ALTERATION IN BLOOD BIOCHEMICAL PROFILE IN PREGNANT AND NON-PREGNANT CROSSBRED COWS FOLLOWING EXOGENOUS SUPPLEMENTATION OF GNRH, HCG AND PROGESTERONE RELEASING INTRAVAGINAL DEVICES.

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ABSTRACT

The present study was designed to evaluate the effect of GnRH, hCG, Progesterone impregnated device on blood biochemical profile in crossbred cows. Repeat breeding crossbred cows (n=32), lactating and negative to white side test were randomly divided into four groups; Group-1(Control, n=8), Group-2(GnRH @ 10µg i.m, n=8), Group-3 (hCG@1500 IU i.m., n=8), Group-4(Progesterone impregnated device @958 mg, n=8). All the treatments were given on 5th day post breeding and in group-4 intravaginally implanted device was withdrawn on 9th day of estrous cycle. Blood samples were collected on day 0, 5, 10, 15, and day 20 of estrous cycle and plasma was separated for biochemical estimation like plasma glucose, cholesterol, total protein, albumin and globulin. There were significantly higher plasma glucose and cholesterol level in pregnant animals as compared to non-pregnant animals of all groups. Plasma glucose level had significantly increased between day 0 and day 5 of estrous cycle compared to control group of pregnant animals. There was no significant difference found in plasma cholesterol, total protein and globulin level in all the groups on day 0, 5, 10, 15 and of day 20th of estrous cycle. There was significantly ($P<0.05$) higher plasma albumin level on day estrus in all groups. The conception rate on day 60 in group-1, group-2, group-3 and group-4 was 37.5%, 50%, 75%, and 37.5%, respectively.It may be concluded that, exogenous supplementation of different hormones like GnRH, hCG and progesterone influenced the levels of glucose and cholesterol in blood, which possibly helped in the biosynthesis of progesterone by the luteal cells during early phases of embryonic development and improved conception rate in crossbred cattle.

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Key words: Blood biochemical profile, Crossbred cow, Pregnant, Non-pregnant.

INTRODUCTION

The reproductive efficiency of dairy cows is affected by a number of disorders like endometritis, anestrous and repeat breeding and causes great economic losses to dairy farmers (Dutta *et al.*, 1988). The basic causes of the reproductive problems in a herd are multiple which includes managemental, nutritional and pathological factors. Normal levels of blood biochemical constituents are of utmost importance for maintaining functional integrity of the reproductive system. Variations in certain blood constituents during different reproductive phases alter the function of genital organs. Nutrient deficiency may impair the enzymatic function and cellular metabolism of reproductive organs and induce reproductive disorders (Thavani *et al.*, 2012).

The present work was undertaken to compare the blood biochemical profile of pregnant and non pregnant cross bred cows.

