Fetal Sex Determination in the Mare Between 55 and 150 Days
Investment and Reward

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Equine fetal sexing is the determination of sex of an equine fetus after 55 days of gestation. A considerable amount of time needs to be spent learning the various techniques described in this paper, but the investment is usually well rewarded if applied in certain types of practices (i.e. adequate concentration of horses and breeding operations, type of clientele, and type of services provided by the competition). Different techniques for determination are required for the different stages of gestation. Technique number one, performed between 55 and 90 days of gestation, involves finding the genital tubercle (precursor to the penis in the male and precursor to the clitoris in the female) and determining its location relative to other fetal structures. The genital tubercle appears as a hyperechoic bilobulated equal sign 2-3 millimeters in length and develops between the hind legs on the ventral midline of both sexes at 50-55 days. Migrations of the tubercle that indicate the sex of the fetus begin about day 54-55. A determination cannot be made before 55 days because the migration toward the umbilical cord in the male or toward the anus in the female has not yet occurred. Using technique number one the veterinarian should be able to make a diagnosis 95% of the time on the first exam with 99% accuracy. The exam should take from a few seconds to 5 minutes to perform depending on the experience of the veterinarian.

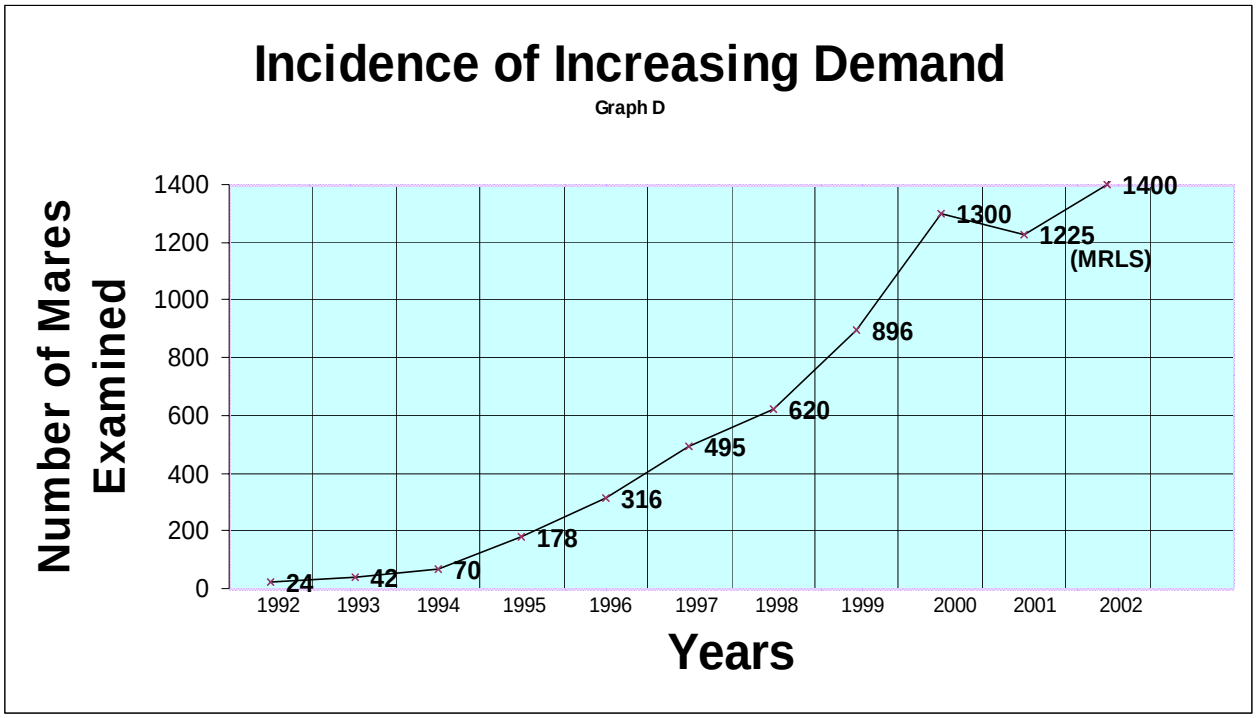
Technique number two performed between 90 days and 150 days of gestation involves finding the external genitalia of the fetus (penis, glans penis, prepuce and gonads in the male and mammary gland, teats, clitoris and gonads in the female). At this stage (90 – 150 days) the veterinarian should be able to formulate a diagnosis 90% of the time on one examination with 99% accuracy. It should take from a few seconds to 10 minutes to perform the exam again depending on the experience of the practitioner.

