WHETHER PLASMA ENDOCRINE AND BIOCHEMICAL PROFILE CAN PREDICT EARLY PREGNANCY IN MARES

M.I. Rabari, R.G. Shah, P.H. Tank And A.J. Dhami

Department of Animal Reproduction, Gynaecology and Obstetrics

College of Veterinary Science and Animal Husbandry

Anand Agricultural University, Anand-388001

Corresponding author : ajdhami@aau.in

Received 4-1-2013 Accepted 14-3-2013

ABSTRACT

This work was carried out on 32 mares selected randomly from different areas of Saurastra region of Gujarat state during March-August, 2011. The mares bred naturally were subjected to per rectal examinations between day 20 and 150 post-breeding together with periodic blood sampling and were divided into pregnant (22) and non-pregnant (10) groups. The pregnant mares were further distributed in three stages as per the gestation days, viz., i) Stage-I: 0-35 days of gestation (4), ii) Stage-II: 36-75 days of gestation (7), iii) Stage-III: 76-150 days of gestation (11). The mean plasma progesterone level (ng/ml) of pregnant mares was significantly higher in stage-III (20.34 ± 2.13) followed by stage-I (15.55 ± 3.54) and II (13.51 ± 1.81), whereas in non-pregnant mares significantly lower mean P_4 level (0.97 ± 0.15) was observed. The mean plasma estradiol-17β concentration (pg/ml) was significantly higher ($P< 0.05$) in pregnant mares of stage-III (289.82 ± 73.05) than those in stage-I (28.50 ± 4.52) and II (43.14 ± 11.90), and even in non-pregnant ones (23.80 ± 1.82). The mean plasma total cholesterol level was significantly ($P<0.05$) higher in non-pregnant mares than the pregnant mares of stage-II, I and III. The mean plasma protein levels did not differ significantly between various stages of pregnancy and non-pregnancy. The mean plasma glucose level was significantly higher at stage-III of pregnancy (101.40 ± 5.38 mg/dl) and calcium level was significantly lower in Stage-I (10.65 ± 0.26 mg/dl) as compared to other stages. The mean plasma inorganic phosphorus (mg/dl) value was significantly higher during stage-III of pregnancy (3.97 ± 0.06 mg/dl). The close interrelationships were observed between cholesterol and triglycerides, progesterone, estradiol, and pregnancy days with glucose level. Thus, the detection of higher level of estradiol-17β (>126 pg/ml) and progesterone (> 8.2 ng/ml) during 76-150 days post-breeding can be used as diagnostic marker for early pregnancy in mares.

Key words: Mare, Early pregnancy, Farmer's door, Hormonal profile, Biochemical profile.

INTRODUCTION

Importance of equines in India is well known. Since the dawn of human civilization, equines have fascinated the mankind for their sheer charm, grace, sensitivity and endurance. In Gujarat, Kathiawari and Marwari breeds of horses have been selected by farmers both for utility and beauty. The reproductive endocrinology of the mare differs radically from that of the ruminant, canine and feline species. The length of gestation varies in mares, ranging from 310 to 380 days (av. 335–340 days) with male conceptus tending to have longer gestation. Progesterone (P_4) has an important role in maintaining uterine quiescence during pregnancy in many species. The plasma concentrations of maternal estrogens also vary during pregnancy. Scanty information is available in India on equine reproduction. Early pregnancy diagnosis is extremely important for improving the reproductive efficiency in equine. Hence, the present study was planned and executed on farmers' mares in Saurashtra region of Gujarat to know whether plasma endocrine and biochemical profile can be used to predict early pregnancy in mares.

MATERIALS AND METHODS

The research work was carried out on apparently healthy field (farmer's) mares (n=32) of Kathiawari and Marwari breeds, aged between 5 and 16 years selected randomly from different areas of Saurastra region of Gujarat state during March-August, 2011. The mares covered naturally by stallions were subjected to per rectal examination between day 20 and 150 post-breeding, and were divided into pregnant (22) and non-pregnant (10) groups. The pregnant mares were further distributed in three stages as per the gestational days, viz., Stage-I: mares between day 0 and 35 of gestation (n=4), Stage-II: mares between day 36 and 75 of gestation (n=7), Stage-III: mares between day 76 and 150 of gestation (n=11). Blood plasma samples were collected randomly from above mares on days 7, 14, 30, 45, 60, 70, 75, 90, 100, 105, 115, 140, 145 and 150 post-breeding and were analyzed for biochemical and hormonal profile. Plasma progesterone and estradiol-17β were estimated by RIA employing standard technique of Kubasik *et al.* (1984) and Robertson *et al.* (1979), respectively. The plasma biochemical constituents of certain metabolites, viz., plasma total protein (Biuret method), total cholesterol (CHOD/ PAP method), triglycerides (GPO/PAP Method), glucose (GOD/POD method) and macro-minerals, viz. calcium (Arsenazol-III method) and inorganic phosphorus (Molybdate UV method) were estimated using standard procedures and assay kits procured from Crest Biosystems, a Division of Coral Clinical Systems, Goa, India. The data generated were analyzed statistically using unequal Completely Randomized Design (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Table 1: Mean (± SE) plasma hormonal, biochemical and mineral profiles of field mares during early stages of pregnancy and non-pregnancy

