EFFICACY OF GnRH TREATMENT IN REPEAT BREEDER BUFFALOES

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CERTIFICATE

This is to certify that the thesis entitle **" *EFFICACY OF GnRH TREATMENT IN REPEAT BREEDER BUFFALOES"*** submitted by **Mr. CHIRANJEEVI B.R.,** I. D. No. **MVNK-1603** in partial fulfillment of the requirements for the award of **MASTER OF VETERINARY SCIENCE** in **VETERINARY GYNAECOLOGY AND OBSTETRICS** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar is a record of bonafide research work carried out by him during the period of his study in the university under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, association ship, fellowship or other similar titles.

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March, 2021

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AFFECTIONATELY

DEDICATED TO

MY FAMILY

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

AI	Artificial Insemination
CL	Corpus Luteum
CR	Conception Rate
et al.	Co-workers
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
i.e.	That is
LH	Luteinizing Hormone
ng	nanogram
ml	Millilitre
@	At the rate
ALP	Alkaline Phosphatase
ACL	Accessory Corpus Luteum
IU	International Units
Viz.	Which are
µg	Microgram
>	More than
<	Less than
USD	United States Dollar
%	Per cent
ELISA	Enzyme-Linked Immunosarbent Assay
birr	Ethiopian currency
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine

Introduction

I INTRODUCTION

Repeat breeding is a major problem adversely affecting the productive and reproductive performance in buffaloes. Repeat breeding results in delayed conception and increased calving interval, loss of milk production, reduction in calf crop, increased cost of treatment and culling of useful breeding animals leading to heavy economic losses to the dairy producers (Bhat and Bhattacharyya, 2012). The incidence of repeat breeding in India has been reported from 5.5 to 33.33% in cattle and 6 to 30% in buffaloes (Saxena, 2004). Jeyakumari *et al*. (2003) has revealed the annual losses incurred as a result of repeat breeding ranged between Rs.2,902.32/- and Rs.3,101.70/- per animal under Indian conditions.

The etiology of repeat breeding appears to be multifactorial and include uterine infections and reproductive tract abnormalities, hormonal dysfunction and nutritional inadequacies, and poor breeding and health management (Purohit, 2008). However, failure of fertilisation (39.7%) and early embryonic death (39.2%) are the two major groups responsible for repeat breeding (Tanabe and Casida, 1949).

In few studies, ovulatory disturbances such as anovulation and delayed ovulation have been recorded as the fertilization failure causing repeat breeding in buffalo (Singh *et al*., 2008 and Purohit, 2008). Lower luteal progesterone at day 5 and 25 appears to be crucial in initiating embryonic deaths. It appears that normal bubaline embryonic growth requires an early development of luteal progesterone and its proper maintenance till implantation period (day25) (Neglia *et al.*, 2009) and throughout the gestation.

Apart from hormones, there are many enzymes whose levels indicate a certain phenomenon or a physiological condition. There is a difference in the enzymatic profile of a normal cyclic and repeat breeders which was documented by researcher (Gandotra *et al.*, 1993). Physiologically increased levels of (Alkaline phosphatase) ALP are found in growth period and during pregnancy (Sato *et al.*, 2005). It was concluded by researcher that, serum ALP values vary in normal cyclic and repeat breeders and can be a diagnostic value and to direct the intervention.

White side test is performed on the cervico-vaginal mucus (CVM) for the detection of uterine infection in animals. White side test can be employed to differentiate subclinical cases of metritis from normal animals. Hence, the white side test is a simple, quick, reliable, accurate test and highly helpful for veterinarians in the field conditions to differentiate the normal healthy animal from subclinical and clinical cases of metritis (Mohankumar *et al*., 2006).

GnRH analogue administration on day of AI helps in higher conception rate by better synchrony of pre- ovulatory LH surge, ovulation and insemination. GnRH at estrus may potentiate conversion of small luteal cells to large luteal cells resulting into development of large sized functional corpus luteum (CL) required for embryo survival by enhancing progesterone secretion (Mandal *et al.*, 2004), 55% Conception rate (CR) (Rao, 2000), 90% CR (Sharma *et al.*, 2008) and administration in mid luteal phase improves pregnancy rates (Mandal *et al*, 2009; Zain and Nakao, 1996).

GnRH have luteotropic and luteoprotective effects, thereby enabling maternal recognition of pregnancy (Mac-Millan *et al*. 1986). It is thought that the chances of embryo

survival by improving luteal function and / or interfering with the luteolytic mechanism may increase by GnRH treatment (Beck *et al*., 1994; Birnie *et al*., 1997; Cam *et al*., 2002). Keeping this background, the present study was undertaken with following objectives: -

1. To study the efficacy of GnRH injections on different days in repeat breeder buffaloes.
2. To study the serum hormonal and biochemical parameters (progesterone and alkaline phosphatase) in repeat breeder buffaloes at different days.
3. To find out the economy of GnRH treatment.

Review of Literature

II REVIEW OF LITERATURE

The review of literature in respect of research topic entitled “Efficacy of GnRH treatment in repeat breeder buffaloes” are discussed under various sub headings as mentioned below.

2.1 White side test

Kavani *et al*. (1984) reported that subclinical endometritis in repeat breeder buffaloes was 10.92 percent.

Kanuya *et al*. (2000) recorded 7.5 % of subclinical endometritis in repeat breeder buffaloes.

Alwan *et al*. (2001) reported that endometritis and metritis may be resulted from inadequate hygienic conditions on postpartum period, during parturition, retained placenta and traumatic lacerations due to dystocia in Iraqi southern buffaloes

Mohankumar *et al*. (2006) found that 40.9, 13.3 and 45.7 % of the buffaloes were normal, clinical and sub clinical endometritis respectively and concluded that white side test effectively investigate repeat breeder syndrome associated with subclinical and clinical endometritis of buffaloes.

Raju *et al*. (2007) reported that 6.1 per cent of subclinical endometritis in repeat breeder buffaloes.

Bhalerao (2014) observed that 15.21, 36.95 and 8.69 % of mild, moderate and intense type of endometritis respectively in repeat breeder buffaloes.

Arjunrao (2017) attempted white side test in repeat breeder buffaloes, where 32, 24 and 4 cases showed mild, moderate and intense grade of endometritis respectively.

Vala *et al*. (2019) found that incidence of endometritis according to histopathological lesions in 50 uterine samples was 72 % (36 genitalia).

Venkateswarulu (2019) revealed that incidence of palpable abnormalities in the genital tract, subclinical endometritis and tubal blockage in 60, 66 and 4 cases of graded Murrah repeat breeder buffaloes was 46.15, 15.00, 50.78 and 3.07 % respectively.

2.2 Gonadotropin releasing hormone (GnRH)

Gonadotropin releasing hormone (GnRH) is a protein hormone produced by hypothalamus. It has the primary effect at pituitary gonadotropes to stimulate the pulsatile release of gonadotropins viz. luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Martinez *et al*., 2003). Mac-Millan *et al*. (1985) suggested that GnRH analogue has both luteotropic and luteoprotective effect which helps in maternal recognition of pregnancy. GnRH at estrus may potentiate conversion of small luteal cells to large luteal cells resulting into development of large sized functional CL required for embryo survival through enhanced progesterone secretion. Mann *et al*. (1995) reported that GnRH administered at mid-luteal phase suppressed the small pulses of PGF2α occurring from day 12 onwards and thus reduces the strength of luteolytic drive.

2.2.1 Efficacy of GnRH injections on day 0 in repeat breeder buffaloes

One of the major factors of repeat breeding in bovines may be delayed ovulation (Erb *et al*., 1976). Normally, ovulation occurs at 15-18 h after oestrus in Indian (Nagpuri)

buffaloes (Gordon, 1996). LH surge could be induced by treatment with GnRH (McDougall *et al*., 1995). The administration of GnRH around the insemination time aims to accelerate and ensure ovulation in buffaloes acting directly on the pituitary stimulating the secretion and release of gonadotrophins such as LH and FSH and promoting the pre-ovulatory LH peak. The enhanced fertilization rate by GnRH usage may be due to reduced variability in ovulation time relative to estrus or by promoting the retention, transport or survival of sperm in the female reproductive tract (Dodamani, 2000). Gonadotropin-releasing hormone (GnRH) increases the pregnancy rate of repeat breeders (Lee *et al*., 1983; Phatak *et al*., 1986; Stevenson *et al*.,1989; Jeffrey *et al*., 1990 and Peters, 2005).

Rao (2000) treated repeat breeding buffaloes with 20 µg GnRH before 8 hrs of insemination and recorded conception rate of 55 per cent compared to 15 per cent in control animals.

Ahmad *et al*. (2002) concluded that GnRH analogue improved the conception rate (42.80%) in Nili Ravi buffaloes when administered as a single dose at the time of AI rather than using in split doses compared to non-treated buffaloes (14.30%).

Samad *et al*. (2002) reported 53.33% conception rate when administered GnRH immediately after AI in repeat breeder buffaloes.

Mandal *et al*. (2004) found that first service conception rate of 50.00 % vs 37.50 % and overall conception rate 87.50 % vs 75.00 % in GnRH (2.5 ml Receptal) treated vs untreated repeat breeder buffaloes. The higher CR was possibly because of better synchrony of pre- ovulatory LH surge, ovulation and insemination. GnRH at estrus may

potentiate conversion of small luteal cells to large luteal cells resulting into development of large sized functional corpus luteum (CL) required for embryo survival by enhancing progesterone secretion.

Batavani and Eliasi (2004) demonstrated that single dose of gonadorelin significantly improved pregnancy rates in buffaloes when administered at the time of AI.

Saho and Nakao (2006) concluded that the major clinical features of repeat breeding buffaloes include a large proportion of heifers, a long interval from calving to treatment, a high incidence of cervicitis, and a high or moderate response to treatment with PGF2α and GnRH or vitamin/mineral mixture.

Markandeya and Patil (2008) revealed that, GnRH treatment in non-infectious cyclic non-breeder cases @ l05 mg intramuscularly during first phase of estrus is effective in 83.33% cows settled with 1.44 services/conception and in 81.81% buffaloes settled with 1.33 services/conception. GnRH treatment has significant effect on timely ovulation and improvement in conception rate in non-infectious cyclic non-breeder animals.

Sharma *et al*. (2008) reported that, the overall highest conception rate within 3 cycles was achieved with the use of GnRH at the time of AI (90%) over the untreated control repeat breeders (40%). Fertility response was better in the treatment with antibiotics and with hormone GnRH, suggesting the role of mild endometritis and endocrine imbalance in causing repeat breeding in buffaloes.

