



RECENT APPROACHES IN THERAPY OF INFERTILITY IN EQUINES

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Equids belong to order **Perissodactyla** which includes family

Rhinocerotidae
Tapiridae and
Equidae

- Equids Domestic Equus caballus
 Feral E.Prezwalski Extinct
 Asiatic wild ass
 African wild ass
 Zebra Plains, Mountain, Grevys

NORMAL EQUINE REPRODUCTION

- Breeding season

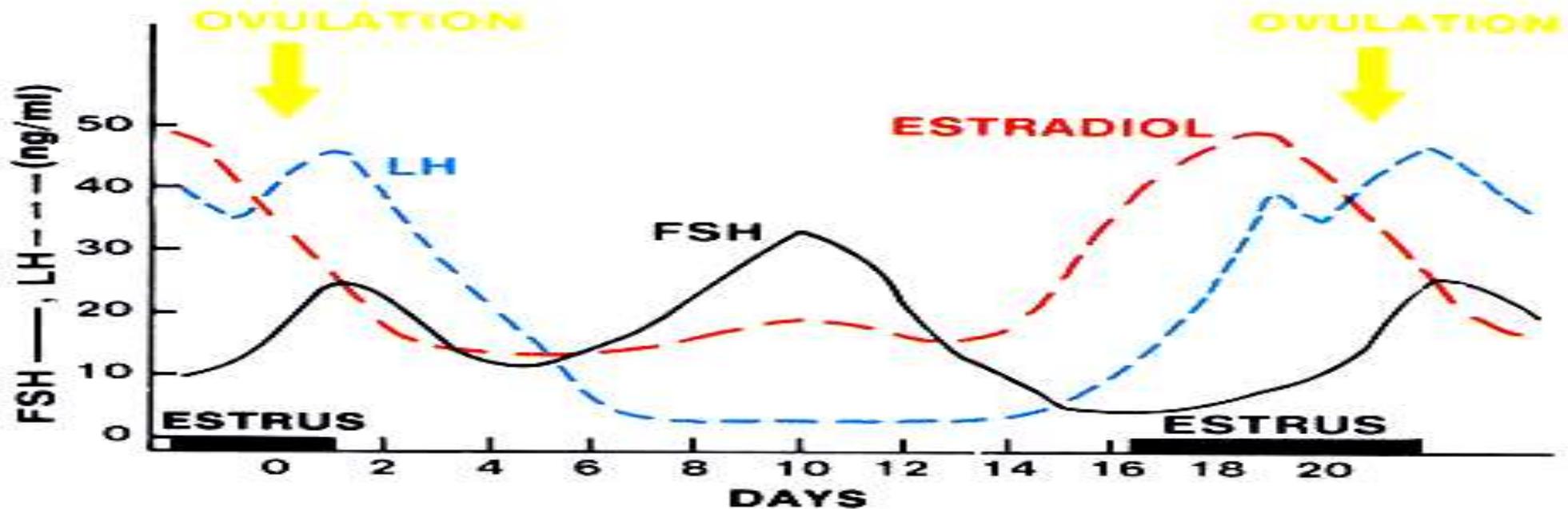
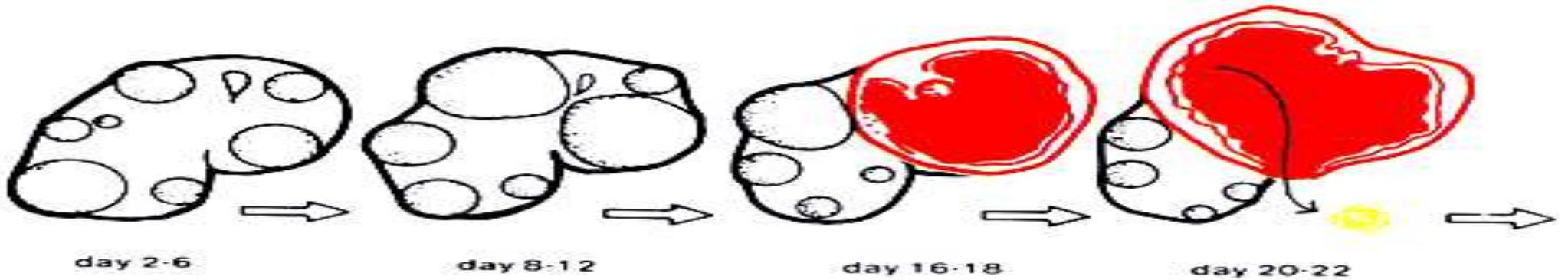
Mares in North Hemisph	15 Feb to 1st Week of July
Mares in South Hemisph	August to December
African wild ass	All year (Sp Apr and May)
Grevys mountain zebra	April to September
Burchells zebra	April - May

