



Comprehensive study on dynamics of early embryonic development in Marwari mares

DEEPAK¹, D JHAMB¹, S S NIRWAN¹, R JUNEJA¹, J SINGH², M GAUR¹ and T R TALLURI^{2✉}

College of Veterinary and Animal Science, Navania, Vallabh Nagar, Udaipur, Rajasthan 313 601 India

Received: 23 August 2021; Accepted: 7 December 2021

ABSTRACT

Horse is a seasonal breeder with reproductive activity being associated with long days, i.e. occurring in spring and early summer. Early equine pregnancy shares many features with that of more intensively assessed domestic animals species, but there are also characteristic differences. In mares, early diagnosis of pregnancy can be done due to the spherical shape. The ultrasonic visualization of conceptus is possible from day 9 of equine pregnancy. In spite of numerous, substantial advances in equine reproduction, many stages of embryonic and foetal morphological development are poorly understood, with no apparent single source of comprehensive information. Hence, in the present study we aimed at studying the dynamics of early embryonic developments in equines, particularly for Marwari breed of horses. The results from the current study revealed that, embryonic vesicles (EV) were first observed on 11th day, fixation occurred after 16.25 ± 0.25 days of ovulation, EV lost spherical shape on 15.83 ± 0.38 days, became oval on 17–18th days and irregular on 19–20th days. Heartbeat, detection of allantoic sac, start of foetal ascent, start of foetal descend and end of foetal descend were detected on 23.91 ± 0.41 , 23.58 ± 0.35 , 38.66 ± 0.28 , 40.66 ± 0.33 and 47.33 ± 0.28 days, respectively. In conclusion, early detection of embryonic vesicles and continuous changes observed in conceptus structure indicate positive pregnancy, viability of embryo and are much beneficial in preventing economical loss of equine owner and stable farm.

Keywords: Allantoic sac, Conceptus, Embryonic vesicle, Marwari mares, Ultrasonography

To have a successful equine breeding program, breeders must successfully manage mares both at pre- and post-breeding to maximize the health of the mare and ensure the delivery of a healthy foal (Bansal *et al.* 2009). Early embryonic deaths, abortion and pre-term birth of foals are major reasons for reproductive losses in the horses (Satue and Gardon 2016). The reasons concerning losses during the first months of pregnancy has special importance in equine reproduction (Talluri *et al.* 2020). Most of these losses occur early in pregnancy and around 15–20% of mares that conceive will lose the embryo before day 50 (Silva *et al.* 2015). Risk pregnancies require close supervision so that adequate treatment can be initiated in time. So it is important that all the breeding mares need to be examined for pregnancy diagnosis as early as possible after breeding, to know whether the valuable mare has conceived after service or not. This will help a lot to avoid economical losses of owner. For most horse breeders, the timeliness and accuracy of pregnancy diagnosis is essential.

Present address: ¹Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Navania, Vallabh Nagar, Udaipur. ²Equine Production Campus, ICAR-NRC on Equines, Bikaner, Rajasthan. ✉Corresponding author email: raotalluri79@gmail.com

Without additional equipment and skills, pregnancy diagnosis in the mare can be difficult. The use of ultrasound enables us to diagnose pregnancy at an early stage (Bansal *et al.* 2009).

Ultrasound has been used for more than two decades for early detection of pregnancy in mares. Pregnancy diagnosis by ultrasound may initially be performed 12 to 18 days after ovulation. The use of transrectal ultrasonography to evaluate the fetus and intrauterine environment in mares during gestation is relatively new and very wide, starting from confirming pregnancy existence, foetal gender determination to monitoring high-risk pregnancies. Ultrasound examination on or before day 16 is also beneficial for the identification and management of twins, scheduling of rebreeding in open mares, and early detection of problems associated with pregnancy (Talluri *et al.* 2021). A follow-up examination with ultrasonography is generally recommended between 25 and 35 days of pregnancy to confirm that the pregnancy is still viable. The fertility potential may be improved by monitoring the early embryonic studies and managing twin pregnancies and slow as well as abnormal growing embryos. (Ginther 1983, Deskur 1985, Kulisa *et al.* 1999). The ultrasonographic scanning enables early identification of non-pregnancy and also of twin pregnancy before the critical stage of

endometrial cup formation (Simpson *et al.* 1982). The real time ultrasonography is able to detect an embryonic vesicle's outline and diameter after ovulation, its mobility within the uterine lumen, embryo's heart beats, width and crown rump length (Gastal *et al.* 1993).