Mandal *et al*. (2009) reported that conception rate of GnRH administered in buffaloes on day of estrus was 87.50% compared to control group was 75.00%.

Dhami *et al*. (2009) revealed that, use of GnRH at the time of insemination definitely improved conception rate by 10-20% in repeat breeding buffaloes.

Ahmed *et al*. (2010) revealed that repeat breeder buffalo-cows responded to the treatments with mineral mixture, GnRH at the time of AI and Lugol's solution with recovery and conception rate of 63.64, 61.54 and 60.00 per cent, respectively.

Abdel-Khalek *et al*. (2012) showed that using PGF2α-PGF2α protocol as therapeutic treatment was more efficient in improving reproductive performance of repeat breeder buffalo heifers with the lowest cost as compared to GnRH treatment at service or GnRH-PGF2α-GnRH protocol.

Rao (2012) concluded that conception rates were significantly (<0.005) enhanced when GnRH administered on 8 hour before artificial insemination (60%) compared to control buffaloes (30%).

Lattoo *et al*. (2013) reported that effect of buserelin injection in buffaloes on pregnancy rates at the time of estrus(treated) was 7.10 (70%) compared to control (untreated) 6.10 (60%).

Savalia (2013) reported that overall conception rate of mid cycle- PG, AI+GnRH in repeat breeders and normal cyclic buffaloes was 70%, 50% and 60% respectively.

Chandra Prasad and Ananda Rao (2014) showed that conception rate of GnRH administration with 5ml (0.021mg) of Receptal (Intervet) on estrus day was 55.5% compared to control group 33.3% in repeat breeder buffaloes.

Al-Saied *et al*. (2015) revealed that pregnancy rate of PGF2α, GnRH, PGF2α+GnRH and PGF2α+Iodine+Oxytetracyclen treated groups were found to be 60.0%, 46.6 %, 71.8 % and 75.0 % respectively.

Pandey *et al*. (2016) concluded that buserelin acetate on the day of first AI leads to an increase in conception rate in buffalo.

Butani *et al*. (2016[a]) concluded that use of GnRH at the time of insemination and progesterone therapy on 4th or 5th day post-insemination definitely improves conception rate by 10-20% depending upon the cause in repeat breeding buffaloes.

Parmar *et al*. (2016) reported first service and overall conception rate of 3 cycles as 37.50 and 62.50 % in Mid-cycle PGF2α; 28.57 and 57.14 % in AI + GnRH; and 42.85 and 71.42 % in repeat breeding and normal cyclic buffaloes.

Abo-Farw *et al*. (2016) showed that conception rate (CR) of GnRH analogue (Receptal) administration at a level of 2.5ml/animal on estrus day was 60% compared to 40% in control groups of repeat breeder buffalo heifers.

Gautam *et al*. (2017) found that repeat breeder buffalo treated with buserelin acetate 20 µg i.m. at the time of breeding resulted in (66.7%) not return to estrus within one month of mating.

Ramlal (2017). concluded that higher conception can be achieved by use of low dose of GnRH with single insemination in both normal cyclic and non-infectious repeat breeder cows and buffaloes.

Tiwari *et al*. (2019) concluded that repeat breeding cattle and buffaloes respond quickly to GnRH along with mineral and vitamin supplementation.

Venkateswarlu (2019) reported that conception rates of repeat breeding Graded Murrah buffaloes injected with GnRH 8 hours before AI at observed estrus recorded to be 40.00 % compared to normal cyclic buffaloes 42.85 %.

Ramya *et al*. (2019) revealed that the conception rates in Group 1 – 10µg of GnRH 8 hours before AI at observed estrus, Group 2 – 10µg of GnRH 8 hours before AI at observed estrus and 13th day of post insemination, Group 3 – 1500 IU of hCG 8 hours before AI at observed estrus, Group 4 – 1500 IU of hCG 8 hours before AI at observed estrus and 13th day of post insemination and Group 5-untreated control were 25.00, 50.00, 62.50, 75.00 and 42.85 per cent respectively. The hCG treatment (Group 4) was more effective in overcome the repeat breeding problem in buffaloes when compared with the GnRH treatment. It was further, observed that hCG groups achieved higher (62.50 and 75.00%) conception rates when compared with that of GnRH treatment groups (25.00 and 50.00%) and control group (42.85%).

Hema (2020) recorded that conception rate in treated group (20µg of GnRH just before AI at observed estrus) was 33.33% compared to untreated control group 20.00 % in repeat breeder buffaloes.

2.2.2 Efficacy of GnRH injections on day 12 post AI in repeat breeder buffaloes

One of the causes of embryonic loss is thought to be the inadequate luteal function (Willard *et al*. 2003). The losses due to early embryonic (<25 days after fertilization) and

late embryonic (between days 25 and 45 of gestation) mortality can be as high as 20-44 and 8-17% respectively (Humblot, 2001). Abnormal corpus luteum (CL) function in early and mid-luteal phase of estrous cycle results in low production of progesterone (Bullman and Lamming, 1978) in peripheral circulation, which may cause early embryonic mortality. GnRH administration between days 11 and 14 post-insemination can boost the function of existing CL followed by an increase in plasma progesterone (Mac-Millan and Thatcher, 1991). This increase in plasma progesterone is due to luteotrophic response of small luteal cells to LH that is released in response to GnRH (Mac-Millan *et al*., 1985).

Treatment with GnRH or hCG during mid luteal phase improves the luteal functions through accessory corpus luteum (ACL) formation as well as by decreasing circulating estradiol (Kerbler *et al*, 1997; Mann and Lamming, 2001) also luteotropic and luteoprotective effects thereby enabling maternal recognition of pregnancy (Mac- Millan *et al*, 1986).

Mann *et al*. (1995) reported that GnRH administered at mid luteal phase suppressed the small pulses of PGF2 α occurring from day 12 onwards and thus reduces the strength of luteolytic drive. This resulted in prolonged life of CL resulting into secretion of sufficient progesterone required for pregnancy maintenance.

Zain and Nakao (1996) concluded that treatment with GnRH at day 14 following breeding improves the pregnancy rate of repeat breeder buffaloes. There was no effect when GnRH was given either at day of insemination or day 6-8 following breeding on pregnancy rate in repeat breeder buffaloes.

Rao (2000) observed conception rate of repeat breeding buffaloes treated with 20 µg GnRH during midluteal stage (between 11 and 14 days of post-AI) was 50 per cent compared to 15 per cent in control animals.

Batavani and Eliasi (2004) demonstrated that single dose of gonadorelin when administered during mid luteal phase after AI significantly improved pregnancy rate in buffaloes.

Vijayarajan *et al.* (2007) revealed that conception rate was significantly higher in GnRH treated group pre-AI (50 %) and post-AI (40 %) as compared to control (30 %) repeat breeder buffaloes.

Mandal *et al*. (2009) revealed that single GnRH administration at day 12 might be sufficient for improvement of conception in buffaloes.

Rao (2012) concluded that conception rates were significantly (<0.005) enhanced when GnRH administered on 12^{th} day (40%) compared to control buffaloes (30%).

Lattoo *et al*. (2013) revealed that positive impact of GnRH administration on progesterone profile during mid luteal phase of estrous cycle which could be used to improve fertility in buffaloes.

Pandey *et al*. (2013) concluded that administration of buserelin acetate or hCG on day 12 post ovulation has beneficial impact on conception rate in buffaloes through an improvement in the post treatment luteal profile as revealed by better development of spontaneous CL, formation of accessory CL and increase in plasma progesterone.

Chandra Prasad and Ananda Rao (2014) showed that administration of GnRH increased the conception rate and maximum conception rate when GnRH administered on 12th day after post insemination in repeat breeder buffaloes.

Abo-Farw *et al*. (2016) concluded that GnRH administration (2.5 ml Receptal) on day 12 of estrus/mating had positive effect on pregnancy rate of repeat breeder buffalo heifers as compared to those injected on day 0 and 10 of mating.

Abo-Farw *et al*. (2020) concluded that early embryonic mortality was associated with reduced circulating concentrations of P_4. The injection of 5 ml receptal (GnRH) on day 12 post-mating increased corpus luteum function, P_4 production, pregnancy rate, and farmer economy of lactating Egyptian buffaloes, in comparing with the injection with hCG (1500 IU/animal) on day 7 post mating and those in the control group.

2.2.3 Efficacy of GnRH injections on day 0 & 12 of post AI in repeat breeder buffaloes

Rao (2000) observed conception rate of repeat breeding buffaloes treated with 20 µg GnRH at 8 hrs before insemination and 20 µg GnRH during midluteal stage (between 11 and 14 days of post AI) was 70 per cent compared to 15 per cent in control animals.

Kumar (2010) showed that significantly (<0.005) enhanced conception rate of GnRH administered on 12 hour before artificial insemination and 12th day (60%) compared to control buffaloes (15%).

Rao (2012) concluded that conception rates were significantly (<0.005) enhanced when GnRH administered on 8 hour before artificial insemination and 12th day (50%) compared to control buffaloes (30%).

Zakiuddin (2013) concluded that GnRH therapy (GnRH on day of estrus + GnRH on day 12 post insemination) could be advocated inspite of higher cost owing to its other benefits in non-infectious repeat breeding buffaloes.

Anbhule (2018) observed that conception rate of GnRH administered on 5 and 12 day of insemination in non-infectious repeat breeder buffaloes was 66.66% compared to control group was 33.33%.

Venkateswarlu (2019) reported higher conception rate in repeat breeding Graded Murrah buffaloes which were injected GnRH 8 hours before AI at observed estrus and 13th day of post insemination 46.67 % compared to normal cyclical buffaloes (control group) 42.85 %.

Ramya *et al*. (2019) revealed that the conception rates in Group 1 – 10µg of GnRH 8 hours before AI at observed estrus , Group 2 – 10µg of GnRH 8 hours before AI at observed estrus and 13th day of post insemination, Group 3 – 1500 IU of hCG 8 hours before AI at observed estrus ,Group 4 – 1500 IU of hCG 8 hours before AI at observed estrus and 13th day of post insemination and Group 5-untreated control were 25.00, 50.00, 62.50, 75.00 and 42.85 per cent respectively. The hCG treatment (Group 4) was more effective in overcome the repeat breeding problem in buffaloes when compared with the GnRH treatment. It was further, observed that hCG groups achieved higher (62.50 and 75.00%) conception rates when compared with that of GnRH treatment groups (25.00 and 50.00%) and control group (42.85%).

Hema (2020) recorded that conception rate in treated group (20µg of GnRH just before AI at observed estrus and 15th day post insemination) was 55.55% while 20.00 % in untreated control group of repeat breeder buffaloes.

