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# Ultrasonographical Measurement of Caspian Mare Embryonic Vesicle and Embryo on Days 8 to 44 after Ovulation

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## Abstract

**Objective-** The aim of this study was to determine the first possible time for pregnancy recognition and several features of developing embryo between days 8 to 44 of pregnancy.
**Design-** Experimental study
**Animals**- Six healthy registered Caspian mares
**Procedures-** Daily ultrasound examinations were conducted from days 8 to 44 (ovulation = day 0) of pregnancy to monitor the conceptus in Caspian mares (No. of pregnancy= 9).
**Results-** Embryonic vesicle was observed for the first time on day 9.67 ± 0.33 (mean ± SEM, range 9-11 days) in 7.17 ± 0.48 mm diameter. The vesicle was fixed in distal part of uterine horn on day 16.44 ± 0.24 (age range 15-17 days). The vesicle was spherical from days 9 to 16 (mean ± SEM growth rate, 2.16 ± 0.14 mm/day), and non spherical from days 16 to 19 with reduced growth rate from day 18-27 (0.60 ± 0.12 mm/day) and then averagely grew at a rate of 1.27 ± 0.15 mm/day until day 44 of pregnancy. Embryo proper and heartbeat were first detected on days 20 ± 0.37 and 23.11 ± 0.35, respectively. Formation of allantoic sac at the same time of decreasing of yolk sac was initiated on day 23.55 ± 0.44 and it was completed on day 35.33 ± 0.55.
**Conclusion and Clinical Relevance-** The dates of occurrence of morphologic features of developing Caspian mare embryo were similar to the previous reports in mares and jennies and also ultrasound measurement of embryo and embryonic vesicle is an accurate tool and simple way for estimating the age of pregnancy in Caspian mares.

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**Key Words**- Transrectal ultrasonography, Embryo, Embryonic vesicle, Caspian mare.

## Introduction

The ability to examine mares reproductive tract with ultrasonography provided the opportunity to diagnose pregnancy earlier than by rectal palpation, effectively manage twins, and detect impeding early embryonic death.[1,2] However, ultrasonography should not be limited to these areas. It can be used to diagnose uterine diseases, such as intrauterine fluid, air debris, cysts, and occasionally abscessation and neoplasia.[3,4] In addition ultrasonographic examination of the ovaries may aid in determining stage of estrous cycle, status of preovulatory follicles, development and morphologic assessment of the corpus luteum, and in interpreting ovarian irregularities, such as anovulatory or hemorrhagic follicles, neoplasia, and periovarian cysts.[4,5]

The diagnosis of pregnancy in mares using ultrasonography was first reported in 1980.[6] This technology has increased veterinarians' ability to estimate gestational age and to evaluate equine pregnancy considerably.[2] Management of twins has improved and it is recommended that mares be examined for presence of twins by ultrasonography per rectum between 14 and 19 days.[7] Success is good for reduction of a twin pregnancy to a singleton if the manual reduction procedure is performed before 30 days of gestation.[8,9]

Caspian ponies are small horses that carry some of the characteristics of ponies. However, they have certain physical characteristics that do not match those of either ponies or larger horses. Caspian ponies are short animals with bay, gray and chestnut as the most common colors. The head is short with a bulging forehead and small ears and the animal has oval feet. This pony has a graceful and perfectly proportioned body and is gentle, intelligent and very willing to work, thus making it very well suited as a riding horse for children.[10] Caspian ponies are perhaps the ancestors of the Arab as well as the wild stock from which hot-blooded horses were derived.[11] The archaeological evidence for the important role and possible historical continuity of a small horse from the pre-Achaemenian period to about the time of Islamic conquest in the 7th century A.D. has been discussed by previous researchers.[12,13] Nowadays, small number of them has been left (almost 70) in Iran.[14] Apart from some physical differences between Caspian and other breed of horses, only one relevant preliminary study was found regarding to the fetometry in Caspian miniature horse by using B-mode real time ultrasound.[15] The present study aimed to determine the earliest day at which the signs of pregnancy can be detected by transrectal ultrasonography and to estimate the gestational age by measurement of conceptus throughout pregnancy in Caspian mare in order to detect any probable differences between Caspian and other types or breed of horses.

