EARLY EMBRYONIC DEATH ASSOCIATED CHANGES IN STEROID HORMONES, LEPTIN, IGF-1, NITRIC OXIDE, ZINC AND COPPER IN MARES

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ABSTRACT

Mares exhibting early embryonic death (EED, n=8), non-pregnancy (NP, n=12) and pregnancy (P, n=10) underwent ultrasound examination and blood sampling until week 4 post-breeding to assess the changes in reproductive and metabolic hormones, nitric oxide (NO), copper and zinc. Progesterone increased ($p<0.05$) and NO decreased ($p<0.05$) in P mares compared to EED and NP mares. Zinc ($p\leq0.05$) at week 1, copper ($p\leq0.05$) at week 2, and NO and copper ($p<0.05$), and estradiol and leptin ($p\leq0.05$) at week 4 varied between NP, P and EED mares. The disturbance in ovarian and metabolic hormones concomitant with inflammatory mediators and/or trace-elements might be responsible for EED in mares.

Keywords: Early embryonic death, IGF-1, Leptin, Nitric oxide, Mare

INTRODUCTION

Any disturbance in developing corpus luteum (CL) will disturb maternal recognition of pregnancy (MRP) in bred mares (Daels *et al*., 1991) and prevents blocking luteolysis by the embryo (Bergfelt *et al*., 1992). Estrogen (E_2) help in signaling the conceptus presence and modulating MRP (Walters *et al*., 2001). Estrogen improves uterine release of IGF-1 that play a role in embryo implantation during early pregnancy (Slater and Murphy, 1999). Leptin acts directly on GnRH neurons (Sullivan and Meonter, 2004) and thus the hypothalamic-pituitary axis during estrous cycle and early pregnancy (Siawrys *et al.,* 2009). The present study aimed to mark the changes in steroid hormones, IGF-1, leptin, nitric oxide (NO), copper and zinc in mares suffering from early embryonic death (EED) as compared to their cyclic non-pregnant (NP) and pregnant (P) counterparts.

MATERIALS AND METHODS

Thirty non-pregnant mares (age, 4-20 yr) were naturally bred with proven fertile stallions 48 h prior to ovulation. Systematic ultrasound scanning per-rectum was done with B-mode scanner (Sonoscape A5V, China) equipped with linear-array 4.5-7.0 MHz transducer until day 28 post-breeding according to the previous characterization (Allen *et al*., 2007). Retrospectively, the mares were categorized into three groups viz., EED (n=8), NP (n=12), and P (n=10).

Weekly collected jugular vein blood samples were centrifuged at 3000 rpm for 15 min and the separated sera were stored at -20°C until assay. A solid phase ELISA assay of progesterone (P_4, EIA-1561), estradiol (EIA-2693), IGF-1 (EIA-4140) and leptin (EIA-1863) were done according to manufacturer instructions. Nitric oxide (Montgomery and Dymock, 1961), zinc and copper (Ventura and King, 1951) concentrations were determined according to the former described methods.

Data were statistically analyzed with one-way ANOVA using SPSS program (Ver. 16). Duncan's multiple range test was used for comparison between means at $p<0.05$.

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RESULTS AND DISCUSSION

An irregularity of embryonic vesicle (EV) borders that appeared smaller in diameter distinguished EED (Fig. 1, upper panel). A circumscribed anechoic EV with dorsal and ventral specular reflections identified normal pregnancy (Fig. 1, lower panel). Embryonic vesicle diameter was larger ($p<0.05$) in Pregnant compared to mares with EED (2.87±0.06 *vs.* 1.83±0.03 cm; Fig. 2). Previous studies reported that the EV lost in mares at Day 11 to 15 (Ginther *et al*., 1985)**,** Day 16 (Papa *et al*., 1998) or prior to Day 20 (McKinnon and Pycock, 2007) post-breeding were smaller than control and floated in a small collection of fluid. Abnormal conceptus shape, reduced conceptual fluids volume and underdeveloped EV designate EED (Rasheed *et al*., 2015). These results signify the impact of real-time ultrasound to diagnose pregnancy and EED earlier than rectal palpation in mares (Rasheed *et al*., 2015).

Pregnant mare had elevated serum progesterone (P_4; $p<0.05$) and lower NO ($p<0.05$) than that of EED and NP mare (Table 1). Also, at 4th week, serum estradiol (E_2; $p\leq0.05$) and leptin ($p<0.05$) levels differed between mare groups during the post-breeding period (Fig. 3).

