

# Controlled Breeding in Horses



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## Early Season Mating

For maiden and barren Thoroughbred mares especially, but also for other breeds that wish to produce early foals.

### Protocol

- From December 15th: Begin to "flush" the mare. Bring her in at night, give her extra feed and leave the lights on in the box or yard until 11.00 pm. This pushes her into the transitional phase that precedes normal ovulatory oestrous cycles.
- On February 1st: Begin giving a daily dose of Regumate® in the morning or evening feed (orally via a syringe) for 10 days. Keep providing the extra light. This allows concentrations of the main sex hormones, FSH and LH, to build up in the mare's pituitary gland.
- On February 10th: Give the last dose of Regumate plus a single intramuscular (i.m) injection of prostaglandin  $F_{2\alpha}$  (i.e. Prosolvin®). This eliminates any secretion of progesterone from the mare's ovaries (corpus luteum) that could stop or delay development of an ovulatory follicle.
- During February 15th-25th: Tease the mare (if a stallion is available) and palpate or scan her ovaries on alternate days. When a dominant follicle reaches  $\geq 35\text{mm}$  in diameter,

give the mare a single intravenous (i.v.) injection of Chorulon and have her covered by the stallion (or artificially inseminated with fresh or cooled semen if preferred) on the same day. The Chorulon stimulates the ripening follicle to rupture and release its egg (ovulation) in 32 - 40 hours. Ovulation occurs 36 hours after the administration of hCG (at which time the mare should also have been mated). This 36 hour interval allows penile debris and excess seminal fluid to be expelled from the uterus before the cervix closes after ovulation, thereby helping to avoid mating-associated endometritis.



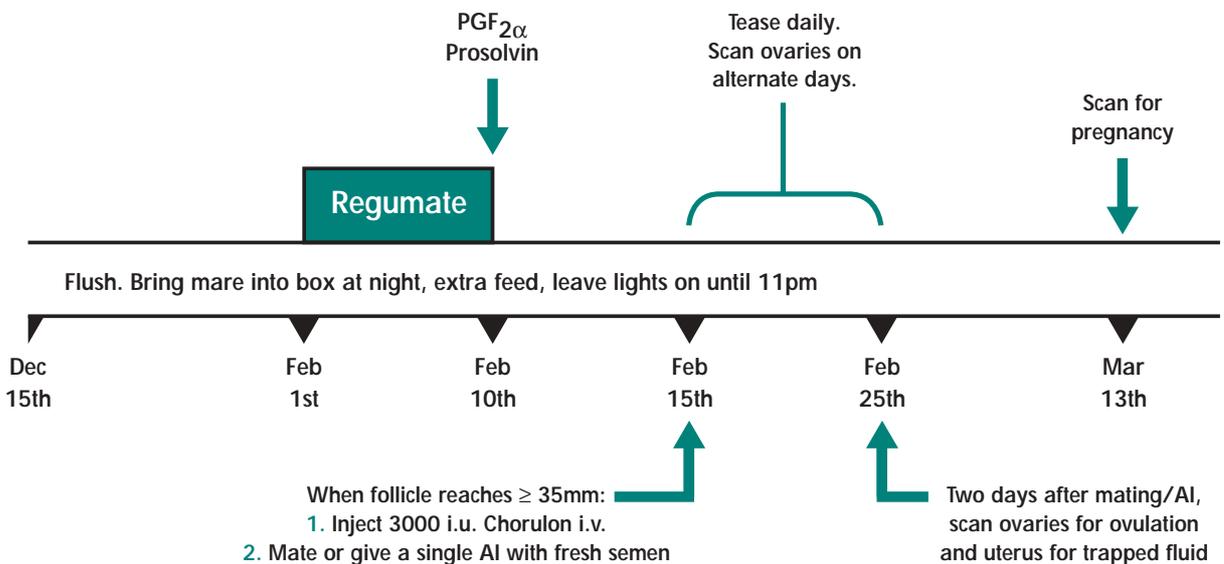
- 2 days later: Palpate or scan the mare's ovaries to confirm that ovulation has occurred and that the uterus has expelled excess fluid. If she has a history of previous early pregnancy losses, daily oral dosing with Regumate to supplement progesterone levels may be commenced on the second or third day after ovulation (see later).

