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Research Article

Correspondence between hormone levels in blood and feces or urine in pregnant cattle

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ABSTRACT

Pregnancy diagnosis is a key to the effective reproductive management of animals. Although transrectal palpation has been established as convenient method to diagnose pregnancy, the measurements of the levels of reproductive hormone has yet to be established. For this, a study was performed to compare the levels of the reproductive hormone in blood with those of matching urine and fecal samples. A total of 30 pregnant cows (10 in each pregnancy trimester) were selected from 226 pregnant cattle based on age, body condition score (BCS) and parity. The reproductive hormones were assayed in blood and corresponding urine and fecal sample through commercial kits by Enzyme Linked Immuno Sorbent Assay (ELISA). A significant variation ($p<0.05$) in progesterone (P_4) and Estradiol (E_2) concentration among trimesters was found in serum, as well as in urine and feces. The values for the Luteinizing Hormone (LH) varied less among the trimesters in all the types of samples. Follicle Stimulating Hormone (FSH) levels varied significantly ($p \leq 0.05$) in serum and fecal sample. A moderate positive (r=0.44) relationship was observed between serum vs urine FSH in the 1st trimester. On the contrary, moderate positive relationship were found between serum vs urine p4 (r=0.40) and serum vs urine FSH in the 2nd trimester and a strong relationship (r=0.54) was found in serum vs fecal FSH which implies that urine and feces could be used for reproductive hormone assay.

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1. INTRODUCTION

Determination of the reproductive status is one major factors for effective management in modern dairy industry. Understanding of basic reproductive biology including endocrinology is necessary to provide proper reproductive management (Wildt *et al.*, 2010). A non-invasive monitoring of hormone spermits frequent sample collection and urine, milk and feces could be used as alternative to blood. Invasive and non-invasive monitoring of ovarian activity and pregnancy diagnosis have been done in domestic animals by determination of

sex steroids in plasma or serum (Batra *et al.*, 1979) and in milk (Gao *et al.*, 1988), urine (Loskutoff *et al.*, 1983; Steven *et al.*, 1991), saliva (Kanchev *et al.*, 1988) and feces (Isobe *et al.*, 2005).

In general, fecal estrogens are measured using specific estrogen antibodies or antibodies against total unconjugated estrogens. Yet, the voided feces of most species contain a higher percentage of free rather than conjugated steroids. Estrogens are end products of the steroid metabolism and therefore the compounds in plasma and feces are similar (Mostl *et al.*, 1984). But progesterone is mainly metabolized by liver before fecal estrogens in ruminants (Schwarzen-berger *et al.*, 1996). About 99% of excreted progesterone is presented as unconjugated compounds (Palme *et al.*, 1996). Steroid hormone metabolites quantified in feces are widely used in studies on wild animals, as a non-invasive, stress-free, economical technique allowing longitudinal studies by permitting frequent sampling of the same individual (Brown *et al.*, 1994). Such characteristics may also support the use of this technique in laboratory and domestic animals. Therefore, this study aims to compare the levels of reproductive hormones (FSH, LH, estrogen, progesterone) in blood, feces and urine samples from pregnant cows in different trimesters of pregnancy, and to correlate the hormone levels in those samples to assess its usefulness as a non-invasive technique for pregnancy diagnosis.

2. MATERIALS AND METHODS

Study area and animals

This is a six month long cross sectional study performed in Military farm, Chattogram, Bangladesh. Thirty cross breed (Holstein Friesian x Indigenous Local) pregnant cows (10 in each trimester) were selected from 226 pregnant cow. Animals enrolled in the study had 3-5 calving with overall BCS of 3-3.5 and 354.33±9.14 kg body weight. The staging of pregnancy was achieved by rectal palpation along with recording the insemination date.

Collection of blood, urine and feces

The blood was collected from all selected pregnant cow via jugular venipuncture from 10 AM to 12 PM, into vacutainers that were kept in rack for smooth coagulation of blood for 2 hours; serum was harvested after centrifugation, and aliquoted in eppendorf tubes and frozen until analysis. Matching fecal samples were also collected from the rectum and transferred into a fecal sample vial, and transported by an ice box to the laboratory for further use. The matching urine samples were collected from midstream urine-immediately after micturition in a sterile plastic beaker. A 10 ml volume was transferred into a falcon tube and transferred to the laboratory. Extraction of fecal steroids was done according to the method described by Mostl *et al.*, (1984) and Hirata and Mori (1995). Extracted fecal samples and urine samples were stored in eppendorf tubes in -20ºC until analysis.