## Materials and Methods

### *Animals*

Our study was carried out in six healthy registered Caspian mares following mating with two Caspian stallions with normal reproductive histories. Their age ranged from 7 to 15 years, height from 100 to 120 cm and weighting between 160 and 230 kg at the beginning of the study. The animals during two breeding seasons were corralled outdoors at the Gharran Caspian breeding center in Bahramjerd, an area located nearly 50 km from south of Kerman in Iran (latitude 29'50''N; longitude 56'50''E, altitude 2860 m above sea level, with an

annual rainfall of 0.4 mm, an average temperature of 27.3 ◦C and a relative humidity of 20%) during the months of April, May and June (2009-2010). Diets were formulated to meet or exceed NRC requirements.

*Estrous detection and ultrasonic examination*

Prior to breeding, the mares were teased daily with a stallion to detect estrus. Daily observation for estrus detection including urination, winking, and lack of resistance of the mare in the presence of a stallion was conducted for each individual mare. Ovarian follicular development was monitored by daily transrectal ultrasound examinations using a portable ultrasound scanner (Kretztechnik AG SA-600V, Seoul, Korea) equipped with a 6.5-8.5-MHz, linear-array, rectal transducer and the day of ovulation was recorded. Breeding was performed every second day, using two registered Caspian stallion, from the first day of detection of an estrous to the day that ovulation was confirmed ultrasonically (ovulation = day 0). Before each examination, transducer was lubricated by a ultrasound gel and covered by a soft plastic sleeve. During the procedure, the operator scanned the uterine horns and ovaries. The cervix, right and left uterine horns and ovaries, and the uterine body were examined. The transducer on the uterus was rotated at different angles in order to obtain suitable images of the embryo.
The mares were daily examined by transrectal ultrasonography from the day 8 to 44 after ovulation when an embryonic vesicle was detected, it was frozen at its maximal size of the height and width diameters were measured with integral electronic calipers. The uterus was divided into 5 segments: the uterine body and the caudal and cranial segments of the left and right uterine horns. The location (uterine segment) of the embryonic vesicle was noted at each examination. The day of fixation was defined as the first day that the embryonic vesicle was consistent in the same uterine segment during subsequent examinations. The embryo proper was first detected as an echogenic spot in the ventral aspect of the yolk sac; thereafter, the crown-rump length (CRL, a straight line between the fetal crown and the origin of tail) and biparietal diameter (BPD, the widest distance between the outer borders of the cranium at an angle of 90° to the falx cerebri) were measured daily. An embryonic heartbeat was subsequently detected as a pulsation within the embryo proper. The first detection of the allantoic sac, the completion of its development, and the ascent and descent of the fetus were noted.
The sonographic scanner was supplied with a capture cart (Play TV 400 USB, Taiwan) for recording images on a notebook and also selected images were printed by a black and white video graphic printer [UP-895 MD/(SYN), Sony Corporation, Japan].

*Statistical analysis*

The data were expressed as mean ± standard error (SEM). A simple linear regression analysis was fitted to evaluate the relationship between gestational age and each of the studied parameters. The various measurements were considered as being dependent on gestational age. Chi square tests were used to discern possible differences between the presence of the embryonic vesicle, fixation, embryo, allantois, amnion, and heartbeat from day 9 to 44 of pregnancy using the SPSS10 for Windows. A 5% of significance level was used.