Early pregnancy loss is generally characterized by the sudden disappearance of the embryonic vesicle between ultrasound examinations. However, signs of impending embryonic loss can be detectable with transrectal ultrasonography. Identification of high-risk pregnancies has aroused the interest of researchers and clinicians because advances in ultrasonographic technology which have facilitated improved pregnancy monitoring, identification of foetal well-being, and diagnosis of placentitis and other diseases in the pregnant mare (Reef *et al.* 1996 and Renaudin *et al.* 1997). The literature regarding features of the early conceptus development in Marwari mare is scarce, hence present study was designed to investigate these characteristics in mares with objectives to study embryonic dynamics related to early pregnancy and conceptus development in mares by ultrasonography.

MATERIALS AND METHODS

Mares: The present study was conducted at College of Veterinary Sciences, Navania, Udaipur. For the current study a total of 12 mares were followed for early conceptus development. All the mares were aged between 4–15 years. And the present study has been carried during the breeding season. All the mares had uniform feeding management and no artificial lighting and no special diet was provided during the current study period. Pre-breeding examination was done with the aid of vaginal speculum and ultrasound machine. Reproductively sound mares were selected for breeding after rectal and ultrasonographic examination of genital organs. For scanning Exago ECM, France ultrasound machine with 5–10 MHz transrectal probe was used.

Ultrasonography procedure: For the routine observation, mares were restrained in trevis with tail deviated with a rope and some mares required nose twitch to control and made them stand quietly and prevent them from kicking the examiner. The mares were examined daily via transrectal ultrasonography from the day when an embryonic vesicle (EV) was first detected till EV size increases beyond the scanning range of the scanner. Largest diameter of EV was recorded during every examination. Location (uterine segment) of the EV was noted at each examination. The day of fixation of EV was defined as the first day that the EV was consistent in the same uterine segment during subsequent examinations. The average period for loss of spherical shape of EV 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 foetal ascent were recorded in experimental

mares. Appearance of umbilical cord formation and start of foetal descend and end of foetal descend were also recorded as described by Abshenas *et al.* (2009).

Statistical analysis: All the results were expressed in Mean $\pm$ SE. Statistical analysis was performed using Statistical Package for Social Science (SPSS® Version 20.0 for Windows®, SPSS Inc., Chicago, USA). The means were compared using Analysis of Variance, Duncan's multiple range test and presented as mean $\pm$ standard error (SE) at the significance level of $P < 0.01$ or $P < 0.05$.

RESULTS AND DISCUSSION

The embryonic dynamics of all the mares of the present study were depicted in the Fig. 1. After detection of embryonic vesicle, all the mares were checked by transrectal ultrasonography daily till day 48 post-ovulation and embryonic developments were recorded.

Fixation of embryonic vesicle: In this present study, the first visualisation of spherical embryonic vesicle was done on day 12 (Fig. 2a) and the fixation of the embryonic vesicle was occurred earliest on day 15 in two mares. The mean days required for fixation of embryonic vesicle in all mares were 16.25 ± 0.25 days. These results are in corroboration with the earlier observation in thoroughbred mares (Abshenas *et al.* 2009). About 70% of fixation of embryonic vesicle occurred in caudal segment of right horn. In another study, Bansal *et al.* (2009) and Paolucci *et al.* (2012) observed the fixation of embryonic vesicle on 17th day and it was slightly higher than present study. Increased size of embryonic vesicle and increased uterine tonicity is associated with decrease in uterine diameter, along with natural bend in the horn at body junction are accountable for fixation of embryonic vesicle (Gastal *et al.* 1993). Contrary result was reported by Bessent and Ginther (1988),