During the embryonic period, P_4 is produced chiefly by the post-ovulatory primary corpus luteum (CL). In this study, the substantially raised serum P_4 in pregnant than mares with EED, chiefly at day 28 compared to NP (coincident with new estrous onset) and EED mares agreed with previous work in mares (Ezzo *et al.,* 2011). During early pregnancy, progressive P_4 increase accompanies the increase in the dimensions and vascularity of growing CL (Sieme *et al*., 2015)**.** The decrease in P_4 concentrations in EED could be due to disturbances in CL development (Bergfelt *et al*., 1992), uterine-induced luteolysis in

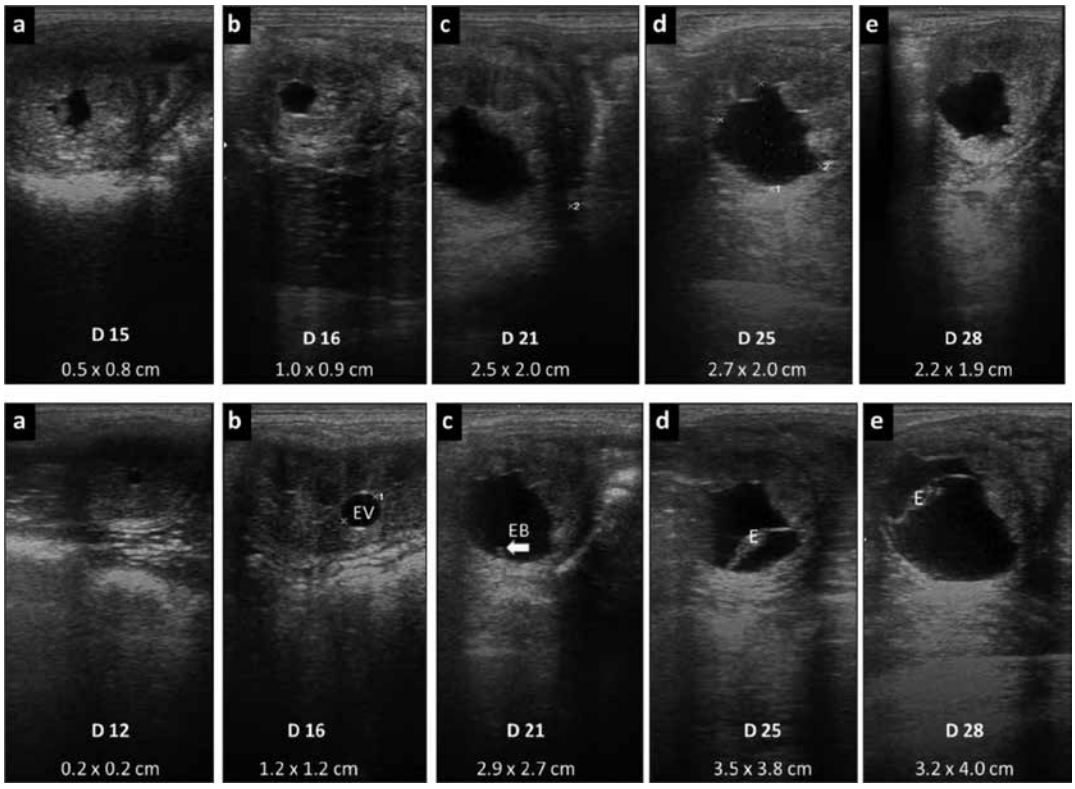

Figure 1: Ultrasound images of early embryonic death in mares. EV, Embryonic vesicle (upper panel); EB, Embryonic bud, and E, Embryonic mass (lower panel)

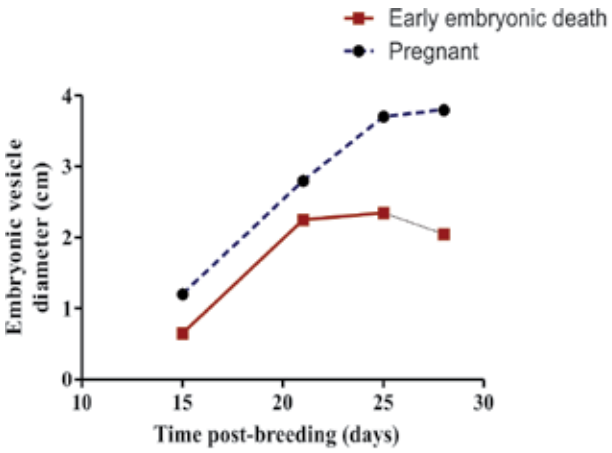

Figure 2: Growth curve of embryonic vesicle in early embryonic death (■) and pregnant (●) mares

association with uterine inflammation, primary luteal inadequacy or other dam-related factors (Ginther *et al*., 1985). The failure of MRP results in CL lysis and EED (McDowell *et al*., 1988). Abnormal conceptus failed to block luteolysis identified in mares with EED prior to Day 20 is characterized by lower P_4, smaller CL diameter and the presence of too small to aged EV that failed in fixation (Bergfelt *et al*., 1992).

