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RESEARCH ARTICLE

Serum Ovarian Steriod Hormones and Some Minerals Concentration in Pregnant Nili-Ravi Buffaloes with or without Pre-Partum Vaginal Prolapse

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ABSTRACT

The present study was designed with the objective to determine the hormones (estradiol, progesterone) and minerals (calcium, phosphorus, magnesium, sodium and potassium) in serum of buffaloes suffering from pre-partum vaginal prolapse. A total of 200 buffaloes were included in this study, half of these were suffering with pre-partum vaginal prolapse (affected group) between 7th to 10th months of gestation while the remaining were normal pregnant buffaloes (healthy group). Blood samples were collected to procure serum from each animal. Serum hormones and minerals were determined by ELISA technique and direct colorimetric method, respectively. The mean serum estradiol and magnesium concentrations were significantly higher ($P<0.01$) whereas progesterone, calcium and phosphorus concentrations were significantly lower ($P<0.01$) in buffaloes suffering with pre-partum vaginal prolapse as compared to healthy pregnant buffaloes. It was concluded that the serum hormones (estradiol and progesterone) and minerals (magnesium, calcium and phosphorus) concentrations differ between the vaginal prolapse affected and healthy buffaloes.

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INTRODUCTION

Buffalo, the thrifty, versatile, adaptable and productive domestic animal has drawn national and international attention in the last few decades and is contributing significantly towards the rural economy of Pakistan. The buffalo has been a poor breeder due to having poor fertility in the majority of environment conditions under which they are raised (Barile, 2005; Rushdi, 2010; Akhtar *et al*., 2010; Tajik *et al*., 2010). This is manifested mainly as late maturity, long postpartum anoestrous intervals, poor expression of estrus, poor conception rates (CR) and long calving intervals (Singh *et al*., 2000). Although, buffalo is of high economic importance for farmers in Pakistan, the reproductive performance is poor due to various diseases of reproductive system, including genital prolapse.

Among pre-partum reproductive disorders, vaginal prolapse is considered to be the major problem causing substantial monetary losses to the farmers. In Pakistan, among the prevalence of various reproductive disorders, repeat breeding showed the highest prevalence (15.69%), followed by anestrous (9.74%), genital prolapse (7.73%), abortion (5.99%), retained placenta (2.58%), uterine torsion (2.39%) and dystocia (2.06%) in buffaloes (Rabbani *et al*., 2010). Incidence of chronic prolapse or eversion of prolapsed mass is considered to be due to weakness of uterine ligaments and vaginal tissue, relaxation particularly in pluripara animals and excessive deposition of perivaginal fat of hereditary predisposition (Noordsy, 1994; Dharani *et al*., 2010).

Various etiological factors considered to be contributing to pre-partum vaginal prolapse include hormone imbalance or deficiency of serum minerals (Ahmed *et al*., 2005). The concentrations and role of some macro-elements, like calcium and phosphorus, in buffaloes with pre-partum vaginal prolapse is well documented (Sah and Nakao, 2003; Akhtar *et al*., 2008). The role of calcium in maintaining the adequate tonicity of vaginal musculature and thus in preventing the occurrence of vaginal prolapse is also well understood. However, no reports are available on serum concentrations of ovarian steroid hormones, like estradiol and progesterone and sodium, potassium in animals of

same species with pre-partum prolapse of the vagina. It was hypothesized that the deficiency or excess of ovarian steroid hormones and electrolyte concentrations in the blood contribute to the occurrence of pre-partum vaginal prolapse in buffaloes. The present study was, therefore, designed with the objective to determine the hormones (estradiol, progesterone) and minerals (calcium, phosphorus, magnesium, sodium and potassium) concentrations in serum of the buffaloes suffering from pre-partum vaginal prolapse and to compare them with their healthy counterparts.

MATERIALS AND METHODS

Experimental area: The present study was conducted on Nili-Ravi buffaloes in district Bahawalpur and Muzaffargarh. The district Bahawalpur is located between latitude 29° and 59°N, longitude 73° and 19°E, while the district Muzaffargarh is located between latitude 30° and 20°N, longitude 71° and 5°E.