2.3 Hormonal studies

2.3.1 Levels of progesterone in repeat breeder buffaloes

Nanda *et al*. (1984) studied that higher levels of serum progesterone on the 20th or 23rd day following impregnation were indicative of establishment of pregnancy in buffaloes. Buffaloes with serum progesterone levels of 1 ng/ml or more on these days were taken as pregnant. The accuracy of this test for diagnosing pregnancy was 75% on the 20th and 83.3% on the 23rd day after artificial insemination.

Bugalia and Sharma, (1990) recorded non-significant serum progesterone levels between fertile (0.49 ± 0.07ng/ml) repeat breeder (0.35 ± 0.05ng/ml) buffaloes in late estrus.

El-belely (1993) reported characteristic temporal changes in concentrations of progesterone and those of plasma and uterine fluid concentrations of calcium, inorganic phosphorus, glucose and total protein, might all interact simultaneously to induce embryonic mortality in the repeat-breeder buffalo cows at around day 12 after mating.

Ahmad *et al*. (2002) reported that mean progesterone concentration in Group A (received GnRH analogue analogue at the time of insemination) was 0.95 ± 0.01 ng/ml, in Group B (received GnRH analogue at the time of insemination and 12 hours post insemination) was 0.94 ± 0.01 ng/ml and in Group C (received normal saline) was 0.93 ±

0.02 ng/ml on the day of estrus (day 0). On 10^{th} day of estrus cycle it was 1.16 ± 0.06 ng/ml in group A, 1.08 ± 0.05 ng/ml in group B and 1.07 ± 0.03 ng/ml in group C.

Dhabale and Sharma (2004) revealed that the non-significant variation in progesterone profile between normal (0.641 ± 0.35 ng/ ml) and repeat breeder (0.810 ± 0.39 ng/ ml) buffaloes during estrus day.

Venkatesan *et al*. (2005) concluded that difference in hormonal concentration of repeat breeders from regular breeders might be responsible for the reproductive disorders in cows and buffaloes.

Kavani *et al*. (2005) studied that progesterone levels were significantly higher ($P<0.05$) in fertile than the infertile cycles on day 14 (2.14 ± 0.21 vs 1.20 ± 0.19 ng/ml) and day 21 (2.57 ± 0.21 vs 0.42 ± 0.04 ng/ml), but not at oestrus (0.37 ± 0.02 vs 0.39 ±0.05 ng/ml) or day 7 (1.29 ±0.08 vs 1.12 ± 0.11 ng/ml) post breeding buffaloes.

Campanile *et al*. (2008) showed that treatment with a GnRH agonist on Day 5 following AI provides a strategy to increase progesterone secretion and the likelihood of pregnancy in buffaloes mated during periods of increasing daylight length.

Vijayarajan *et al*. (2009) found that level of progesterone concentration on day 0,8,16 and 24 post -AI in control and pregnant buffaloes was within the range of 0.20 to 0.38, 1.25 to 1.90, 3.05 to 3.95 and 3.80 to 3.90 ng/ml respectively.

Mandal *et al*. (2009) results showed that progesterone concentration increased significantly (P<0.05) on days 12,13 and 21 but non significantly on days 5 and 6 in buffaloes when GnRH administered at day of estrus than control.

Ahmed *et al*. (2010) results revealed that serum progesterone level was 1.44 ± 0.39 and 3.66 ±0.84 ng/ml in repeat breeder and normal buffaloes.

Kumar (2010) reported that progesterone profiles in conceived repeat breeding buffaloes following GnRH treatment on 0, 7 and 14 days were 0.212 ± 0.007, 1.630 ± 0.020, 3.149 ± 0.027 ng/ml compared to conceiving animals of control group 0.193 ± 0.009, 1.613 ± 0.012, 3.130 ± 0.035 ng/ml respectively.

Butani *et al*. (2011) revealed that the serum progesterone was significantly (P<0.01) higher in suboestrus (3.36 ±0.50 ng/ml) as compared to anoestrus, repeat breeder and normal cyclic buffaloes (1.24 ± 0.21, 0.45 ± 0.05 and 0.36 ± 0.08 ng/ml) respectively.

Pandey *et al*. (2011) concluded that diameter of pre-ovulatory follicle (POF) in buffaloes has positive impact on plasma estradiol concentration at estrus, post-ovulation luteal profile and conception rate. The diameter of CL can be used as an indicator of luteal function at early but not at mid or late luteal phase of estrus cycle in buffaloes.

Ergene (2012) concluded that application of PRID and GnRH injection after artificial insemination did not significantly improve pregnancy rates despite the fact that serum progesterone concentrations higher in the treatment groups.

Pandey *et al*. (2013) concluded that buserelin acetate and hCG administration on day 12 post-ovulation leads to accessory CL formation, improves luteal profile and consequently increases conception rate in buffaloes.

Lattoo *et al*. (2013) revealed that positive impact of GnRH administration on progesterone profile during mid luteal phase of estrous cycle which could be used to improve fertility in buffaloes.

Savalia *et al*. (2014) showed that CIDR was better than Ovsynch protocol in inducing fertile estrus in anestrus buffaloes while mid-cycle PG treatment was superior over AI ± GnRH in repeat breeders and all four treatment protocols significantly influenced plasma progesterone profile but not the protein or cholesterol.

Pandey *et al*. (2015) concluded that buserelin acetate or hCG administration on day 5 post-ovulation has a positive effect on luteal tissue and subsequently increases progesterone synthesis in buffalo. These tropic hormones have a beneficial impact on conception rate, however; study is warranted in a large number of buffaloes with the dose rate used in the present study. The pregnant buffalo had greater concentrations of progesterone than non-pregnant buffalo in the present study. The greater conception rate in treatment groups could be due to accessory corpus luteum formation in a greater proportion of buffalo.

Nakrani *et al*. (2015) reported that repeat breeding cows and buffaloes had significantly ($P<0.01$) higher plasma progesterone (4.73±0.37 and 4.43±0.43 ng/ml) at the time of PGF2α injection suggesting that they all had midcycle functional CL on the ovary.

Parmar *et al*. (2016) reported that mid-cycle PGF2α and AI ± GnRH protocols improved conception rate and plasma progesterone level in repeat breeding buffaloes though there was no significant influence on plasma protein and cholesterol profile.

Abo-Farw *et al*. (2016) indicated that GnRH analogue (Receptal) administration at a level of 2.5ml/animal on day 12 postmating improves pregnancy rate of repeat breeder buffalo heifers in comparison with day 0 or day 10 in term of positive effects on enhancing luteal function by elevating progesterone level.

Raj *et al*. (2016) analysed that mean values serum progesterone at AI and on day 10 in Graded Murrah buffaloes were 0.67 ± 0.12 ng/ml and 3.04 ± 0.06 ng/ml. serum progesterone concentration at the time of AI and pregnancy status was negatively correlated indicating that when progesterone level drops < 0.3 ng/ml (basal level) at the time of AI, the chances of the animal becoming pregnant were more.

Kumar and Purohit (2017) concluded that the hormonal therapies on day 5 post insemination increase the plasma progesterone concentration and conception rate in repeat breeding dairy cattle.

Deshpande (2017) studied that Group I (n=8), Group II (n=8) and Group III (n=8) were treated with Inj. GnRH (Buserelin acetate) @ 20 ug, Inj. hCG @ 1500 IU and Inj. hCG @ 3000 IU on day 7 after AI, respectively while Group IV buffaloes were untreated. Mean serum progesterone concentration of Group I, II, III and IV was 2.53 ± 0.26, 2.57±0.39, 3.77 ± 0.58 and 2.54±0.28 ng/ml, respectively on day 7 while 5.37 ± 0.91,

5.99±1.06, 7.34 ± 0.90 and 3.96±0.27 ng/ml, respectively on day 14 in repeat breeder buffaloes.

Roza *et al.* (2019) concluded that combination of GnRH and PGF2α gives a clear appearance of estrus, progesterone hormone levels and optimal buffalo blood profile.

Abo-Farw *et al*. (2020) concluded that early embryonic mortality was associated with reduced circulating concentrations of P_4. The injection of 5 ml receptal (GnRH) on day 12 post-mating increased corpus luteum function, P4 production, pregnancy rate, and farmer economy of lactating Egyptian buffaloes, in comparing with the injection with hCG (1500 IU/animal) on day 7 postmating and those in the control group. Treatment with GnRH may reduce the early embryonic loss by increasing diameter of corpus luteum to increase P_4 level in repeat breeder buffaloes during early post-mating period.

2.4 Biochemical studies

2.4.1 Levels of Alkaline phosphatase in repeat breeder buffaloes

Alkaline phosphatase is a zinc-containing dimeric enzyme, it plays an integral role in metabolism within the liver and in development within the skeleton (Tamas *et al*, 2002). The enzyme concentrations in the blood depend on various physiological status such as age, gender, blood type and pregnancy.

Derashree *et al*. (1984) reported that alkaline phosphatase was significantly higher in repeat breeder than normal cyclic buffaloes.

Sinha *et al*. (1986) revealed that the higher alkaline phosphatase activity observed in repeat breeder cattle during oestrogenic phase might indicate the possible early mobilization of glycogen there by resulting in depletion of energy stock in the endometrium and death of the embryo due to starvation during progestational phase.

Gandotra *et al*. (1993) reported that alkaline phosphatase value of repeat breeder buffalo found to be 80.8±16.5 (n mol phenol produced /min/ml) compared to normal cyclic animal 107.4±17.0 (n mol phenol produced /min/ml).

Eissa (1996) reported that mean alkaline phosphatase concentration in follicular fluid of buffalo cows during proestrus, oestrus, metestrus and diestrus were found to be 135.33 ± 7.51 IU/L, 142.62 ±8.31 IU/L, 131.54 ± 6.63 IU/L and 198.44 ± 11.53IU/L respectively.

Dhabale and Sharma (2002) concluded that the concentration of ALP and ACP was higher during estrus affecting fertility in repeat breeder animals.

Kavani *et al*. (2005) revealed that significantly higher alkaline phosphatase on all days post service in fertile than infertile cycles but did not vary between days /stages within the cycle in Surti buffaloes.

Talvelkar *et al*. (2008) indicated that alkaline phosphatase value in buffalo was higher during gestation than on the 'O'day. This increase could be attributed to the higher concentrations of circulating estrogen and hepatic lipidosis. The parathyroid over activity might play some part in higher ALP activity during pregnancy, which is related to maternal

mobilization of Ca from the skeleton. A reduction of ALP on the day of parturition may be ascribed to the withdrawal effect of progesterone and estrogen.