## Benefits

- The protocol is accurate. Experiments in Europe, America and Australasia have shown that as many as 85% of barren and maiden mares will ovulate normally during the 12 day period after Regumate withdrawal. Conception rates to a single, planned mating within this period are high.
- The protocol is cost effective. It allows pre-planned concentration of management and veterinary effort around the most propitious time to achieve conception.

- The protocol is biologically sensible. It minimises use of the stallion at a time when his fertility is also lowered by seasonal changes, it minimises the chances of uterine infection in the mare and it maximises the chances of the desired early foal next year.
- Even if the mare fails to conceive to this first mating or insemination, having begun to cycle she will return to oestrus spontaneously in 16-18 days and so provide a second chance for re-mating before mid-March.

## Early Season Mating



# The Single Mare Owner -

## sending the mare to the stud

The ability of Regumate to synchronise oestrus in mares enables the single mare owner to schedule the mare's oestrus period at a time that can be organised well in advance, facilitating the transport and boarding plans for the mare.

### Protocol

- Before starting this protocol, the mare should be in transitional oestrus or she should be cycling normally.
- During the breeding season administer Regumate orally for 10 days, starting on a Monday and giving the last dose on the 10th day (e.g. a Wednesday of the following week). Also on the 10th day of Regumate treatment administer a single intramuscular injection of PGF<sub>2α</sub> (Prosolvin).
- On the following Monday (14 days after the start of Regumate treatment) the mare should be transported to the stud farm or breeding centre and her ovaries should be scanned for follicular development on the day of her arrival.
- The mare should then be covered naturally or artificially inseminated later during the same week of her arrival at the stud farm.

- After the day of ovulation has been determined, the mare may be transported back to her original training yard or stud farm.
- Around day 33 after the start of Regumate treatment (i.e. 15 days after ovulation), the mare should be scanned for pregnancy.

### Benefits

- Mare transport and the duration of the mare's stay at the stud farm where she is to be covered can be arranged well in advance.
- The cost of boarding the mare at the stud farm is minimised as the mare receives her Regumate treatment at home.
- The frequency of veterinary examinations is reduced and the need for a teaser stallion is eliminated.



# Artificial Insemination (AI)

For non-Thoroughbred horse or pony mares, and for non-racehorse Sport horse Thoroughbreds, wishing to benefit from the practical advantages of AI using fresh, cooled or frozen-thawed semen.

## Protocol

- Beginning any time after March 15th (Day 1 e.g. a Monday). Commence a daily dose of Regumate (in the feed or orally via a syringe) for 10 days. This temporarily stops the mare cycling and increases the concentrations of sex hormones (FSH and LH) in her pituitary gland.
- Day 10 (e.g. a Wednesday): Give the last dose of Regumate plus an i.m. injection of prostaglandin F<sub>2α</sub> (Prosolvin) to destroy any luteal tissue that may still be present in the mare's ovaries.

Then either;

- On Day 15 (e.g. a Monday): Palpate or scan the mare's ovaries. If a dominant follicle of  $\geq 35$ mm diameter is present, place the order for fresh or cooled extended semen to be available the next day. Give the mare a single i.v. injection of 3000i.u. Chorulon to induce the ovulation process.
- On Day 16 (e.g. a Tuesday): Inseminate the mare with 5 - 10ml of extended, fresh or cooled semen

containing 300 - 500 million motile spermatozoa. Do not reinseminate the mare next day, even if excess cooled semen is available.

- On Day 18 (e.g. a Thursday): Scan the mare's ovaries and uterus to confirm ovulation and check if fluid has accumulated in the uterine lumen. If it has, give an i.v. injection of 20i.u. Oxytocin to help expel it.
- On Day 33 (e.g. a Friday): Scan the uterus for pregnancy at 15 days after ovulation.

Or;

- On Day 16. Blindly (i.e. without palpation or scanning) inseminate the mare with 5 - 10ml extended fresh or cooled semen containing 300 - 500 million spermatozoa, provided her cervix feels relaxed and oestrus-like. Give an i.v. injection of 3000i.u. Chorulon to induce maturation and ovulation of the follicle within 48 hours.
- On Day 37. Palpate or scan the mare for pregnancy. If not pregnant, a new ripening dominant follicle should be present in her ovaries to allow the Day 16 or 17 protocols to be repeated.

## Benefits

- The protocol is accurate and conception rates are high - between

65 and 75% when using the blind insemination method and up to 85% when scanning to monitor follicle development.

