HORMONAL AND BIO-CHEMICAL PROFILE AND EARLY PREGNANCY DIAGNOSIS BY ULTRASONOGRAPHY IN THOROUGHBRED MARES*

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This work was carried out on 45 Thoroughbred mares of private stud farm of North Gujarat during breeding season. The mares bred naturally were subjected to per rectal examination and ultrasonography for the pregnancy status and were divided into pregnant (n=35) and non-pregnant (n=10) groups. The pregnant mares were further distributed in three Groups as per the gestation days, viz., i) Group-I: mares between 0-35 days of gestation (n=12), ii) Group-II: mares between 36-75 days of gestation (n=12), iii) Group-III: mares between 76-150 days of gestation (n=11). The mean plasma progesterone level (ng/ml) of pregnant mares was significantly (P<0.01) higher in Group-III (29.32 ± 2.48) than in Group-I (17.29 ± 1.66) and II (23.62 ± 2.31), whereas in non-pregnant mares (Group-IV) it was significantly lower (1.68 ± 0.35). The mean plasma estradiol-17â level (pg/ml) was significantly higher (P< 0.05) in pregnant mares of Group-III (240.91 ± 32.13) than those in Group-I (91.92 ± 14.28), II (86.00 ± 7.15) and IV (74.50 ± 6.37). The mean plasma total cholesterol level (mg/dl) was significantly (P<0.05) higher in non-pregnant mares than in pregnant mares of all three Groups. The mean plasma protein (g/dl) and glucose (mg/dl) levels did not differ significantly between various Groups of pregnant and non pregnant mares. The non-pregnant mares showed significantly higher mean plasma calcium level (mg/dl) than the pregnant ones. The mean plasma phosphorus (mg/dl) value was significantly higher in Group-III of pregnancy. The embryonic vesicle first became visible by ultrasonography as a fluid filled structure, recognized as non-echogenic sphere of 13.0 ± 4.0 mm on day 14 of pregnancy in 2 mares. The embryos as well as the fetal heart beats were detected for the first time on 28th day post-covering. The close relationships were observed between cholesterol and triglycerides, progesterone and estradiol, and gestation days with glucose level. In conclusion, the pregnancy can be diagnosed in Thoroughbred mares at the earliest on day 14th post-covering by scanning the embryonic vesicle, and around gestational days 76-150 by detecting higher levels of estradiol-17â (>155 pg/ml) and progesterone (>11 ng/ml).

Key words: Thoroughbred mare, early pregnancy diagnosis, hormonal profile, biochemical profile, ultrasonography.

In early pregnancy, by day 6, the equine conceptus migrates to the uterus where it derives nutrients from the uterine hystotrophs secretions. This is essential for the initial maintenance of pregnancy. If the mare is pregnant, then ovarian progesterone production from one or more number of corpora lutea (CLs) has to be maintained until at least day 75. If there is a failure of the functional CL during this time, then the mare will abort. The plasma concentrations of maternal estrogens also vary during pregnancy (Haluska and Currie, 1988; Naber *et al.,* 1999). Moreover, the trans-rectal ultrasonography is also now used to diagnose early pregnancy in almost all species of animals (Kamble *et al.,* 2005). Therefore, the aim of this study was to evaluate applicability of ultrasonography in diagnosis of early pregnancy in Thoroughbred mares together with the determination of steroid hormones and biochemical constituents in pregnant and non-pregnant mares.