Estrous cycle

Mare Av. 21 days cycle length 5-7 days Ovulation: 24 to 48 hours prior to end of heat

Diestrus: 14 – 16 days

Donkey mares cycle length 5 to 7 days



Mating Behavior (Estrus signs)

- The mare will allow the stallion to **smell and bite**.
- She will
 - **extend her hind legs,**
 - **lift her tail to the side and**
 - **lower her rump.**
 - The erect clitoris will be exposed frequently by contractions (**winking**) of the labia.
 - The vulva will be elongated and swollen, with the labia partly everted.
- The mare should be teased by a stallion for accurate detection.
 - Attempts to fight the stallion indicate she is not in estrus even though some other signs of estrus are apparent.



- **Some peculiarities of reproduction**

- Seasonality

Uterus

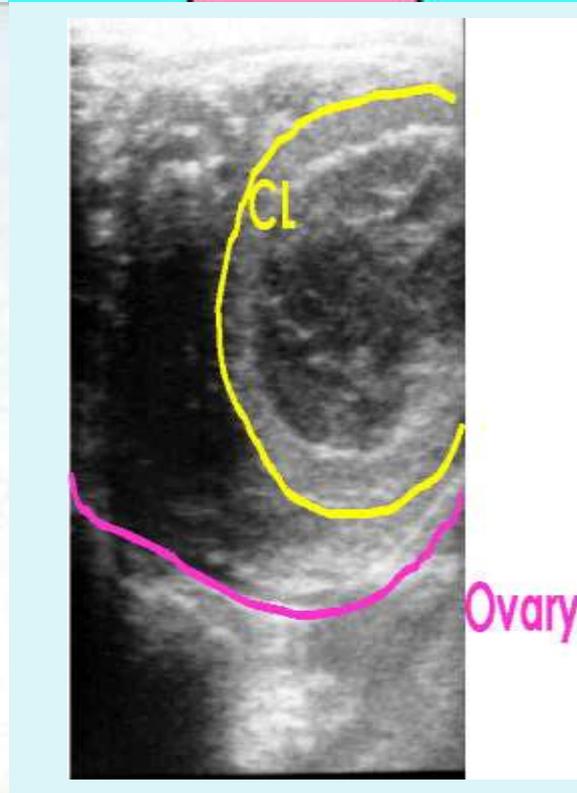
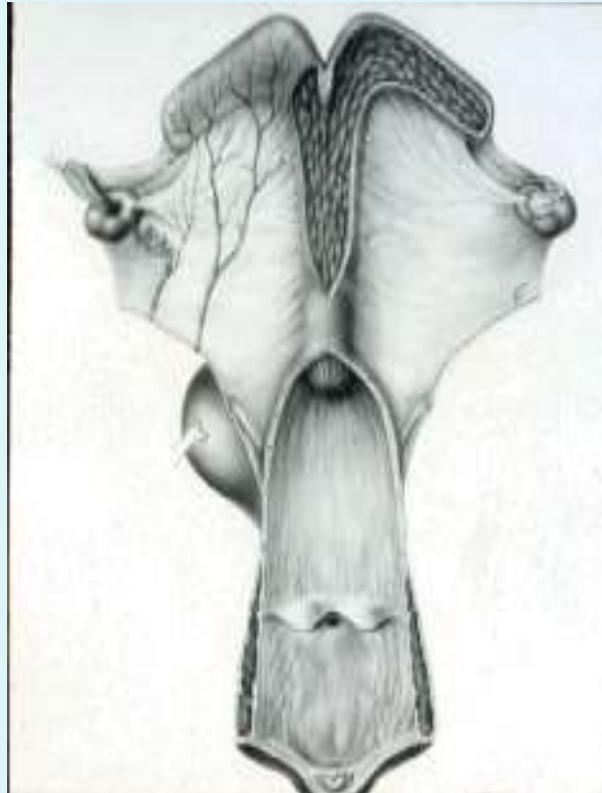
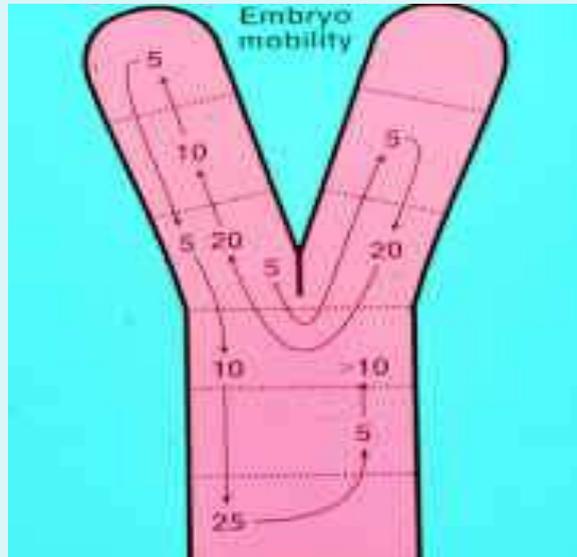
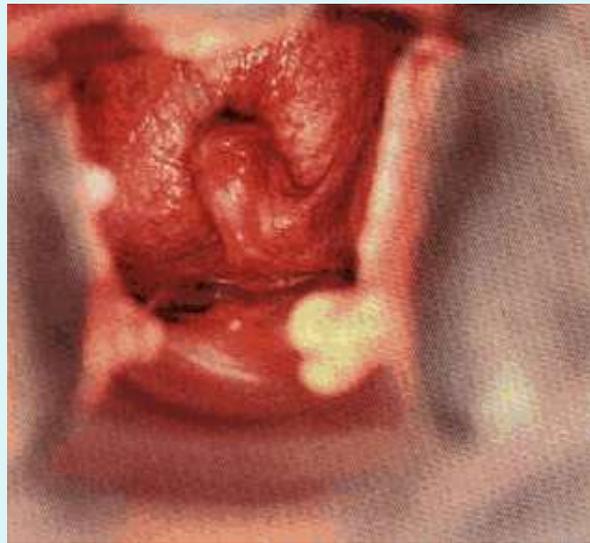
Cervix