Tabatabaei and Mamoei (2011) reported that plasma concentration of alkaline phosphatase was higher ($P<0.05$) than in small and large follicles. ALP concentration difference between large follicles and plasma was not significant ($P>0.05$) in buffalo.

Khan *et al*. (2011) observed that greater alkaline phosphatase activity were found to be 27.5 ± 3.08 U/dl in acyclic compared to 14.0 ± 1.09 U/dl cyclic buffaloes.

Acar *et al*. (2013) recorded that mean concentration of alkaline phosphatase of Anatolian water buffalo in luteal phase and follicular phase were found to be 181.0 ± 51.97 U/L and 77.73 ± 17.26 U/L in serum compared to 263.60 ± 50.56 and 177.33 ± 20.95 in follicular fluid.

Abdulkareem (2013) reported Alkaline phosphatase level in Iraqi riverine buffaloes (300-310 days) around calving and 60 days post-partum (PP) period were 103.72 ± 11.19 and 85.88 ± 9.23 U/L respectively.

Abd Ellah *et al*. (2013) reported that mean serum alkaline phosphatase of late pregnant buffaloes (7 to 10 month) was 151.26 U/L and Reference interval was 71.80-327.91 U/L.

Neama (2015) indicated that the blood enzymes (AST, ALT and ALP) activities in Egyptian buffaloes were insignificantly higher during pregnant period than in lactation

period. The concentrations of plasma IGF-1, thyroid hormones and leptin were higher during pregnant period than in lactation period.

Yogesh (2015) concluded that means of two groups were statistically nonsignificant, repeat breeding buffalo showed marginally elevated levels of serum alkaline phosphatase activity through the oestrus cycle.

Butani *et al*. (2016[b]) recorded serum alkaline phosphatase, alanine aminotransferases and aspartate aminotransferases activities increased significantly ($P<0.01$) on T2 supplementation in repeat breeder buffaloes.

Chaurasia *et al*. (2016) concluded that serum alkaline phosphatase and acid phosphatase activities in anestrus group were significantly lower than normal cyclic and repeat breeders.

Aggarwal *et al*. (2016) suggested that ALP showed higher values in Tharparkar during different seasons but the magnitude of increase in ALP was during summer season among different breeds of cattle and Murrah buffaloes.

Patel *et al*. (2016) reported that serum concentrations of ALP in pregnant and non-pregnant lactating Banni buffaloes were found to be 171.26 ± 20.67 U/L and 158.39 ± 10.37 U/L respectively.

Anita (2018) showed alkaline phosphatase and aspartate aminotransferase activities significantly elevated in repeat breeding and decreased in anoestrum compared to normal cyclic Murrah buffaloes.

Chaudhary and Patel (2019) conducted trail for 90 days to evaluate the effect of supplementing appropriate mineral mixtures (AMMs) on their nutrient intake, blood profile, estrus occurrence, and conception, by using 20 anestrus and 20 repeat breeder buffaloes. These animals were randomly allotted to treatments viz., (Ionic mineral mixture) T1 and (T1 ± 25% extra zinc in the chelate form) T2. serum alkaline phosphatase decreased non-significantly whereas those of alanine and aspartate aminotransferases enzyme activities remained mostly unaltered.

2.5 Economy of GnRH treatment

Bartletta *et al*. (1986) reported that cost components associated with unsuccessful inseminations included costs of delayed conception, extra inseminations, extra veterinary service and losses due to culling. Lactations with repeat-breeder syndrome were associated with a loss of approximately $385. An estimated extra cost of $140 was associated with a second insemination, $279 with three inseminations, $429 with four inseminations and $612 with five inseminations.

Morgan and Lean (1993) showed that greatest profit is achieved by using GnRH in repeat breeder cows. Cost of repeat breeder treatment with GnRH was 3 $.

Shamsuddin *et al*. (2007) found that GnRH treatment on day of AI for 13 repeat breeder cow/heifers, conceived 10 animals having the treatment cost GnRH was 9.3$.

Zakiuddin (2013) found that GnRH treatment on day of estrus and GnRH on day of estrus + GnRH on day 12 post insemination having the cost of treatment as Rs. 162.25 and Rs. 324.50 per repeat breeder buffalo respectively.

Hailu *et al*. (2015) indicated that farmers would be benefitted if bought GnRH analogue (Buserelin acetate) for treatments of repeat breeding dairy cows rather than living with problem. The cost is effective and affordable to farmers to pay 25 to 50 birr (2.20-4.40 $) to the 10 / 20 µg of the hormone rather than losing 140 USD.

Biradar (2018) concluded that single GnRH treatment was most economical treatment of repeat breeder cattle, as one dose of GnRH (Rs.125/-) can save costs (Rs. 2500-2700/-) incurred on the repeated cycle.

Materials and Methods

III MATERIALS AND METHODS

The research study entitled “Efficacy of GnRH treatment in repeat breeder buffaloes” was carried out to study the efficacy of GnRH injections on different days, serum progesterone and alkaline phosphatase parameter on different days and economy of GnRH treatment.

3.1 Location of research work

The present study was carried out in 32 repeat breeder buffaloes presented to Veterinary Clinical Complex of Veterinary College Nandinagar, Veterinary Hospital APMC Bidar, Veterinary dispensaries and different dairy farms of Bidar district of Karnataka state.

3.2 Selection of animals for research

The repeat breeder buffaloes in the age group of 6-8 years with history of 2 to 3 calvings were selected.

3.3 Screening of animals for uterine infection

A total of 73 repeat breeder buffaloes presented for treatment were screened for uterine infection by gynaeco-clinical examination. The cervico-vaginal mucus collected aseptically by aspiration using a sterile plastic sheath was subjected to white side test, wherein 1ml of vaginal mucus discharge in a clean test tube was mixed with equal volume of 5% NaOH and heated upto boiling point. After cooling the intensity of colour changes were interpreted as follows (Pateria and Rawal,1990), (Plate 1).

Table 1 Interpretation of white side test.

Sl. No.	COLOUR	INTERPRETATION
1	Cloudy or no colour	Negative
2	Light yellow	Mild infection
3	Yellow colour	Moderate infection
4	Dark or deep yellow	Severe infection

3.4 Experimental design

Out of 73 repeat breeder buffaloes, 41 were positive for white side test and were not included in the study. However, 32 repeat breeder buffaloes which were negative divided equally into four groups randomly and assigned to different treatments as below:

Group I Inj. Buserelin acetate (Gynarich, Intas Pharmaceuticals, Ahmedabad, India) 2.5 mL was injected intramuscularly immediately after artificial insemination (AI) to buffaloes.

Group II Inj. Buserelin acetate 2.5 mL was injected intramuscularly on 12^{th} day after AI to buffaloes.

Group III Inj. Buserelin acetate was injected twice one after AI and second on 12^{th} day after AI @ 2.5 ml to buffaloes.

Group IV **(Control group)** Inseminated without any hormonal injections when buffaloes presented at estrous period.

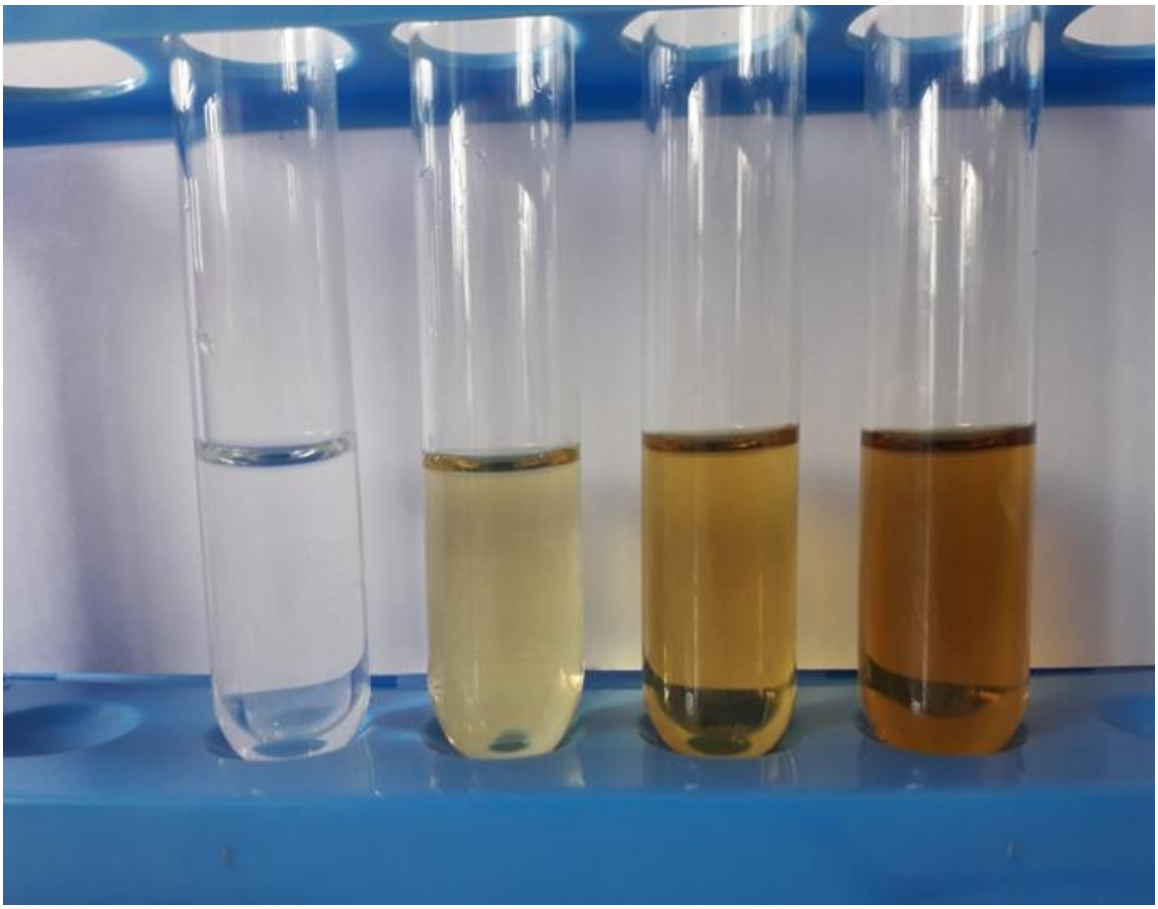


Plate 1. White side test for screening of sub-clinical endometritis (from left to right)

Note: Negative (no colour change), mild (light yellow), moderate (yellow) and severe (deep yellow)

3.5 Artificial insemination

All the buffaloes were inseminated with thawed semen at 37°C for 30 seconds by following aseptic measures by recto-vaginal method with semen being deposited in mid cervix.