## Results

On average, the embryonic vesicle was first detected on day 9.67 ± 0.33 with 7.17 ± 0.48 mm diameter (Table 1). In individual mares, the embryonic vesicles were detected on days 9 (55.6%), 10 (66.7%) and 11 (100%) and their diameters were 6.60 ± 0.81, 7.78 ± 0.29 and 10.11 ± 0.43 mm, respectively. After detection, the embryonic vesicle was observed in different uterine parts until the day 16.44 ± 0.24 of pregnancy. Fixation occurred in 1 (11.1%), 4 (44.4%) and 9 (100%) mares on days 15, 16 and 17, respectively (Table 1).
The shape of embryonic vesicle was spherical from the day of first detection (day 9) to the day 16 (Table 1 & Fig. 5B), oval from days 16 to 18 (Fig. 5C), and irregular from days 18 to 19 (Fig. 5D & E). The growth rate was 2.16 ± 0.14 mm/day on days 9 to 16 and reduced to 0.60 ± 0.12 mm/day from days 18 to 27, and then averagely grew at a rate of 1.27 ± 0.15 mm/day until day 44 of pregnancy.
The embryo proper was an echogenic spot (consistently in the ventral hemisphere of the yolk sac) that first detected on day 20 ± 0.37 (age range, 18-21; Table 1, Fig. 5F), with an average length of 6.05 ± 0.39 mm. The embryo grew at an average rate of 0.80 ± 0.05 mm/day up to the day 44. Embryonic heartbeat was first detected on day 23.11 ± 0.35 (Table 1).
Development of the allantoic sac was first detected on day 23.55 ± 0.44 and was completed on day 35.33 ± 0.55 (Table 1). Concurrent with development of the allantoic sac, the embryo proper migrated from the ventral to the dorsal pole of the embryonic vesicle, began to descend ventrally (start of formation of the umbilical cord) on day 35.33 ± 0.55, and (the fetus) reached the ventral aspect on day 40.1 ± 1.3 (Table 1and Fig. 5K & L).

**Table 1.** First day of detection (mean ± SEM), diameter at first detection (mm) and age range (day) of appearance of several ultrasonographic features of the developing embryo in Caspian mares (N = 9).

| Feature | **Age range** (day) | **First detection** (day) | **Diameter** (mm) |
|---|---|---|---|
| **Embryonic vesicle** | 9-11 | 09.67 ± 0.33 | 07.17 ± 0.48 |
| **Fixation** | 15-17 | 16.44 ± 0.24 | 21.33 ± 0.50 |
| **Loss of spherical shape** | 16-19 | 18.00 ± 0.33 | 24.77 ± 0.97 |
| **Embryo proper** | 18-21 | 20.00 ± 0.37 | 06.05 ± 0.39 |
| **Heartbeat** | 22-25 | 23.11 ± 0.35 | --- |
| **Start of allantoic sac** | 22-25 | 23.55 ± 0.44 | --- |
| **Start of fetal ascent** | 22-25 | 23.55 ± 0.44 | --- |
| **End of fetal ascent** | 34-38 | 35.33 ± 0.55 | --- |
| **Start of fetal descent** | 34-38 | 35.33 ± 0.55 | --- |

The relationship between the ultrasound fetal measures, the gestational age, the time of pregnancy at which each fetal measure can be detected and the equations of prediction are shown in Fig. 1–4. All the fetal measures were significantly ($P < 0.001$) correlated to the gestational age. However, the height ($r^2 = 0.85$) and width ($r^2 = 0.90$) diameter of embryonic vesicle and the BPD ($r^2 = 0.92$) and the CRL ($r^2 = 0.92$) of the embryo correlated with the gestational age.
Equation for estimation of gestational age from ultrasound measurements are presented in Table 2. The equation for estimating gestational age by embryonic vesicle height ($y=0.87x+1.40$; Fig. 1), embryonic vesicle width ($y=0.63x+6.03$; Fig. 2), CRL ($y=1.12x+13.83$; Fig. 3) and BPD ($y=1.63x+16.90$; Fig. 4) were also effective for estimating gestational age.