Hormone assays

The level of reproductive hormone in serum sample and the corresponding fecal and urine samples were assayed by ELISA using commercially available kits for Progesterone (Human, Germany), estradiol (Human, Germany), FSH (Omega Diagnostics Ltd., United Kingdom) and LH (Omega Diagnostics Ltd., United Kingdom), according to the instruction provided by the manufacturer.

Data analysis

Data retrieved in the study were stored in MS Excel 2007, sorted, and exported to STATA-11 (STATA Corporation, USA). The values for the reproductive hormones are expressed as mean ± standard error of mean (SEM). A Pearson's product-moment correlation was run to assess the relationship between serum hormone level with urine or fecal hormone level and the correlation coefficient (r) determined. Significant differences were considered at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

Table 1 depicts the sex steroids and gonadotropins in serum with corresponding urine and feces in the different trimesters of pregnancy. The values of sex steroid hormones (P_4 and E_2) significantly varied among different trimesters (P_4, p=0.001; E_2, p<0.001). P_4 was found highest in 2nd trimester (17.26±2.18 ng/ml) whereas E_2 was highest in 3rd trimester (135.69±11.82 pg/ml). The P_4 level generally increased and reaches to a peak in 2nd trimester and then decline in the third trimester as found in Henricks *et al.*, (1972). This constant rise in the 2nd trimester might be due to the full functional activity of corpus luteum (Hafez, 1962). Besides, in a previous study of Henricks *et al.*, (1972) found, during 14 days before parturition, estrogen could increased up to 2660 pg/ml at parturition.

In relation to gonadotropins, the values for FSH varied significantly (p=0.05) among the different trimesters, but no differences were found in case of LH. Serum LH was remained closely in same margin in 1st and 2nd trimester and then increases a little bit in the third trimester which was coincided with the earlier findings of

Table 1. Sex steroid and gonadotropins level in matched serum, urine and feces in the three trimesters of pregnancy in cross bred cows

Hormone	1st trimester (Mean±SEM)	2nd trimester (Mean±SEM)	3rd trimester (Mean±SEM)	*p*
Serum				
Progesterone (ng/ml)	8.85±1.03	17.26±2.18	15.37±0.83	0.001
Estradiol (pg/ml)	53.96±3.85	75.83±3.44	135.69±11.82	<0.001
FSH (mIU/ml)	22.50±2.07	19.83±0.78	16.6±1.83	0.05
LH (mIU/ml)	9.56±0.91	9.72±1.06	10.13±4.35	0.98
Urine				
Progesterone (ng/ml)	2.87±0.406	2.79±0.451	6.15±1.069	0.002
Estradiol (pg/ml)	60.17± 5.59	135.27± 25.51	161.85± 19.11	0.001
FSH (mIU/ml)	21.29± 2.43	13.27± 2.105	16.59± 2.53	0.07
LH (mIU/ml)	7.65± 0.44	7.23± 0.93	4.79± 0.55	0.01
Feces				
Progesterone (ng/ml)	5.62± 0.7	10.35± 0.96	9.64± 1.14	0.003
Estradiol (pg/ml)	168.69± 60.43	581.65± 65.79	460.26± 90.53	0.001
FSH (mIU/ml)	40.02± 3.69	38.42± 2.64	50.70± 3.42	0.02
LH (mIU/ml)	13.26± 1.49	14.44± 1.58	14.33± 0.66	0.78

Edgerton and Hafs (1973). The level of FSH in pregnant cattle also agreed in line as found earlier by Bolt and Rollins (1983). Sex steroids in urine significantly varied in different trimesters, as it was also found in urine´s LH but not for urine´s FSH. Urine P_4 and E_2 were higher in 3rd trimester of pregnancy.

The level of sex steroid hormone concentrations in feces of pregnant cattle followed a similar pattern as described for blood and urine. Sex steroid hormones in feces differed significantly among the trimesters. Fecal P_4 and E_2 presenting the highest values in the 2nd trimester. FSH level of significantly differed among trimesters (p=0.02), contrasting to LH whose variations were not statistically different.