3.5.1 Follow up

All the buffaloes were inseminated in the subsequent 1st and 2nd estrus periods if repeated after treatment.

3.6 Blood sample collection

Blood was collected from the jugular vein of all buffaloes on 0th (day of insemination), 12th, 20th and 40th day post insemination in sterilized vacutainer with coagulant. The serum was separated by centrifugation (3000 rpm for 20 min) and collected in sterile vials and preserved at -20°C until analysis.

3.7 Pregnancy diagnosis

Pregnancy of buffaloes was diagnosed by estimating serum progesterone on day 40.

3.7.1 Hormonal studies

Serum progesterone concentrations of experimental buffaloes were measured by enzyme-linked immunosorbent assay (ELISA) using CALBIOTECH progesterone ELISA kit as described by Radwanska *et al*. (1978).

3.7.2 Principle of the test

The samples and progesterone enzyme conjugate were added to the wells coated with anti-progesterone monoclonal antibody. Progesterone in the test serum sample was allowed to compete with a progesterone enzyme conjugate for binding sites. Unbound progesterone and progesterone enzyme conjugate was washed by washing buffer. Upon the addition of the substrate, the intensity of colour was inversely proportional to the concentration of progesterone in the samples. A standard curve was prepared relating colour intensity to the concentration of the progesterone.

3.7.3 Reagents preparation

1. *Working reagent A*: Progesterone - enzyme conjugate solution: - Progesterone enzyme conjugate was diluted 1:21 with assay diluent in a suitable container. A slight excess of solution was made.
2. *Wash buffer*:1x wash buffer was prepared by adding the contents of the bottle (25ml, 20X) to 475ml of distilled or deionized water and was stored at room temperature.

3.7.4 Assay procedure

Prior to assay, all reagents were brought to room temperature and mixed gently before use, (Plate 2).

1. The desired number of coated strips were placed into the holder.
2. 20 µl of progesterone standards, control and test serum samples were pippeted.
3. 100 µl of progesterone enzyme conjugate was added to all wells.

Plate 2. From top to bottom: Microtips, Micropipettes, Solution holding boats, ELISA plate and ELISA reagents for ELISA

4. Incubated for 60 minutes at room temperature (18-26°C).
5. Liquid was removed from all wells. Wells were washed thrice with 300mL of 1x wash buffer and blotted on absorbent paper towels.
6. 100 µl of TMB substrate was added to all wells.
7. Incubated for 15 minutes at room temperature.
8. 50 µl of stop solution was added to all wells. The plates were shaken gently to mix the solution, (Plate 3).
9. Absorbance was read on ELISA reader at 450 nm within 15 minutes after adding the stop solution, (Plate 4).

3.7.5 Calculation of results

The standard curve was constructed as follows:

1. Progesterone standard value was checked on each standard vial and the standard curve was constructed by plotting the absorbance of progesterone standards on vertical axis versus concentrations on horizontal axis on a linear graph paper.
2. The absorbance for controls and each unknown sample was recorded from the curve.

3.8 Biochemical studies

Serum alkaline phosphatase was estimated by the IFCC method using the commercially available kit from Transasia Bio-medicals Ltd. duly following the procedure as prescribed by Bessey *et al*. (1946), with a semi-automatic biochemical analyzer (Microlab-300, Elite Tech Ltd).

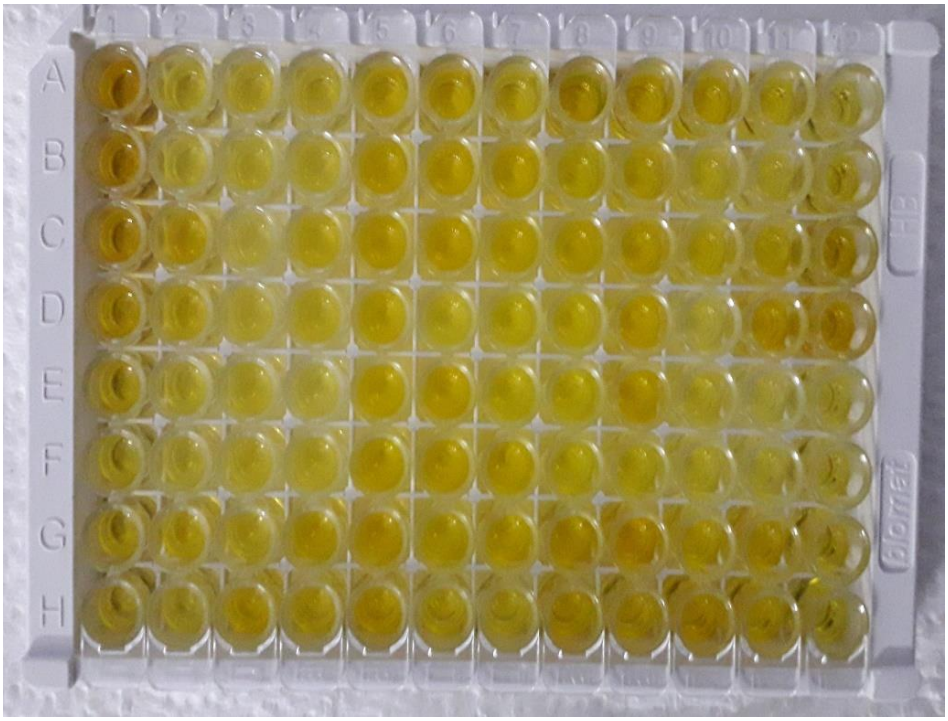


Plate 3. ELISA plate showing colour change from blue to yellow after adding stopping solution

Plate 4. ELISA Reader (Biotek) for estimation of serum progesterone

3.8.1 Principle of assay

The method was according to IFCC recommendation. This method utilised 4-nitrophenyl phosphate as the substrate. Under the optimised conditions ALP present in the sample catalysed the following reaction.

$$\text{AMP} + \text{4-NPP} + H_2O \xrightarrow[\boldsymbol{Mg}^{2+}/\text{Alkaline Ph}]{\text{ALP}} \text{4-nitrophenol} + \text{phosphate}$$

The values are expressed as IU/L.

3.8.2 Procedure of test

1. 1000 µl of reagent solution was pippeted into a test tube and 20 µl of serum sample was added to it.
2. This sample and reagent mixture was aspirated into the automatic analyser.
3. The sample was analyzed at 405 or 415 nm wavelength by kinetics method and readings are given in IU, (Plate 5).

3.8.3 Calculations of result

The calculation of the concentration of ALP was made from the following formula.

$$\text{ALP activity (IU/L)} = \text{Abs/min.} \times 2764$$

Where, Abs/min is the absorption of the solution at 405 nm wavelength per minute and 2764 is the factor.

3.9 Calculation of economy of GnRH treatment

Cost of treatment was calculated based on the price of Buserelin acetate (GnRH) vial. The cost of Busereline acetate (GnRH) (Gynarich) was Rs 660.00 per 10 ml vial.

3.10 Statistical analysis

The data was analyzed by standard statistical procedures as described by Snedecor and Cochron (1994).

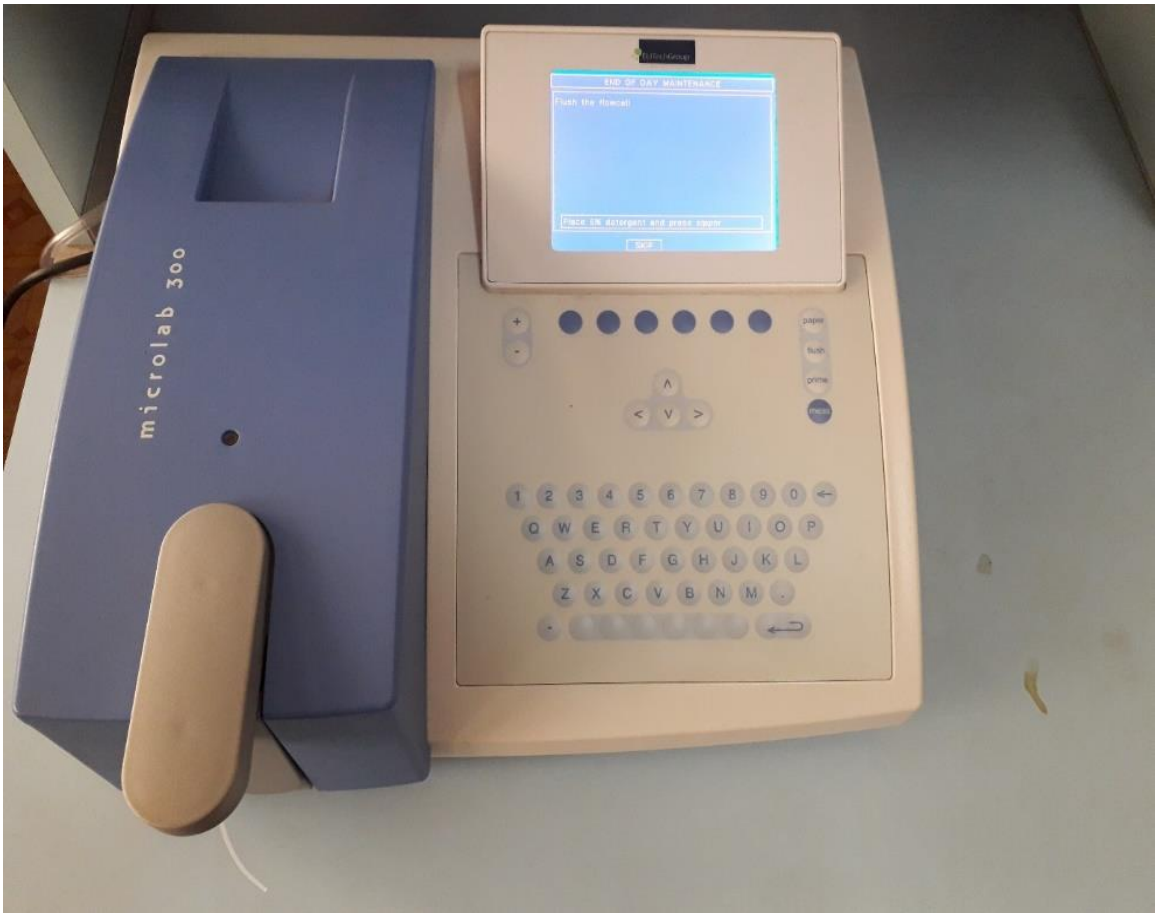


Plate 5. Biochemical analyser (Microlab 300) for estimation of Serum alkaline phosphatase

Results

IV RESULTS

The results of research work entitled “Efficacy of GnRH treatment in repeat breeder buffaloes” are mentioned as below.