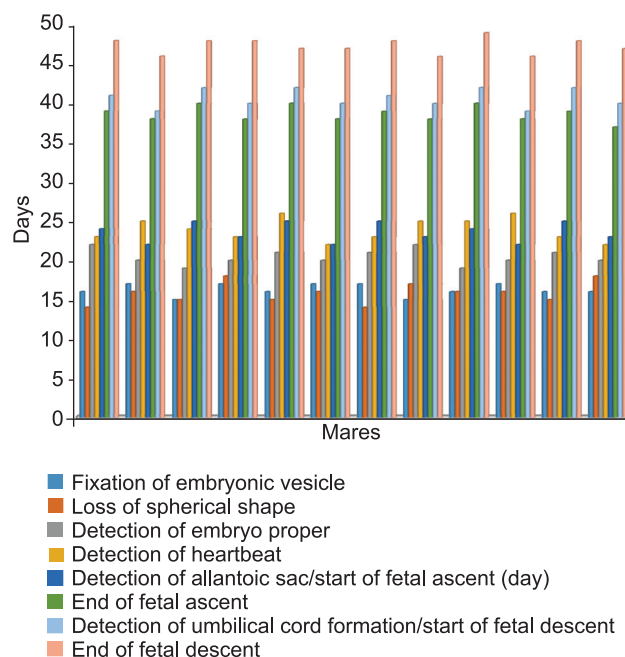


Fig. 1. Embryonic development in experimental mares.

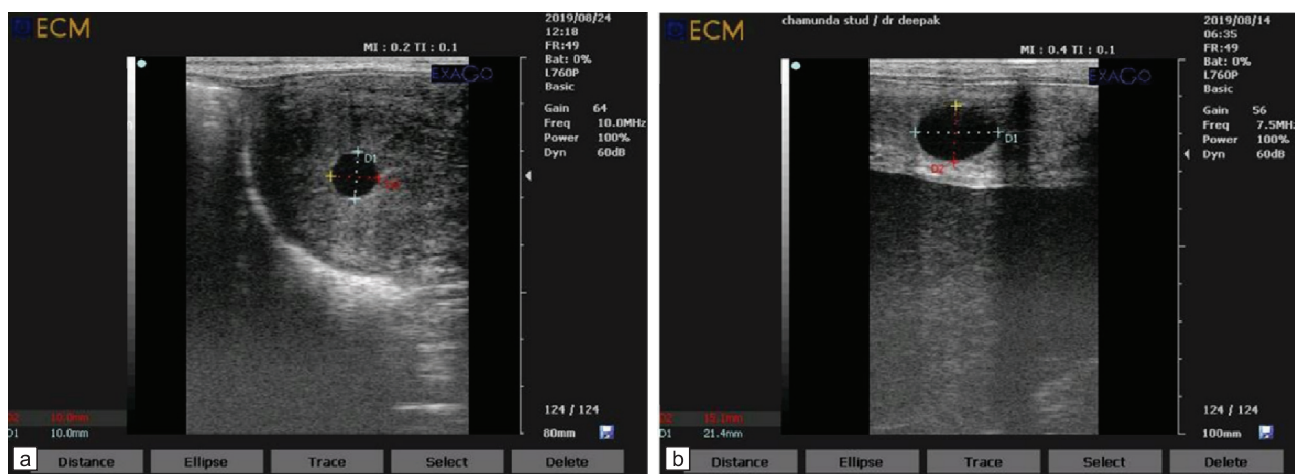


Fig. 2. (a) Ultrasonogram depicting 12 days pregnancy spherical embryonic vesicle measuring 10.0 mm; (b) Ultrasonogram depicting 16 days pregnancy oval shape embryonic vesicle in the uterine body measuring 21.4 mm.

where they observed fixation in 14.7 days in ponies.

Loss of spherical shape: The spherical shape of EV exists up to 16 days, later it became oval on 17–18th day. The overall mean days required for the loss of spherical shape of an embryonic vesicle were observed to be 15.83 ± 0.38 (Fig. 2b). Loss of spherical shape of an embryonic vesicle was earliest detected in two mares on day 14. These findings are in agreement with finding of Abshenas *et al.* (2009). The spherical shape of embryonic vesicle is due to cellular glycoprotein capsule that surrounds the vesicle which provides resilience to embryo during transuterine migration phase, further loss of the spherical shape is attributed to increased uterine tone and embryonic vesicle growth (Maria, 2015). Contrary to our results, Abshenas *et al.* (2009) observed irregular EV from 18–19th day in mare.