Around 150 days of gestation the fetus begins to assume an anterior presentation. This means that the head will be easily accessible but the pelvic area will be out of reach. The fetus is much larger now and does not change positions often. Therefore, rectal scanning for the determination of sex after 150 days is very difficult. The percent of diagnosis between 150-200 days would be very low (5% - 25% of attempts made by rectal examination).

Technique number three involves performing a transabdominal scan and finding external genital after 150 days of gestation. In my experience, determining sex transabdominally has a very low percentage of diagnosis and is very time consuming. Additionally, clipping of the abdomen is sometimes required and access to a more powerful ultrasound machine is helpful. Because of the low percentage of diagnosis, the extra amount of time required and clipping and ultrasound machine requirements, I strongly recommend that technique number one and two be used for determination of sex in the equine.

The information obtained from the fetal sexing procedure is simply a management tool to be used by the owner or manager. Depending on the sire, the dam, or both, the fetal gender may affect the value of the foal. Consequently, this affects the value of the mare. (For example: If the value of the mare changes depending on the gender of the foal then appraisals, sales reserves, insurance coverage and collateral limits for loans could all be affected). This knowledge of the fetal gender could impact various management decisions such as where to foal the mare. (If you want to race a New York bred colt it is impractical to send a mare carrying a filly to New York to foal). Some other considerations include: Cash flow predictions that could be determined earlier. Are you going to have a certain individual for sale (income) or an individual to race (expense)? Perhaps an owner wants a particular sex from a particular cross, if that breeding was successful he could breed the following year to a different stallion or try again to the same stallion if he didn't get the sex desired from the first mating. In this way the mating list could be determined much earlier. Perhaps if a mare is carrying a colt, the size of the fetus may be of some concern and precautions at foaling may be taken. Each year as the demand for fetal sex determination increases, there seems to be more new reasons for horsemen wanting to know the sex of the fetus.

If a practitioner is the only one in his area able to accurately perform a sexual determination, the procedure can develop into a new worthwhile profit center for the practice. The following is a graph of increasing demand when fetal sex determination was introduced into the area.



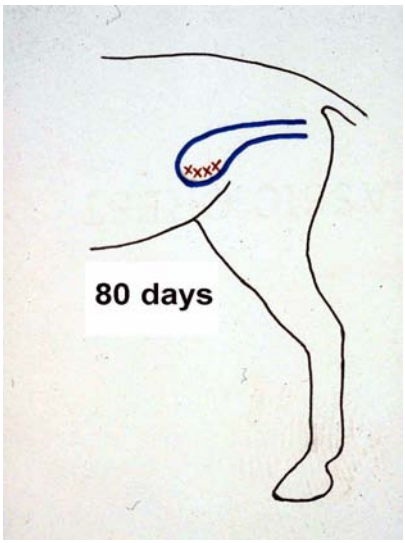
Materials Needed:

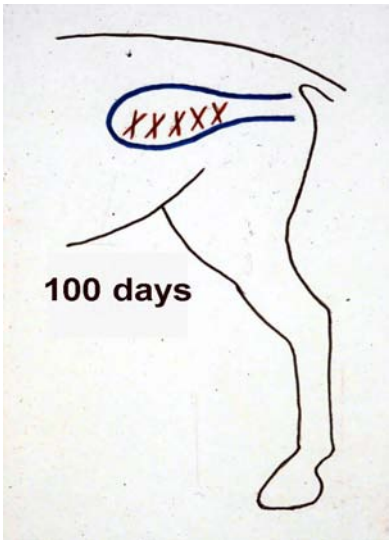
1) Quality ultrasound machine with a 5-MHz linear array rectal transducer (ALOKA 500 SSD or equivalent)
    a. Settings – if the overall gain, near gain or far gains are set too high, the contrast between the fetus and background is less and the tubercle is more difficult to see.
2) Rectal palpation essentials:
    a. Lube, sleeves
    b. Restraint – stocks, lip chain, twitch, etc.
    c. Tranquilizers - Depending on the situation, tranquilization is acceptable, but this may cause the uterus to relax and drop away from the examiner making it more difficult to reach. I use 200 mg of Xylazine (Sedazine®) and 10 mg of Butorphanol Tartrate (Torbugesic® CIV) mixed IV.
    d. Propantheline bromide may be used to prevent rectal straining – 30 mg IV.
3) Ultrasound stand on wheels – for close eye level viewing.
4) Subdued lighting – for good screen visualization.
5) Hat – to remove surrounding glare
6) Fly spray – (if needed) to keep mare movement to a minimum.
7) Video or printers are helpful to verify the diagnosis and for record keeping.

