

STUDIES ON EMBRYONIC DEVELOPMENT IN THOROUGHBRED MARES

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ABSTRACT

This study was planned to determine the embryonic developments in 12 Thoroughbred mares. The follicular growths were recorded in mares from day 8 postpartum till ovulation daily by using ultrasound machine with 7.5 MHz transrectal probe. All mares which were detected with largest follicle size and a characteristic uterine pattern of edema (cart-wheel like appearance) were mated with stallion every second day. Ultrasonography was carried out daily from day 14th post-mating till day 40 (ovulation = day 0) for detection of early of pregnancy and to monitor the conceptus. Embryonic vesicle was observed for the first time on day 14 in all mares with mean size of 10.25±0.59 mm. The fixation of vesicle, loss of spherical shape of vesicle, detection of embryo, detection of heart beats, detection of allantoic sac and start of fetal ascent were observed on 16.75±1.52, 20.66±1.86, 25.08±2.23, 28.16±2.45, 27.83±2.43 and 27.83±2.43 days post-mating, respectively. It was concluded that ultrasonic detection of early pregnancy and embryonic developments studies is an accurate tool and simple way for estimating the age of pregnancy in Thoroughbred mares.

KEY WORDS : Thoroughbred mares, Preovulatory follicle, Embryonic development, Ultrasonography**INTRODUCTION**

The "Thoroughbred" is a horse breed best known for its use in horse racing. Thoroughbreds are used mainly for racing, but are also bred for other riding disciplines such as show jumping, combined training, dressage, polo, and fox hunting. The main objective of the equine breeder is to get maximum number of foals from breeding mare in its lifetime using the limited breeding season efficiently and it can be achieved if one foal is obtained every year during the breeding life of that mare. A strategy to obtain an interval of less than 12 months between foaling is to breed mares during foal-heat (Schilela *et al.*, 2001). Because of the long gestation, the mare should conceive within a month after the previous parturition to foal every year. The horses particularly Thoroughbreds are very costly, so it is important not only to know whether the valuable mare is ovulating during foal heat but also the mare has conceived or not after service at an early stage. Similarly, very limited stallions are available for breeding and the covering charges of stallions are very high (approximately Rs. 75,000 per covering). Therefore, there is a very strong pressure from breeders on veterinarians to bred the mares exactly just before ovulation to avoid stress to the stallion during breeding season (Kamble *et al.*, 2005).

The use of ultrasound to evaluate the fetus and intrauterine environment in mares during gestation is relatively new and very wide, starting from confirming pregnancy existents, fetal gender determination to monitoring high risk pregnancies. The fertility potential may be improved by monitoring the early embryonic studies and managing twin pregnancies and slow as well as abnormal growing embryos. Death of embryos resulting from twin pregnancy is a serious problem in Thoroughbreds. Draft and Thoroughbred mares have greatest incidence of multiple ovulations (Kulisa *et al.*, 1999). Multiple ovulation may result in multiple pregnancy, and consequently in early embryonic mortality, underdevelopment of fetuses, abortions or births of underdeveloped foals. Twin

pregnancies cause dystocia and even the mare can be lost. By monitoring multiple and twin pregnancy the reduction of twin pregnancies to single pregnancies by manual crushing prevents the waste of the breeding season of mares (Horoz *et al.*, 2002) which may increase overall reproductive efficiency in mares. By ultrasonography the embryonic vesicle is detected as 17-33 mm in diameter during 14-15 day post ovulation. The heart beat is commonly detected at about day 22nd, being this feature is one manner for determining embryo viability. The allantois is recognized on day 24th and concurrent with its expansion and contraction of the yolk sac (Kilicarlan *et al.*, 1996). Most of the embryonic deaths (5-24%) occur during 11 and 50th day post ovulation (Papa *et al.*, 1998). So ultrasonography is carried out till 40th day post-ovulation. Keeping in view the present research was planned to study the embryonic developments in Thoroughbred mares.

MATERIALS AND METHODS

The present work was carried out on twelve Thoroughbred mares from "Manjari Horse Breeder's Farm" at Manjari, Taluka Haveli, District Pune. The recently foaled mares without any difficulty in foaling were randomly selected. All the mares were between 7-15 years of age and weighing between 500 and 600 kg. All the mares were fed green fodder (Lucerne), dry (hay) and concentrate (HH Growth- L feed, 4 kg per mare divided three times in a day). All the mares had free access to drinking water. The foal heat was detected with visual external symptoms like urination, winking of clitoris, vaginal discharge, relaxation of vulva and reddening of vaginal mucosa. The mares were teased with stallion for detection in estrous which includes lack of resistance of the mare in the presence of a stallion as well as allowing stallion to mount prior to scanning. The opening of the cervix was visualised by application of vaginal speculum. The experimental mares were examined by rectal palpation and ultrasonographically to reveal the presence of intrauterine fluid accumulation, detection of cyst and follicular growth on either of the ovaries since day 8 post partum. All mares which were detected with largest follicle size and a characteristic uterine pattern of edema (cart-wheel like appearance) were mated with stallion. Mating was performed every second day, using two registered stallion. The ovulation was confirmed ultrasonographically (ovulation, day 0), on next day by observing rupture of preovulatory follicle. The flushing of uterus was done with one to two litres of warm, sterile saline (Normal saline) and a suspension of antibiotics Benzyl penicillin (3 gm), Gentamycin (30 ml) and 7.5 % Sodium bicarbonate (25 ml) were instilled into the posterior uterine body to remove fluid and resolve any infection, after 6-8 hrs of mating. After treatment, temporary Caslick operation (vulvoplasty) was done to prevent pneumovagina. The early pregnancy diagnosis was carried out first on 14th day post mating by ultrasonography.