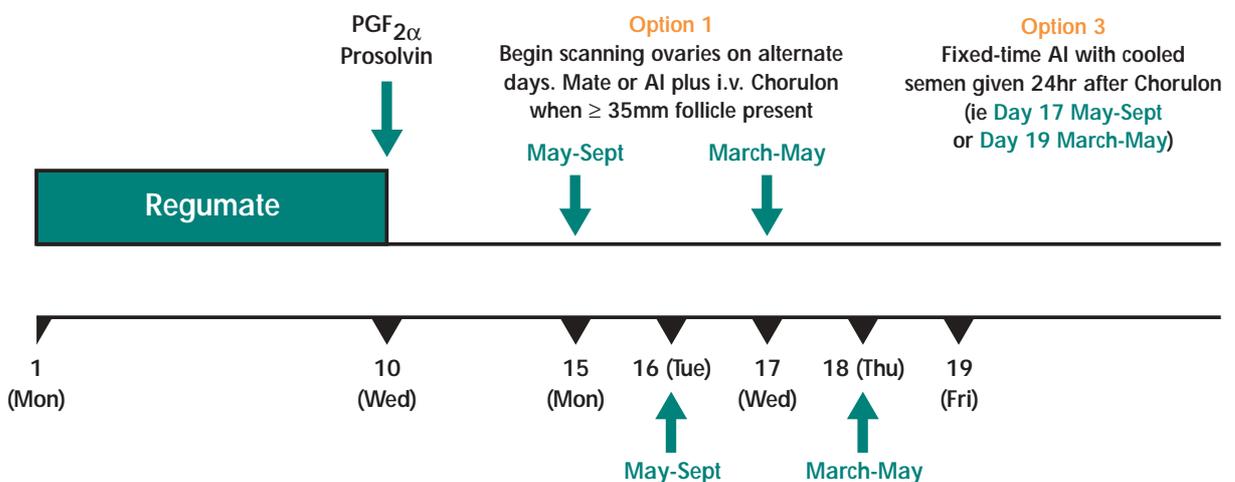
- Only one natural mating or artificial insemination of a small volume (5 - 10ml) of extended fresh or cooled semen is required on a single day that can be decided well in advance by starting the treatment protocol on the appropriate day.
- Eliminates the need for teasing and natural mating and thereby helps to prevent the spread of venereal or other infectious diseases. AI is more bacteriologically friendly to the mare and is physically safer for both the mare and stallion.
- Saves money on animal transport and veterinary examinations.

- If the mare fails to conceive to her first service, the protocol provides for another high conception chance on a predetermined single day for re-mating or re-insemination.

## Recommendations

- Although costs may be reduced using these protocols, it is beneficial to examine the mare at least once before Regumate therapy and once after insemination, to rule out or treat any signs of uterine infection, especially in older mares (>14yo).
- An early scan for pregnancy is recommended in larger breeds of mares (e.g. Thoroughbreds) in case of twin pregnancy.

## Single Mating or AI during the Breeding Season



**N.B.** Early in the breeding season (March-May) it takes longer for follicles to develop.

Ref: Studies conducted at Equine Fertility Unit, Nmkt.

## Fixed-Time AI with Frozen-Thawed Semen

Frozen-thawed semen has a reduced lifespan in the mare's reproductive tract compared to fresh or cooled semen. This necessitates insemination of the frozen-thawed semen close to (i.e. within 6 - 12 hours) the time of ovulation. Repeated scanning or palpation of the mare's ovaries to attempt to judge impending (or recently occurred) ovulation can be time wasting, expensive and frustrating. It is much better replaced by a single fixed-time insemination linked to planned hormone treatments to synchronise and control the time of ovulation.



