Sometimes it is necessary to utilize an estrus synchronization program where estrus detection is not required. This can be due to labor constraints (inadequate manpower to properly heat check) or in individual animals

estrus may not be displayed due to psychological reasons. Utilizing progestogens to synchronize estrus, in combination with estradiol or GnRH to synchronize follicular waves, estrus and ovulation can be synchronized so tightly in cattle that it is not necessary to check the cows for heat. Cows can simply be bred at the prescribed time after completion of the hormonal therapy. This type of procedure is also effective in other ruminants. Unfortunately it is not yet practical to attempt synchronization of ovulation in mares.

Induction of estrus during anestrus in monestrous animals

Monestrous animals are species (canine) that only have a single estrus interspersed with long periods of anestrus. Utilizing progestogen and gonadotropin therapy, some success has been achieved in achieving estrus in dogs that were in the anestrus state.

Some of the protocols used (in general terms)

Progestogen therapy (Syncro-Mate-B, MGA, Progesterone, etc.)

These modes of estrus synchronization, cyclicity induction, estrus suppression and birth control are uses in all species. Many of the progestogen protocols include the use of other hormone drugs to achieve their goal. With regards to estrus synchronization and cyclicity induction, these methods tend to be the most complex, but frequently they will result in higher estrus response rates and tighter synchrony of the animals than natural methods or treatments based primarily on prostaglandins.

- A) Treatment of heifers with Syncro-Mate-B implants. Norgestomet is a potent Progestogen.
 - 1) Norgestomet silastic implant containing 6mg norgestomet is implanted SQ on the back of the ear,
 - 2) 2ml Norgestomet and Estradiol is injected at time of implant
 - 3) The estradiol induces a rapid decrease in FSH and LH, resulting in follicular atresia, initiating a new follicular wave
 - 4) Induction of endometrial oxytocin receptors
 - 5) PGF2 alpha production
 - 6) Regression of CL, if present, due to estradiol inducing oxytocin receptors and PGF release (not 100%)
 - 7) FSH rebound in 4 to 5 days initiates a new follicular wave
 - 8) Endometrial glandular development
 - 9) Removal of implant at 9 days
 - 10) Estrus at 24 to 48 hours after implant removal
 - 11) Low fertility of first pubertal heats
 - 12) Possibly poor oocyte maturation
 - 13) Pregnancy rate about 50% with timed AI
 - 14) Can be used in sheep and goats, 1/2 of an implant (3mg)

- B) Treatment of heifers with Melengestrol acetate (MGA) + Lutalyse
 - 1) MGA .5mg per head per day, added to feed
 - 2) Feed MGA for 14 days
 - 3) There may be a transient decrease in FSH and LH release, but follicular atresia may not occur. There is a rebound increase in LH release
 - 4) Follicular recruitment and follicular waves may be prolonged and the dominant follicle tends to persist for an extended period. The persistent dominant follicle's oocyte is less likely to be fertilized and develop normally, adversely affecting fertility if the animal is bred at the 1st heat after MGA withdrawal
 - 5) Endometrial glandular development
 - 6) Removal of MGA from feed after 14 days
 - 7) Estrus at 3 to 7 days after last MGA feeding