4.1 White side Test

Out of 73 repeat breeder buffaloes, 41 (56.17%) were positive and 32 (43/73%) negative for white side test (Table 2).

4.2 Conception rate

4.2.1 Group –I (AI + GnRH)

Out of 8 repeat breeder buffaloes, 3 were conceived in first and 2 in second estrous cycle with 62.50% overall conception rate (Table 3).

4.2.2 Group -II (AI + GnRH on day12)

Out of 8 repeat breeder buffaloes, 2 were conceived in first and 1 in third estrous cycle with 37.50% overall conception rate (Table 3).

4.2.3 Group - III (AI + GnRH on day 0 and 12)

Out of 8 repeat breeder buffaloes 3 were conceived in first and 1 in second estrous cycle with 50.00% overall conception rate (Table 3).

4.2.4 Group IV (Control)

Out of 8 repeat breeder buffaloes, 1 was conceived in first and another 1 was conceived in third estrous cycle with 25.00% overall conception rate (Table 3).

Table 2. White side test for screening of uterine infection in repeat breeder buffaloes

Sl. No.	No. of repeat breeder buffaloes screened	No. of repeat breeder buffaloes found positive	No. of repeat breeder buffaloes found negative
01	73	41 (56.17%)	32 (43.83%)

Figure 1. White side test for screening of uterine infection in repeat breeder buffaloes

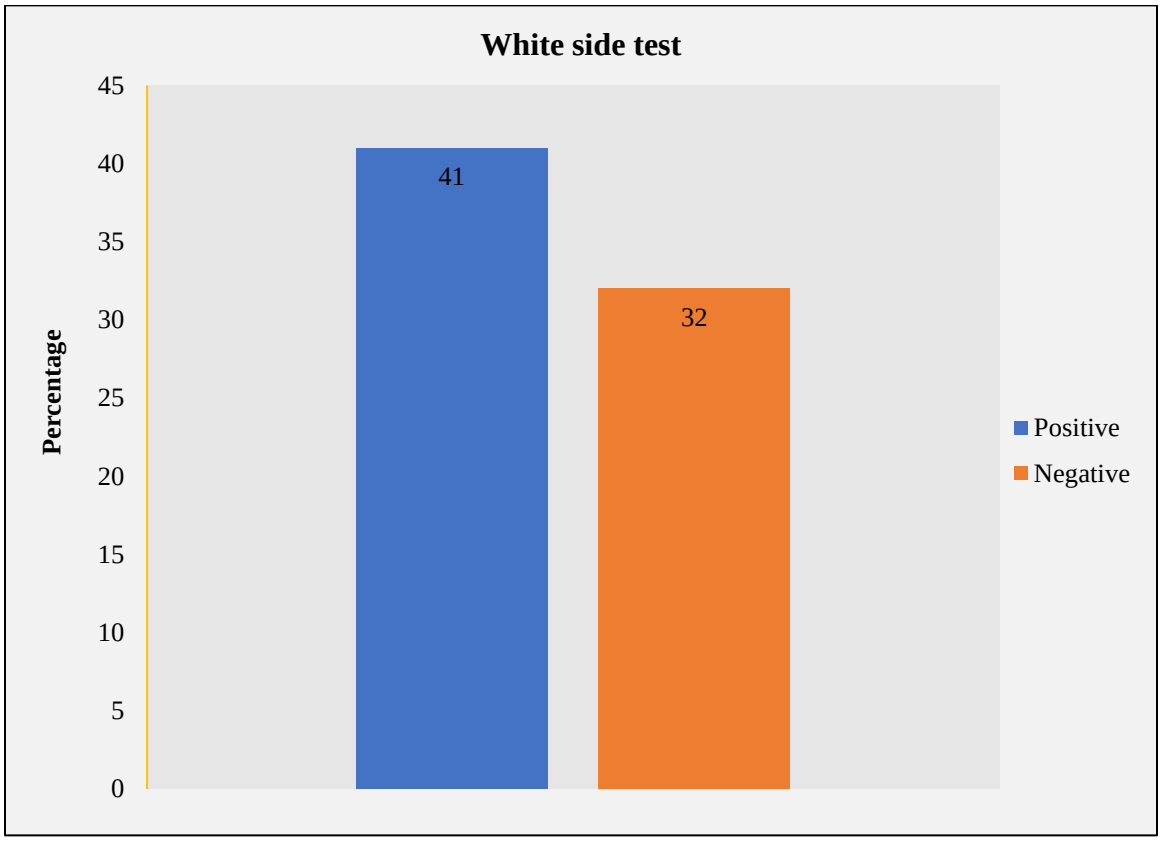
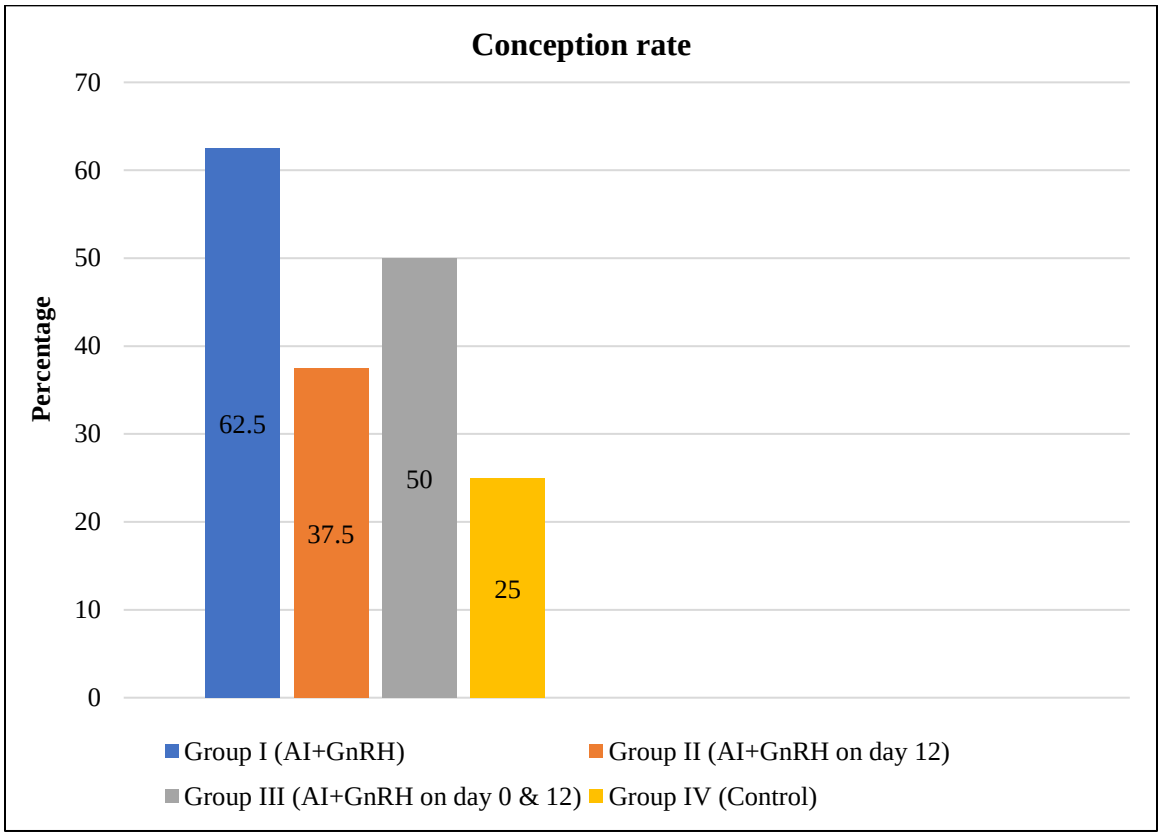


Table 3. Conception rate of repeat breeder buffaloes in different treatment groups

Sl. No.	Treatment groups	No. of repeat breeder buffaloes treated	No. of repeat breeder buffaloes conceived in oestrus cycle			Total no. of repeat breeder buffaloes conceived	Overall conception rate
			I	II	III		
1	Group-I AI + GnRH	08	3	2	-	5/8	62.50
2	Group-II AI + GnRH on day 12	08	2	-	1	3/8	37.50
3	Group -III AI + GnRH on day 0 and 12	08	3	1	-	4/8	50.00
4	Group -IV Control	08	1	-	1	2/8	25.00

Figure 2. Conception rate of repeat breeder buffaloes in different treatment groups



4.3 Overall conception rate

Higher conception rate of 62.50% was noticed in repeat breeder buffaloes of Group I (AI+GnRH) followed by 50.00% in Group III (AI + GnRH on day O and 12), and 37.50% in Group II (AI + GnRH on day 12). Lower conception rate of 25.00% was noticed in control group of repeat breeder buffaloes (Table3).

The conception of repeat breeder buffaloes were confirmed by per rectal examination on day 60 in treated and untreated control groups.

Artificial insemination plus GnRH was more effective in treating repeat breeding buffaloes than AI + GnRH on day 0 & 12 and AI + GnRH on day 12 protocols.

4.4 Comparison of progesterone levels in treated and control repeat breeder buffaloes

The progesterone levels (ng /mL) in treated and control repeat breeder buffaloes are depicted in Table 4.

4.4.1 Group – I (AI + GnRH)

The progesterone levels (ng/mL) were lower (0.60 ± 0.08) on day 0 and then increased to 5.19 ± 0.58 on day 12 and reduced to 4.95 ±1.05 on day 20 and again increased to 6.44 ± 1.45 on day 40 in repeat breeder buffaloes.