Fallopian tubes

Embryonic signals and mobility

Ovulation & CL

Teasing program



Special equine fertility problems

- Impositions of the breeding season
- Poor fertility during the transition period-Irregular cycles
- Prolonged diestrus
- Early embryonic deaths-Absorptions
- Fluid accumulations post mating-PMIE
- Failure of timely ovulation
- Poor fertility at foal heat
- Abortions due to twins and Herpes Virus infections

Advances in infertility treatments

- **Artificial lighting -16 h light 8 h darkness**

60 W incandescent bulb one month ahead of the transition period enhances resumption of cyclicity during January and regular cycles in February and majority of mares in estrus and 65% conceiving in March in the Northern hemisphere (Bosh et al., 2009; Lane et al., 2016)

Advent of the blue light masks has also been shown to hasten early season estrous cyclicity in barren and maiden mares (Murphy et al., 2014).



Hormone assays

- Prolonged diestrus: The mares failure to show ovulatory estrous cycles during the breeding season was the common cause of occurrence of “prolonged diestrus” – spontaneous prolongation of the life of the cyclical corpus luteum (CL) in the absence of pregnancy (Stabenfelt et al., 1974). The condition occurs at least once during the year in as many as 25% of mares when the CL persists in the ovary and continues to secrete progesterone for some 80–90 days.

- Till 1970s prolonged diestrus could not be diagnosed by rectal palpation since the persisting luteal tissue lay deep within the ovarian stroma. The advent of ultrasonography widened the possibility of detection of corpus luteum however, assay of the plasma progesterone is the surest method of detection of luteal tissue.
- Early assay methods for the steroid hormones, both estrogen and progesterone, involved gas-liquid chromatography (GLC) or radioimmunoassay (RIA). Both techniques required expensive laboratory-based counting equipment and were unsuited to rapid clinical measurements and diagnoses.

- Currently, the development of ELISA and automated systems can rapidly and accurately diagnose the persistence of active luteal tissue in mares' ovaries and treat them accordingly with prostaglandins.



Oral progestagens

- Allyl trenbolone (altrenogest) to mimic the biological actions of endogenous progesterone and progestagen (Webel, 1973) paved the way for its use in mares.
- The ability of altrenogest, but the failure of four other synthetic progestagens, namely medroxyprogesterone acetate, hydroxyprogesterone hexanoate, norgestomet and megesterol, to maintain early pregnancy in the absence of endogenous progesterone (McKinnon et al., 2000) paved the way for its use in mares more frequently to regulate pregnancy and estrus.