- 8) The heifers are NOT bred on this heat due to oocyte age, poor oocyte quality and low fertility of the MGA heat.
 - 10) Poor fertility if overcome by breeding on the second heat after MGA treatment
 - 11) PGF2 alpha is administered at 17 to 19 days after the last MGA feeding, Approx. 10 to 14 days after the MGA estrus
 - 11) Pregnancy rates are about 65% after breeding at the PGF2 alpha induced heat.
 - 12) This program works best with observation of heifers for heat and breeding each heifer 12 to 24 hours after she is observed in estrus.
- C) Treatment of cattle, sheep, goats and deer with progestogen impregnated sponges or progesterone releasing intravaginal devices such as the CIDR-B device.
- 1) Natural or synthetic progesterone impregnated sponge containing 30 to 60 mg progesterone analog or the CIDR device containing 1.38 or 1.9 gram of natural progesterone is placed in the vagina
 - 2) Estradiol capsule can be attached to the intravaginal device or an injection of estradiol can be administered at the time of vaginal insert placement. GnRH can be used instead of estradiol. GnRH may also initiate a new follicular wave by causing luteinization of a dominant follicle
 - 3) Estradiol causes a rapid decrease in FSH and LH, causing follicular atresia
 - 4) Induction of endometrial oxytocin receptors and PGF2 alpha production and CL regression if estradiol is administered
 - 5) FSH rebound in 4 to 5 days initiates a new follicular wave
 - 6) Endometrial glandular development
 - 7) Administration of a prostaglandin 12 to 24 hours prior to vaginal insert removal (or at the time of insert removal) assures regression of the corpus luteum
 - 8) Removal of device at 7 days if estrogen or GnRH is given at device insertion, (cattle)
 - 9) Removal of device at 8 days (cattle), 14 days (ewes), or 16 days (does) if only progesterone is used
 - 10) In sheep and goats eCG (PMSG) or eCG / hCG (PG600) can be given to induce follicular development and ovulation; this improves fertility and contributes to twinning
 - 11) Bull, buck or ram exposure may also increase fertility
 - 12) Estrus occurs at 36 to 72 hours after implant removal, depending on supplemental treatments
 - 13) Pregnancy rate vary depending on species and season
- D) Treatment of mares with the oral progestogen Altrenogest (Regu-Mate)
- 1) Label indication is suppression of estrus with a 'predictable' occurrence of estrus following drug withdrawal,
 - 2) Used in transitional mares (mares with good follicular activity - follicles of 25mm or greater), mares with anovulation, prolonged estrus, or weak estrus
 - 3) Can be used to suppress estrus prior to scheduled breeding
 - 4) If breeding during January and February, mares should have been maintained under lights, usually initiated in November
 - 5) Treatment continues for 15 days
 - 6) Estrus behavior will usually begin in 48 to 72 hours after the last dose of progestogen
 - 7) Pregnancy rate vary depending on season, typically very good

GnRH therapy (Cystorelin, Fertagyl, Factrel)

GnRH will cause ovulation or luteinization of dominant or large follicles with LH receptors. It accomplishes this by causing the release of LH from the anterior pituitary gland. GnRH is not used by itself to synchronize estrus,

but rather it is used as an adjunct to estrus synchronization. The two primary reasons for using GnRH in an estrus synchronization program are to synchronize follicular development or induce ovulation.

When GnRH is used to synchronize follicular development it is given 7 days prior to the time of progestogen removal and/or prostaglandin injection. At this time GnRH causes ovulation or luteinization of large follicles. This will cause a new group of follicles to begin development (a new follicular wave) at approximately 2 days after GnRH injection. If large follicles are not present or are atretic, small follicle will continue to grow and be ready to ovulate at the scheduled heat. The goal is to have as many animals as possible with a new dominant follicle that is ready to ovulate at the time of progestogen removal and/or prostaglandin injection. In other words GnRH is not used to synchronize estrus, but it is used to synchronize follicle development, hopefully causing more synchronous ovulation.

GnRH can also be used to induce ovulation at the time a heifer, cow, or mare is in heat or due to be in heat, further increasing the chance for synchronizing ovulation with insemination.

There are several prostaglandin/GnRH based estrus synchronization programs for cattle.

Select-Synch, Ov-Synch, Co-Synch, Modified Co-Synch, and a few others

The basic protocol for these is:

- 1) Day 0 inject 100mcg (2ml) GnRH, inducing a LH surge and luteinization of the dominant follicle(s) and atresia of the subordinate follicles. Progesterone levels rise.
- 2) A new follicular wave is initiated in about 2 days
- 3) On day 7 inject prostaglandin; luteolysis of the original CL and the luteinized follicles allow estrus to occur
- 4) There are then 4 options
 - A. Breed 12 to 18 hours after observed heat, estrus usually occurs at about 48 hours after prostaglandin injection (referred to as Select-Synch).
 - B. Inject a second dose of GnRH 48 hours after the prostaglandin injection and breed 18 hours after the second GnRH injection, no heat detection required (referred to as Ov-Synch).
 - C. Inject a second dose of GnRH at 60 hours (timing varies by practitioner) after the prostaglandin injection and breed at the same time as the second GnRH injection, no heat detection required (referred to as Co-Synch). (Not recommended by A.R.T., as the pregnancy rate is lower than the other protocols)
 - D. Breed cows 12 to 18 hours after observed heat, then at 60 hours after the prostaglandin injection and inject all cows not observed in heat with GnRH and breed at the same time (referred to as modified Co-Synch)
- 5) GnRH / Prostaglandin synchronization methods do have the potential to initiate postpartum cyclicity in cows if there is adequate follicular activity and a dominant or large follicle.
- 6) GnRH / Prostaglandin synchronization methods are not a good choice in peri-pubertal heifers unless a progestogen is added to the protocol, such as using a CIDR-B insert at the time of the first GnRH injection and pulling the CIDR at the time of the prostaglandin injection (referred to as CIDR-Synch). Other methods that utilize progesterone compounds and do not rely on GnRH to synchronize follicular waves, probably work better in heifers.