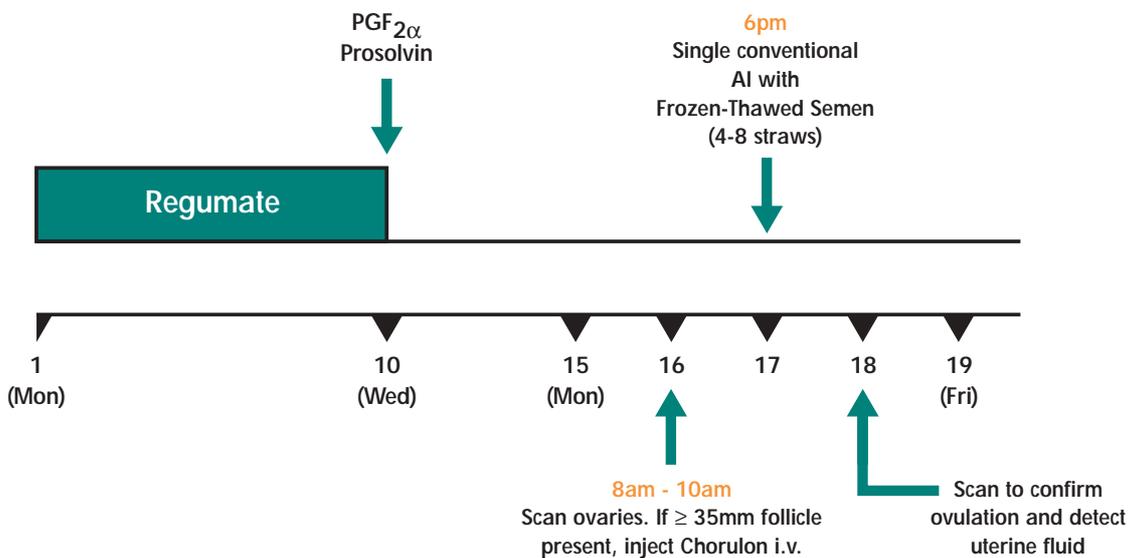
### Protocol

- On Day 1. Commence a 10-day course of daily oral Regumate administration.
- Day 10. Give the final dose of Regumate together with an i.m. injection of prostaglandin  $F_{2\alpha}$  (Prosolvin) to destroy any luteal tissue that may be persisting in the ovaries.
- Day 16 (Between 8.00 and 10.00 am). Either: Scan or palpate the ovaries to seek a dominant follicle of  $\geq 35$ mm diameter. Or: Go ahead "blindly" on the assumption (i.e. +85% chance) that a dominant and responsive follicle will be there. Give an ovulation-inducing i.v. injection of 3000 i.u. Chorulon.
- On Day 17 (Around 6.00 pm). Ideally at around 32 hours after the Chorulon injection administered the previous day, give the mare a single insemination of the frozen-thawed semen after first checking sperm motility under the microscope. Sperm motility  $>40\%$  is required to obtain acceptable pregnancy rates.
- On Day 18 (Optional). Scan the mare's ovaries to confirm ovulation and her uterus to check for fluid accumulation. Give 20i.u. Oxytocin i.v. if uterine fluid is evident.
- On Day 34. Scan for pregnancy (i.e. Day 16 after ovulation). If not pregnant, repeat the protocol used on Days 16 and 17 previously.

## Benefits

- Gives a single accurate fixed-time insemination that can be planned well in advance.
- Matches insemination time with ovulation, thereby maximising the chance of conception.
- Allows for a further fixed-time, single, optimised insemination if conception does not occur first time round.
- Eliminates the need for multiple inseminations and thereby uses fewer straws of semen.
- Saves time and money on management and veterinary examinations.

## AI with Frozen-Thawed Semen during the Breeding Season



## High Fertility Utero-tubal Junction-AI (UTJ-AI) for frozen-thawed semen

Exciting new experiments<sup>1</sup> have demonstrated that, by using the videoendoscope for insemination a very low dose of stallion spermatozoa is required (i.e. 1-5 million, compared to 300-500 million, in a conventional insemination dose). Semen is deposited directly onto the little papilla at the tip of the uterine horn which connects the uterus to the oviduct (the uterotubal junction or UTJ). Very high conception rates (65-80%) can be achieved using either fresh or frozen-thawed semen. The technique takes the semen close to the site of fertilisation in the oviduct and so overcomes the huge losses of spermatozoa during their long swim up the uterine horns after conventional insemination into the uterine body.

### Protocol

- Between Days 1 and 10: As for conventional AI, administer Regumate daily for 10 days plus a single i.m. injection of PGF<sub>2α</sub> (Prosolvin) on Day 10 to destroy any luteal tissue left in the ovaries.
- Between Days 14 and 16: Commence scanning examinations of the follicular development in the mare's ovaries.



- Between Days 15 and 20:
  1. Further scanning examinations to monitor the development of a  $\geq 35$ mm follicle.
  2. Give an injection of Chorulon to hasten maturation and ovulation of the follicle when it's diameter is  $\geq 35$ mm.
  3. Videoendoscopic UTJ-AI is performed 32 hours after Chorulon to deposit only 1 straw of frozen-thawed semen directly on to the UTJ.
  4. A further scan next day is required to confirm ovulation.
- Around Day 20: Return the mare to her training yard or stud farm.
- Around Day 37 (i.e. Day 16 after ovulation): Palpate or scan the mare for pregnancy.