Experimental animals: A total of 200 buffaloes between 7^{th} to 10^{th} month of gestation were included from experimental area, out of these half were suffering with pre-partum vaginal prolapse (affected group) while the remaining were normal pregnant buffaloes (healthy group). These animals were were kept under similar management conditions. All buffaloes were clinically free of diseases, ranging from 5 to 9 years of age with parity ranged from 2 to 6. In both groups, gestation period was divided in three parts, i.e. below 8^{th}, from 8^{th} to 9^{th} and above 9^{th} months of gestation. In each group; 34, 30 and 36 buffaloes were belonging to the period below 8^{th} month of gestation, from 8^{th} to 9^{th} month and above 9^{th} month of gestation, respectively.

Serum collection and analysis: Blood samples without anticoagulant were collected from each animal from May to September, 2006 for the collection of serum which then stored at -20°C until analysis. Serum estradiol and progesterone concentrations were determined by using ELISA technique. Estradiol and progesterone were determined by using commercially available kits i.e. Bio-Check, Inc. Serum calcium was determined by direct colorimetric method by using a commercially available kit (Cat # 606635; Wiener Lab, Argentina) whereas phosphorus was determined by using the commercially available kit (Cat # 600074438; Dia Sys GmbH-Germany). Magnesium (Cat # 0512110), sodium (Cat # 0602010) and potassium (Cat # 0509010) were determined by using commercially available kits (Centronic GmbH-Germany).

Statistical analysis: The values (mean±SE) of hormones and minerals of two experimental groups were subjected to two factor completely randomized design (Steel *et al*., 2006). The values were considered significant at P<0.01.

RESULTS

The mean serum estradiol concentrations were significantly higher (P<0.01) in buffaloes suffering with pre-partum vaginal prolapse as compared to healthy pregnant buffaloes in different periods of gestation (Table 1). Mean serum estradiol concentrations in vaginal prolapse affected buffaloes differed non-significantly in different periods of gestation. In healthy group buffaloes the estradiol concentrations differed non-significantly through different periods of gestation (Table 1).

The mean serum progesterone concentrations were significantly lower (P<0.01) in vaginal prolapse affected buffaloes in comparison with healthy group buffaloes in different periods of gestations. The serum progesterone concentrations differed non-significantly within vaginal prolapse affected group buffaloes and healthy group buffaloes through different periods of gestation (Table 1).

The mean serum calcium and phosphorus concentrations were considerably lower (P<0.01) in buffaloes suffering with pre-partum vaginal prolapse as likened to healthy pregnant buffaloes in different periods of gestation. Mean serum calcium and phosphorus concentrations in vaginal prolapse affected buffaloes differed non-significantly in different periods of gestation. In healthy group buffaloes the calcium and phosphorus concentrations differed non-significantly over different periods of gestation (Table 2).

There was non-significant difference in magnesium concentrations during different periods of gestation in healthy and vaginal prolapse affected buffaloes. The mean serum magnesium concentration were significantly higher (P<0.01) in buffalo with vaginal prolapse during different periods of gestation in comparison with healthy pregnant buffaloes. The serum sodium and potassium concentrations differed non-significantly during different periods of gestation in healthy and affected buffaloes.

Table 1: Serum estradiol and progesterone (Mean±SE) of healthy and vaginal prolapse affected buffalo during different periods of gestation

Period of gestation	Estradiol (pg/ml)		Progesterone (ng/dl)	
	Affected	Healthy	Affected	Healthy
Below 8 month (n=34)	170.4±4.8^a	86.2±3.7^b	2.6±0.1^a	4.8±0.3^b
From 8 to 9 month (n=30)	171.9±5.3^a	88.7±4.1^b	2.6±0.9^a	4.8±0.9^b
9 months to term (n=36)	172.1±6.3^a	88.2±4.7^b	2.6±0.4^a	4.7±0.7^b

Values sharing different superscripts in the same row of a parameter differ significantly (P<0.01).

Table 2: Serum calcium, phosphorus, magnesium, sodioum and potassium (Mean±SE) of healthy and vaginal prolapse affected buffalo during different periods of gestation

Mineral	Animal group	Below 8 month (n=34)	From 8 to 9 month (n=30)	9 months to term (n=36)
Calcium (mg/dl)	Affected	6.70±0.09^a	6.68±0.12^a	6.64±0.15^a
	Healthy	9.31±0.17^b	9.33±0.22^b	9.30±0.16^b
Phosphorus (mg/dl)	Affected	3.10±0.11^a	3.06±0.08^a	3.09±0.07^a
	Healthy	6.01±0.13^b	5.98±0.12^b	5.99±0.16^b
Magnesium (mg/dl)	Affected	2.33±0.01^a	2.35±0.01^a	2.33±0.02^a
	Healthy	2.17±0.02^b	2.15±0.01^b	2.17±0.01^b
Sodium (mmol/l)	Affected	133.6±1.12^a	133.9±1.19^a	133.9±1.16^a
	Healthy	133.1±1. 09^a	134.11±1.15^a	133.5±1.07^a
Potassium (mmol/l)	Affected	4.56±0.07^a	4.55±0.09^a	4.55±0.14^a
	Healthy	4.49±0.23^a	4.53±0.18^a	4.53±0.13^a

Values sharing different superscripts in the same column of a parameter differ significantly (P<0.01).