Induction of ovulation in animals with a mature dominant follicle is possible with GnRH.

- 1) In cattle an injectable GnRH is effective in inducing a LH surge that should cause ovulation if the GnRH is administered at the time of expected estrus. As long as progesterone levels are low (baseline < 1 ng/ml), and a healthy dominant follicle is present, pregnancy may be achievable even in animals that are not displaying estrus.

GnRH should be given at the very start of estrus for maximum effectiveness in animals displaying heat.

- 2) In mares injectable GnRH has not been effective in inducing ovulation of large (>=30mm) follicles. Therefore an implant has been developed, "Ovuplant" that releases GnRH continually over several days. The resulting increase in circulating LH will cause ovulation in 86% of implanted mares within 48 hours. Ovuplant contains 2.1 mg of GnRH analog that is released over 4 to 5 days.

All of the above methods of estrus induction can be used to synchronize the heat period of large numbers of animals for efficient artificial insemination.

Prostaglandin Therapy, "Short Cycling" and estrus synchronization with prostaglandin F2 alpha
The normal estrus cycle in most farm animals is 18 to 21 days. By causing early CL regression with prostaglandin F2 alpha (PGF), the length of the estrus cycle can be shortened in mares, cows, ewes and does. There are a few important points to consider when using prostaglandin preparations: The CL of the mare and ruminants is refractory to PGF2 alpha treatment for about 5 days after ovulation. Animals that had a heat only 5 days ago will probably not respond. Also, since prostaglandins exert their effect on a mature and functional CL they are only effective in a cycling animal. Animals that are pre-pubertal, and those in post-partum anestrus or seasonal anestrus will not respond. Due to these factors the response rate to a single PGF injection will vary from 0% to 75% maximum. It is rare, except in a small group, to ever get a response rate that exceeds 75%. Prostaglandins are not very effective in swine since their CL is only responsive to PGF2 alpha very late in the estrus cycle when natural PGF2 alpha is released from the uterus anyway.

After administration of a prostaglandin preparation, CL regression is complete within 48 hours for a responsive CL. If a dominant (estrogen active) follicle, of ovulatory size, is present, the animal will exhibit estrus, typically within 48 to 72 hours after injection. Earlier estrus occurs if CL regression was already occurring, in spite of the PGF injection (0 to 36 hours.). A longer interval to estrus will occur if there is not a dominant follicle present (84 hours to 9 days). If the CL was refractory to PGF treatment, estrus will occur at the normal interval for that animal (10 to 21 days after PGF injection).

Prostaglandin F2 alpha analogs are used extensively as an inexpensive and effective method to synchronize estrus for AI in cycling ruminants. Since simple PGF injection protocols (utilizing only PGF) do nothing to synchronize the follicular cycle, heat detection is usually done. Numerous protocols have been used in cattle including:

- A) Single injection methods: The following methods will reduce material and semen costs but usually require labor for heat detection.
 - 1) Inject a single dose in all cattle, observe for estrus, and breed 12 hours after standing heat is observed. This method is used when minimization of materials and semen costs is necessary. All animals not bred by day 10 or 11 post-injection can be injected with a second dose of PGF and heat detection and breeding can continue.
 - 2) The best (my opinion) single injection method includes observing the cattle for estrus for 5 or 6 days prior to PGF injection. Breed all cattle at 12 hours after seen in heat. On day 6 or 7 inject PGF into all cattle not yet observed in heat. Continue to breed at 12 hours after observed heat. This protocol effectively deals with refractory CLs, since all animals that are to be injected with PGF would have had a heat over 6 days prior to injection.

Timed AI at 75 to 80 hours post injection, of all animals not yet observed in heat, has been utilized with this protocol, and perhaps a few animals will become pregnant as a result. But, many animals that do not respond by 80 hours may be anestrus or not

yet in heat and too early to breed. A better way to deal with animals that have not responded is to treat them with a CIDR-B protocol.