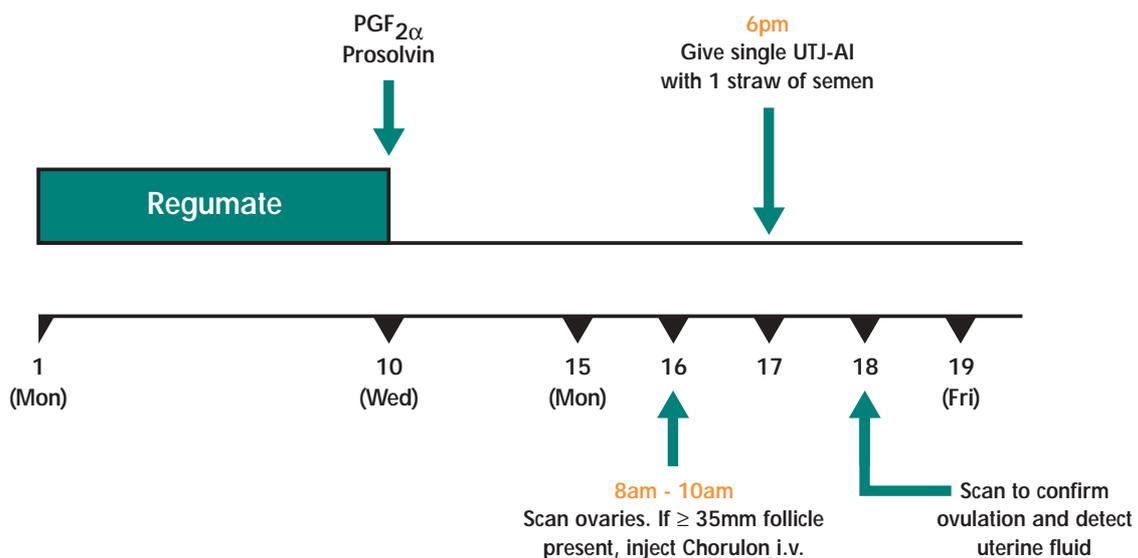
## Benefits

- Pre-arranged Regumate/Prosolvin induction of oestrus to enable expert veterinary supervision of the mare around the crucial time of insemination and ovulation.
- Maximum conception chances with only a single straw of frozen-thawed semen, instead of the 4-8 straws required normally for conventional insemination.
- Improves greatly the chance of conception when using frozen-thawed semen of moderate or poor quality.

- Saves time, money and semen whilst greatly increasing the chances of pregnancy.
- In future, the method will permit the use of sex-sorted spermatozoa to greatly increase the chances of producing a colt or filly, whichever is desired.

<sup>1</sup> Experiments performed at The Equine Fertility Unit, Mertoun Paddocks, Newmarket, Suffolk CB8 9BH (Morris et al, 2000 JRF 118, 95-100).

## UTJ-AI with Frozen-Thawed Semen during the Breeding Season



# Embryo Transfer

For successful embryo transfer, the oestrous cycles of the donor and recipient mares must be synchronised so that ovulation in the donor mare occurs between 0 and 3 days before (not after) ovulation in the recipients. This may be achieved simply and effectively by the use of Regumate, Prosolvin and Chorulon.

## Protocol

- On Day 1: At any time during the breeding season (mid-March to mid-October) begin giving a daily oral dose of Regumate to the donor mare for 10 days. She may remain in training throughout the period but should not be competed whilst receiving the Regumate or for 8 days following treatment.
- On Day 3: Similarly, begin a 10-day course of Regumate to the recipient mares.
- On Day 10: Give the last dose of Regumate to the donor mare, plus an i.m. injection of PGF<sub>2α</sub> (Prosolvin) to destroy any luteal tissue that may still be present in her ovaries.
- On Day 12: Give the last dose of Regumate and an injection of Prosolvin to the recipient mares.
- On Day 16: Begin scanning the donor and recipient mares. When the donor has a dominant follicle of ≥35mm diameter, mate or inseminate her once and give an i.v. injection of 3000i.u. Chorulon to stimulate ovulation within 48 hours. Rescan the donor the next day and the day after to determine accurately the day on which ovulation has occurred (Day 0).
- **One day after mating the donor:** Scan the recipient mares and give an i.v. injection of 3000i.u. Chorulon to any that exhibit a ≥35mm follicle.
- **Two days later:** Scan the Chorulon-treated recipient mares to confirm ovulation.
- **Donor mare, 7 days after ovulation:** Flush her uterus to recover the embryo at the early blastocyst stage. Transfer this, surgically or non-surgically, to the most appropriate recipient mare that ovulated 1 - 2 days after the donor.
- **From the day of transfer:** Begin administering a daily dose of Regumate to the recipient. Cease this treatment if she is scanned not pregnant at days 14-16 after ovulation. If pregnant, continue Regumate to around Day 60 and thereafter reduce the dose by one half for 4 days, and then to a quarter for the next 4 days, and finally cease treatment altogether. This extra progestagen helps the recipient uterus to nurture the very young "foreign" embryo and it prevents the mare from unwittingly returning to oestrus.

