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Evaluation of the effect of GnRH analogue, Progesterone and Tolfenamic acid on biochemical profiles and conception rate in repeat breeder crossbred cows

Sudhanshu Pratap Singh, Ankesh Kumar, Prakrutik Prafulchandra Bhavsar, Mukesh Sahu, Praveen Kumar and Sushil Kumar

Abstract
The present study was focused to study the effects of parenteral administrations of GnRH analogue-Buserelin acetate, Progesterone and Tolfenamic acid on biochemical parameters viz., serum glucose, serum cholesterol and serum total protein in repeat breeding animals. A total of 32 repeat-breeder crossbred cows were selected and divided in 4 equal groups (n=8). Group 1 was kept as positive control *i.e.* without any treatments, Group 2 animals were treated with Buserelin acetate, Group 3 animals were treated with Buserelin acetate + Progesterone and Group 4 animals were treated with inj. Buserelin acetate + Progesterone + Tolfenamic acid . Biochemical analysis was done by commercially available diagnostic kits. The mean blood glucose and serum protein concentration differed non- significantly without any changes in the normal range. The mean value of total serum cholesterol concentration on day 0 varies significantly ($p<0.05$) in group 2 & 4 compared to other groups. Moreover, on other days, cholesterol mean value was significantly ($p<0.05$) higher in group 4 as compared to other groups. Highest conception rate was observed in group 4 in which combinations of drugs were used.

Keywords: GnRH analogue, progesterone, Tolfenamic acid, conception rate, cholesterol

Introduction

Repeat breeding (RB) is a considerable problem in cattle breeding which leads to large economic losses due to increasing in the number of inseminations, increasing in the length of calving interval and moreover increasing in culling rates. A repeat breeder is generally defined as any cow that has not conceived after three or more services associated with true estrus [1].

The bovine conceptus produces interferon-tau (IFNτ) which prevents luteolysis over and above functional corpus luteum (CL) produces progesterone which is very much essential for supporting pregnancy [2]. There are two crucial periods of bovine pregnancy. The first period is first week after breeding and second period is from day 8 to 28, at which maternal recognition of pregnancy (MRP) takes place. Approximately 32% of total embryonic loss reported in this second period [3]. This leads the problem of repeat breeding.

Several factors like nutritional stress [4], heat stress [5], transportation stress [6] or other stress promotes secretion of prostaglandin F2α from the uterine endometrium. This can cause lysis of the functional corpus luteum and leads to early embryonic death [7]. The two cyclooxygenase (COX) enzymes convert arachidonic acid into prostaglandin-H2 which is further converted into PGF2α through the enzyme prostaglandin-F-synthase. The NSAIDs exhibits anti-inflammatory activity mainly by the inhibition of the cyclo-oxygenase (COX) enzyme, results in inhibition of prostaglandin synthesis [8]. Besides tolfenamic acid, several studies also evaluated the effects of NSAIDs of Flunixine meglumine [9, 10] and meloxicam [11, 12]. Whereas administration of tolfenamic (NSAIDs) acid has significantly improved embryo transfer rate and pup delivery in mice [13].

Administration of GnRH or GnRH analogue before artificial insemination induces preovulatory LH (luteinizing hormone) surge which controls ovulation, while post inseminations with supplementation of exogenous P4 support early embryonic development [11]. At days 3 to 5 post-ovulation, the embryos usually enter the uterus, undergoing genomic activation and increases in P4 concentration; therefore, this may be a physiologically important time in the cattle, so administration of a low dose of P4 on days 4, 5 and 6 of the estrous cycle increase the conception rate among repeat breeder cattle [14]. This extend the life span of the bovine corpus luteum (CL) so this is one of the strategies aimed at reducing embryo loss by inhibiting the PGF2α in the endometrium during the critical period [15, 16], Inhibition of PGF2α enhances the CL lifespan and avoiding detrimental and toxic effects of PGF2α on the embryo [15].