- 3) If timed AI must be used for labor saving reasons, inject a single dose of PGF in all cattle, use timed AI at 75 to 80 hours post injection (no estrus detection). This method is used when semen cost is of no concern, minimization of labor is necessary and conception rate to the timed AI is not critical. Only expect about 65 to 70% of the animals to actually respond and exhibit estrus. Timing of the AI will be poor for many of the animals bred. Overall conception rates of 35 to 50% are expected. Clean-up bulls should be used to breed the balance of the cattle.
- B) Double injection methods:
- 1) Inject first dose of prostaglandin. Expect about 65% to 70% of the animals to respond and exhibit estrus in 2 to 5 days. The animals are not bred at this time.
 - 2) Inject a second dose of prostaglandin 11 days after the first dose. All animals that had a heat after the first dose should respond and exhibit estrus after the second dose. Animals that did not respond after the first dose due to refractory CLs (too soon after the previous estrus) should also respond to the second dose of prostaglandin and exhibit estrus. The only animals that will not respond to the second dose are anestrus. Heat detect after the second dose of PGF and breed 12 hours after standing heat is observed. Timed AI at 75 to 80 hours post injection (no estrus detection) can be employed if desired.
 - 3) If desired, animals seen in heat after the first dose can be inseminated. Obviously these animals would be excluded from the second injection of prostaglandin.
- C) Estrus synchronization using GnRH in combination with Prostaglandin, "Ov-Synch", "Select-Synch", etc. are discussed above.
- D) In mares PGF is used to induce a fertile heat but it will not synchronize ovulation due to the long and variable length of estrus.
- E) Prostaglandins have some other uses:
- 1) Early abortion of mis-mated farm animals. In cattle and horses the PGF treatment should be delayed for 6 or 7 days to account for the CL's refractory period. In swine treatment should be delayed for 14 post-ovulation. Usually a single luteolytic dose of PGF is all that is required. Sometimes heifer calves are treated at weaning time with PGF to assure that there are no pregnancies in early maturing individuals.
 - 2) Early abortion of mis-mated bitches (although with much greater difficulty than in farm animals). Requires twice a day (BID) treatment for at least 4 days. There are many side effects.
 - 3) Synchronization of farrowing in swine
 - 4) Treatment of uterine infections associated with a functional CL
 - 5) Treatment of canine pyometra

Gonadotropin therapy

Although gonadotropins (FSH, LH, eCG or hCG) are not used extensively in estrus synchronization, there are some gonadotropin applications, related to estrus control, that are worth mentioning.

PG600 from Intervet is a commercial preparation of eCG (also called PMSG) in combination with hCG. PG600 is used in postpartum sows, pubertal gilts, and anestrus sheep. The eCG part of this product has very potent FSH activity and will stimulate follicular development if given during diestrus or the proestrus parts of the estrus cycle. Follicular development is also stimulated by eCG during the transition from anestrus to proestrus in non-cycling animals. The hCG part of this product has LH activity and promotes final maturation of large graafian follicles. Using this product may hasten or stimulate an estrus.

PG600 is used in pre-pubertal and pubertal gilts at 6 to 8 months of age to initiate an estrus. This will shorten the time to puberty in later maturing gilts and can be used to synchronize gilts for AI. Response to treatment is usually over 65%. PG600 is given to sows at the time of weaning pigs to induce estrus, reduce the number of anestrus sows post-weaning, and shorten the time interval from weaning to first estrus. Most gilts and sows treated with PG600 will be in heat within 4 days.

During progesterone-based synchronization procedures in small ruminants PG600, given at the time of progesterone removal and prostaglandin administration may induce twins or even triplets, which is particularly desirable in sheep.

FSH and PMSG have been used in embryo recipient cows at sub-superovulation dosage to induce 2 or 3 ovulations, thus boosting progesterone levels and theoretically pregnancy rates. There has been some success using this technique.

FSH or PMSG (not PG600) are used for superovulation in cattle, sheep and goats. PMSG has a longer duration of action (half-life of about 2 days) than FSH (half-life of 30 to 60 minutes). PMSG is effective with a single injection. FSH based superovulation requires several injections, usually twice a day for up to 5 days.