4.4.2 Group - II (AI + GnRH on day 12)

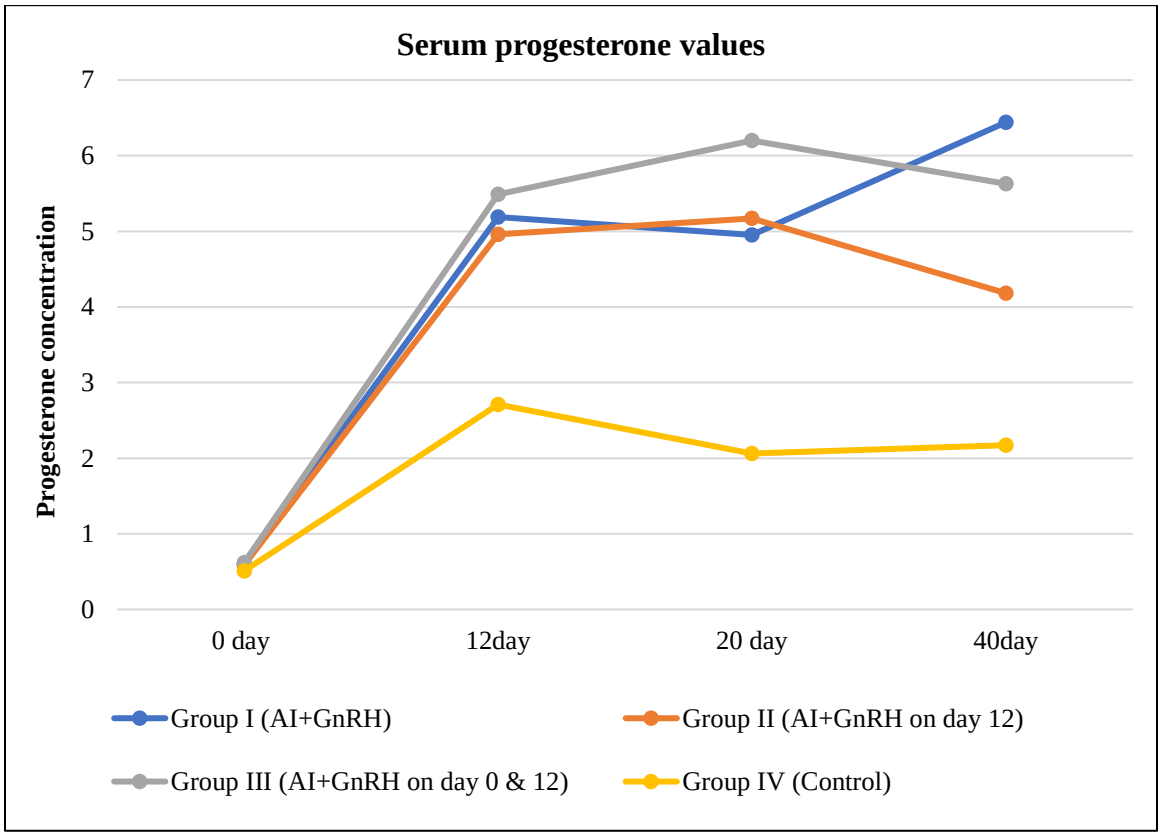
The progesterone levels (ng/mL) were higher (5.17 ± 0.86) on day 20 followed by 4.96 ± 0.24 on day 12, then 4.18 ± 1.52 on day 40 and 0.59 ± 0.08 on day 0 in repeat breeder buffaloes.

Table 4. Comparative analysis of serum progesterone(ng/ml) values between treated and untreated control repeat breeder buffaloes

Group	0th day	12th day	20th day	40th day
Group -I (n=08) AI + GnRH	0.60 ± 0.08^{aA}	5.19 ± 0.58^{bB}	4.95 ± 1.05^{bB}	6.44 ± 1.45^{bA}
Group -II (n=08) AI + GnRH on day 12	0.59 ± 0.08^{aA}	4.96 ± 0.24^{bB}	5.17 ± 0.86^{bB}	4.18 ± 1.52^{bA}
Group -III (n=08) AI + GnRH on day 0 and 12	0.62 ± 0.07^{aA}	5.49 ± 0.68^{bB}	6.20 ± 1.24^{bB}	5.63 ± 1.69^{bA}
Group -IV (n=08) Control	0.51 ± 0.08^{aA}	2.71 ± 0.21^{bA}	2.06 ± 0.56^{bA}	2.17 ± 0.80^{bA}

Means bearing different superscripts differed significantly ($P<0.05$) within the groups (a, b) and between the groups (A, B).

Figure 3. Comparative values of serum progesterone(ng/ml) between treated and untreated control repeat breeder buffaloes



4.4.3 Group - III (AI + GnRH) on day 0 and 12)

The progesterone levels (ng/mL) in repeat breeder buffaloes were 0.62 ± 0.07 on day 0, 5.49 ± 0.68 on day 12, 6.20 ± 1.24 on day 20 and 5.63 ± 1.69 on day 40 respectively.

4.4.4 Group - IV (control)

The progesterone levels were higher 2.71 ± 0.21 on day 12 followed by 2.17 ± 0.80 on day 40 then 2.06 ± 0.56 on day 20 and 0.51 ± 0.08 on day 0 in repeat breeder buffaloes.

The repeat breeder buffaloes in control group, showed significantly low serum progesterone concentration on day 12 and 20 post-AI when compared to three treatment groups. Further, the progesterone levels were also significantly higher on days 12^{th}, 20^{th} and 40^{th} post AI compared to day 0 within the groups.

4.5 Progesterone levels (ng/mL) on different days in pregnant and non-pregnant repeat breeder buffaloes

The progesterone (ng/ml) values in pregnant and non-pregnant between treated and control repeat breeder buffaloes were presented in Table 5.

The serum progesterone concentration on 12th, 20th or 40th days following treatment with GnRH on day 0 or day 12 or on both days along with insemination was higher in pregnant ($P < 0.05$) and non-pregnant ($P > 0.05$) animals compared to control animals. Further progesterone was also higher in pregnant animals compared to non-pregnant animals in all the groups except on day 0 and 12 in the control group.

Table 5. Comparative analysis of serum progesterone(ng/ml) values in pregnant and non-pregnant between treated and untreated control repeat breeder buffaloes

Groups	0th		12th		20th		40th	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant
Group I (n=08) AI + GnRH	0.53 ± 0.11^{X}	0.72 ± 0.13^{X}	5.84 ± 0.70^{BY}	4.11 ± 0.75^{Y}	7.10 ± 0.26^{aBY}	1.38 ± 0.22^{bX}	9.40 ± 0.23^{aBZ}	1.51 ± 0.35^{bX}
Group II (n=08) AI + GnRH on day12	0.52 ± 0.08^{X}	0.63 ± 0.12^{X}	5.66 ± 0.27^{aBY}	4.55 ± 0.18^{bZ}	8.04 ± 0.58^{aBYZ}	3.46 ± 0.12^{bY}	9.23 ± 1.05^{aBZ}	1.15 ± 0.17^{bX}
Group III (n=08) AI + GnRH on day 0 and 12	0.53 ± 0.11^{X}	0.72 ± 0.08^{X}	6.62 ± 0.52^{BY}	4.37 ± 1.04^{Y}	8.44 ± 0.67^{BYZ}	3.96 ± 1.86^{XY}	10.10 ± 0.33^{aBZ}	1.17 ± 0.32^{bXY}
Group IV (n=08) Control	0.38 ± 0.10^{X}	0.56 ± 0.11^{X}	2.67 ± 0.55^{AY}	2.73 ± 0.26^{Y}	4.56 ± 0.32^{aAYZ}	1.22 ± 0.20^{bX}	5.81 ± 0.36^{aAZ}	0.96 ± 0.12^{bX}

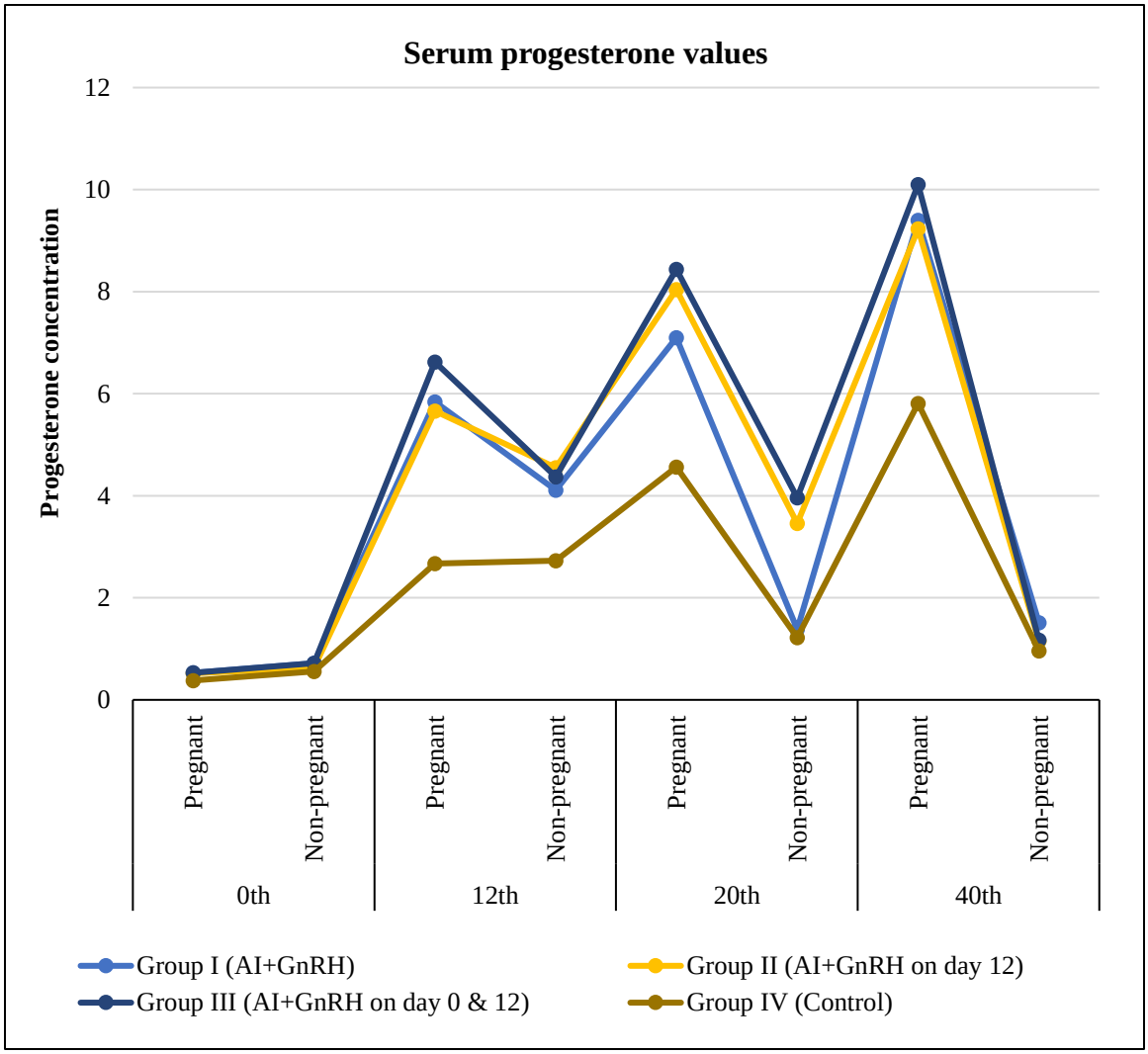
Means bearing different superscripts differed significantly ($P<0.05$).

[a, b] Within the groups, within the days between the pregnant and non-pregnant animals.