Glucose is an important biochemical factor which regulate and modulate the metabolic functioning of the cell at different levels, it also acts at hypothalamus-pituitary gonadal axis and affect the reproductive function in animals, moreover, it is essential to regulate the quality of oocytes [17, 18]. It was noted that glucose within the oocyte regulates meiotic maturation and the glucose transporter (GLUT) content increased in rat ovary following gonadotropin use [19, 20]. Thus, Glucose called as central nutrient in metabolism and must for reproductive tract function. Cholesterol is a precursor of reproductive hormone and also a biochemical factor which represents the condition of lipid metabolism and have a positive relationship with the health of the animals [21]. Demand for cholesterol for the biosynthesis of progesterone, estrogen by the avascular granulosa cells increases during the LH surge [22]. Low levels of cholesterol with reduced levels of steroidogenesis [23]. Although, the high incidences of repeat breeding and anestrus are associated with the deficiencies of cholesterol. Serum concentration of protein reflects the nature of the uterine environment which is one of the most important factors for embryo development [24]. The dynamic of uterine lumen exhibits a marked difference at the stages of the estrous cycle as a consequence of ovarian steroidal regulation of endometrial secretion. Intakes of high protein diet alter the pH and the concentrations of other ions in uterine secretions, but only during the luteal phase of estrous cycle [25]. A study report showed that if the higher amount of rumen degradable protein fed to heifers reduces fertility by altering the uterine pH [24].

Materials and Methods

Preparation of animals before commencement of treatment

The study was conducted on 32 apparently healthy, 3-8 years old repeat breeding crossbred cattle. All animals were dewormed with Fenbendazole @ 7.5 mg per kg body weight 60 days prior to the commencement of experiment and they were supplemented with 40 gm mineral mixture daily prior to experiment.

Grouping of animals and treatment

Selected animals were randomly divided into four groups (each group containing 8 animals). In group 1, animals were inseminated on spontaneous estrous without any treatment, in group 2, animals were treated with injection Buserelin acetate @ 20 µg IM at the time of artificial insemination (AI), in group 3, animal were treated with injection Buserelin acetate @ 20 µg IM at the time of AI followed by Injection P4 @100mg IM on days 4,5,6 after AI and in group 4, animals were treated with injection Buserelin acetate @ 20 µg IM at the time of AI, Injection P4 @100mg IM on days 4,5,6 and Inj. Tolfenamic acid @4 mg/kg body weight IM On days 16,17,18 after AI. All the animals were inseminated on spontaneous heat.

Blood collection and biochemical assay

Blood samples were collected from all the cows at day 0, 7, 16, 17, 18 of the oestrous cycle. Estrous day assumed as day '0'. After restraining of the cow 10 ml of blood was collected aseptically from jugular vein. 2ml blood sample transferred into fluoride vial for serum glucose estimation. 8 ml blood sample transferred into clot activator vial for separation of serum for estimation of total cholesterol, total protein and progesterone. Blood sampling done in clot activator vial was allowed to clot in slanting position for about one hour at room temperature. Then it transferred into a centrifuge tube with the help of micro pipette and centrifuge for 15 minutes at 3000 rpm. Blood sample of fluoride vial was also centrifuge for plasma separation. The serum and plasma samples transfer in eppendorf tube with the help of micropipette and kept at (-20) degree centigrade for further use.

Estimation of glucose, cholesterol and protein was carried out according to manufacturer's protocol. Glucose was estimated by Glucose Test Kit by GOD-POD method. Cholesterol was estimated by Cholesterol Test Kit by CHOD-PAP method and the plasma total protein in blood serum was determined by using diagnostic kit manufactured by Coral Clinical Systems.

Statistical analysis

Repeated Measures ANOVA was used to analysis the obtained result. The multiple comparisons between group, day and interaction for different parameters were done by using Tukey test at 5% level of significance. The analysis was done using JMP 9.0 software.

Results and Discussion

Total Blood Glucose

The mean blood glucose concentration was observed with non-significant difference among all groups (group 1, group 2, group 3 and group 4) on day '0', 7, 16, 17 and 18 (Table-1).

The mean blood concentration of glucose reported in the present study was nearly similar to that of [26–28]. Administration of GnRH and leads to significant differences in mean serum concentration of glucose, but the observations were found similar in the present study [29]. Flunixin meglumine at the dose rate of 1.1 mg/kg body weight in dogs for 5 days intravenously causes non-significant changes in the blood glucose concentration [30], though, there is an increased in blood glucose level after using dexamethasone and prednisolone in normal cows [31]. Supplementation of flaxseed to cow was observed to have higher value of glucose compared to present study and similar higher value was also observed in a study by [32, 33]. Some studies also showed the lower value with respect to present study [34, 35].

Circulating glucose concentration were lower in severe negative energy balance (SNEB) compared to mild negative energy balance (MNEB) in mares [36].