- Altrenogest (0.22% solution, Merck) is administered orally 1 mL per 110 pounds body weight (0.044 mg/kg) once daily to mares with a history of any sort of pregnancy loss without any attempt to assay for progesterone/progestagen concentrations in their blood. It has also been advocated for postponement of foal heat and estrus and ovulation control (Macpherson and Blanchard, 2005). Estrus suppression is also an indication for the administration of altrenogest, but it should not be used in mares with uterine disease.



Ovulation failures

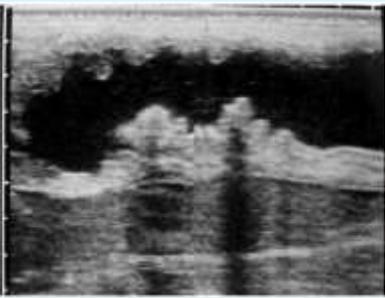
- Ovulation failures are frequent in mares and GnRH analogs have shown to have poor effects until administered continually and hCG have the potential dangers of development of antibodies and Theilers disease.
- The GnRH analogue, deslorelin acetate (2.1 mg), when incorporated into a slow-release subcutaneous implant, proved highly efficacious in hastening and inducing ovulation some 40–42 h after administration (McKinnon et al., 1993; Meyers et al., 1997).

- The implant should be removed once ovulation has occurred (Mc Cue, 2002) otherwise it results in extended inter-ovulatory period (Henderson et al., 2012).



- A number of injectable GnRH analogues have now been produced commercially (Ferris et al., 2012; Finan et al., 2016) and are used widely and to good effect throughout the horse breeding world to induce ovulation.





Post breeding endometritis (Fluid accumulations)

Traditionally saline has been infused followed by oxytocin injections.

DMSO Mares infused with 30% solution of 30-60 mL of DMSO after breeding tended to have higher pregnancy rates than mares infused with saline (Ley et al. 1989).

N-Acetylcysteine (mucolytic)

- Mares received a 0.6% solution of N-acetylcysteine (ACE) either the treatment cycle before (n = 10) or in the 48 h before breeding (n = 10) in addition to conventional treatments. Infusion before breeding was associated with higher than expected pregnancy rates as 17 of 20 mares (85%) conceived and foaled (Le Blanc, 2010).

Biofilm Associated Bacterial Endometritis In Mares

- Infusion of buffered chelators such as 250–500 ml of Tricide or Tris-EDTA into the uterus on day 1, and lavage of the uterus to remove the solution out within 24 h and examination of the efflux has been suggested (Le, Blanc, 2010).

Table 1. Antibiotic and Non-Antibiotic Combinations For The Treatment Of Biofilm Associated Bacterial Endometritis In Mares

Tris EDTA- final concentration in the syringe should be 50 mM Tris and 3.5 mM EDTA

Note: Tris-EDTA and Tricide are similar; however Tricide is not equivalent to Tris-EDTA in regards to bacterial killing

To make Tris-EDTA: 16oz bottle of Dechra Triz-EDTA crystals; add 8 oz of sterile water (this is different than the bottle instructions). The 2x concentration of Tris-EDTA solution will be further diluted by the antibiotics below to the proper final concentration.

Antibiotic	Drug Amount	Tris EDTA	QS	Final volume	Notes:
Amikacin (250 mg/ml)	4 mls (1 gram)	30 mls	16 mls sterile fluid (Saline, LRS, Sterile H ₂ O)	60 mls	10 mls of 8.4% sodium bicarbonate should be added to the amikacin
Ceftiofur (1 gram reconstituted in 20 mls)	20 mls (1 gram)	30 mls	10 mls sterile fluid (Sterile H ₂ O)	60 mls	
Ciprofloxacin (10 mg/ml)	40 mls (400 mg)	40 mls	0	80 mls	Split between two syringes

H₂O₂- 1% final concentration in the syringe

A 3% stock solution is available at many drug stores and veterinary distributors

Antibiotic	Drug Amount	H ₂ O ₂	QS	Final volume	Notes:
Amikacin (250 mg/ml)	4 mls (1 gram)	20 mls	26 mls sterile fluid (Saline, LRS, Sterile H ₂ O)	60 mls	10 mls of 8.4% sodium bicarbonate should be added to the amikacin
Ciprofloxacin (10 mg/ml)	40 mls (400 mg)	20 mls	0	60 mls	