Table 2 depicts the correlations between hormonal levels in serum and the corresponding urine and fecal samples in different trimesters. Little to moderate correlation existed between serum P_4 with urine (r= -0.29) or fecal (r= -0.33). It implies that fecal P_4 decrease moderately with increased level of serum P_4 in 1st trimester. In the 2nd trimester,the correlations between serum and urine P_4 was a bit higher (r= 0.40) compared to the 1st trimester while a slight negative correlation (r= -0.15) was found between serum and fecal P_4 in the 2nd trimester. On the other hand, this trend was inversed in the 3rd trimester (r= -0.25 and 0.21, respectively).

Weak correlation existed between serum and urine E_2 (r= -0.22) or serum and fecal E_2 (r= -0.14) in 1st trimester. It implies that very little decreases occur in urine and fecal E_2 with any serum E_2 increase in the 1st trimester. In the 2nd trimester, the correlation between serum and urine E_2 was positive (r= 0.19) and between serum and fecal E_2 (r= 0.02) in the 2nd trimester. On the other hand, this trend was both small negative in 3rd trimester (r= -0.06 and -0.03, respectively).

A moderate positive correlation existed between serum and urine FSH (r= 0.44) or serum and fecal FSH (r= 0.25). So, urine FSH increased moderately with increased serum FSH levels in the 1st trimester. These correlations were lower in the 2nd trimester for serum and urine FSH, although still moderately positive (r= 0.42) while it was a small positive (r= 0.03) in case of serum FSH and fecal P_4 of 2nd trimester. On the other hand, these measurements were negatively correlated (r= -0.26) in case of serum and Urine FSH, but a strong positive correlation (r= 0.54) persisted between serum and fecal FSH.

Little correlation was found between serum and urine (r= 0.10) or fecal LH (r= -0.14). In the 2nd trimester the correlation between serum and urine LH was slightly higher (r= 0.13) than in 1st trimester and there was a

Table 2. Relationship in reproductive hormone level between serum with corresponding urine and feces in various trimesters

Trimester	Correlation between	r	Strength of association
1st	Serum P4 and Urine P4	-0.29	Little
	Serum E2 and Urine E2	-0.22	Little
	Serum FSH and Urine FSH	**0.44**	**Moderate**
	Serum LH and Urine LH	0.10	Little
	Serum P4 and Fecal P4	**-0.33**	**Moderate**
	Serum E2 and Fecal E2	-0.14	Little
	Serum FSH and Fecal FSH	0.25	Little
	Serum LH and Fecal LH	-0.14	Little
2nd	Serum P4 and Urine P4	**0.40**	**Moderate**
	Serum E2 and Urine E2	0.19	Little
	Serum FSH and Urine FSH	**0.42**	**Moderate**
	Serum LH and Urine LH	0.13	Little
	Serum P4 and Fecal P4	-0.15	Little
	Serum E2 and Fecal E2	0.02	Little
	Serum FSH and Fecal FSH	0.03	Little
	Serum LH and Fecal LH	0.21	Little
3rd	Serum P4 and Urine P4	-0.25	Little
	Serum E2 and Urine E2	-0.06	Little
	Serum FSH and Urine FSH	-0.26	Little
	Serum LH and Urine LH	0.07	Little
	Serum P4 and Fecal P4	0.21	Little
	Serum E2 and Fecal E2	-0.03	Little
	Serum FSH and Fecal FSH	**0.54**	**Strong**
	Serum LH and Fecal LH	-0.24	Little

small positive correlation (r= 0.21) between serum and fecal LH of 2nd trimester. On the other hand, a very weak correlation exists among these traits (p= 0.07 and -0.24 respectively) in 3rd trimester. A positive correlation between the levels of serum P_4 and fecal P_4 metabolites has been reported by Kornmatitsuk *et al.*, (2007) within individual (r=0.73-0.88, $p < 0.001$) and pooled data (r=0.78, $p < 0.001$). Moreover, a good correlation was also reported to exist between serum E_2 and urine E_2 in a earlier study by Monk *et al.*,(1975).

4. CONCLUSION

In this study the correlations between serum reproductive hormones and matching urinary and fecal hormonal metabolites was established. A very strong positive correlation exists between serum FSH and fecal FSH in 3rd trimester which possibly indicate a good marker of 3rd trimester using hormonal assay. The sample size was too small and it needs further intensive study on large scale to make up the gaps of the study.

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