**Table 2.** Regression equations of gestational age prediction from fetal ultrasonographic measurements from 9 to 44 days of pregnancy in Caspian mares (N = 9, p<0.001 for all equations)

| Measurements (x) | Equation | $R^2$ | Range of validity | |
|---|---|---|---|---|
| | | | days | measurements |
| **EVH** | y=0.87x+1.40 | 0.85 | 9-44 | 5-55 |
| **EVW** | y=0.63x+6.03 | 0.90 | 9-44 | 5-67 |
| **CRL** | y=1.12x+13.83 | 0.92 | 19-44 | 5-28 |
| **BPD** | y=1.63x+16.90 | 0.92 | 19-44 | 1-16 |

EVH, Embryonic vesicle height; EVW, Embryonic vesicle width; CRL, Crown rump length; BPD, biparietal diameter

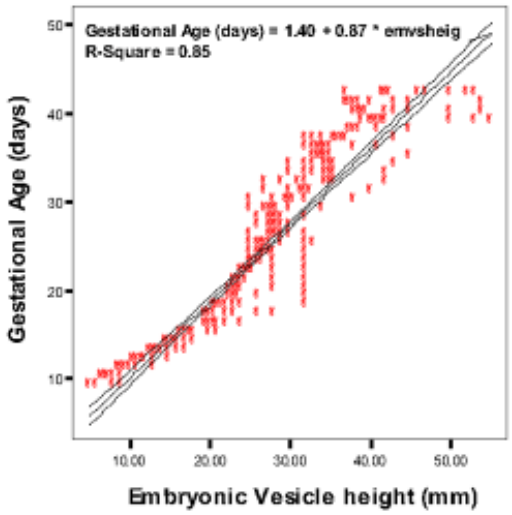


**Figure 1.** Relationship between the embryonic vesicle height and the gestational age of Caspian mares (N = 9, No. of sonographic observations = 303).

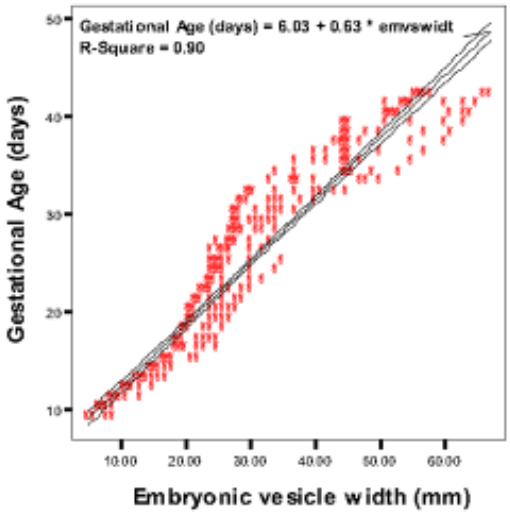


**Figure 2.** Relationship between the embryonic vesicle width and the gestational age of Caspian mares (N = 9, No. of sonographic observations = 303).

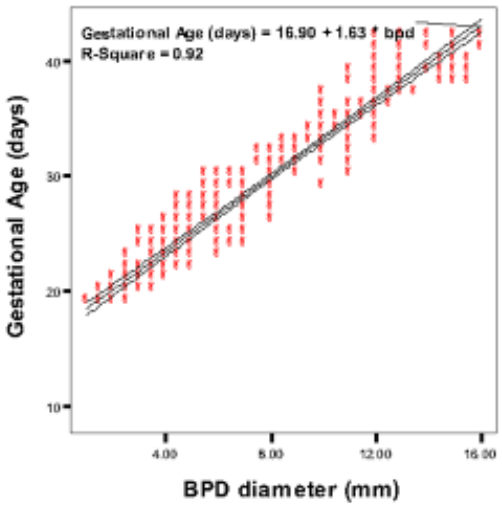


**Figure 3.** Relationship between the biparietal diameter (BPD) and the gestational age of Caspian mares (N = 9, No. of sonographic observations = 216).

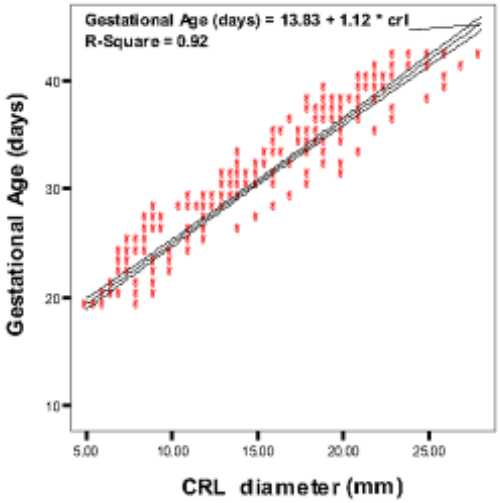


**Figure 4.** Relationship between the embryonic vesicle height and the gestational age of Caspian mares (N = 9, No. of sonographic observations = 303).