DISCUSSION

Pre-partum prolapse of vagina is frequently observed complication of late pregnancy in buffalo. Estrogen and progesterone play an important role in maintaining pregnancy (Thota *et al*., 2003). A disturbance in their ratio may lead to gestational abnormalities.

The increased serum estradiol concentrations and lowered progesterone concentrations in affected buffaloes have been recorded during this study. The increased in estradiol concentration has been reported in cows (Vicenti *et al*., 1992) and sheep (Sobiraj, 1990) with vaginal prolapse. There could hence be a causal relationship between the high estrogen concentrations in the maternal blood and the clinical picture of vaginal prolapse. The increase in estradiol concentrations in affected animals coupled with decrease in progesterone concentrations of these animals may be responsible for causing relaxation and odematization of the parturient canal resulting in vaginal inversion and prolapse. The decrease in progesterone concentrations has earlier been reported (Zicarelli, 2000) in buffaloes suffering with vaginal prolapse. The description on successful progesterone therapy in pre-partum vaginal prolapse in buffaloes (Sah and Nakao, 2003) and ewes (Bhattacharyya *et al*., 2006) has been reported indicative of the role of low serum progesterone concentrations in causing pre-partum vaginal prolapse. The causes of these hormonal irregularities are to be found partly in the dam but probably mainly in the fetus. Nakamura *et al*. (2003) reported that excess biologically inactive conjugated oesterone sulphate can be converted *in vitro* into biologically active 17- β estradiol via a 17- β-OH dehydrogenase and an aryl sulphate present in the cells of myometrium. *In vivo* the origin of large quantities of oesterone sulphate is unclear. The prenatal rise in fetal ACTH of the adrenal cortex brings about a rise in the hydrocortisone concentration, which in turn causes a large increase in the placental synthesis of oestrogen (Benediktsson, 1995). A dysfunction of this mechanism-perhaps genetically mediated-could also be the cause of excessively raised 17 β-oestradiol concentrations in the maternal blood.

The serum analysis of healthy and vaginal prolapse affected buffalo revealed that calcium and phosphorus concentrations in the sera of affected buffaloes were significantly lower compared with that of their healthy counterparts. Similar findings have been reported previously (Mandali *et al*., 2002) and Ahmed *et al*. (2005). The calcium phosphorus ratio did not change in the affected buffaloes, however if the serum estradiol concentrations of the affected buffaloes are also seen in conjunction with the calcium concentrations of affected buffaloes it may be possible that relaxation of the musculature by increased estradiol concentrations and reduced tonicity of the muscles by decreased serum concentrations of calcium may synergistically cause this condition. The previously reported calcium and phosphorus values in healthy pregnant dry buffalo in late gestation are in close agreement with those recorded during this study in the dry pregnant healthy buffaloes but much lower in the affected buffalo (Hagawane *et al*., 2009). The magnesium concentrations in sera of affected and healthy buffaloes were also different. The serum concentrations of magnesium were higher in affected buffaloes and correspondingly decreased concentrations of serum calcium in the affected buffaloes can be explained on the grounds that where the decreased serum calcium concentrations result in loss of tonicity of muscles of vagina and uterus, the magnesium increases in response to decreasing concentrations of calcium to combat this phenomena (Radostits *et al*., 2010).

The serum sodium and potassium concentrations in affected and healthy buffalo were not different; however the serum sodium and potassium concentrations were in normal range in both groups of buffaloes. The serum sodium and potassium concentrations values for affected and healthy buffaloes both are close to those reported by Prasad (1992). The values reported by Vicenti *et al*. (1992) for sodium in cows affected with vaginal prolapse and healthy cows are also in agreement to this study but in total disagreement for the values of potassium which were reported to be much higher for cows.

Based on information obtained from this study, it was concluded that the serum hormone and mineral concentrations differed between the vaginal prolapse affected and healthy buffaloes.

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