Detection of embryo proper: The embryo proper was detectable by overall mean on day 20.41 ± 0.28 in the mares (Fig. 3a), while earliest detection of embryo proper was at 19 days in two mares. Ginther (1985) reported detection of embryo proper on 19th days post-mating, which is earlier

than present study. The days required for detection of embryo proper in the present study are in close agreement with Abshenas *et al.* (2009). Paolucci *et al.* (2012) reported on day 20 ± 0.3 and observed day 19.9 ± 1.9 , respectively. The more days required for detection of embryo proper in studies reported (Pycock 2007, Bansal *et al.* 2009, Wasudeo 2014, Bika *et al.* 2015). Similar to present study, Pycock (2007) reported that the allantois was hypoechoic region below the embryo proper.

Detection of heartbeat: The embryonic heart beat was first detected on day 22 in two mares with an average of 23.91 ± 0.41 days in all the mares. Similar finding was observed on days 23.5 ± 1.3 , 23.11 ± 0.35 and 24 ± 2.4 respectively in mares (Meira *et al.* 1998, Abshenas *et al.* 2009, Paolucci *et al.* 2012). Earlier detection of embryonic heartbeat was observed in the present study than the results cited by Wasudeo (2014) on day 28.16 ± 2.45 , Crisci *et al.* (2014) on day 25 ± 1.1 , Bika *et al.* (2015) on day 25 of pregnancy. In another study, Curran and Ginther (1995) also stated that a heartbeat can be seen as a flickering in

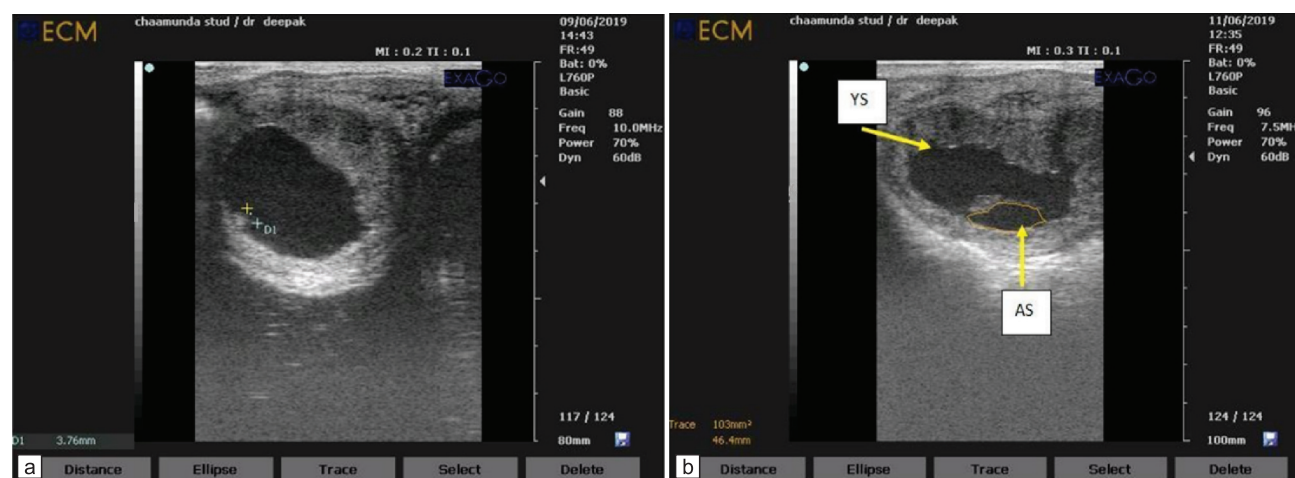


Fig. 3. (a) Ultrasonogram depicting 20 days pregnancy irregular shape embryonic vesicle; first detection of embryo proper measuring 3.76 mm; (b) Depicting 27 days pregnancy; embryo proper was in lower third portion of vesicle. Yolk sac (YS) and Allantoic sac (AS) are shown.