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MATERIALS AND METHODS

This study was conducted on 32 repeat breeding crossbred cows at Instructional Dairy Farm, Nagla, G. B. Pant University of Agriculture and Technology, Pantnagar- 263 145, District Udham Singh Nagar (Uttarakhand) from January to April 2015. All animals were thoroughly examined per-rectally to rule out any anatomical defect of genitalia and ovarian abnormalities. They were kept under uniform feeding and managemental conditions. Cows included in the study were in lactational phase, negative to white side test and had more than two unsuccessful inseminations within the current lactation. Cows were inseminated twice (12 hrs interval) at normal estrus. White side negative animals were randomly divided into four groups (each group containing 8 animals). Group-1(Control, n=8), Group-2(Buserelin acetate @ 10µg i.m, n=8; Receptal VET, MSD-Animal health), Group-3 (hCG@1500 IU i.m., n=8, Chorulon®,MSD-Animal health), Group-4(Progesterone impregnated device @958 mg, n=8, TRIU-B, Virbac–Animal health, India). All the treatments were given on 5th day post breeding and in group-4 intravaginally implanted device was withdrawn on 9th day of estrous cycle post breeding. The plasma samples from the blood of all the animals were collected during hormonal treatment on 0, 5, 10, 15 & 20th days of estrous cycle. The blood was collected aseptically from jugular vein with the help of sterilized disposable syringe and 18G needles in Heparinized vials and for plasma glucose estimation 2ml blood was collected in vial containing sodium fluoride (@10mg/ml) as anticoagulant. After collection, the blood samples were brought to laboratory in thermocol box containing ice packs. Plasma was separated by centrifuging blood samples at 3000 rpm for 15 min. transferred into sterilized vials and stored at -20°C for plasma biochemical constituents like glucose, cholesterol, total protein, albumin and globulin estimation. Plasma glucose (mg/dl) level was estimated by GOD/POD method (Glucose xidase/ Peroxidase) with the help of span diagnostic kit. The plasma total protein and albumin in blood was determined by the method of Young (1997)using diagnostic kit manufactured by SPAN diagnostics Ltd., Surat, India. The plasma globulin content was arrived at by subtracting the value of albumin content from total protein content in plasma. Plasma cholesterol (mg/dl) level was measured by CHOD-PAP method. Pregnancy was confirmed through per rectal and ultrasonography on day 60 days post artificial insemination using rectal transducer of 5.0 MHzfrequency. The data were analyzed statistically using Analysis of Variance (ANOVA) (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

In the present study, the plasma glucose concentration (mg/dl) in pregnant animal of group-1, 2, 3 and 4 were significantly ($P<0.05$) higher as compared to non- pregnant animal of the same group on day 0, 5, 10, 15, and day 20 of estrous cycle. Parmer *et al.* (1986) reported higher level of glucose during the luteal phase.Higher blood glucose levels directly increased the progesterone production by increasing the pulse and mean concentration of LH or indirectly stimulate progesterone release during early luteal phase by increasing insulin level (Richards *et al.*, 1989).

The plasma cholesterol concentration (mg/dl) in pregnant animal of group-1, 2, 3 and 4 were significantly ($P<0.05$) higher as compared to non-pregnant animal of the same group on day 0, 5, 10, 15, and day 20 of estrous cycle. All values were within normal range of 65-220 mg/dl (Radostits *et al.*, 2007). Highshoe *et al.,* (1991) reported increased demand for cholesterol for the biosynthesis of progesterone, estrogen by the avascular granulosa cells under the influence of LH surge. Joe Arosh *et al.*, (1998) suggested the association of low levels of cholesterol with reduced levels of steroidogenesis. High incidence of repeat breeding and anestrus are associated with the deficiencies of cholesterol. Agarwal *et al.* (1982) also reported no cyclic change in the cholesterol levels on day 1, 13, and 16 of estrous cycle. However, Shah *et al.* (2003) observed non-significant differences in the mean levels of total cholesterol in postpartum fertile and infertile surti buffaloes.

Total protein level of pregnant and non-pregnant animals did not differ significantly in all groups.Low level of plasma total protein resulted in the deficiency of certain amino acids required for the biosynthesis of gonadotropins and gonadal hormones (Vohra *et al.*, 1995; Joe Arosh *et al.*, 1998) might cause reproductive hormonal disturbances in animals leading to inactive ovaries (Roberts, 1971).Rise of plasma total protein might be associated with high level of estrogenic activity. Chandrahar *et al.* (2003) and Das and Bisnoi (2005) reported non-significant difference in plasma total protein of cow.

There was no significant difference observed in plasma albumin levels (g/dl) between pregnant and non-pregnant animals of all groups on day 0, 5, 10, 15 and day 20 of estrous cycle. Khan *et al.* (2010) reported that high level of albumin in normally cyclic cows may be due to increased demand for amino acids and protein for the biosynthesis of GnRH and LH to initiate ovulation.Radostits *et al.* (2007) reported that the normal range of serum albumin in cattle was 2.1-3.6 g/dl. So, all the animals were having normal serum albumin level. It is generally accepted that albumin levels are positively related, with productive and reproductive performance (Rowlands *et al.*, 1977; Payne and Payne, 1987).

