

Original Research Article

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Evaluation of the Effect of GnRH Analogue, Progesterone and Tolfenamic Acid on Serum Progesterone Profile and Conception Rate in Repeat Breeding Crossbred Cattle

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ABSTRACT

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Secretion of prostaglandin $F_{2\alpha}$ at the time of maternal recognition of pregnancy leads to embryonic loss. Early embryonic losses are one of the important causes of repeat breeding which increases the calving interval and leads to economic losses to farmers. Non-steroidal anti-inflammatory drugs inhibit the synthesis of $PGF_{2\alpha}$. The present study was designed to study the effect of treatment by GnRH analogue - Buserelin acetate, Progesterone (P_4) and Tolfenamic acid on 32 repeat-breeding crossbred cattle aged 3-8 years. All the animals were randomly divided into 4 equal groups ($n=8$). Group 1 was taken as positive control i.e. inseminated on spontaneous estrous without any therapy. Group 2 animals were treated with injection Buserelin acetate @ 20 μ g intramuscular (IM) at the time of artificial insemination (AI). Group 3 animals were treated with inj. Buserelin acetate @ 20 μ g intramuscular at the time of AI followed by Injection P_4 @ 100mg IM on days 4, 5, 6 after AI. Group 4 animals were treated with inj. Buserelin acetate @ 20 μ g IM at the time of AI, inj. P_4 @ 100mg IM on days 4, 5, 6 and inj. Tolfenamic acid @ 4 mg/kg body weight IM on days 16, 17, 18 after AI. Blood samples were collected from all the cows at day 0, 7, 16, 17, 18 of the estrous cycle for the study of serum progesterone profile. The conception rate observed in groups 1, 2, 3 and 4 were 12.5%, 37.5%, 50% and 75% respectively. Highest conception was observed in group 4 in which, combinations of Buserelin acetate, Exogenous P_4 and Tolfenamic acid was administered. The study concluded that combination of Buserelin acetate, Exogenous P_4 and Tolfenamic acid therapy helps to maintain the P_4 level and significantly increases the conception rate by 6 times than control group.

Introduction

The bovine conceptus produces interferon-tau ($IFN\tau$) which prevents luteolysis. In addition to that functional corpus luteum (CL)

produces more progesterone which is essential to supports pregnancy (Spencer and Bazer, 2002). There are two crucial periods of bovine pregnancy. The first period is the first week of after breeding and second is day 8 to

28, when maternal recognition of pregnancy (MRP) takes place. Approximately 32% of total embryonic loss occurs in this period (Wiltbank *et al.*, 2016). This leads to a repeat of oestrous. Repeat breeding (RB) is a considerable problem in cattle breeding which leads to large economic losses due to more inseminations, increased calving interval and increased culling rates. Repeat breeding has been defined as the failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities (Bartlett *et al.*, 1986).

Several factors like nutritional stress (Bender *et al.*, 2014), heat stress (Sakatani, 2017), transportation stress (Merrill *et al.*, 2007) or other stress promotes secretion of prostaglandin $F_{2\alpha}$ from the uterine endometrium. This can cause lysis of the functional corpus luteum and leads to early embryonic death (Hockett *et al.*, 2004). The two cyclooxygenase (COX) enzymes convert arachidonic acid into prostaglandin-H₂ which is further converted into $PGF_{2\alpha}$ through the enzyme prostaglandin-F-synthase. The NSAIDs exhibits anti-inflammatory activity mainly based on the inhibition of the cyclooxygenase (COX) enzyme, results in inhibition of prostaglandin synthesis (Malm and Borisch, 2015).

Several studies evaluated the effects of NSAIDs like Flunixin meglumine (Kasimanickam *et al.*, 2019, 2018) and meloxicam (Amiridis *et al.*, 2009; McDougall *et al.*, 2016). Administration of the NSAID tolfenamic acid has significantly improved embryo transfer rate and pup delivery in mice (Schlapp *et al.*, 2015).

Administration of GnRH or GnRH analogue before artificial insemination induce preovulatory LH (luteinizing hormone) surge which controls ovulation, or post inseminations with supplementation of

exogenous P_4 to support early embryonic development (Amiridis *et al.*, 2009). At days 3 to 5 post-ovulation, the embryos usually enter the uterus, undergoing genomic activation and increases in P_4 concentration; therefore, this may be a physiologically important time in the cattle, so the administration of a low dose of P_4 on days 4, 5 and 6 of the oestrous cycle increase the conception rate among repeat breeder cattle (Ferguson *et al.*, 2012).