Postpartum induction of estrus using natural methods:

- A) Nutrition; Increasing the level of energy in the diet 30 to 45 days prior to expected breeding will hasten the onset of estrus cyclicity at puberty and after parturition. This is referred to as a "nutritional flush".
- B) Weaning; Weaning the offspring early will initiate estrus cyclicity in several species. This is particularly valuable in swine where weaning will initiate the first post-partum estrus within 5 to 10 days.
- C) Exposure to males; this is referred to as the "Ram Effect" in sheep. Exposure to males will increase the percent of post-partum females that cycle early. This effect is also effective in early breeding of seasonally polyestrus species and increasing the percent of females that respond to estrus synchronization. Sterile teaser males can be used in artificial insemination programs.

Inducing estrus during seasonal anestrus using natural methods:

- A) Mares and Lights; by manipulating the hours of daylight using artificial lighting, mares can be made to cycle earlier in the year than normal. The transition from anestrus to estrus in mares typically occurs in February or March. In December and January, in the Northern Hemisphere, there are only about 8 to 10 hours of daylight. The long hours of darkness result in long periods of melatonin secretion that suppress the hypothalamus and pituitary glands. By exposing mares (and stallions) to 14 to 16 hours of light, using artificial light in the morning and evening, the onset of the breeding season can be hastened by 30 or more days. It is recommended that the lighting be increased incrementally by about 30 minutes per week, but abrupt transition to 15 hours of light is also effective. Typically artificial lighting is initiated around the first day of December in order to commence fertile estrus activity in mid January.

- B) Felines and Lights; Felines such as the domestic cat, are spring breeders, and like mares, will also respond to artificial lengthening of daytime. 12hr / 12hr light / dark stimulation, during seasonal anestrus should be effective although 15 hours of light may be better. If maintained under artificial light queens will start to exhibit estrus within 2 to 6 weeks.
- C) Small ruminants and Dark; In fall breeding species such as sheep, goats and deer, decreasing daylight hours and the resulting increase in melatonin secretion has the opposite effect and stimulates hypothalamic GnRH secretion. I am not aware of the commercial application of artificial shortening of exposure to daylight as a means of hastening the breeding season or inducing out of season breeding. If year around breeding is desired, it may be advisable to maintain a constant 12hr/12hr light / dark cycle in conjunction with hormonal therapy. Instead of relying exclusively on photo period control, typically progestogens are used in fall breeding species to induce out of season breeding.

Suppression of estrus cycles, Contraception and Population control

- A) Ovariectomy and Ovario-hysterectomy
- B) Birth control: Primarily progestogen therapy in one form or another is used:
 - 1) Megestrol (Ovaban) in dogs, 5mg and 20 mg tablets.
 - a. Proestrus regimen is begun as soon as blood spotting is noticed. Administer 1 mg/pound BW daily for 8 days. Will stop follicular development, prevent ovulation and prevent functional CL development. Suppresses the hypothalamic-pituitary axis, via negative steroidal hormone feedback on the hypothalamus, preventing FSH and LH release by the pituitary gland.
 - b. Treatment with low dose Megestrol during anestrus will prevent the onset of estrus. Daily treatment with .25 mg/pound BW for 32 days is effective. Once again, moderate elevation in this progestogens blood level will suppress FSH and LH release from the pituitary and prevent or delay the onset of proestrus and estrus.
 - c. Bitches will return to estrus in 4 to 6 months after either treatment regimen. The nearly normal anestrus period length after megestrol therapy indicates that therapy will mimic diestrus.
 - 2) Megestrol (Ovaban) in cats:
 - a. Like canines a proestrus regimen is begun as soon as sexual behavior is noticed. Daily treatment with 1 mg/pound BW (.5mg is effective for most queens) for 3 days will stop follicular development, prevent sexual behavior, and prevent mating. Since queens are induced ovulators it is wise to isolate the queen from the tom in case therapy is started too late.
 - b. Treatment with low dose Megestrol during anestrus will prevent the onset of estrus. Once weekly treatment with 2.5 to 5 mg is effective. Treatment should be limited to 10 weeks maximum.
 - c. Return to estrus is quite variable and depends on the time of year, due to the seasonality of the feline reproductive cycle.
 - 3) Repository Progesterone (Reprogest) injections can be used in the feline instead of Ovaban. Use a dose of 3mg/lb on a weekly basis as needed to prevent estrus. Other injectable and oral progestogens are available and can be used if an effective dosage regimen is available. Progesterone, like all gonadal steroid hormones, will suppresses the

hypothalamic-pituitary axis, via negative steroidal hormone feedback on the hypothalamus, preventing FSH and LH release by the pituitary gland. As a rule of thumb, therapy with any progestogens should never be extended beyond 10 weeks or approximately the length of gestation in any species.