There were no significant differences observed in plasma globulin levels (g/dl) between pregnant and non-pregnant animal of all groups. Sharma *et al.*(1984) and Cetin *et al.* (2002) observed non-significant difference in plasma globulin of cows.

It may be concluded that, exogenous supplementation of different hormones like GnRH, hCG and progesterone influenced the levels of glucose and cholesterol in blood, which possibly helped in the biosynthesis of progesterone by the luteal cells during early phases of embryonic development and improved conception rate in crossbred cattle.

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TABLE 1: PLASMA GLUCOSE CONCENTRATION (MG/DL) OF DIFFERENT GROUPS ON DIFFERENT DAYS OF ESTROUS CYCLE/PREGNANCY

Group/ days	Group-1 (Control) (n=8)		Group-3 (n=8)		Group-4 (n=8)	
	Pregnant (n=3)	Non-pregnant (n=5)	Pregnant (n=6)	Non-pregnant (n=2)	Pregnant (n=3)	Non-pregnant (n=5)
Day 0	51.53*±2.26	38.44±1.44	53.76*±1.42	42.55±2.05	52.14*±1.18	40.50±1.30
Day 5	57.13*±1.91	43.10±0.95	59.73*±o.87	48.19±1.30	57.25*±1.50	45.33±0.69
Day 10	55.92*±1.61	42.59±0.90	56.82*±0.80	47.10±0.50	54.37*±1.21	44.26±0.50
Day 15	54.05*±1.15	40.85±0.81	55.92*±0.77	45.23±1.62	54.06*±1.09	42.96±0.73
Day 20	52.52*±1.34	39.35±1.06	54.21*±1.25	43.45±1.04	51.83*±1.63	41.56±1.21

Mean bearing * differ significantly between pregnant and non-pregnant animal of respective groups at 5% level

TABLE 2: PLASMA CHOLESTEROL CONCENTRATION (MG/DL) OF DIFFERENT GROUPS ON DIFFERENT DAYS OF ESTROUS CYCLE/PREGNANCY

Group/ days	Group-1 (Control) (n=8)		Group-2 (n=8)		Group-3 (n=8)		Group-4 (n=8)	
	Pregnant (n=3)	Non-pregnant (n=5)	Pregnant (n=4)	Non-pregnant (n=4)	Pregnant (n=6)	Non-pregnant (n=2)	Pregnant (n=3)	Non-pregnant (n=5)
Day 0	107.93*±3.65	94.62±3.48	110.74*±2.82	94.24±4.60	108.77*±1.95	98.52±2.51	112.16*±4.85	94.98±3.75
Day 5	109.66*±3.27	95.12±3.65	111.86*±2.75	95.79±5.03	110.85*±2.59	99.92±1.57	115.15*±5.54	96.67±4.52
Day 10	110.45*±3.52	99.51±1.22	113.80*±3.11	103.52±2.71	113.89*±3.12	101.20±0.80	117.23*±5.91	101.53±2.89
Day 15	112.60*±3.60	99.94±1.70	116.76*±3.20	105.24±3.33	116.34*±2.91	102.51±2.49	119.11*±4.49	102.51±3.23
Day 20	112.98*±4.85	95.61±3.00	118.50*±2.22	98.51±5.08	117.15*±2.80	98.14±0.84	118.50*±4.38	97.30±4.33

Mean bearing * differ significantly between pregnant and non-pregnant animal of respective groups at 5% level

TABLE 3: PLASMA TOTAL PROTEIN CONCENTRATION (G/DL) OF DIFFERENT GROUPS ON DIFFERENT DAYS OF ESTROUS CYCLE/PREGNANCY.