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This work was carried out on the apparently healthy Thoroughbred farm mares (n=45), aged between 5 and 16 years, of a private stud farm of North Gujarat during breeding season (February-June, 2012). The mares covered naturally by stallions were subjected to per rectal examination and ultrasonography on different days of covering starting from day 14 to know the pregnancy status, and were divided into pregnant (n=35) and non-pregnant (n=10) groups. The early pregnant mares were further distributed in three pregnancy stages as per the gestational days, viz., Group-I: mares between day 0 and 35 of gestation (n=12), Group-II: mares between day 36 and 75 of gestation (n=12), Group-III: mares between day 76 and 150 of gestation (n=11), whereas the Group-IV comprised of non-pregnant mares (n=10). Pregnancy diagnosis by ultrasonography was carried out first on day 14 post-covering in mares and then on days 18-22, 28-30. 35, 45-48, 56 and 60-63. Ultrasonic examinations were done with a sequential liner array transducer using ultrasound coupling gel, which produces a real time B-mode image (ALOKA SSD – 500, SN - M10408, Japan). The scanner was equipped with a 5 MHz transducer designed for intra-rectal insertion in mare. In order to keep the probe hygienic and to protect it from moisture a plastic sleeve was pulled over the probe and the plastic sleeve was filled with ultrasound gel to exclude any air bubble which cause undesirable reflections and thus affect the image quality. Blood plasma samples collected from above mares/days were analyzed for biochemical and hormonal profile. Plasma progesterone and estradiol-17â were estimated by RIA employing standard technique of Kubasic et al. (1984) and Robertson et al. (1979), respectively. The plasma biochemical constituents, viz., plasma total protein (Biuret method), total cholesterol (CHOD/ PAP method), triglycerides (GPO / PAP Method), glucose (GOD/POD method), calcium (Arsenazol-III method) and inorganic phosphorus (Molybdate UV method) were estimated using standard procedures and assay kits procured from Crest Biosystems, Goa, India. The data generated were analyzed statistically using unequal completely randomized design.

The mean plasma progesterone (ng/ml) concentrations of Thoroughbred pregnant mares in Group-I, II, III and non-pregnant mares (Group-IV) were 17.29 ± 1.66, 23.62 ± 2.31, 29.32 ± 2.48 and 1.68 ± 0.35 ng/ml, respectively. The corresponding plasma estradiol-17â (pg/ml) levels were 91.92 ± 14.28, 86.00 ± 7.15, 240.91 ± 32.13 and 74.50 ± 6.37 pg/ml, respectively. The differences were significant (P<0.01) among the Groups of pregnant and non-pregnant mares for both progesterone and estradiol-17â. The mean levels of both in Group-III mares were significantly higher than those of Group-II, I and IV. The mean progesterone levels showed a gradual and significant increase in pregnant mares of Group-I to III, whereas the levels of estradiol were statistically similar among Group-II, I and IV. Thus, the detection of higher values of progesterone (> 11 ng/ml) and estradiol-17â (> 155 pg/ml) confirmed the pregnancy in individual Thoroughbred mares, whereas the lower levels were suggestive of non-pregnancy.

The mean progesterone level found in Group-I mares corroborated with the finding of Makawiti et al. (1983), who recorded the value of 13.3 ± 1.3 ng/ml in pony mares during 0-35 days of gestation. On the contrary, Borst et al. (1985) and Abo El-Maaty (2011) found higher progesterone levels (6.7 to 30.0 ng/ml) during early pregnancy. Progesterone levels in Group-II mares were higher than those reported by Holtan et al. (1975) (8-10 ng/ml during 45-55 day of pregnancy), though it corroborated with their earlier report (Holtan et al., 1973). The high progesterone levels noted during second and third month of pregnancy are generally due to formation of secondary corpora lutea after regression of primary corpus luteum. The mean plasma progesterone value found in Group-III mare is in agreement with the value of 32.5 ng/ml reported during 72 to 153 days by Haluska and Currie (1988), although Naber et al. (1999) recorded much lower progesterone values of 8-10 between 70 to 200 days of pregnancy. The progesterone levels in non-pregnant mares of the present study corroborated well with the report of Vries and Holst (1983). The present findings of estradiol-17â are in close agreement with the report of Stabenfeldt et al., (1991), whereas Haluska and Currie (1988) recorded comparatively lower levels of estradiol-17â (< 10 pg/ml)

during 0 to 76 days of pregnancy. The higher estradiol-17â levels observed in the present study might be attributed to the breed and management difference.

The mean plasma total cholesterol and triglycerides levels were significantly (P< 0.05) higher in non-pregnant mares (Group-IV, 86.70 ± 5.56 and 39.93 ± 2.69 mg/dl, respectively) than those of early pregnant mares (Group-I to III, 69.50 ± 2.02 to 78.74± 2.15 and 23.95 ± 3.21 to 26.24 ± 2.89 mg/dl). The levels of both the traits showed gradual increasing trend in Group-I to III. These observations are in close agreement with the report of Watson *et al.* (1993). Meliani *et al.* (2011), however, found lower levels of triglycerides (12 mg/dl) in early pregnant mares.