Ceragyn Infusion Product- >30% final volume in the syringe

Antibiotic	Drug Amount	Ceragyn	QS	Final volume	Notes:
Amikacin (250 mg/ml)	4 mls (1 gram)	20 mls	26 mls sterile fluid (Saline, LRS, Sterile H ₂ O)	60 mls	10 mls of 8.4% sodium bicarbonate should be added to the amikacin
Ciprofloxacin (10 mg/ml)	40 mls (400 mg)	20 mls	0	60 mls	

DMSO- 30% final concentration in the syringe

99% stock solution is used for calculations below

Antibiotic	Drug Amount	DMSO	QS	Final volume	Notes:
Ceftiofur (1 gram reconstituted in 20 mls)	20 mls (1 gram)	20 mls	20 mls sterile fluid (Sterile H ₂ O)	60 mls	
Ciprofloxacin (10 mg/ml)	40 mls (400 mg)	20 mls	0	60 mls	

Modulation of the inflammatory response for PMIE

- Single dose dexamethasone administered within one hour of mating and daily prednisolone administration given before and after mating have improved pregnancy rates in mares with uterine fluid (Bucca et al. 2008; Papa et al. 2008). A single injection of dexamethasone administered within one hour of mating (50 mg, IV; approximately 0.1 mg/kg) combined with routine post breeding therapies have also shown improvement in pregnancy rates in mares with post breeding endometritis (Bucca et al., 2008).

Placentitis

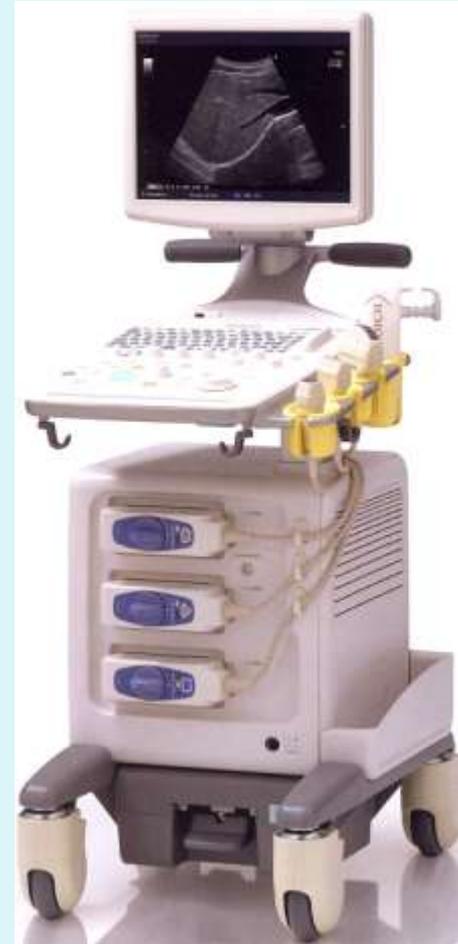
- Affects 3-5% of pregnant mares
- Vaginal discharge, udder enlargement and birth of septic foals
- Antibiotics such as Gentamicin 6.6 mg/kg IV q 24 h
- Pentoxifylline (brand name tablet flexital sun Pharma or tablet Flowpent Abott labs 400 mg tablets) to be administered orally at dose of 8.5 mg/kg every 12 h
- and dexamethasone 40, 35, 25 mg q 24 h give over 6 days decreasing dose every 48 h