4) Norgestimate / estradiol and other progestogen / estrogen combinations are used in humans. The result of therapy is similar to megestrol in preventing follicular development and ovulation by suppressing the hypothalamic-pituitary axis. The cycle of progestogen / estrogen allows normal menstrual cycles to occur.

5) Altrenogest (Regu-Mate) therapy in Mares

6) MGA treatment of heifers or ewe lambs in the feedlot

Treatment can be extended throughout the entire feeding period. Results in increased feed consumption, increased rate of weight gain and reduced injury due elimination or decrease in sexual activity.

C. Anti-progesterone therapy: RU486 is a progesterone receptor antagonist (mimics progesterone by binding to receptors but has no biological activity). Prevents attachment and implantation due to decreased progesterone effects in the uterus.

Induction of ovulation

A) hCG and LH therapy

- 1) Direct action on the LH receptors in the follicular thecal cells and granulosa cells
- 2) Only mature, dominant follicles will respond by ovulating
- 3) If administered prior to a new follicle achieving dominance, it will cause atresia of the growing follicles
- 4) A regressing dominant follicle may luteinize without ovulation if atresia is not advanced
- 5) A growing dominant follicle that is not mature (no LH receptors on granulosa cells, may partially luteinize without ovulation

B) GnRH therapy

- 1) Binds to GnRH receptors on gonadotrope cells (cells that secrete the gonadotropin LH & FSH) in the anterior pituitary gland
- 2) Strong stimulation of LH Secretion and LH production
- 3) Non-pulsatile, prolonged LH release (over 1-2 hours) causes an elevation in circulating LH levels that resembles the normal LH surge
- 4) GnRH should always be administered as a single injection. Even a single injection causes gonadotrope depletion. Repeated injections are not effective.
- 5) The resulting LH level may cause ovulation if a mature dominant follicle(s) is present.

C) Estrogen therapy

- 1) If used alone, an estrogen injection to induce estrus and ovulation is an antiquated mode of therapy.
- 2) Is occasionally effective due to late follicular phase positive feedback of estrogen on GnRH secretion (LH surge). If a mature follicle is present it may induce a fertile estrus and ovulation.
- 3) Also effective in inducing signs of heat
- 4) Proper timing of therapy is difficult without constant ultrasound monitoring of follicular growth or without synchronizing the follicular wave cycle. Should be used as an adjunct to follicular wave and estrus synchronization with GnRH/PGF or Progestogen therapy such as Syncro-Mate-B.

- 5) Estrogen should be given just prior to expected estrus to induce an LH surge and ovulation.

Induction of superovulation

“Brute force methods”, circumventing the negative feedback of estrogen and inhibin on gonadotropin secretion by direct administration of gonadotropins

- A) FSH
 - 1) Administered for 3 to 5 days using twice a day injections (BID) starting during mid diestrus day 9 to 11 post-estrus. It is best to start injections at the initiation of a new follicular wave.
 - 2) Prostaglandins are administered with the FSH on the 3rd or 4th day of therapy to induce estrus. Progestogen therapy can also be used to induce or synchronize estrus during FSH treatment.
 - 3) FSH preparations are partially purified extracts of pituitary glands
 - 4) Most FSH preparations are “contaminated” with LH
 - 5) FSH:LH ratio appears to be important in superovulation success
 - a. Too much LH will lower embryo recovery and / or lower embryo quality
 - b. FSH:LH ratios of 5:1 to 10:1 appear to be effective in most British and Continental breeds of cattle. FSH-P (Schering), FSH-P (Sioux Biochemical)
 - c. Preparations with very high FSH:LH ratios, i.e. $\geq 100:1$, are available, Folltropin (Vetrepharm, Canada), Ovagen (ICP, New Zealand), Super Ov (AUSA)
 - d. Very high FSH:LH ratios may lower overall embryo yield but will increase embryo quality.
 - e. Some LH is required for follicular and oocyte maturation but it probably does not need to be supplied with the FSH injections
 - f. Too high of a LH content will cause pre-mature luteinization of follicles, which may lower oocyte quality and fertility.
 - g. Too high of a LH content may also cause premature rise in progesterone which may prevent the normal estrus LH surge or cause a premature LH surge.
 - h. NOTE: it appears that some cattle will only respond to FSH preparations with higher LH content.
 - 6) Usual dose in cattle is 25 to 50 mg of FSH Armour Units, (1AU = ~1mg) or 200 to 400 mg of NIH FSH P1 (Folltropin) divided into 8 injections administered BID for 4 days. Start cattle on day 9 to 11 post-estrus.
 - 7) Usual dose in sheep and goats is 15 to 25 mg of FSH Armour Units divided into 6 to 8 injections administered BID for 3 to 4 days. Start sheep on day 11 to 13 and goats on day 13 to 15 post-estrus.
 - 8) Variability in response is the rule with FSH superovulation. There is significant variability between breeds, between animals, and even between consecutive superovulation procedures performed on an individual animal.
 - d. Breeds and individuals that are prone to twinning tend to yield more embryos
- B) Human Menopausal Gonadotropin (Pergonal, Serono)
 - 1) FSH and LH purified from the urine of menopausal women.
 - 2) FSH:LH ratio of 1:1
 - 3) Can be used in livestock but tends to be expensive.
 - 4) Pergovet (Serono) is available in Europe for superovulation of livestock.
 - 5) FSH-P is probably more appropriate for most animals
 - 6) Can be tried in animals that have not responded to higher FSH:LH ratio products.