The mares were examined daily by transrectal ultrasonography from the day 14 till day 40 postmating when an embryonic vesicle was detected. The location (uterine segment) of the embryonic vesicle was noted at each examination. The day of fixation of embryonic vesicle was defined as the first day that the embryonic vesicle was consistent in the same uterine segment during subsequent examinations. The average period for loss of spherical shape of embryonic vesicle was detected when shape of vesicle became irregular. The average days for detection of proper embryo was considered when an echogenic spot in the ventral aspect of the yolk sac was recorded. The average days required for detection of heart beat of embryo was recorded when the pulsation within embryo proper was noted. The average days for first detection of allantoic sac and the start of fetal ascent were recorded in experimental mares.

RESULTS AND DISCUSSION

In the present work, the early pregnancy was diagnosed with transrectal ultrasonography using 7.5 MHz probe on day 14 post mating. The pregnancy was confirmed after detection of embryonic vesicle. The embryonic vesicle was detected in all 12 (100%) experimental mares on day 14 with transrectal ultrasonography and the mean diameter of embryonic vesicle was 10.25±0.59 mm.

The size of embryonic vesicle detected varied between 7 mm and 13 mm. In 7 out of 12, (58.33%) mares the size of embryonic vesicle detected was in the range of 10-12 mm. This result of detection of early pregnancy on day 14 post-mating was in close agreement with Lemma *et al.* (2006) and Bansal *et al.* (2009), who reported detection of embryonic vesicle on day 14 post breeding in mares. Ginther (1985) reported detection of the embryonic vesicle on 11th day and Paolucci *et al.* (2012) observed embryonic vesicle on 8th day post-mating in mares. The mean diameter of embryonic vesicle in the present study was slightly less than that observed by Bansal *et al.* (2009). Paolucci *et al.* (2012) observed 4.6±1.1 mm embryonic vesicle size on day 8 in mares. The results of the present study for accuracy of early pregnancy diagnosis are in line with Kamble *et al.* (2005) and Abshenas *et al.* (2009). Slightly lower accuracy was observed by Omran and Rasheed (2013) and Palmer and Driancourt (1980). The variation in the detection of embryonic vesicle may be due to the differences in days after ovulation, frequency of probe, technician and experience of the operator.

In the present study, the fixation of vesicle, loss of spherical shape of vesicle, detection of embryo, detection of heart beats, detection of allantoic sac and start of fetal ascent were observed on 16.75±1.52, 20.66±1.86, 25.08±2.23, 28.16±2.45, 27.83±2.43 and 27.83±2.43 days, respectively. The result of the present study for fixation of embryonic vesicle is in accordance with the results of Bansal *et al.* (2009) and Paolucci *et al.* (2012). The days required for loss of spherical shape of vesicle were slightly more than the observations of Gastal *et al.* (1993). Abshenas *et al.* (2009) observed the shape of vesicle as spherical from 9 to 16 days, oval from 16 to 18 days and it becomes irregular from 18 to 19 days in mares. The days required for detection of embryo proper in the present study are slightly higher than the results observed by Abshenas *et al.* (2009) and Paolucci *et al.* (2012)

The higher days were required for detection of embryonic heart beat in the present study than the results cited by Abshenas *et al.* (2009) and Paolucci *et al.* (2012), who observed 23.11 ± 0.35 and 24±2.4 days, respectively. The days required for detection of allantoic sac are more than the observations cited by Abshenas *et al.* (2009) as 23.55 ± 0.44 days. In the present study, the days required to detect start of fetal ascent were more than that documented by Abshenas *et al.* (2009), who reported that the fetal ascent was started from 23.55 ± 0.44 and completed by on average 35.33±0.55 days in mares. The variation in the days required for the embryonic development like fixation of vesicle, loss of spherical shape, detection of embryo proper, detection of heart beats, detection of allantoic sac and start of fetal ascent may be due to the differences in breed, body weight, type and frequency of probe used and operator's experience.

CONCLUSION

Mating the mares in foal heat is best strategy for getting foal per year. Use of transrectal ultrasonography is the best tool in detecting the pre ovulatory follicle size, uterine edema, ovulation, early pregnancy diagnosis and embryonic developments in mares which improves the fertility.

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