Plasma profile	Pregnant mares			Non-pregnant mares (n=10)
	Stage I (0-35 days) (n=4)	Stage II (36-75 days) (n=7)	Stage III (76-150 days) (n=11)	
Progesterone (ng/ml)	15.55±3.54^{bc} (8.2 – 23)	13.51±1.81^{b} (10 – 20.5)	20.34±2.13^{c} (16 – 34)	0.97±0.15^{a} (0.34 – 1.6)
Estradiol-*17ß* (pg/ml)	28.50±4.52^{a} (18 – 39)	43.14±11.90^{a} (23 – 126)	289.82±73.05^{b} (45 – 700)	23.80±1.82^{a} (14 – 36)
Cholesterol (mg/dl)	64.81±2.58^{a}	74.05±3.50^{ab}	64.15±3.22^{a}	86.15±5.58^{b}
Triglycerides (mg/dl)	27.64±2.38^{ab}	30.26±3.65^{b}	29.33±1.86^{ab}	20.56±2.56^{a}
Total protein (g/dl)	9.87±0.21	10.31±0.23	10.57±0.27	9.69±0.28
Glucose (mg/dl)	77.80±0.70^{a}	83.09±1.98^{a}	101.40±5.38^{b}	80.26±1.49^{a}
Calcium (mg/dl)	10.65±0.26^{a}	11.36±0.32^{b}	11.98±0.11^{b}	11.77±0.17^{b}
Phosphorus (mg/dl)	2.99±0.09^{a}	3.04±0.19^{a}	3.97±0.06^{b}	2.91±0.16^{a}

Means bearing uncommon superscripts within the row differ significantly (P<0.05).

The mean plasma progesterone level was significantly higher in stage-III pregnant mares than stage-I and II, whereas it was significantly lower in non-pregnant mares (Table 1).

The mean progesterone levels found during stage-I (15.55 ± 3.54 ng/ml) corroborated with the finding of Makawiti *et al.* (1983), who recorded plasma progesterone value as 13.3 ± 1.3 ng/ml during 0-35 days of gestation in pony mares, whereas Abo EI-Maaty (2011) observed lower progesterone concentrations. On the contrary, Borst and Smidt (1985) found higher progesterone levels (6.7 to 30.0 ng/ml) during 15-19 days of pregnancy. Increased progesterone levels following conception might be associated with the development of viable blastocyst which might stimulate progesterone secretion by the corpus luteum or cause changes in the utilization or metabolism of progesterone. On contrary to the present findings, Naber *et al.* (1999) and Terblanche and Maree (1999) recorded lower progesterone levels of 4-6 ng/ml and 4-10 ng/ml between 45 to 70 and 30 to 60 days, respectively and of 8-10 and 7-10 ng/ml between 70 to 200 and 60 to 110 days of pregnancy, respectively. The high progesterone levels noted during second and third month of pregnancy are generally due to formation of secondary corpora lutea after regression of first corpus luteum (Niekerk *et al.,* 1975). The higher progesterone values found during stage-III of pregnancy in present study are in agreement with the report of Haluska and Currie (1988). They reported plasma progesterone value of 32.5 ng/ml during 72 to 153 days of gestation. The progesterone levels in non-pregnant mares of the present study corroborated well with the reports of Ginther *et al.* (1980) and Vries and Holst (1983) as 0.55 ± 0.08 ng/ml and < 2 ng/ml, respectively.