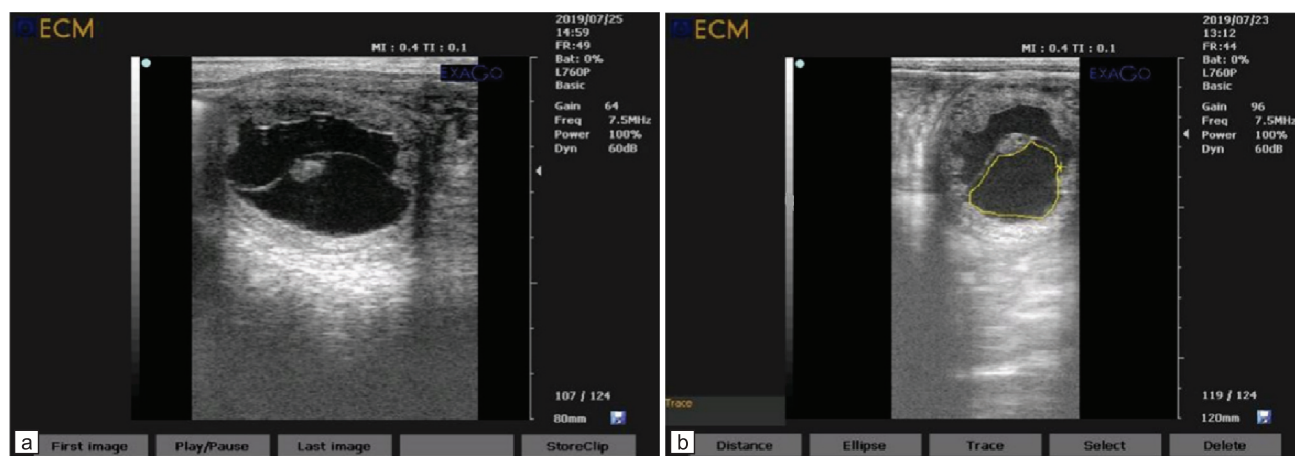


Fig. 4. (a) Depicting embryo proper in the middle of embryonic vesicle on day 30; (b) Depicting embryo proper in upper third part of embryonic vesicle on day 37.

embryo proper beginning on day 25. The detection of heartbeat was observed earlier than present study by Gastal *et al.* (1993) on day 21.8 ± 0.3 in jennies, he further revealed that heart beat is sign of confirmation of pregnancy and viability.

Detection of allantoic sac/Start of foetal ascent: Detection of allantoic sac/start of foetal ascent was first reported on day 22 in three mares with an average detection on 23.58 ± 0.35 days in all the mares (Fig. 1) and migration of embryo proper from ventral to dorsal direction in embryonic vesicle with increase in allantoic cavity and reduction in yolk sac (Figs 3b, 4a and 4b). It is close with the observations cited by Abshenas *et al.* (2009), who reported 23.55 ± 0.44 days in mares where as Gastal *et al.* (1993) and Meira *et al.* (1998) observed 19 to 23 and 24.4 ± 1.7 days in Jennies, respectively.

End of foetal ascent: Foetal ascent was complete on day 37 in one mare in the current study and end of foetal ascent was reported as an average of 38.66 ± 0.28 days in all the mares (Figs 1 and 4b), which was higher than foetal ascent on 35.33 ± 0.55 and 36.8 ± 1.6 day of gestation in jennies observed by Abshenas *et al.* (2009) and Meira *et al.* (1998), respectively.

Detection of umbilical cord formation/ Start of foetal descent: Detection of umbilical cord formation/ start of foetal descent was observed in the mares with an overall mean of 40.66 ± 0.33 days (Figs 1 and 5a) and the earliest detection was on day 39 in two mares. In the present study the end of foetal descent was observed on overall mean day 47.33 ± 0.28 days, with earliest detection on day 46 in three mares. Contrary to our study, Abshenas *et al.* (2009) reported that start of formation of umbilical cord was on 35.33 ± 0.55 day.

End of foetal descent: In the present study the end of foetal descent was observed on overall mean day 47.33 ± 0.28 days (Fig. 5b), with earliest detection on day 46 in three mares. The result of foetal descent in present study was in close agreement with the result of Ginther (1992) who reported that fetus was on ventral floor by day 49. However, in similar study by Abshenas *et al.* (2009) reported that embryo proper reached the ventral aspect on day 40.1 ± 1.3 . The variation in the days required for the embryonic development like fixation of vesicle, loss of spherical shape, detection of embryo proper, detection of heartbeats, detection of allantoic sac and start of foetal ascent may be due to the differences in breed, body weight,