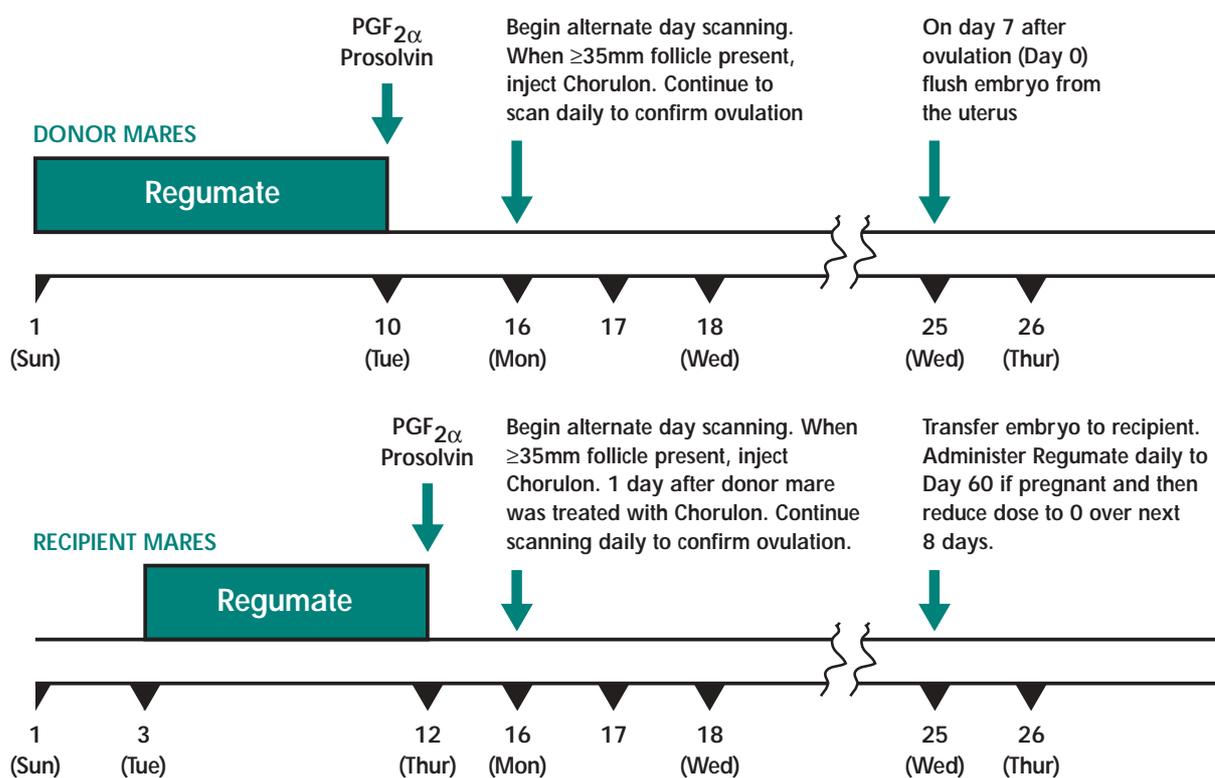
For further embryo recovery attempts:

- **On the day of embryo recovery:** Give the donor mare an i.m. injection of prostaglandin  $F_{2\alpha}$  (Prosolvin) immediately after the uterine flush to induce luteolysis and a return to oestrus in 3 - 4 days.
- **Two days after embryo recovery:** Similarly inject the unused recipient mares with Prosolvin to induce luteolysis.
- Thereafter, repeat the donor insemination, ovulation induction treatments and embryo recovery and transfer manipulations as above.