The mean plasma estradiol-17β concentrations were significantly higher (P< 0.05) in pregnant mares of stage-III than those in stage-I and II, and in non-pregnant mares, which were however at par (Table 1). The plasma estradiol-17β levels in non-pregnant mares ranged from 14.0 to 36.0 pg/ml, whereas in pregnant mares of 7 to 150 days, it ranged from 18.0 to 700.0 pg/ml. To confirm the pregnancy on the basis of estradiol-17β levels, the level >126 pg/ml may be considered as positive for pregnancy. The present findings of estradiol-17β are in close agreement with the report of Terqui and Palmer (1979) and Stabenfeldt *et al.* (1991). They found comparable values of estradiol-17β levels during the gestational days 0 to 35. They further observed increase in the concentration of estradiol-17β levels by day 80 of gestation, these levels were higher than the levels observed in non-pregnant mares. The estradiol-17β levels reported by Papa *et al.* (1998) were 30.73 to 96.61 pg/ml during 4 to 30 days of gestation; which in close agreement with the present findings. Similarly, Amer *et al.* (2007) also found significant (P<0.05) increase in the plasma estradiol-17β levels from 21 to 45 days of gestation. Whereas estradiol-17β levels (< 15 pg/ml and < 10 pg/ml) recorded by Nett *et al.* (1973) and Haluska and Currie (1988) during 0 to 76 days of pregnancy were comparatively lower than present findings. The higher estradiol-17β levels observed in the present study might be attributed to the breed and management difference. Pregnant mares also have high concentration of estrogens in their circulation. Beginning on approximately day 40 of gestation, the primary CL and possibly accessory CLs, under the influence of eCG, produce large quantities of estrogens (Evance *et al.,* 1997). This is also due to the oestrogen surge on day 39 due to stimulation of follicular growth by eCG (Terqui and Palmer, 1979). In the non-pregnant mare, the maturation of Graffian follicles is characterized by an increase in both oestradiol- 17β and oestrogen conjugate whereas, there is no increase in oestradiol-17β associated with the increase in oestrogen conjugates concentrations before day 70 of gestation, suggesting that the oestrogens secreted in early pregnancy may be from a source other than antral follicles (Nett *et al.,* 1975).

The mean plasma total cholesterol levels were significantly higher in non-pregnant mares than the pregnant mares of all three stages. There was non-significant difference in the cholesterol concentration between various stages of early pregnancy **(**Table 1**)**. In the present study, mares had significantly (P< 0.05) higher mean total cholesterol levels during non-pregnancy are in agreement with the study of Watson *et al.* (1993), who also found higher total cholesterol concentration in non-pregnant than the pregnant mares.

The mean triglycerides level was significantly lower during non-pregnancy as compared to the stages of early pregnancy (Table 1). The triglycerides level reported by Watson *et al*. (1993) is in agreement with present finding of non-pregnant mares (24.48 ± 0.49 mg/dl). However, they reported higher plasma triglycerides level in pregnant mares (50.04 ± 0.76 mg/dl) as compared to the present findings. Similarly, Harvey *et al*. (2005) observed the highest levels of triglycerides during mid gestation. The increase in the triglyceride concentration was due to increased concentration of low density lipoproteins. On the contrary to the present findings, Meliani *et al.* (2011) found lower triglycerides values in early pregnant mares (12 mg/dl). The fatty acids metabolism from the aspect of energy imbalance in horses, especially during pregnancy and up to 8-10 weeks of lactation period, is not fully elucidated.

The mean plasma protein levels did not differ significantly between various stages of pregnancy and non-pregnancy. The mean plasma glucose (mg/dl) level was significantly higher at stage-III of pregnancy (101.40 ± 5.38) than at stage-I (77.80 ± 0.70). The mean calcium level (mg/dl) was significantly lower in Stage-I pregnancy (10.65 ± 0.26) as compared to other stages (Table 1). This finding of calcium did not match with the report of Unanian *et al.* (1999), who failed to see any change in blood calcium level during pregnancy. On the contrary to the present findings, Ali *et al.* (2004) observed lower calcium (8.76 ± 0.44 mg/dl) level. The lower plasma calcium level during early pregnancy may be due to its utilization by the body for the production of milk compared to non-pregnant/non-lactating mares.

The mean plasma inorganic phosphorus (mg/dl) values were significantly higher during stage-III of pregnancy. The mean phosphorus levels showed an increasing trend from stage-I to III with the values of 2.99 ± 0.09, 3.04 ± 0.19 and 3.97 ± 0.06 mg/dl, respectively (Table 1). Calcium and inorganic phosphorus represent 70 per cent of the organisms total content of minerals (Harrison, 1998); its biological function is well known, nevertheless in horses, its homeostatic regulation is not yet clear (Breidenbach *et al.,* 1998). The negative effect of calcium and phosphorus deficiency on reproduction in cattle has been reported by Hurley and Doem (1989).

Thus, it appears that the detection of higher mean values of plasma progesterone > 8.2 ng/ml and estradiol-17β levels > 126 ng/ml may confirm the pregnancy in field mares whereas lower levels are suggestive of non-pregnancy.

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