Glucose is also essential for gonads to maintain the quality of oocytes, development of embryo [17, 18]. There were no significant changes seen in blood serum glucose concentration and this might be due to no effects of GnRH on metabolic profile of animals. A study also showed the decreased in blood glucose concentration in cows with advancement of pregnancy in winters [37].

Table 1: Mean (± SE) blood glucose concentration (mg/dl) in different groups on 0, 7th, 16th, 17th and 18th day of estrous cycle in repeat breeder cows.

Group	Day 0	Day 7	Day 16	Day 17	Day 18
1	56.32 ± 0.84	56.13 ± 0.84	55.71 ± 0.94	56.02 ± 1.32	56.08 ± 1.34
2	54.01 ± 1.54	55.29 ±1.61	54.59 ± 1.65	55.11 ± 1.99	56.12 ± 2.01
3	52.53 ± 1.77	53.65 ± 2.28	53.37 ± 2.04	55.11 ± 2.20	54.94 ± 2.58
4	55.29 ± 1.21	56.05 ± 1.09	57.45 ± 1.24	57.74 ± 0.98	57.59 ± 1.31

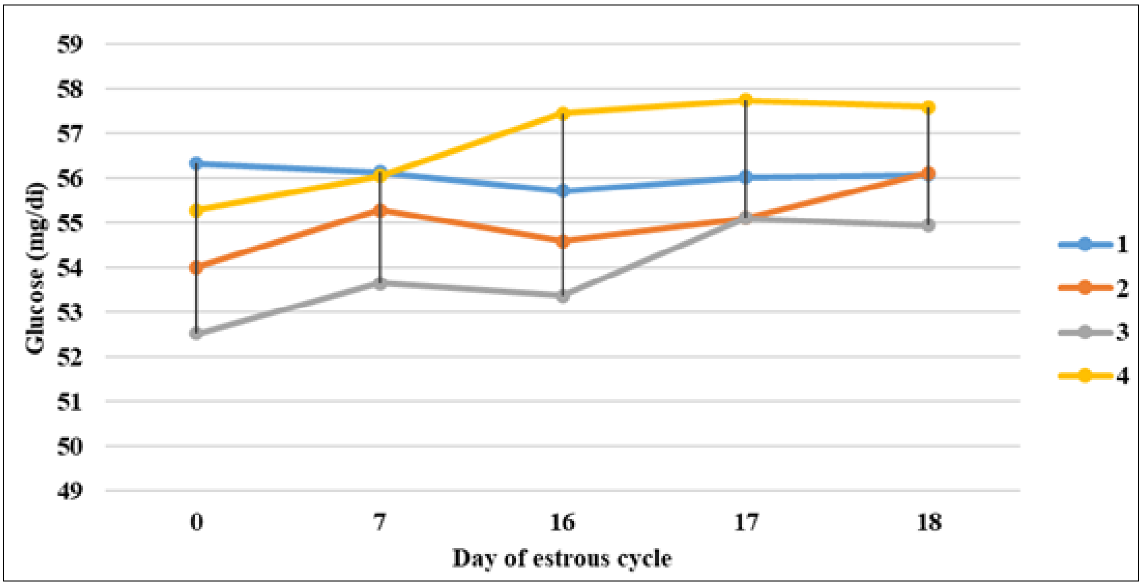

Fig 1: Mean (± SE) blood glucose concentration (mg/dl) in different groups on 0, 7th, 16th, 17th and 18th day of estrous cycle in repeat breeder cows.

Total Serum Cholesterol

The significant difference was observed in mean total serum cholesterol concentration between groups 4 and 3 as compared to group 1 and 2 on day '0'. There was observed significantly ($p<0.05$) higher level of serum cholesterol on days 0,7,16,17& 18 in group 4 compared to all three groups i.e. group 1, 2& 3 (Table-2).

The mean serum cholesterol concentration of treated animal found in the present experiment were in agreement with reports of [26, 32, 34, 37] but the mean serum cholesterol concentration of non-treated animal group was lower. However, lower concentration has been reported by [38] in buffaloes during different stages of estrous cycle.

The mean serum cholesterol concentration in the present experiment showed the higher values as compared to results reported by [39–41], in fertile (112.10±3.57) and repeat breeder cows (126.10±9.74) [33].

Effect of GnRH, hCG and progesterone impregnated device on blood biochemical profile in repeat breeder crossbred cows had the lower value of cholesterol compared to the present study [42].