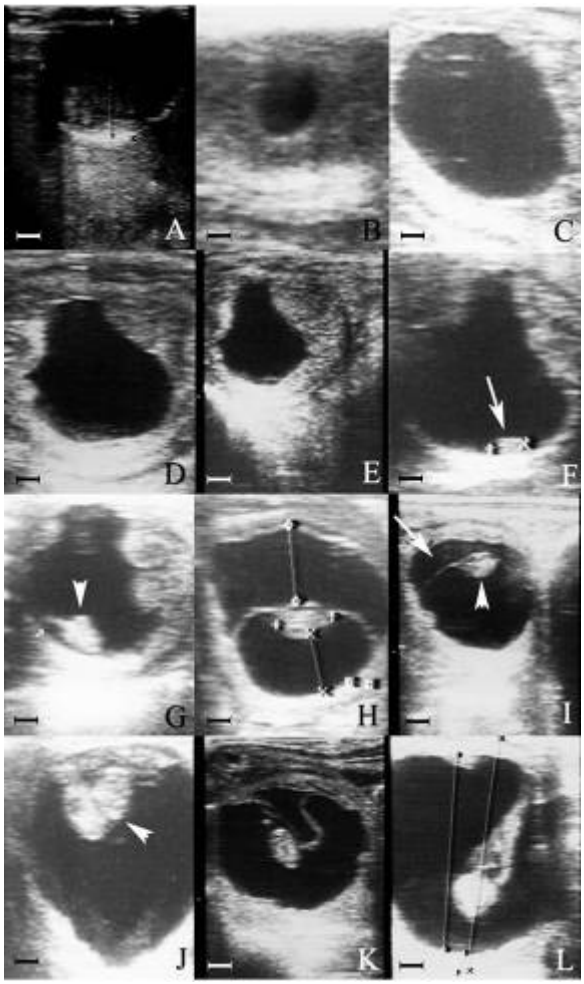


**Figure 5.** Ultrasonography (7.5 MHz) of the conceptus during early pregnancy in Caspian mares (ovulation = day 0).
A: preovulatory follicle; B: spherical yolk sac on day 10; C: oval yolk sac on day 16; D & E: irregular yolk sacs on days 18 and 19, respectively; F: embryo proper on day 21 (arrow); G: allantoic sac (arrow) ventral to the embryo proper (arrow head) on day 23; H & I: allantoic sac (ventral to the embryo proper, arrow head) and yolk sac (dorsal, arrow) on days 30 and 32, respectively; J: ascent completion on day 34; K: start of fetal descent on day 36 and L: end of fetal descent on day 42. Scale bar = 5mm.

## Discussion

To the best of our knowledge, this is the first study to evaluate the relationship between the gestational age and ultrasonographic measured embryonic characteristics in Caspian mares. The average days of first detection of embryonic vesicle (9.67±0.33 days) by transrectal ultrasonography (7.5 MHz) reported in the present study is earlier than that reported in mares (11-14 days),[5] and jennies (11.5 days).[16] In both later studies, the mares were daily scanned rectally using a 5 MHz transducer from day 9 after ovulating. In the present study, the probability of detection increased as the vesicles were enlarged in diameter on day 11 that is similar to the reports of previous studies.[17] Although the vesicle has been detected as early as day 9 in the mare,[2,16] in another study in mares, first detection ranged from day 11 to day 14.[17] The vesicle was more likely to be detected in the uterine body or caudal segments of the uterine horns than the cranial segments of the horns, consistent with previous reports for both Jennies,[5,17] and mares.[2] Although the location of vesicles was determined only once daily in the present study but their location were changing into the uterine horns, this phenomenon was in agreement with the previous observations in both jennies and mares which had substantial mobility between days 9 and 15.[2,5,16] This intrauterine movement can enhance metabolic exchange between the endometrium and embryo,[16] and seems to be necessary for the maintenance of pregnancy in the mare.[1,9,18]