- 7) Can be tried in animals that have been superovulated numerous times and a suspected immunity to porcine FSH is preventing a good response to FSH-P
- C) ECG (PMSG) in conjunction with anti-PMSG antibody
- 1) Administered as a single injection on day 9 to 11 days post estrus
 - 2) Prostaglandins are administered 48 hours after PMSG injection
 - 3) Anti-PMSG antibody is given with the PGF injection
 - 4) Progestogen therapy can also be used to induce or synchronize estrus
 - 5) PMSG has a high LH activity

“Subtle methods” of superovulation are used to overcome the negative feedback of estrogen and inhibin on gonadotropin (FSH) secretion

- D) Anti-estrogen antibodies, Fecundin, is used to induce twins or triplets in ewes
- E) Anti-Inhibin antibodies are in development
- F) Anti-estrogen therapy, using clomiphene citrate, has been tried in livestock with moderate success. Can result in superovulation in women.
 - 1) Clomiphene citrate is the most commonly used anti-estrogen drug.
 - 2) Used primarily in humans as a “fertility drug”
 - 3) Clomiphene has affinity for estrogen receptors in the hypothalamus, anterior pituitary and elsewhere, but very weak estrogen activity.
 - 4) Inactivation of estrogen receptors in the hypothalamus via competitive binding results in a marked decrease in estrogen activity, releasing GnRH secreting neurons and the anterior pituitary gonadotropes from the negative feedback action of estrogen.
 - 5) Removal of negative estrogen feedback on the hypothalamus results in an increase of pulsatile GnRH secretion and a resulting increase in production and secretion of LH and FSH. Does not remove the negative influence of inhibin on FSH secretion from the pituitary. FSH increase is usually moderate.
 - 6) Removal of negative estrogen feedback on the pituitary results in an increase in secretion of FSH, which is the main desirable effect.
 - 7) Clomiphene is administered for 5 days during the follicular stage of the cycle to stimulate follicular development. hCG may be administered after cessation of clomiphene to induce ovulation.
 - 8) Follicle development is followed with ultrasound to assess development and coordinate the timing of clomiphene withdrawal and hCG administration.
 - 9) Can produce ovarian over-stimulation or superovulation.

Induction of abortion

- A) Prostaglandins are used during the first trimester in cattle sheep, horses and dogs
- B) Various combinations of estradiol, corticosteroids, oxytocin and prostaglandins are used during late pregnancy.

Induction of parturition

Estradiol, prostaglandin, oxytocin and dexamethasone are all hormones that are used to induce parturition at or near term in domestic animals. It is best if current methods are referred to for the specific species to be induced. Methods vary tremendously between species.

Induction of lactation

Chronic administration of progesterone and estrogen over a 2-week period is effective (sometimes) in cattle. The hormones can be given as injections or as implants.