Fetal Development

Days	Visualization
55-60	Fetus is very small, genital tubercle is difficult to see and may or may not be fully migrated.
60-70	Fetus is easily accessible; tubercle is distinct and fully migrated **(Easiest and most ideal time for fetal sex determination)**
70-80	Fetal tubercle is distinct but slightly more difficult to reach.
*80-90	Difficult to access fetus, tubercle is less distinct, genitalia development is just beginning. **(Most difficult time)**
90-100	Fetus is generally accessible, genitalia is not very well developed. (Difficult to differentiate genitalia at times)
100-110	Genitalia becoming more evident.
110-120	Fetus very accessible and genitalia is well developed. **(Ideal time in technique two)** for determination
120-140	Genitalia is well developed but posterior of fetus may be difficult to access at times.
**140-150	At times fetus has anterior presentation with posterior out of reach.
150+	Usually the fetus has anterior presentation and posterior is out of reach. Transrectally this stage is with a difficult low % of diagnosis 5-25%
150 – Term	Transabdominal – low percentage diagnosis, time consuming, need to clip abdomen, more powerful ultrasound machine is helpful.

* At 80-90 days the fetus is often difficult to reach due to the position of the uterus in the posterior abdomen. At approximately 80 days the allantoic fluid of the pregnancy pulls the uterus over the rim of the pelvis. The fetus is small, falls to the most ventral portion of the uterus, and is very difficult to reach. As the uterine contents (fetus and fluids) increase in size, the uterus actually elevates more in the abdominal cavity and becomes easier to reach and view the fetus.

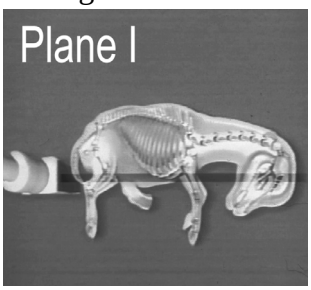




** Mares that are 130-150 days that are out of reach should be viewed again for possible position change.

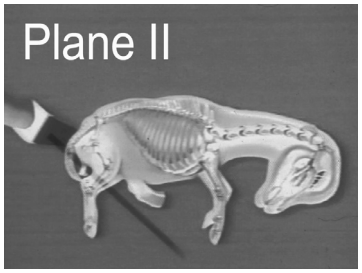
PLANE I
Ultrasound Cuts A Frontal Plane

fig 1

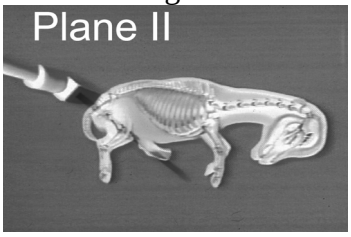


PLANE II
Ultrasound Cuts A Plane Perpendicular to Axis of Spine in Pelvic Area

female fig 5a

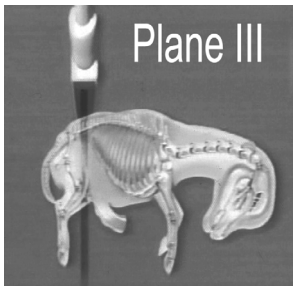


male fig 5b



PLANE III
Ultrasound Cuts Plane Perpendicular to Axis of Spine in Posterior Abdominal Area

fig 11



#1 **Plane 1**	**#5a, 5b** **Plane II**	**#11** **Plane III**
#2a, 2b, Slide of Male 60 Days Frontal Plane	**#6** 60 Day Male	**#12** 60 Day Male Tubercle
#3 134 Day Female Mammary Gland	**#7a, 7b, 7c** T-T Triangle	**#14** 140 Day Male Prepuce
Various oblique views	**#8** 80 Day Clitoris	**#15** 94 Day Prepuce and Penis
#4a Anus Near Tail	**#9** 124 Day Mammary Gland	**#17** Male Gonads 125 Days
#4b Clitoris in Cleavage	**#10a, 10b, 10c** Shaft of Penis	**#18** Female Gonads
#16 Flank View 109 Day Shaft and Glans Penis	**#13** Bones of Hind Limbs and Pelvis	