In our study embryonic fixation was detected on day 16 (range, 15 to 17d). In previous reports, the average of embryonic fixation was day 16 in jennies.[16,17] This fixation time was approximately 1 day later in jennies than in ponies (15.6 versus 14.7).[5] In horse mares, fixation usually occurs between days 15 and 17; on rare occasions, fixation occurs as early as day 13 or as late as day 18.[2,16,19]

The embryonic vesicle had an average of 19.44 mm in diameter; this seems slightly lower than in previous studies, consistent with previous observations in jennies of 21.7 mm, [5] and 21.8 mm.[17] In the present study, the site of fixation was consistently the caudal segment of either right uterine horn, close to its junction with the uterine body independent of side of ovulation. The increase of vesicular diameter and maximal uterine tone probably was important in fixation. In mares, fixation usually occurred in the caudal aspect of the uterine horn; the natural bend present in the horn at this location combined with increasing uterine tone and expansion of the vesicle prevent continued movement of the vesicle.[17] However, in one study, fixation occurred in the medial segment of the right uterine horn in 2 of 21 Jennies and in the caudal aspect of either uterine horn in the remainder.[17]

The vesicle was spherical from detection to Day 15, oval from Days 15 to 17, and then irregular until Day 28. Similarly, the vesicle had an irregular shape from Days 17 to 31 in Jennies,[17] and from days 18 to 27 in mares.[17] The plateau in vesicle growth and its irregular shape are attributed to an increase in uterine tone limiting expansion of the vesicle. [16,17] Furthermore, it has been suggested that at the day 17, equine embryonic vesicle is able to adapt itself to the irregular endometrial folds. The change from a spherical shape into an irregular one, observed in the ultrasound image of the vesicle during the period of apparent stability, may have made the detection of the actual growth difficult.[17]

Growth of the embryonic vesicle was very rapid during the mobility phase (2.16 mm/d), relatively slow from days 18 to 26 (0.6 mm/d, plateau), and then moderate from days 26 to 44 (1.27 mm/d). Similar growth patterns were reported in the mare,[6,19] and jennies,[16] including a plateau phase between days 18 to 25 and 19 to 28, respectively.

The day of first detection of the embryo proper (days 18 to 21), its echogenic appearance, its location in the ventral hemisphere of the yolk sac, its increase in crown-rump length and the

first detection of the embryonic heartbeat (day 24.11) were consistent with that of previous reports in mares,[17] and Jennies.[16,17] The ascent of the embryo from the ventral to the dorsal pole of the embryonic vesicle occurred between days 22 and 38 and was always associated with the concurrent development of the allantoic sac and the regression of the yolk sac, as previously reported in the mares,[17] and Jennies.[16] The subsequent descent of the fetus to the ventral pole of the vesicle was associated with formation of the umbilical cord. All of these aspects of development were similar to those of previous reports in mares,[17] and Jennies.[16,17]
In a previous study, the allantois initially becomes visible during days 22–24,[16] and in two separate studies in Jennies and mares, on an average on days 21 to 27.[17] In our study, because of the use of 7.5 MHz frequency, allantois was seen earlier, on average on day 23.55 (in three mares it was seen on day 22). The heartbeat becomes detectable simultaneous with embryo observation or a few days after it in mares.[17] Occasionally, the heartbeat is detectable on day 24, and more often about day 26 of pregnancy. Although, the regular heartbeat begins about day 20.[7] In this study, the heartbeat usually became detectable a few days after embryo observation on day 24.11. Certainly, heartbeat detection at this time is difficult and time-consuming, because of the difficulty in fixation of the embryo image for counting. In this study, the heartbeat was observed few days after the first day of detection of the embryo (24.11 ± 0.35 day). Others have reported embryonic heartbeats in Jennies on days 21 to 26 and mares on days 26 of gestation.[16] In the present study, an accurate record of heartbeats was not possible until days 22 and 24 because of the small heart size and high beat frequency.
In conclusion, the ultrasonic appearance of the conceptus on days 10 to 44 in the Caspian mares was very similar to that of previous reports in the mare and Jennies. Pregnancy detection was evident with 100% confidence only after day 12 after ovulation. However, on day 23 the diagnosis has an added value, as it is possible to monitor the embryo proper and heart beatings , being this feature are one manner for determining embryo viability. The age of embryo in Caspian mares can be accurately estimated by ultrasound measuring the CRL, BPD, embryonic vesicle width and height. However, it is still possible to estimate the fetal age by measuring different fetal structures instead of the entire conceptus. Embryonic vesicle height and width, CRL and BPD provided a good index of fetal development because they showed high correlations with gestational age, enabling long periods of observation from 9–44 days after ovulation.