Uterine lavage for uterine infections

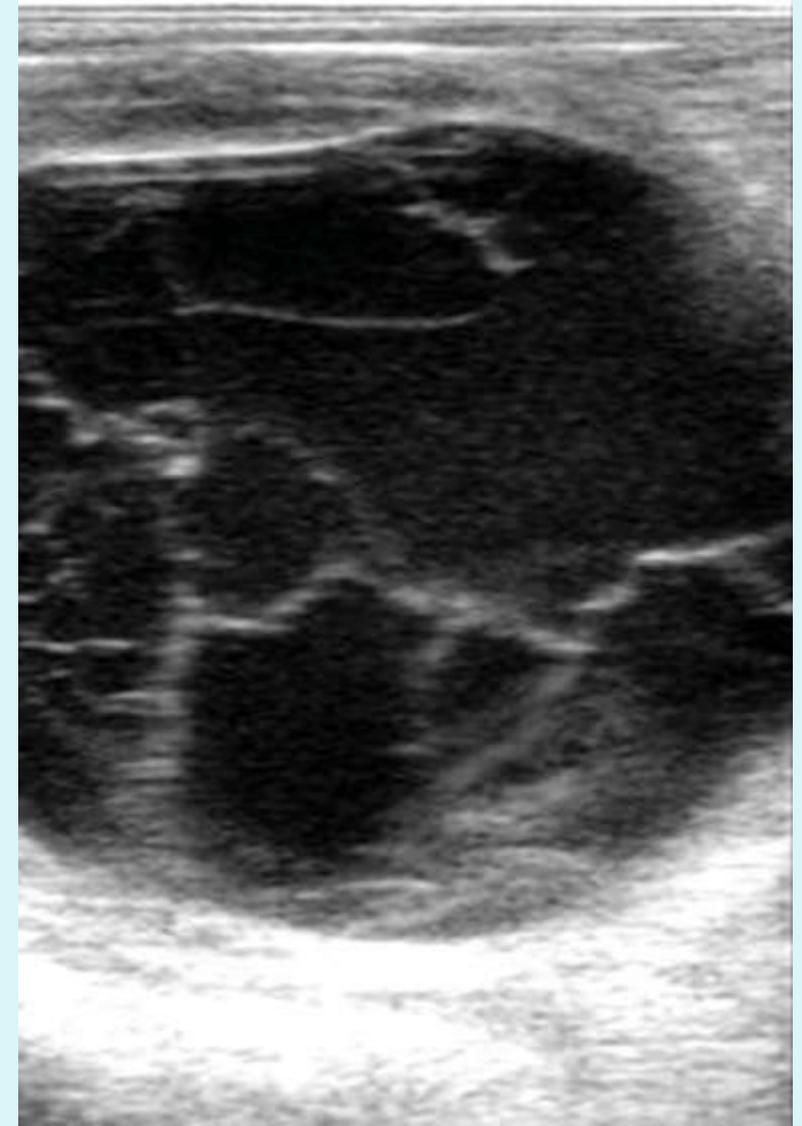
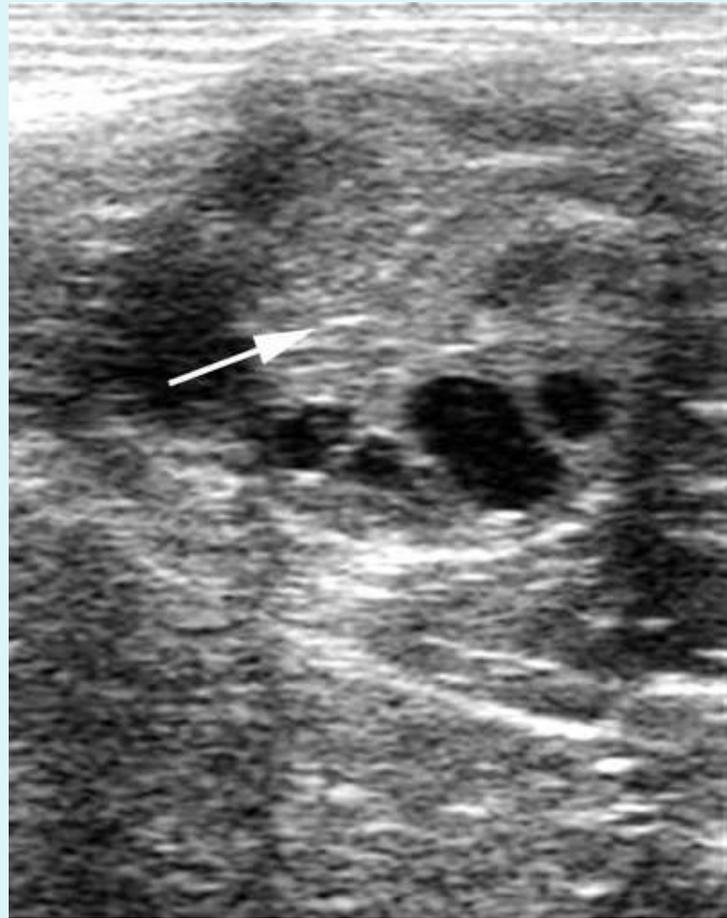