This extends the life span of the bovine corpus luteum (CL) so this is one of the strategies aimed at reducing embryo loss by inhibiting the $PGF_{2\alpha}$ in the endometrium during the critical period (Binelli *et al.*, 2001; Pugliesi *et al.*, 2011), Inhibition of $PGF_{2\alpha}$ enhances the CL lifespan and avoiding detrimental and toxic effects of $PGF_{2\alpha}$ on the embryo (Binelli *et al.*, 2001).

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 (Table 1).

Blood collection and hormonal assay

Assuming estrous day as day 0, blood samples was collected in clot activator from all the cows at day 0, 7,16,17,18 of the cycle to harvest for hormonal estimation (Table 1). Level of P₄ in blood was estimated by Radio-Immuno Assay (RIA) Progesterone assay kit (M/S Beckman Coulter, Brea, CA) catalogue no. IM 1188 as per the suggested protocol. In brief all reagents were brought to the room temperature (20-25°C) before assaying.

For estimation of serum progesterone, 50µl standard and 50µl serum sample were taken into antibody coated tubes. 500µl ¹²⁵I-labelled serum progesterone was added to each antibody-coated tube.

Two additional non-coated tubes were prepared for total activity computation containing 500µl tracer (¹²⁵I-labelled serum progesterone) and was set-aside until counting. The contents of the tubes were mixed with a vortex and incubated for 1 hr for serum progesterone estimation at room temperature, while continuously shaking (300-350 rpm).

The incubation mixture was carefully aspirated while aspirator tip touched the bottom of the antibody coated tube so that all the liquid was removed. The radioactivity was measured with SR-300 fully automatic gamma counter.

Statistical analysis of the effect of different treatments between the groups and within the group on different days and serum P₄ concentration in of repeat breeder cows was studied by repeated measures ANOVA. 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

Conception rate

The conception rates observed in the present work in all four groups were 12.5%, 37.5%, 50% and 75% respectively in group 1, group 2, group 3 and group 4. The highest conception rate was observed in group 4.

In a similar study by Amiridis *et al.*, (2009) reported highest conception rate of 33.76 % in repeat breeder cows which is lower conception rate than the present study. (Aguiar *et al.*, 2013) they reported conception rate of 66.70% with the use of meloxicam- a NASID and this is comparable with the present finding while they obtained 49% Conception rate in the control group which is higher than the present report. Archbald *et al.*, (1993) used GnRH at the time artificial insemination and reported the conception rate ranging from 33% - 40% this is comparable to the present finding (Fig. 2).

While using progesterone in a low dosage by (Ferguson *et al.*, 2012) in an experiment observed conception rate of 45% which slight lower than the present finding.

Highest conception rate in present study was observed in group 4 i.e. 75%, where treatment had been given with combinations of GnRH analogue (Busereline acetate), Exogenous progesterone and non-steroidal anti-

inflammatory drug (Tolfenamic acid). All the treatments were given at different days of estrus cycle when the chances of conception failure are more i.e. Buserelin acetate on day 0 when time of ovulation depend on concentration of LH which is govern by release of GnRH, then Progesterone injected on day 4, 5 and 6 when embryo travels from horn to uterus and Tolfenamic acid was given on day 16, 17 and 18 when MRP process are going on.

Least conception rate observed in group 1 in which no treatment was given and kept as a control. The combined administration of GnRH, Progesterone, and NSAID was effective in treatment of the repeat breeder cows.

Serum progesterone profile

In group 1

The mean total P4 concentration on day 0, 7, 16, 17 and 18 was 0.31 ± 0.06 , 2.31 ± 0.27 , 3.99 ± 0.23 , 4.63 ± 0.29 and 4.40 ± 0.31 ng/ml, respectively. The significant ($p<0.05$) differences were observed between days, significantly higher level were observed on days 16,17 and 18 compared to days 0 and 7.