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## چکیده

# اندازه گیری اولتراسونوگرافی وزیکول رویانی و رویان مادیان کاسپین در روزهای ۸ تا ۴۴ پس از تخمک گذاری

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**هدف**- باور محققین بر اینست که اسب نژاد کاسپین جد مستقیم اسب های اولیه می باشد. تشخیص آبستنی در مراحل ابتدایی یکی از ضروریات برنامه ریزی های باروری و گسترش یک نژاد خاص است. هدف از مطالعه حاضر تشخیص اولین زمان ممکن آبستنی و برخی خصوصیات جنین در حال رشد بین روز های ۸ تا ۴۴ بعد از تخمک گذاری است.

**طرح مطالعه**- مطالعه تجربی

**حیوانات**- ۶ مادیان کاسپین ثبت شده و سالم

**روش کار**- با استفاده از دستگاه اولتراسونوگرافی معاینات روزانه بین روز های ۸ تا ۴۴ آبستنی (تخمک گذاری = روز صفر) انجام شد تا برخی خصوصیات وزیکول رویانی و رویان در مادیان های کاسپین آبستن (تعداد آبستنی کار شده = ۹) بررسی گردد.

**نتایج**- وزیکول رویانی برای اولین بار در روز ۰/۳۳ ± ۹/۶۷ ( متوسط ± میانگین خطای استاندارد، با دامنه ۱۱-۹ روز) و با قطر ۰/۴۸ ± ۷/۱۷ میلی متر مشاهده شد. این وزیکول در روز ۰/۲۴ ± ۱۶/۴۴ و در بخش عقبی شاخ رحمی ثابت گردید (دامنه سن ۱۷-۱۵ روز بود). وزیکول از روز ۱۶-۹ دایره ای شکل (متوسط ± میانگین خطای استاندارد میزان رشد، ۰/۱۴ ± ۲/۱۶ میلی متر در روز) و غیر دایره ای شکل از روز ۱۹-۱۶ با میزان رشد کاهشی تا روز ۲۷-۱۸ (۰/۱۲ ± ۰/۶۰ میلی متر به ازای هر روز) و سپس بطور متوسط ۰/۱۵ ± ۱/۲۷ میلی متر در روز تا روز ۴۴ آبستنی رشد کرد. توده رویان و ضربان قلب جنین اولین بار در روز ۳۷ ± ۲۰ و ۰/۳۵ ± ۲۳/۱۱، به ترتیب تشخیص داده شد. تشکیل کیسه آلانتوئیسی همزمان با کاهش کیسه زرده و در روز ۰/۴۴ ± ۲۳/۵۵ آغاز شد و در روز ۰/۵۵ ± ۳۵/۳۳ کامل گردید.

**نتیجه گیری و کاربرد بالینی**- زمان رخداد ایجاد ساختار های رویان در حال رشد مادیان کاسپین مشابه با گزارشات قبلی در مادیان و الاغ می باشد. ضمنا می توان بیان کرد معاینه اولتراسونوگرافی از طریق راست روده ای یک ابزار دقیق و ساده در راه تشخیص سن آبستنی مادیان کاسپین است.

**کلید واژگان**- اولتراسونوگرافی، رویان، وزیکول رویانی، مادیان کاسپین.