Treatment of pathologic conditions

- A) Cystic ovaries in cattle
- 1) LH deficiency is usually a post-partum problem at the time of re-initiation of cyclicity.
 - 2) A dominant follicle matures, but absence of a normal LH surge prevents ovulation
 - a. Complete lack of a LH surge results in a follicular cyst, which may be estrogen active and cause nymphomania
 - b. A deficient LH surge may result in partial luteinization of a pre-ovulatory follicle and a luteal cyst may form. A normal cycle may follow if the luteal cyst is capable of responding to uterine PGF release.
 - c. Cystic CLs are normal CLs with a cystic cavity. They can form from an ovulated follicle or an un-ovulated follicle. Cystic CLs respond to uterine PGF release and normal cyclicity occurs.
 - 3) Continued FSH stimulation may cause the follicle to continue to grow
 - 4) Cysts are not necessarily static, cysts may regress and new cysts develop.
 - 5) Multiple cysts are common
 - 6) Genetic predisposition
 - 7) Nutritional (energy) deficiency may predispose to this condition, i.e. a lactational catabolic state.
 - 8) Therapy for follicular cysts:
 - a. hCG or LH, direct effect upon the cystic follicle, causing luteinization of the cyst
 - b. GnRH injection inducing an endogenous LH surge like release of LH from the anterior pituitary, causing luteinization of the cyst
 - c. Manual rupture of the cyst is possible with a thin walled follicular cyst, this is not recommended as it may cause hemorrhage, scarring, and does not cause luteinization of the cyst.
 - d. Luteal cysts and cystic CLs by definition are already luteinized and should respond to prostaglandin therapy. If in doubt about the degree of luteinization of a cyst, it is best to first administer GnRH or hCG, and then follow that with prostaglandin 6 or 7 days later, to induce estrus.
- B) Male Hormone Therapy; There are few indications for hormone therapy in the male.
- 1) Administration of GnRH to newborn males with cryptorchidism
 - a. Testicular descent is influenced by testosterone
 - b. GnRH will increase testosterone production in a newborn calf at a time when testosterone production is decreasing.
 - c. The continued testosterone stimulation may cause final descent of the retained testicle.
 - d. Only occasionally effective but worth trying.
 - 2) Testosterone injections to increase libido (Don't use this therapy)
 - a. Effective in increasing libido if animal is truly deficient in testosterone.
 - b. Decreased libido is frequently associated with injury or chronic pain.
 - c. Testosterone injections will inhibit gonadotropin secretion and result in decreased spermatogenesis.
 - 3) FSH or PMSG for treatment of oligospermia or azoospermia (decreased numbers or complete lack of sperm cells in the ejaculate)
 - a. May be effective if the cause of decreased spermatogenesis is truly a deficiency in hypothalamic or pituitary function. Usually oligospermia or azoospermia have predisposing causes such as genital or gonadal infection, injury, endocrine pathology or a genetic basis.
 - b. Treatment must be continued for longer than the full length of the spermatogenesis cycle, or at least 60 days to 90 days before evaluation of therapy can be made.
 - c. Weekly injections of PMSG are typically used. FSH must be injected daily.

Non-Reproductive Hormone Therapy

- A) Growth promotants, used in feeder cattle.
- B) Anabolic steroids in debilitation of all species
- C) Antineoplastic therapy
 - 1) Treatment of breast cancer and other estrogen responsive neoplasia (human)
 - a. Selective antiestrogen or selective estrogen receptor modulator drugs such as Tamoxifen can allow remission of breast cancers that are stimulated by estrogen. Tamoxifen is a non-steroidal estrogen receptor-binding agents that block estrogen from binding and activating the receptor in mammary gland cells. (Although non-steroidal the molecule resembles the estrogen molecule).
 - b. These drugs are primarily used as adjuncts to surgery or chemotherapy to prevent metastasis or recurrence.
 - c. Obviously cannot be used in breeding animals or pregnant animals.
 - d. Not approved for animal use.
 - 2) Treatment of benign prostatic hyperplasia and prostatic neoplasia
 - a. Castration is therapy of choice, not desirable in some animals or humans
 - b. Estrogen therapy is sometimes effective but results in decreased spermatogenesis and has other side effects such as prostatic metaplasia and further enlargement of the prostate
 - c. Anti-androgen drugs would be an ideal treatment but are only experimental at this time. (Flutamide is an anti-androgen drug that competitively inhibits testosterone activity.
 - d. GnRH agonists can be used to down regulate or paralyze the pituitary gonadotrope cells and inhibit LH secretion. Without adequate LH the testicles will stop testosterone production, which allows hormone responsive prostatic hyperplasia and/or cancer to undergo remission. Lupron is a brand name for leuprolide, which is a potent GnRH agonist, available for use in men. Lupron is available as a depot injection.