Elevated serum E_2 at 4th week post-breeding in pregnant and EED mares accompanied embryo existence. A former study pointed high serum E_2 in early pregnant mares compared to normal cyclic and lactating mares (Ezzo *et al*., 2011). Mares exhibit E_2 up-surge from day 21 to 45 compared to day 14 of

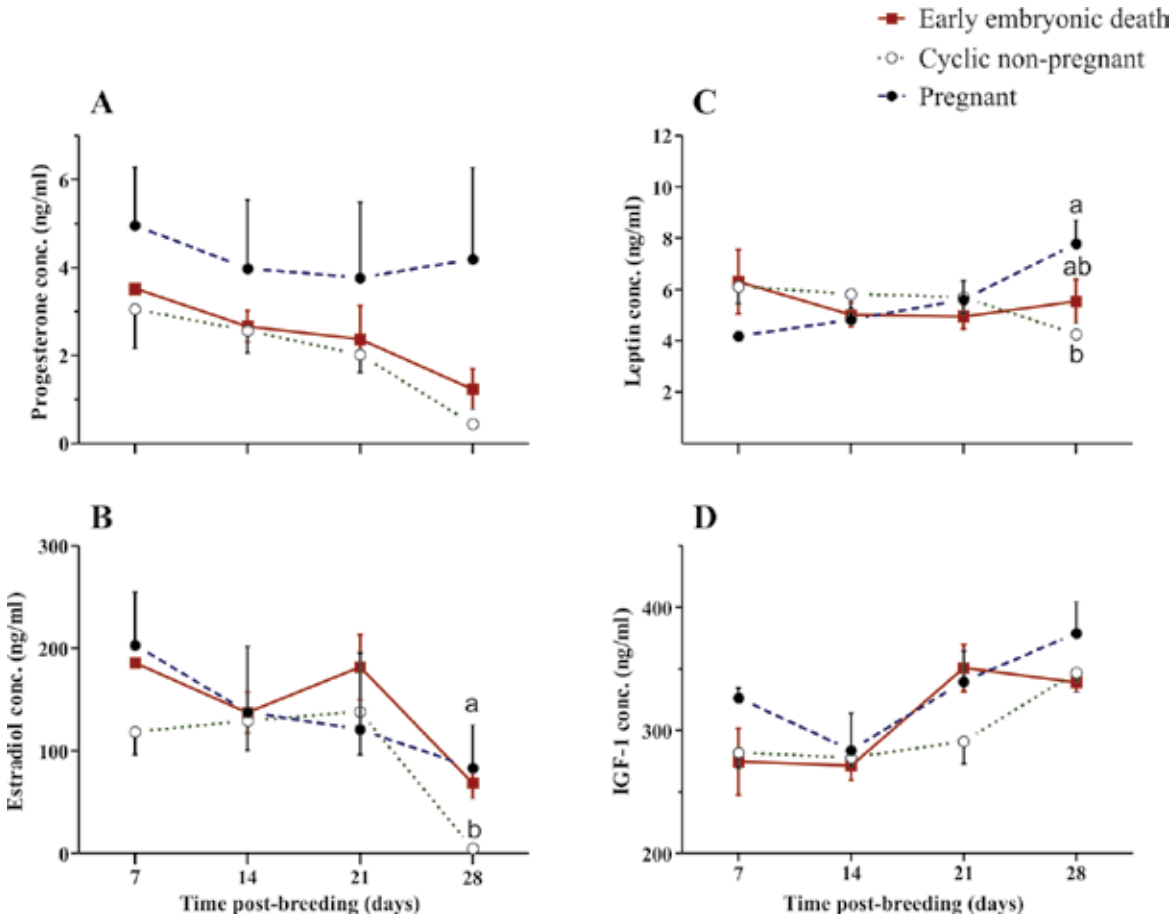

Figure 3: Changes in serum hormones in early embryonic death (■), cyclic non-pregnant (○) and pregnant (●) mares