In group 2

The mean total P4 concentration on day 0, 7, 16, 17 and 18 was 0.37 ± 0.06 , 2.47 ± 0.27 , 5.06 ± 0.47 , 6.37 ± 0.40 and 7.08 ± 0.52 ng/ml, respectively. The significant differences were observed between days, significantly ($p<0.05$) higher level were observed on days 17 and 18 compared to days 16, 7 and 0. The non-significant difference was observed among days 16, 17 and 18 (Fig. 1).

In group 3

The mean total P4 concentration on day 0, 7, 16, 17 and 18 was 0.42 ± 0.05 , 2.81 ± 0.23 ,

4.67 ± 0.46 , 5.69 ± 0.49 and 6.44 ± 0.58 ng/ml, respectively. The significant differences were observed between days, significantly ($p<0.05$) higher level were observed similarly as in previous group on days 17 and 18 compared to days 16, 7 and 0.

In group 4

The mean total P4 concentration on day 0, 7, 16, 17 and 18 was 0.44 ± 0.06 , 2.71 ± 0.22 , 5.14 ± 0.48 , 7.13 ± 0.46 and 8.41 ± 0.54 ng/ml, respectively. The significant differences were observed between days, significantly higher level were observed similarly as in previous group on days 17 and 18 compared to days 16, 7 and 0 (Table 2).

The significantly ($p<0.05$) highest level of progesterone was observed on day 18 in group 4 and group 2 among all the treatment groups and levels were 8.41 ± 0.54 and 7.08 ± 0.52 ng/ml respectively. The rising trend of serum progesterone was observed in all the treatment groups on days 0, 7, 16, 17 and 18 and significant differences ($p<0.05$) was observed on days 17 and 18 between groups.

On the day 17 and 18 of group 4, on the administration of tolfenamic acid the mean serum progesterone concentration was 7.13 ± 0.46 and 8.41 ± 0.54 ng/ml respectively which was significantly higher between the treatments which might have luteotropic effect on corpus luteum, leading to increase in serum progesterone concentration.

The similar observations were reported by (Jaroszewski *et al.*, 2009; Maithani, 2017; von Krueger and Heuwieser, 2010). The reason of this highest serum progesterone levels in group 4 of the present experiment was the combine administrations of GnRH analogue, Progesterone and Tolfenamic acid.

Table.1 Dose and dosage schedule of drugs in different treatment group

Cattle groups (N= 8 in each group)	Treatment	Day of treatment	Day and time of blood collection
Group-1 (Control)	No treatment	No treatment	At days 0, 7, 16, 17, 18 of estrous cycle
Group- 2	Inj.Buserelin Acetate @ 20 µg IM	At the time of AI	At days 0, 7, 16, 17, 18 of estrous cycle
Group -3	Inj.Buserelin Acetate @ 20µg IM	At the time of AI	At days 0, 7, 16, 17, 18 of estrous cycle
	Inj.Progesterone@100mg IM	On days 4,5,6 after AI	
Group -4	Inj.Buserelin acetate @ 20 µg IM	At the time of AI	At days 0, 7, 16, 17, 18 of estrous cycle
	Inj.Progesterone@100mg IM	On days 4,5,6 after AI	
	Inj.Tolfenamic acid @4 mg/kg b. wt. IM	On days 16,17,18 after AI	

Table.2 Mean (\pm SE) serum P₄ concentration (ng/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	0.31 \pm 0.06 ^I	2.31 \pm 0.27 ^H	3.99 \pm 0.23 ^{FG}	4.63 \pm 0.29 ^{EF}	4.40 \pm 0.31 ^{EF}
2	0.37 \pm 0.06 ^I	2.47 \pm 0.27 ^H	5.06 \pm 0.47 ^{DEF}	6.37 \pm 0.40 ^{BCD}	7.08 \pm 0.52 ^{AB}
3	0.42 \pm 0.05 ^I	2.81 \pm 0.23 ^{GH}	4.67 \pm 0.46 ^{EF}	5.69 \pm 0.49 ^{CDE}	6.44 \pm 0.58 ^{BC}
4	0.44 \pm 0.06 ^I	2.71 \pm 0.22 ^{GH}	5.14 \pm 0.48 ^{CDEF}	7.13 \pm 0.46 ^{AB}	8.41 \pm 0.54 ^A

The values bearing the different superscripts across the rows and columns differ significantly from each other, (P<0.05)

Table.3 Conception rate in different group of repeat breeder cows after treatment