All values observed in the present study were within normal range of 65-220 mg/dl [43]. Demand for cholesterol increases during the biosynthesis of progesterone, estrogen by the avascular granulosa cells under the influence of LH surge [44]. It is suggested that the low levels of cholesterol is related with reduced levels of steroidogenesis [23].

Increase in cholesterol level in serum causes greater availability of cholesterol in ovarian follicular fluid, and luteal tissue which has been associated with increase in E2 synthesis by the follicles result into better LH surge which finally helps in improving the embryo quality and similarly increase in P4 synthesis within CL [45].

Table 2: Mean (± SE) serum cholesterol concentration (mg/dl) in different groups on 0, 7th, 16th, 17th and 18th day of estrous cycle in repeat breeder cows

Group	Day 0	Day 7	Day 16	Day 17	Day18
1	148.22±1.99^{JK}	154.06±1.83^{HI}	159.11±2.00^{EFG}	160.71±2.85^{DEF}	160.69±2.85^{DEF}
2	150.69±1.68^{IJ}	155.07±2.01^{GHI}	158.95±2.20^{EFG}	161.53±2.22^{DE}	162.86±2.60^{CDE}
3	145.27±4.11^{K}	152.73±2.91HI^{J}	156.68±3.30^{FGH}	162.48±2.68^{CDE}	164.85±3.87^{BCD}
4	153.95±2.78^{HI}	162.21±3.11^{CDE}	166.55±3.22^{ABC}	168.55±3.28^{AB}	170.35±3.32^{A}

The values bearing the different superscripts across the row and column differ significantly from each other, P<0.05

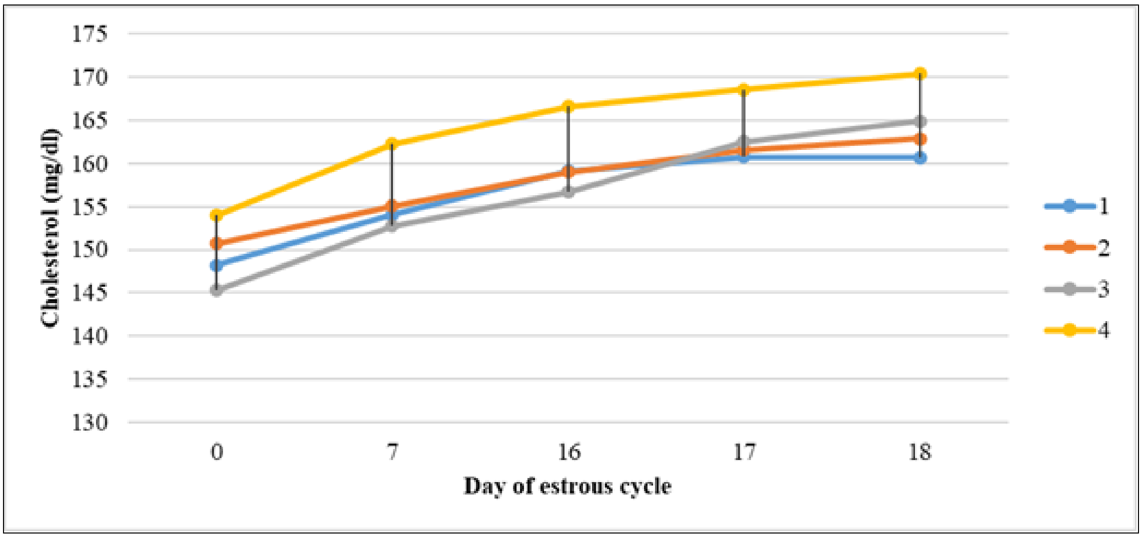

Fig 2: Mean serum cholesterol concentration (mg/dl) in different groups on 0, 7th, 16th, 17th and 18th day of estrous cycle in repeat breeder cows

3.3 Total Serum Protein

There have been non-significant differences observed among the entire treatment group on day 0, 7, 16, 17, &18 in the present study (Table-3).