## Benefits

- A cheap and practical protocol that can be used on mares at any stage of the oestrous cycle to provide good synchronisation of ovulation in donor and recipient animals.
- Saves time and money and reduces the need for veterinary examinations.
- Maintains a good degree of synchronisation between donor and recipients to allow repeated embryo recovery attempts from the donor mare.
- Allows the donor mare to remain in active training and requires few veterinary manipulations.

## Embryo Transfer - April to September



## To Avoid Foal Heat Mating & Treat Lactation-Related Anoestrus

Most foaling mares show oestrus and ovulate between 8 and 12 days after foaling. Mating at this foal heat gives a reduced conception rate, an increased early abortion rate, and it carries with it an increased risk of establishing a severe mating-induced endometritis which is difficult to resolve. Foal heat mating is therefore best avoided. Eliminating the need to tease the mare with a stallion around this time also saves the foal-proud mare from anxiety and stress. The following treatment protocol can then be used to preserve a 12-month foaling interval.

### Protocol

- **On Day 15 after foaling:** Take a blood sample for progesterone assay to confirm that ovulation has occurred as expected around Day 11 or 12 and the mare is now in **dioestrus**.
- **On Day 16 after foaling:** If the progesterone assay reveals the mare has not yet ovulated and she is in **anoestrus**, start her on a 10-day oral course of Regumate.
- **On Day 21 after foaling:** In those mares that did ovulate normally at the foal heat, give an i.m. injection of prostaglandin  $F_{2\alpha}$  (Prosolvin) to induce luteolysis and a return to oestrus.
- **On Day 26 after foaling:** Give the last dose of Regumate to the **anoestrous** mares plus an i.m. injection of prostaglandin  $F_{2\alpha}$  (Prosolvin) in case some of them have ovulated while being given the Regumate.  
  
Begin teasing and scanning the ovaries of the **dioestrous** mares given Prosolvin on Day 21 after foaling. Give a single mating or insemination when a dominant follicle of  $\geq 35$ mm diameter is present, coupled with an i.v. injection of 3000i.u. Chorulon to hasten ovulation.
- **On Day 30 after foaling:** Scan the ovaries of the mated/inseminated mares to confirm ovulation. Scan also the ovaries of the **anoestrous** Regumate-treated mares to assess follicular development. If this is not progressing, assume the mare has entered a state of true lactation-related anoestrous and commence thrice daily injections of low doses of GnRH (Receptal) at regular intervals (e.g. 3ml Receptal i.m. or i.v. at 8.00 am, 4.00 pm and midnight) to stimulate follicular growth (W.R. Allen. Pers. Comm.). Continue the treatment for up to a maximum of 10 days, or until a dominant follicle of  $\geq 35$ mm diameter develops in the ovaries. At this point, inseminate or mate the mare once and give an i.v. injection of 3000i.u. Chorulon to hasten ovulation.

## Benefits

- Avoids the considerable risk of infection in the mare's uterus, the stress to the mare and her foal incurred during teasing and mating at around the time of the foal heat.
- Shortens the dioestrous interval in mares that have ovulated and provides a pre-determined time to concentrate veterinary attention on the expected time of ovulation. Therefore, it provides an optimum time for a single mating or insemination and so minimises the bacterial challenge to the post-foaling uterus.
- Identifies at an early stage the small percentage of mares (~10%) that fail to ovulate at the foal heat. This allows effective treatment to be initiated before they pass into a profound and usually intractable lactation-related dioestrus
- Helps to maintain a 12 month foaling interval in mares and it reduces early pregnancy loss rates.



## Pregnancy Support

Progesterone is the principal hormone of mammalian pregnancy. It keeps the cervix closed to maintain the uterus as a sterile environment for the embryo, it keeps the uterus "quiet" until it is time for the strong muscular contractions that bring about birth, and it stimulates the myriads of tiny secretory glands in the endometrium to produce the protein-rich secretions ("uterine milk") that are an essential component of the embryo's diet.

In the pregnant mare there are 3 sources of progesterone; i) the primary corpus luteum, which develops from the ovulation that gave rise to the pregnancy and which begins to decline in secretory activity from as early as 16 - 20 days after conception; ii) the secondary corpora lutea, which develop in the mare's ovaries between days 40 and 150 of gestation due to combined stimulation by pituitary gonadotrophin Follicle Stimulating Hormone (FSH) and placental gonadotrophin equine Chorionic Gonadotrophin (eCG); iii) the placenta, which secretes appreciable quantities of progesterone from around day 80 of gestation and which remains the principal supplier of progesterone throughout the remainder of gestation.