PROCEDURE – TECHNIQUE 1

- The procedure involves a thorough evacuation of the feces from the rectum to allow easy manipulation of the transducer.
- Determine positioning of the fetus by scanning the entire fetus: (Fig. 1, 5a, 11)
  a. The skull is a good anterior marker.
  b. The heart is a good ventral marker.
  c. Ribs coming off of vertebrae and base of tail are good dorsal markers.
  d. The tail is a good posterior marker.
- When the position of the fetus has been determined, proceed to the posterior part of fetus until the transducer has gone completely off of the posterior part of the fetus. (Fig. 5a)
- Gradually, ease back on the fetus with the transducer cutting a plane perpendicular to the axis of the spine of the fetus. (Fig. 5a, 5b)
- A cross-section of the tail head should be picked up on the dorsal aspect of the fetus. It will appear as a hyperechoic round mass with very little muscle tissue around it. (Fig. 7c, 8)
- Ventrally, two tibias (hyperechoic round structures, with no muscle mass) should be seen forming a triangle with the tail head. (Fig. 7a, 7b, 7c)
- If the fetus is a female, there will be a hyperechoic tubercle within the tibia-tail head triangle with the tubercle slightly toward the tail head. The female tubercle is

difficult to consistently identify other than within the tibia-tail head triangle. (Fig. 7a, 7b, 7c)

- If the fetus is a male, nothing will be seen within the tibia-tail head triangle.
- If nothing is seen in the triangle, move the transducer gradually anteriorly along the fetus keeping the same perpendicular plane (Plane II or III) to the axis of the spine of the fetus. (Fig. 5a, 5b)
- A hyperechoic equal sign should appear between the two tibias (The tibias moving anteriorly change to the stifles or femurs). (Fig. 6, 12)
- ***TIP: Remember tibias have no muscle mass around them and femurs do have muscle mass around them.***
- Moving further anteriorly, the large round abdomen will be seen. In the male the tubercle can often be seen on the outside ventral wall of the abdomen just posterior to the urachus, which is seen as a dark hole 4 – 5 mm in diameter. (Fig. 2a, 3, 15)
- Moving the transducer back and forth over the posterior area in this plane usually exhibits a visible tubercle.
- The male tubercle can also be readily seen from a frontal plane (Plane 1) (Fig. 1) this plane exhibits the front legs, ventral abdomen, and hind legs with the tubercle appearing slightly anterior to a line drawn between the hind legs (usually femurs or stifles). (Fig. 2a, 2b)

PROCEDURE – TECHNIQUE 2

- **After 90+ days gestation, the tubercle is less distinct.**
- Proceed to the posterior aspect of the fetus. Find where the posterior muscles of the buttocks come together and form a definite cleavage on the ventral midline. (Fig 3, 10c)
- Follow the cleavage posteriorly to the tail head.
- If the fetus is a female, a clitoris will appear as a small round structure in the cleavage shortly before the tail head is reached. (Fig. 8, 4b)
- ***TIP: <u>Caution</u>: If the small round structure is too close to the tail head, it could be the anus.*** (Fig. 4a)
- If you follow the midline or cleavage line anteriorly, you will encounter the mammary gland in the female (Fig. 9) or the prepuce and penis in the male. (Fig. 10a, 10b, 10c)
- A mammary gland appears as a triangular structure with slightly denser tissue than surrounding muscle tissue. It may have two bright hyperechogenic teats and slightly dense areas in each half of the mammary gland; it may also have a translucent division between halves of the mammary gland. (Fig. 9)
- In the male, the prepuce may appear as a cone shaped structure with a hyperechoic area within the cone. The hyperechoic area is the penis itself. (Fig. 14, 15)

- Sometimes the actual shaft of the penis can be seen with a hyperechoic distal segment. (Fig. 10a, 10b, 10c)
- Occasionally the shaft and glans penis can be seen on a Flank view. (Fig. 16)
- If the prepuce is viewed from a cross-section across the posterior ventral abdomen, it will appear as a cone shaped structure off of the ventral abdominal wall just posterior to the urachus. (Fig. 14, 15)
- The gonads can be viewed from a plane III view of the posterior abdomen near the urachus. (Male Fig. 17, Female Fig. 18)