The mean serum concentration of total blood protein was observed in the present study is 6-7 g/dl, which is comparable with the reports of other scientists [46]. The mean total serum protein concentration observed in the present study is at par with the reports of different studies [42, 47, 48]. On the contrary the values of mean total serum protein were lower than [35]. Similar lower values of mean total protein concentration during different stages of estrous cycle in buffaloes were also reported by [38]. The present finding is in agreement with report of in which they showed that the value of serum total protein concentration does not differ significantly following administration of Flunixin Meglumine by repeatedly at 12-hour interval for 12 days in crossbred goats [49]. Flunixin Meglumine at the dose rate of 1.1 mg/kg body weight in foal and observed decreased in protein level with non-significant difference [50]. Administration of Flunixin Meglumine at the dose rate of 1.1 mg/kg body weight in dogs for 5 days by intravenously and the observed serum protein concentrations were not consistent with the present findings [30]. The total protein level in pregnant and non-pregnant with non-significant difference in all the treatment groups on different days of estrus cycle i.e. 0, 5, 10, 15 and day 20 [29].

Use of Flunixin Meglumine there was no any direct effect on protein level [49]. The values of protein level were decreased if it is given repeatedly due to Gastro-enteropathy which causes loss of protein.

Our findings are also comparable to the findings, who also observed the non-significant difference in serum protein levels between stages of the estrous cycles in cows [34].

Whereas, the values of mean serum Protein concentration in fertile (7.09±0.27) and repeat breeder cow (7.92±0.31) were higher as compared to present study [33].

Table 3: Mean (± SE) serum protein concentration (mg/dl) in different groups on 0, 7th, 16th, 17th and 18th day of estrous cycle in repeat breeder cows.

Group	Day 0	Day 7	Day 16	Day 17	Day 18
1	6.25±0.06	6.39±0.08	6.40±0.23	6.05±0.21	6.34±0.15
2	6.26±0.07	6.37±0.06	6.38±0.05	6.49±0.06	6.52±0.05
3	6.32±0.03	6.44±0.10	6.56±0.15	6.90±0.32	6.73±0.17
4	6.30±0.03	6.34±0.05	6.32±0.05	6.34±0.05	6.40±0.05

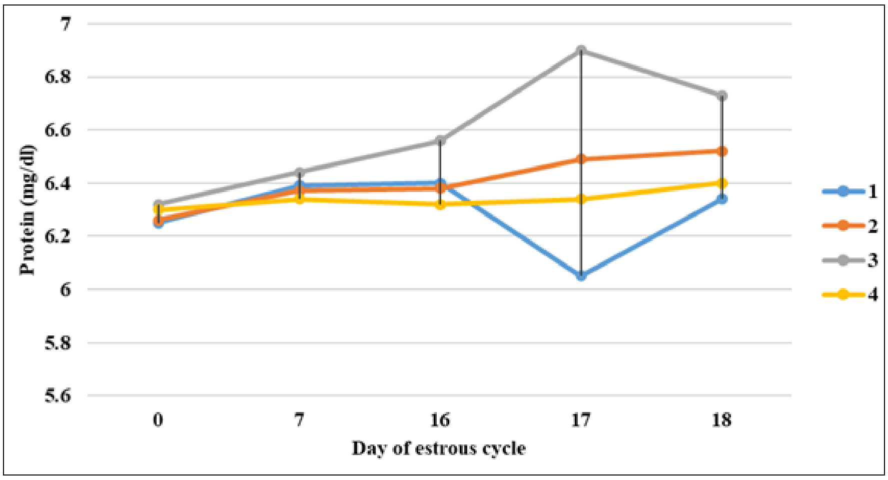

Fig 3: Mean serum protein concentration (mg/dl) in different groups on 0, 7th, 16th, 17th and 18th day of estrous cycle in repeat breeder cows.

Conception Rate
The conception rate was observed increased in the entire treated group with respect to non-treated group and the maximum conception was found in the group-4, where combinations of all treatment were given [51]. The combinations in group-4 were Buserelin acetate, Exogenous P_4 and Tolfenamic acid, these therapies help to maintain the P_4 level and significantly increases the conception rate by six times than control group, moreover, the level of cholesterol found high in pregnant animal with respect to non-pregnant shows that cholesterol level supports the pregnancy. The conception rate in groups 1, 2, 3 & 4, were 12.5%, 37.5%, 50%, 75% respectively.

Conclusion
In partial report of this study, conception rate had been reported increased in all the treated group with respect to non-treated group and the maximum conception was observed in the groups where combinations of all treatment were given as well as the level of cholesterol was also observed significantly higher in group 4 as compared to other groups. Moreover, the serum cholesterol level were also observed higher in pregnant animals with respect to non-pregnant animals. There are non-significant changes observed in glucose and protein level, but significantly increased in the level of serum cholesterol which acts as a precursor of steroid hormone following treatment might have supported the increased in conception rate.

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