The plasma total protein levels in Group-I, II, III and IV mares were 7.20 ± 0.18, 6.98 ± 0.15, 7.52 ± 0.76 and 6.77 ± 0.24 g/dl, respectively. The corresponding values of blood glucose were 81.60 ± 2.73, 84.97 ± 0.85, 102.18 ± 4.26 and 80.62 ± 1.12 mg/dl. The levels of both were significantly (P<0.05) higher in Group-III than those in other groups. These findings on protein are in agreement with those of Fazio *et al.* (2011). Further, the non-pregnant mares showed significantly higher mean plasma calcium level (12.73 ± 0.18 mg/dl) than those in pregnant mares of Group-I to III (10.76 ± 0.20, 10.91 ± 0.17 and 11.63 ± 2.97 mg/dl, respectively). The mean plasma inorganic phosphorus levels were 3.25 ± 0.14, 2.90 ± 0.11, 3.76 ± 0.11 and 3.13 ± 0.25 mg/dl in Group-I to IV, respectively, being significantly higher only in Group-III. These findings are comparable with the report of Meliani *et al.* (2011)

and can be due to lesser calcium utilization by the body in absence of pregnancy or milk production.

The trans-rectal USG carried out at the earliest on day 14 of covering revealed the embryonic vesicle as a fluid filled structure, first recognized as non-echogenic sphere of 9 and 17 mm in two mares (Plate 1) and then the shape became more triangular at around day 18. The mean size of embryonic vesicles observed on the day 14, 18-22, 28-30, 35, 45-48, 56, 60-63 of pregnancy was 13.0 ± 4.0, 28.0 ± 0.82, 36.5 ± 1.41, 52.0 ± 0.0, 69.0 ± 5.0 ,74.0 ± 0.0 and 92.2 ± 2.2 mm, respectively. Embryo was located at bottom of vesicle (Plate 2) and the entire hypoechoic structure seen was the yolk sac. The embryonic vesicle looked like the size of a tennis ball by day 45. After day 60, the pregnancy was like an elongated football. From 4 months onwards, fetus could be palpated per rectally. Kahn and Leidl (1984) and Bansal *et al.* (2009) also observed embryonic vesicle between 13 and 19 mm on day 14 of the pregnancy in mares. Further, Kahn and Leidl (1984) detected embryo for the first time near the floor of the vesicle on day 21. However, we could not detect it even on day 22, but the embryos as well as the fetal heart beats were detected for the first time on 28th day post-covering (Plate 2). The mean size of embryo was 11.2 ± 0.95 mm during days 28-30 of gestation. The embryos increased in size with advancing gestational days from 35, 45-56 to 60-63 with the corresponding size as 22.0 ± 0.00, 29.67 ± 0.41 and 54.8 ± 0.63 mm, respectively. This growth pattern in the developing embryos corroborated with the observations of Kahn and Leidl (1984).
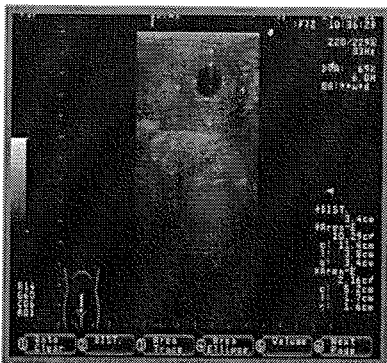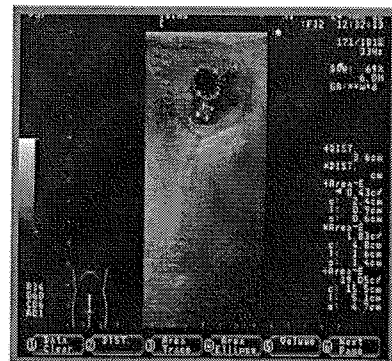

Plate 1: Sonogram 14th day of pregnancy

Plate 2: Sonogram 28th day of pregnancy

In conclusion, the pregnancy can be diagnosed in Thoroughbred mares at the earliest on day 14th post-covering by scanning the embryonic vesicle, and around gestational days 76-150 by detecting higher levels of estradiol-17â (>155 pg/ml) and progesterone (>11 ng/ml).

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