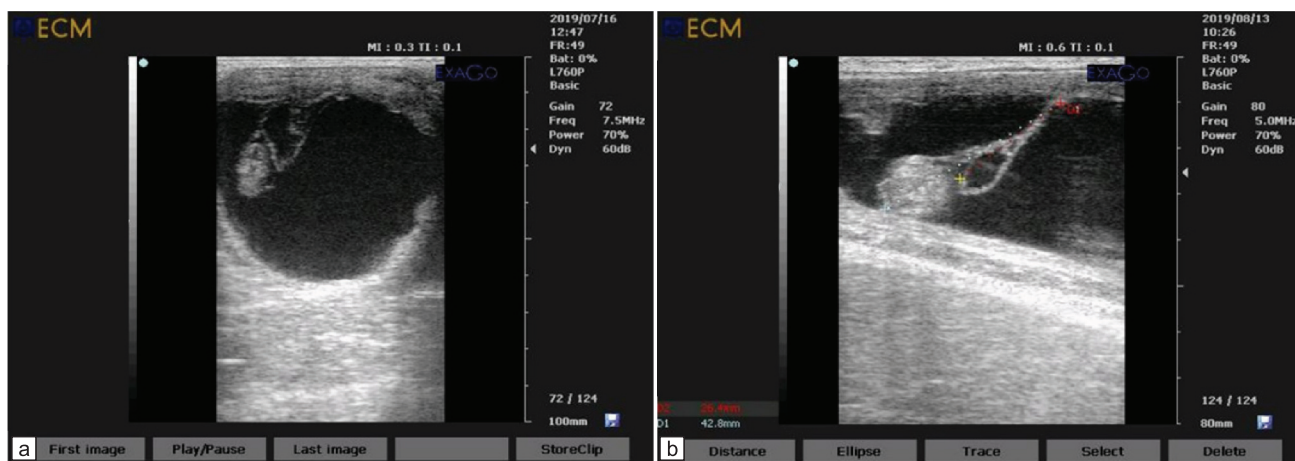


Fig. 5. (a) Depicting umbilical cord formation and start of foetal descend on day 40; (b) Depicting end of foetal descend on day 46.

type and frequency of probe used and operators experience. Schnobrich *et al.* (2013) justified that transrectal ultrasonography is useful as assessing viability at all stages of gestation. In early gestation it can be used to assess embryonic anatomy of the growing conceptus to make sure development is consistent with normal values.

In the current study we have reported the various embryonic dynamics which are very much essential in the context of identifying the stage of the pregnancy and foetal wellbeing and this may be used as ready reckoner for the clinicians and researchers working in this area. Early detection of EV and continuous changes noticed in its structure indicated positive pregnancy and viability of embryo. It is possible through transrectal ultrasonography to diagnose twin pregnancy and early abortions like conditions and helpful in preventing excessive economical loss to equine owner.

ACKNOWLEDGEMENTS

The authors would like to thank the Dean, College of Veterinary and Animal Sciences, Vallabh Nagar, Navania for providing excellent research facilities for carrying out the present research study and also thank owners of Chamunda and Veer Gujar stud farms for providing their mares for the current study.