Table 1: Serum hormonal, nitric oxide and mineral profile (Mean±SE) in mares with different pregnancy outcome

Parameter	Early embryonic death (EED)	Cyclic non-pregnant (NP)	Pregnant (P)
Progesterone, ng/ml	2.45±0.47[b]	2.02±0.57[b]	4.22±0.26[a]
Estradiol, ng/ml	143.4±27.2	97.8±31.2	136.4±25.0
Leptin, ng/ml	5.45±0.31	5.5±0.4	5.60±0.79
IGF-1, ng/ml	309.0±20.9	299.5±16.1	332.2±19.6
Nitric oxide, µmol	14.63±0.46[a]	14.01±0.50[a]	12.58±0.33[b]
Copper, µg/dL	185.4±66.21	263.4±48.9	224.5±41.4
Zinc, µg/dL	254.6±6.5	234.4±24.2	214.4±11.2

[a vs. b] $p<0.05$

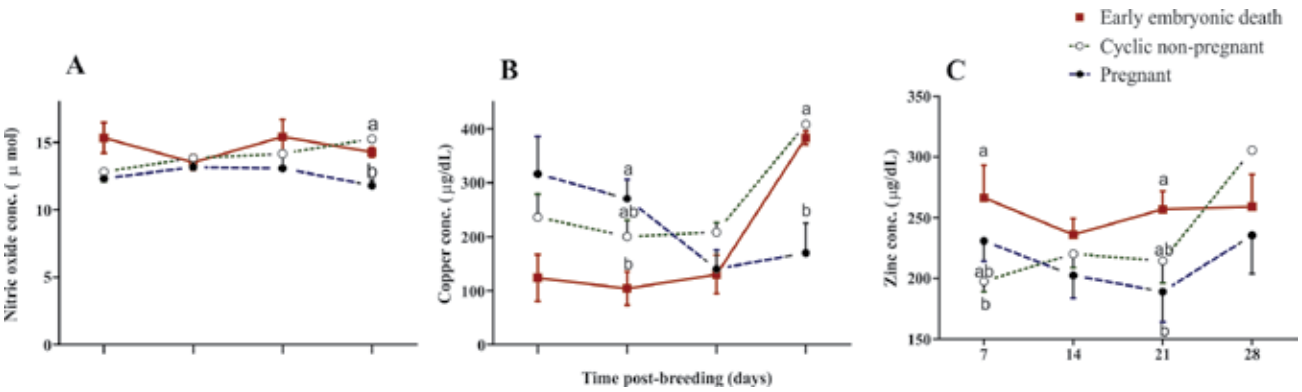

Figure 4: Changes in serum nitric oxide, copper and zinc in early embryonic death (■), cyclic non-pregnant (○) and pregnant (●) mares

gestation (Amer *et al*., 2008). This increase might be attributed to eCG stimulation of ovarian steroid synthesis during day 35-45 of pregnancy (Daels *et al*., 1991). An embryonic death (after day 35) without luteolysis maintains serum E_2 for more 10 to 14 days (Daels *et al*., 1991).

An increment of serum leptin in pregnant over NP at 4th week suggested its role in embryonic and/or placenta development. Elevated leptin (Schubring *et al*., 1997) from the placenta origin (Masuzaki *et al*., 1997), pointed to its regulation of maternal nutrition, uterine blood flow and embryo maintenance during the period of reduced sensitivity to leptin during pregnancy (Mounzih *et al*., 1998).

Elevated serum NO in mare with EED compared to their pregnant counterparts were recorded at week 4 ($p<0.05$) after mating (Fig. 4). High NO after EED might help to clear the uterus from remnants of absorbed embryo and to prepare for the next estrous (Kotp *et al*., 2015).

Serum copper concentrations varied ($p<0.05$) at 2nd week between NP and pregnant mares, and at the 4th week between NP and EED mares (Fig. 4). Serum zinc was elevated ($p<0.05$) in EED at 1st week compared to NP (Fig. 4). The differences in serum copper and zinc levels might be due to inflammatory like reaction associated with implantation and pregnancy establishment (Meyer and Ahlswede, 1978). Copper

metabolism in pregnant mares is expected to vary due to appetite and intake changes, and the extra-nutritional requirements of fetus (Ali *et al*., 2013). Plasma copper concentration was greater in pregnant than lactating mares (Ali *et al*., 2013).

In conclusion, the disturbances in reproductive and metabolic hormones act in collaboration with inflammatory mediators and/or trace elements to cause EED in mares.

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