The advent of the ultrasound scanner has greatly increased the accuracy of early pregnancy diagnosis in the mare and studies in recent years have

indicated that up to 25% of horse embryos are lost between fertilisation and 120 days of gestation, with a further 3 - 5% losses from then until term. There could be a number of reasons for these losses, including chromosomal abnormalities that occur accidentally at the time of fertilisation and age-related degenerative changes in the endometrium. A deficiency of essential supplies of progesterone could be another cause. This might stem from failure of transmission of the vital "maternal recognition of pregnancy signal" from the young embryo between days 10 and 16 after ovulation, from a simple failure of the primary corpus luteum or a failure of development of secondary corpora lutea before the primary structure has faded away.

Many studies have demonstrated that Regumate is the only synthetic progestagen capable of maintaining early pregnancy in the mare in the absence of any progesterone support from the maternal ovaries (McKinnon et al, 1988; McKinnon et al, 2000<sup>\*</sup>). It is simple and safe to administer and it produces no untoward side effects. Hence, in mares with a history of previous pregnancy losses in which progesterone deficiency was considered to be the likely cause, the following protocol can be initiated.

## Protocol

- Scan the mare's ovaries for ovulation, and her uterus to rule out post breeding fluid accumulation 1-2 days after mating.
- From the 2nd or 3rd day after ovulation in the mated or inseminated mare, begin feeding a daily oral dose of Regumate.
- Scan for pregnancy during days 14 - 16 after ovulation. If pregnant, continue administering Regumate. If not pregnant, cease Regumate administration to allow the mare to return to oestrus and be re-mated.
- Continue feeding Regumate daily to around Day 120 of gestation. Thereafter, reduce the dose by half for 4 days, then to a quarter for 4 days, then stop altogether. At this stage, the placenta should be producing adequate supplies of progesterone directly onto the endometrium as the principal target tissue (W.R.Allen Pers.Comm.).

## Benefits

- An efficacious and practical method of administering supplemental progesterone to pregnant mares.
- Can prevent pregnancy loss in mares in which progesterone deficiency is the underlying cause of the problem and does no harm in those in which progesterone deficiency is not involved.

## References:

- \* McKinnon A O, Squires E.L., Carnevale E.M., Herment M.J. (1988) *Theriogenology* 29, 1055-1063.
- \* McKinnon A O, Lescun T B, Walker J H, Vasey J R and Allen W R, *Equine Vet J.* (2000) 32 (1) 83-85.



## Suppression of Unwanted Oestrus Behaviour

In Sport horse mares (eventers, showjumpers, dressage horses, polo ponies, endurance horses and carriage-driving horses) the period of sexual receptivity (oestrus) may interfere significantly with training schedules or competition performance.

Likewise, some childrens' ponies and riding horses show unruly and sometimes aggressive and dangerous behaviour when they are in oestrus. Daily administration of Regumate will usually suppress this unwanted sexuality and provide a more stable, steady and safer horse.

### Protocol

- Regumate may be given for shorter intervals (e.g. 10 - 20 consecutive days) to suppress sexual behaviour during important training periods, thereby allowing the true oestrus to occur during Days 3 - 8 after the stopping Regumate administration, with an injection of 3000i.u. Chorulon given on Day 6 or 7 to hasten ovulation and the onset of dioestrus.
- Alternatively, Regumate may be given for much longer periods (e.g. over 100 consecutive days) without adverse side effects and with a normal oestrus and fertile ovulation occurring as expected 7 - 10 days after stopping the Regumate.

- Studies are in progress to determine the true withdrawal period for Regumate but until these results are available, the standard "8-Day Rule" applies where a horse should not have been administered Regumate during the 8 days before the competition. Thus, Regumate should be used to synchronise the mare's oestrous period so that it does not coincide with the competitive event and she has already entered a natural dioestrus by the time the event takes place.



- The last dose of Regumate should be given 14 days before the competition.

### Benefits

- A stable and consistent female horse that will perform at her best in whatever discipline she is entered. "Mareishness" is eliminated.
- A safer, more stable and more reliable child's pony that will perform at her best. Brings peace of mind to rider and parents.

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