Particulars	GROUP-1	GROUP-2	GROUP-3	GROUP-4
Total no. of animal	8	8	8	8
Pregnant animal	1	3	4	6
Conception rate	12.5%	37.5%	50%	75%

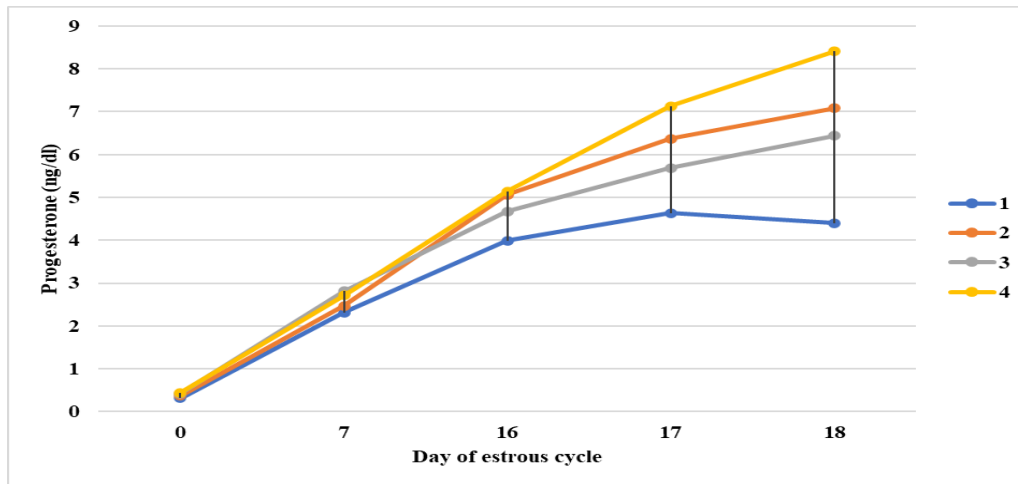


Figure.1 Mean serum P₄ concentration (ng/dl) in different groups on 0, 7th, 16th, 17th and 18th day of estrous cycle in repeat breeder cows

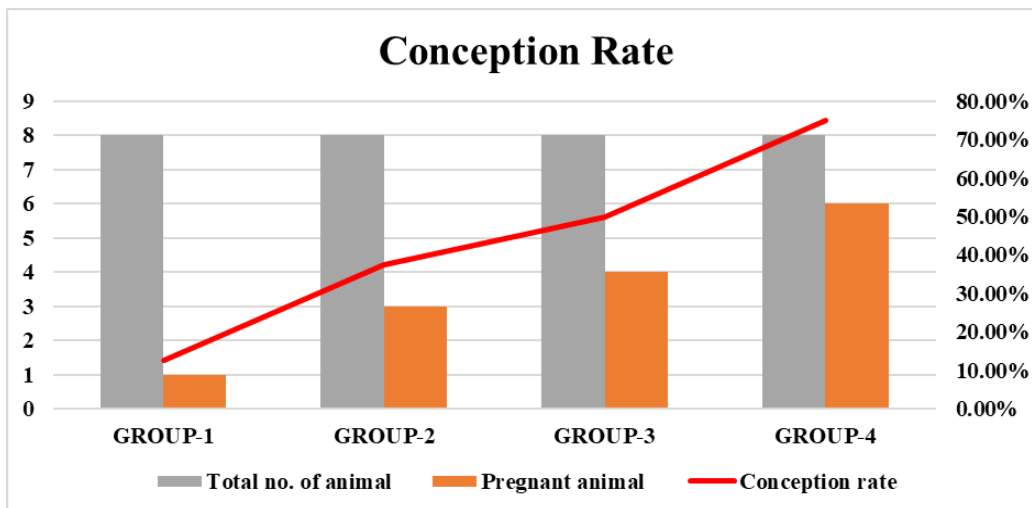


Figure.2 Conception rate in different group of repeat breeder cows after treatment

The increasing of serum progesterone was observed in all treatment groups simultaneously increasing in conception rate. Since, higher concentration of progesterone in early pregnancy directly related to embryonic growth rate and pregnancy. The significant increase in progesterone was observed in group 4 where combined administrations of Buserelin acetate, Progesterone and Tolfenamic acid were given. Hence, it is concluded that combination therapies of Buserelin acetate, Progesterone and Tolfenamic acid may be beneficial in the treatment of repeat breeding problems.

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