REFERENCES

- Abshenas J, Babaei H, Mahdavi I, Alimolaei M and Ali S. 2009. Ultrasonographical measurement of Caspian Mare embryonic vesicle and embryo on days 8 to 44 after ovulation. *Iranian Journal of Veterinary Surgery* **4**(1): 85–93.
- Bansal R S, Pal Y and Pareek P K. 2009. Ultrasonographic imaging for early pregnancy diagnosis in mares. *Indian Journal of Animal Reproduction* **30**(2): 52–53.
- Bessent C and Ginther O J. 1988. Comparison of early conceptus mobility between mares and jennies. *Theriogenology* **29**(4): 913–20.
- Bika C S, Nakhashi H, Babulal S and Jhamb D. 2015. Ultrasonographical investigations during early phase in thoroughbred pregnant mares. *Veterinary Practitioner* **16**(2): 190–95.
- Crisci A, Alessandra R, Duccio P, Micaela S, Jennifer C O and Francesco C. 2014. Clinical, ultrasonographic, and endocrinological studies on donkey pregnancy. *Theriogenology* **81**: 275–83.
- Curran S and Ginther O J. 1995. M-Mode Ultrasonic assessment of equine foetal heart rates. *Theriogenology* **44**: 609–17.
- Deskur S. 1985. Twinning in thoroughbred mares in Poland. *Theriogenology* **23**(5): 711–18.
- Gastal E L, Santos G F, Henry M and Piedade H M. 1993. Embryonic and early foetal development in donkeys. *Equine Veterinary Journal* **25**(15): 10–13.
- Ginther O J. 1983. Effect of reproductive status on twinning and on side of ovulation and embryo attachment in mares. *Theriogenology* **20**(4): 383–95.
- Ginther O J. 1985. Dynamic physical interactions between the equine embryo and uterus. *Equine Veterinary Journal* **17**(3): 41–47.
- Ginther O J. 1992. *Reproductive Biology of the Mare Basic and Applied Aspect*, 2nd ed. Cross Plains, Tex. Equiservices. pp. 1–642.
- Kulisa M, Pieszka M and Frybes O. 1999. Ciążebliźniacze w hodowlikonipelnejkriangielskiej w Polsce w latach 1987–1992 (Multiple pregnancies in thoroughbred horses breeding in Poland in 1987–96 years. *Medycyna. Weterynaryjna* **55**(10): 689–93.
- Meira C, Ferreira J C, Papa F O and Henry M. 1998. Ultrasonographic evaluation of the conceptus from days 10 to 60 of pregnancy in jennies. *Theriogenology* **49**: 1475–82.
- Paolucci M, Palombip C, Sylla L, Stradaoli G and Monaci M. 2012. Ultrasonographic features of the mule embryo, fetus and foetal-placental unit. *Theriogenology* **77**(2): 240–52.
- Pycock J F. 2007. *The Pregnant Mare: Diagnosis and Management, Pregnancy Diagnosis in the Mare*. Chapter 52. (Eds.) Samper J C, Pycock J F and McKinnon A O. Current therapy in equine reproduction. Saunders st. Louis Missouri. pp. 337.
- Reef V B, Vaala W E, Worth L T, Sertich P L and Spencer P A. 1996. Ultrasonographic assessment of foetal well-being during late gestation: Development of an equine biophysical profile. *Equine Veterinary Journal* **28**: 200–8.
- Renaudin C D, Troedsson M H T, Gillis C L, King V L and Bodena A. 1997. Ultrasonographic evaluation of the equine placenta by transrectal and transabdominal approach in the normal pregnant mare. *Theriogenology* **47**: 559–73.
- Satue K and Gardon J C. 2016. Pregnancy Loss in Mares, Genital Infections and Infertility, Atef M. Darwish, Intech Open. DoI: 10.5772/63742. Available from: <https://www.intechopen.com/chapters/50404>.
- Schnobrich M R, Riddle W T, Stromberg, A J and LeBlanc M M. 2013. Factors affecting live foal rates of thoroughbred mares that undergo manual twin elimination. *Equine Veterinary Journal* **45**(6): 676–80.
- Silva E S M, Meira C, Pantoja J C F and Filho J N P P. 2015. Ultrasonography of the conceptus development from days 15 to 60 of pregnancy in non-cyclic recipient mares. *Ciênc. Rural, Santa Maria* **45**(3): 512–18.
- Simpson D J, Greenwood R E, Ricketts S W, Rossdale P D, Sanderson M and Allen W R. 1982. Use of ultrasound echography for early diagnosis of single and twin pregnancy in the mare. *Journal of Reproduction and Fertility Supplement* **32**: 431–39.
- Talluri T R, Ravi S K, Singh J and Tripathi B N. 2020. Reproductive indices of Manipuri horses reared under arid zone region. *Indian Journal of Animal Sciences* **90**(10): 1414–17.
- Talluri T R, Chandan Singh and Ved Prakash. 2021. Pregnancy outcomes in thoroughbred mares inseminated with fresh semen from either stallion or jack at foal heat or subsequent estrus. *Indian Journal of Animal Sciences* **91**(2): 99–102.
- Wasudeo A R. 2014. 'Early pregnancy diagnosis and embryonic developments in thoroughbred mares.' M.V.Sc. Thesis, MAFSU, Nagpur, Maharashtra.
- Zhao M O, Kose M, Kurar E and Semacan A. 1996. Early detection of pregnancy in mares. *Ataturk Universitesi Veteriner Bilimleri Dergisi* **7**(1): 67–75.