Ultrasonography

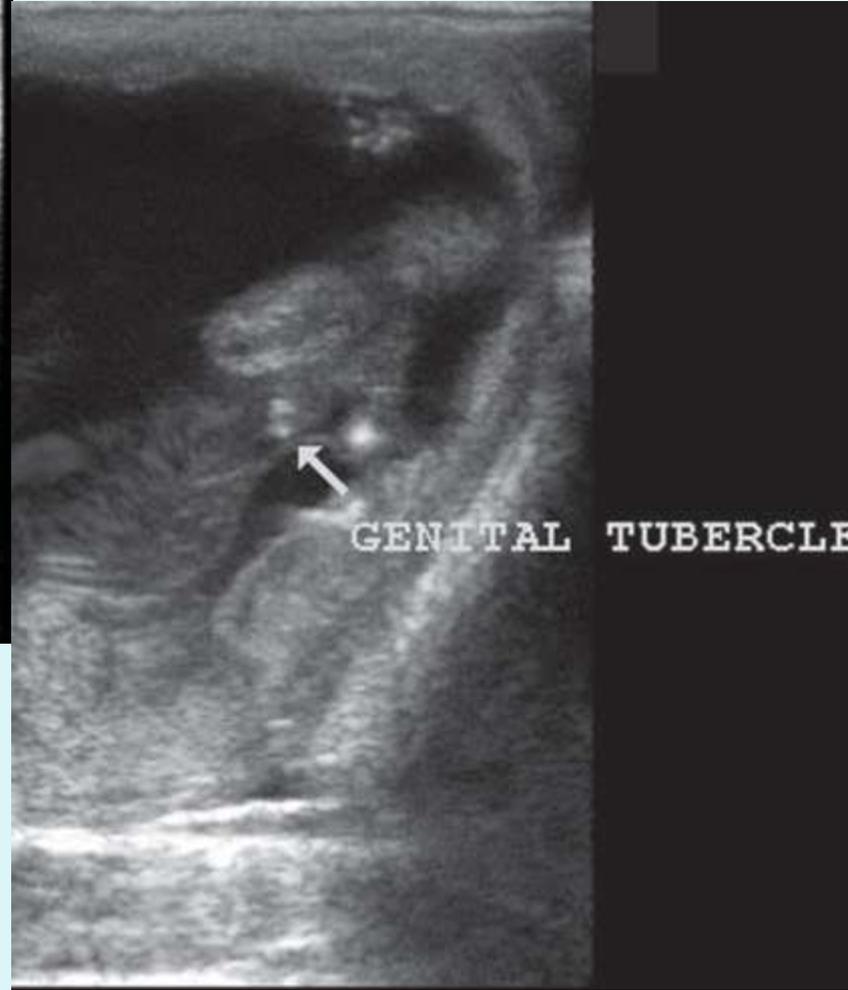
- Ultrasound is now used with certainty for detection of ovulation, early pregnancy, twins and uterine luminal fluid accumulations.
- Perhaps the most important of all from the stud management point of view, ultrasound can accurately determine that a mare was definitely not pregnant as early as 14 days after ovulation, thereby enabling the clinician to confidently administer prostaglandin to induce luteolysis and bring her back into estrus for remating.
- Other applications include evaluation of uterine involution (Dadarwal et al., 2004) and fetometric studies or evaluation of placentitis in pregnant mares (Cummins et al., 2008).



Follicle, CL and anovulatory follicle with echogenic strands



Endometrial folding, Twin pregnancy, 25 Day fetus and fetal sex determination (Male)