Group/ days	Group-1 (Control) (n=8)		Group-2 (n=8)		Group-3 (n=8)		Group-4 (n=8)	
	Pregnant (n=3)	Non-pregnant (n=5)	Pregnant (n=4)	Non-pregnant (n=4)	Pregnant (n=6)	Non-pregnant (n=2)	Pregnant (n=3)	Non-pregnant (n=5)
Day 0	6.74±0.27	6.30±0.14	7.02±1.61	6.39±0.21	6.78±0.19	6.14±0.34	6.98±0.290	6.20±0.11
Day 5	6.50±0.23	6.46±0.17	6.93±0.16	6.50±0.22	6.65±0.19	6.20±0.35	6.90±0.30	6.37±0.12
Day 10	6.31±0.14	6.26±0.15	6.77±0.20	6.27±0.28	6.49±0.20	6.03±0.42	6.72±0.264	6.18±0.135
Day 15	6.13±0.16	6.14±0.18	6.60±0.23	6.14±0.22	6.43±0.18	5.91±0.44	6.63±0.28	6.08±0.16
Day 20	6.24±0.20	6.03±0.21	6.60±0.21	6.04±0.24	6.36±0.17	5.83±0.48	6.50±0.26	5.99±0.18

No significant difference observed between pregnant and non-pregnant animal of respective groups.

TABLE 4: PLASMA ALBUMIN CONCENTRATION (G/DL) OF DIFFERENT GROUPS ON DIFFERENT DAYS OF ESTROUS CYCLE/PREGNANCY

Group/ days	Group-1 (Control) (n=8)		Group-2 (n=8)		Group-3 (n=8)		Group-4 (n=8)	
	Pregnant (n=3)	Non-pregnant (n=5)	Pregnant (n=4)	Non-pregnant (4)	Pregnant (n=6)	Non-pregnant (n=2)	Pregnant (n=3)	Non-pregnant (n=5)
Day 0	3.72±0.13	3.24±0.23	3.69±0.13	3.36±0.22	3.71±0.83	3.39±0.50	3.72±0.14	3.37±0.18
Day 5	3.50±0.12	3.08±0.20	3.55±0.10	3.23±0.18	3.56±0.58	3.22±0.37	3.50±0.12	3.17±0.14
Day 10	3.26±0.12	2.97±0.20	3.35±0.11	3.18±0.17	3.30±0.56	3.11±0.28	3.26±0.66	3.06±0.12
Day 15	3.09±0.66	2.88±0.20	3.15±0.60	3.10±0.22	3.14±0.29	3.05±0.30	3.12±0.26	3.01±0.13
Day 20	2.98±0.57	2.95±0.91	3.00±0.40	3.39±0.23	3.00±0.28	3.37±0.42	3.01±0.208	3.31±0.16

No significant difference observed between pregnant and non-pregnant animal of respective groups.

TABLE 5: PLASMA GLOBULIN CONCENTRATION (G/DL) OF DIFFERENT GROUPS ON DIFFERENT DAYS OF ESTROUS CYCLE/PREGNANCY

Group/ days	Group-1 (Control) (n=8)		Group-2 (n=8)		Group-3 (n=8)		Group-4 (n=8)	
	Pregnant (n=3)	Non-pregnant (n=5)	Pregnant (n=4)	Non-pregnant (n=4)	Pregnant (n=6)	Non-pregnant (n=2)	Pregnant (n=3)	Non-pregnant (n=5)
Day 0	3.02±0.16	3.05±0.33	3.32±0.14	3.03±0.39	3.06±0.20	2.75±0.15	3.25±0.23	2.83±0.26
Day 5	3.00±0.13	3.38±0.34	3.37±0.10	3.27±0.35	3.08±0.20	2.97±0.25	3.40±0.22	3.19±0.20
Day 10	3.05±0.17	3.28±0.31	3.41±0.19	3.08±0.39	3.19±0.23	2.91±0.13	3.46±0.198	3.11±0.19
Day 15	3.04±0.12	3.25±0.31	3.45±0.22	3.04±0.35	3.29±0.18	2.86±0.13	3.51±0.26	3.07±0.23
Day 20	3.26±0.14	3.07±0.24	3.60±0.24	2.65±0.40	3.36±0.17	2.46±0.59	3.49±0.27	2.68±0.31

No significant difference observed between pregnant and non-pregnant animal of respective groups.

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