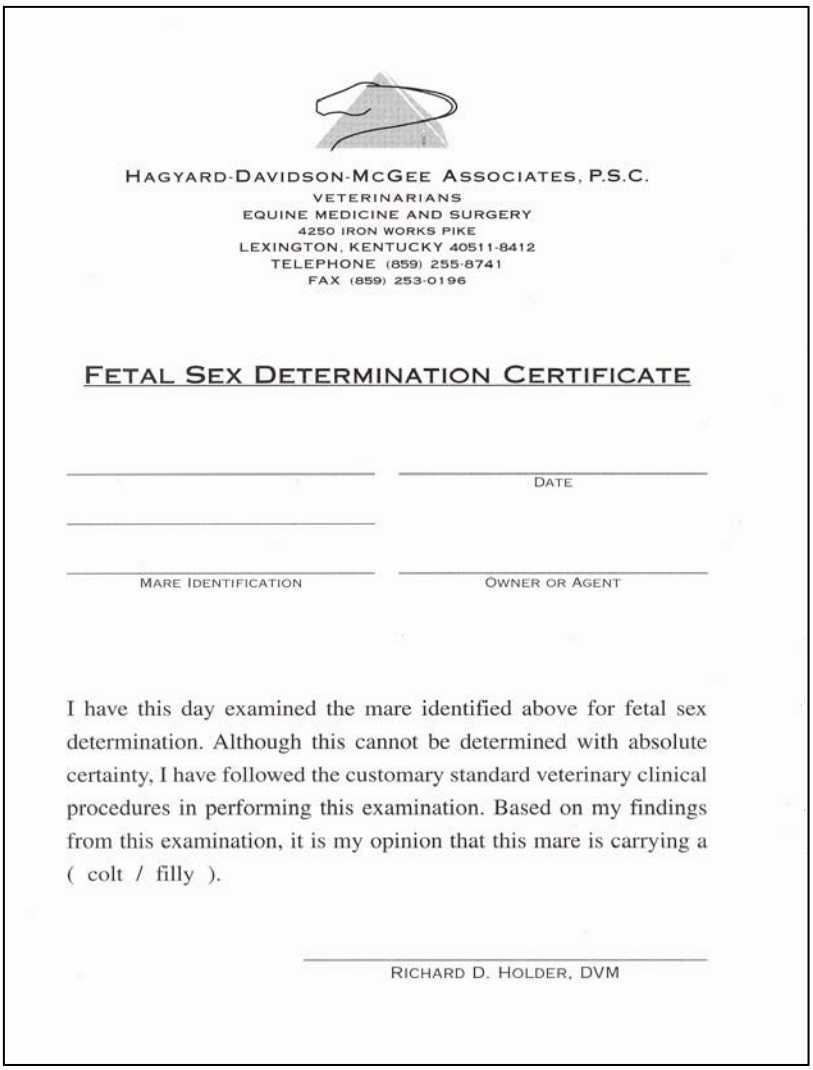
As the fetus gets older, the genitalia become more developed and more easily differentiated from surrounding tissue. However as the fetus develops, it becomes more difficult to access the posterior area. After day 150, the fetus begins to have an anterior presentation, which puts the posterior pelvic area out of reach. Also, because of its size, the fetus is less mobile and less frequently rotates the posterior pelvic area to a more accessible position. A fetal sex determination has been diagnosed at 184 days on transrectal exam, but this is very unusual and possibly not a good sign if the fetus is in a posterior presentation this late in gestation.

There are a few precautions to consider:

1. It is mandatory to keep good records of determination.
2. Don't guess – guessing leads to errors, errors lead to lack of confidence in procedure.
3. Certificates can be given but there are no guarantees given.

It should be emphasized that these techniques are for gender identification only and not for gender control. It would be difficult to have a mare successfully pregnant at 60 days, terminate the pregnancy, and be successfully re-mated that season.

Learning these sex determination techniques provide a worthwhile service to clients but requires many hours of actual sonographic visualization of the equine fetus between 55 – 150 days of gestation. Only when the sonographic cross-sectional anatomy of the fetus is learned, will confidence of an accurate sexual determination be achieved.



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FETAL SEX DETERMINATION CERTIFICATE

DATE

MARE IDENTIFICATION

OWNER OR AGENT

I have this day examined the mare identified above for fetal sex determination. Although this cannot be determined with absolute certainty, I have followed the customary standard veterinary clinical procedures in performing this examination. Based on my findings from this examination, it is my opinion that this mare is carrying a (colt / filly).

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SUPPLEMENTAL READINGS

Ginther OJ. Ultrasonic Imaging and Animal Reproduction: Horses Book 2.

Holder RD. A Guide To Equine Fetal Sexing 55 – 150 days 2002: Aloka, Jim Chrzan, 10 Fairfield Boulevard, Wallingford, CT 06492-7502, ph: (740) 927-6147 , Hagyard Davidson and McGee 4250 Iron Works Pike, Lexington, Kentucky 40511, ph: (859) 2558741 (Instructional Video).

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Renaudin CD, Gillis CL, Tarantal AL. Transabdominal Ultrasonographic Determination of Fetal Gender in the Horse, During Mid-gestation; UCD School of Veterinary Medicine (Instructional Video)