Color doppler

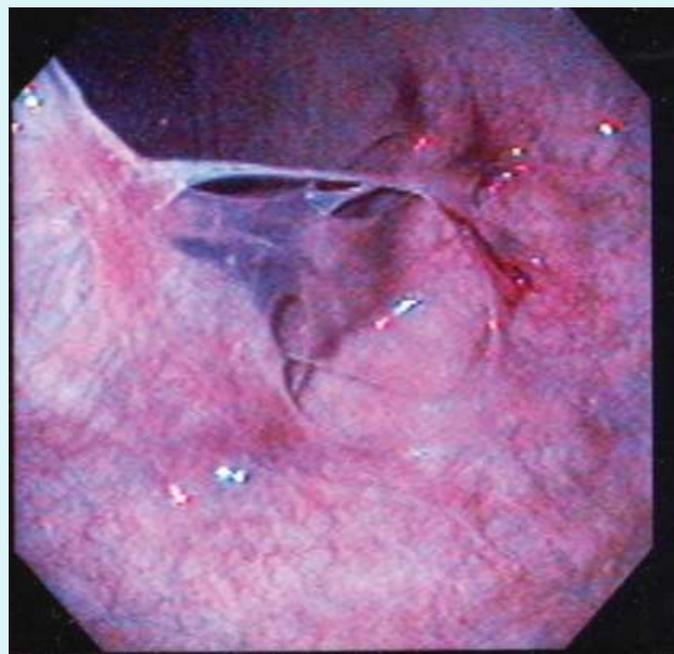
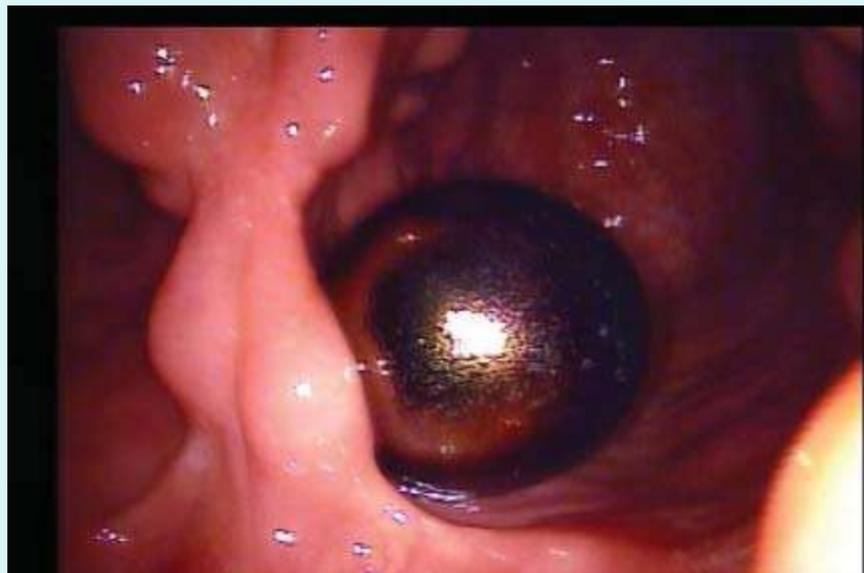
- Doppler capabilities allowing the assessment of blood flow in the reproductive tract (Ginther, 2007). These can measure the absence, presence, direction and quantify blood flow to assess development and function of the corpus luteum, predict ovulation, indicate the presence of the fetal circulation in the early pregnancy and measure blood flow indices (such as velocity, flow volume and resistance) in the uterine artery during pregnancy, or following the administration of vasoactive drugs (Bollwein et al., 2002; Gastal et al., 2006; Bailey et al., 2012; Klewitz et al., 2015; Brogan et al., 2016).

Hysteroscopy and laparoscopy

- The rapid advances in endoscopy technology over the past 20 years, both rigid and flexible, have benefited equine reproduction in a number of ways.
- Endometrial cysts and fibrous adhesions resulting from injudicious infusion of irritant solutions into the uterus can be assessed and sight directed endometrial biopsies can be recovered.
- Laparoscopic Examination of the Uterus and Ovaries can be performed

- Virtual laser photo-ablation of large, fluid-filled endometrial cysts placed where they might impede free movement of the young embryo throughout the uterine lumen between Days 10 and 16 after ovulation (Allen and Wilsher, 2018).
- Ablation of intrauterine fibrous adhesions (Bracher et al., 1994).

- As with many other reproductive procedures in the mare, her straight and easily penetrable cervix combined with the straight body and horns of the uterus make the whole process of hysteroscopy and laser surgery very simple and fruitful in diagnostic and reparative terms.
- Low dose hysteroscopic insemination of fresh or frozen/thawed spermatozoa (e.g. 1–5 million) suspended in only 200 μ L of TALP medium directly onto the external surface of the utero-tubal papilla (Morris et al., 2000).



- A more recent application has been to use laparoscopy to apply a prostaglandin E2 gel preparation, designed to soften and dilate the cervix in pregnant women to facilitate labor, to the whole length of the equine oviduct (Allen et al., 2006) to presumptively unblock the oviducts of infertile mares.

- Application of the gel to the oviduct induces relaxation of the circular and contraction of the longitudinal smooth muscle fibers in the wall of the oviduct, thereby mimicking the PGE₂ normally released by the equine embryo to “drive itself” down the oviduct (Weber et al., 1991; Weiber et al., 1995) and through the very tight utero-tubal junction to enter the uterus on Day 6–6.5 after ovulation (Battut et al., 1997).

- The careful selection of mares for treatment with oviductally applied PGE2 gel, based entirely on the veterinary exclusion of other possible causes of their persisting infertility, has given very impressive results, especially in mares treated 4 or 5 days after a previous mating or insemination and which then exhibit a normal 14 days pregnancy when scanned 10 days later.

Places for passing endoscopic instruments



• THANKS