[A, B] Within pregnant or non-pregnant animals, within the days between the groups.

[XYZ] Within the groups, within pregnant or non-pregnant animals, between the days.

Figure 4. Comparative values of serum progesterone(ng/ml) in pregnant and non-pregnant between treated and untreated control repeat breeder buffaloes



4.6 Alkaline phosphatase values (IU/L) in treated and control repeat breeder buffaloes

The values of alkaline phosphatase in treated and control repeat breeder buffaloes are depicted in Table 6.

4.6.1 Group - I (AI + GnRH)

The alkaline phosphatase values (IU/L) were 67.75 ± 5.88 on day 0 and then decreased to 49.87 ± 4.11 on day 12 then increased to 65.62 ± 2.83 to 156.50 ± 32.17 on day 20 and 40 respectively in repeat breeder buffaloes Table 6.

4.6.2 Group - II (AI + GnRH)

The alkaline phosphatase values (IU/L) were higher (130.12 ± 36.82) on day 40 followed by 67.62 ± 3.40 on day 20, then 62.37 ± 4.61 on day 0 and 50.75 ± 5.37 on day 12 in repeat breeder buffaloes Table 6.

4.6.3 Group - III (AI + GnRH)

The alkaline phosphatase values (IU/L) in repeat breeder buffaloes were 68.25 ± 4.39 on day 0, 53.12 ± 4.44 on day 12, 67.50 ± 5.98 on day 20 and 141.62 ± 35.91 on day 40 respectively Table 6.

4.6.4 Group -IV (control)

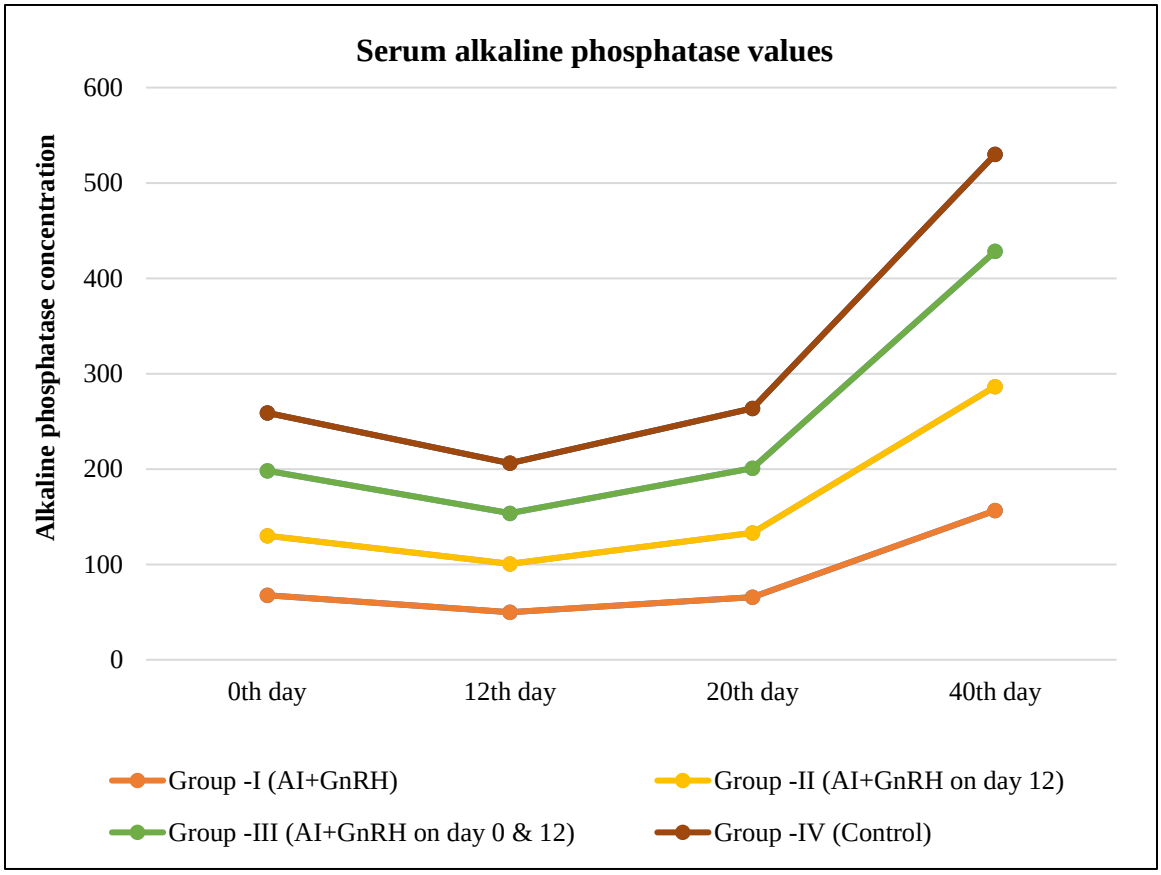
The alkaline phosphatase values (IU/L) were higher 101.75 ± 32.94 on day 40 followed by 62.87 ± 5.56 on day 20 then 60.50 ± 4.71 on day 0 and 52.37 ± 2.26 on day 12 in repeat breeder buffaloes Table 6.

Table 6. Comparative analysis of serum alkaline phosphatase (IU/L) values between treated and untreated control repeat breeder buffaloes

Group	0th day	12th day	20th day	40th day
Group -I (n=08) AI + GnRH	67.75 ± 5.88[a]	49.87 ± 4.11 [a]	65.62 ± 2.83 [a]	156.50 ± 32.17[b]
Group -II (n=08) AI + GnRH on day 12	62.37 ± 4.61 [a]	50.75 ± 5.37 [a]	67.62 ± 3.40 [a]	130.12 ± 36.82[b]
Group -III (n=08) AI + GnRH on day 0 and 12	68.25 ± 4.39 [a]	53.12 ± 4.44 [a]	67.50 ± 5.98 [a]	141.62 ± 35.91[b]
Group -IV (n=08) Control	60.50 ± 4.71 [a]	52.37 ± 2.26 [a]	62.87 ± 5.56 [a]	101.75 ± 32.94[a]

Means bearing different superscripts differed significantly (P<0.05) within the groups (a, b)

Figure 5. Comparative values of serum alkaline phosphatase (IU/L) between treated and untreated control repeat breeder buffaloes



Serum alkaline phosphatase concentration showed non significant (P>0.05) variation either between the groups or within the groups except on day 40 post-AI in treatment groups, which showed significant (P<0.05) increase compared to previous days.

4.7 Serum alkaline phosphatase (IU/L) values on various days in pregnant and non-pregnant repeat breeder buffaloes

The serum alkaline phosphatase (IU/L) values on various days in pregnant and non-pregnant repeat breeder buffaloes are shown in Table 7.

The serum alkaline phosphatase values did not show any significant changes either between the pregnant and non-pregnant animals or between the groups within pregnant or non-pregnant animals on day 0, 12 and 20. However, on day 40, the alkaline phosphatase values were significantly increased in pregnant animals compared to non-pregnant animals in all the groups.

4.8 Economy of GnRH treatment

The cost of treatment based on price of GnRH in Group I (AI + GnRH) and Group II (AI+ GnRH on day 12) was Rs. 165.00 whereas in Group III (AI + GnRH on day 0 & day 12) was 330.00.

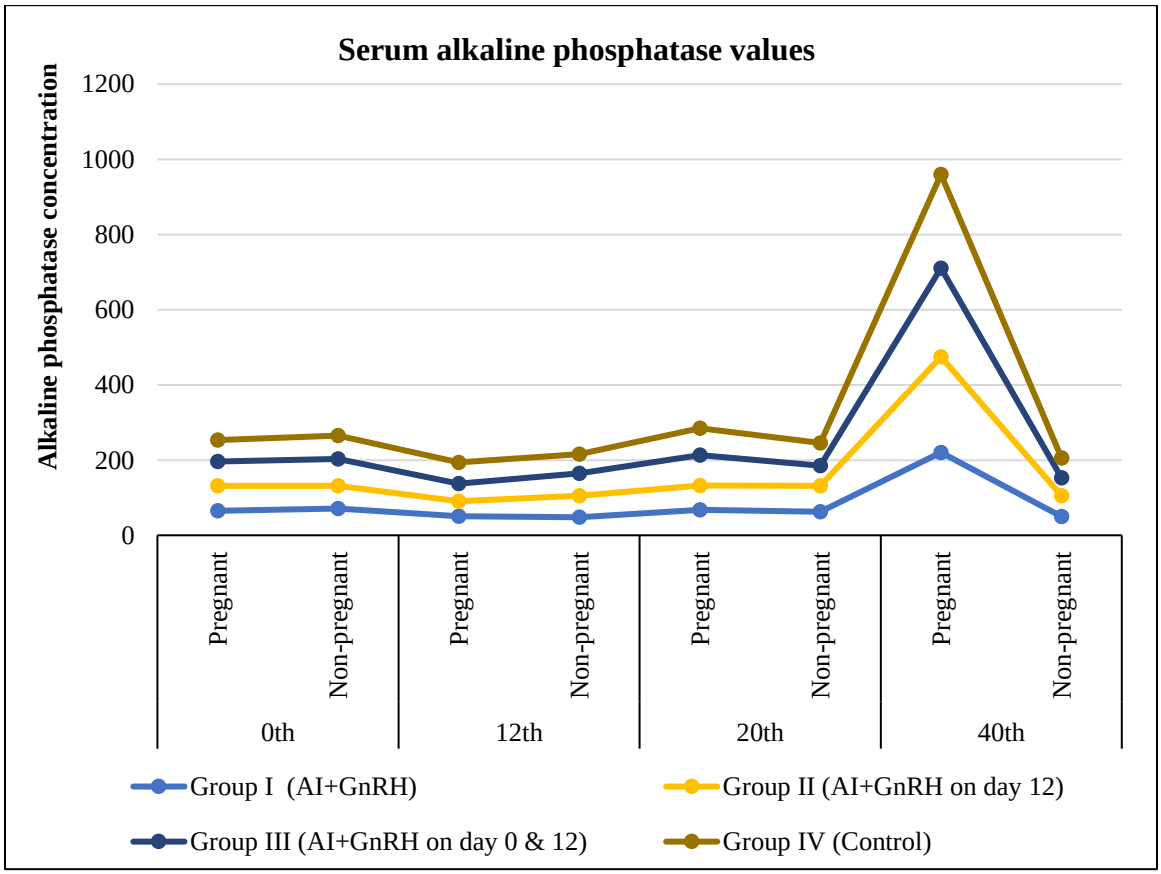
The cost of treatment based on price of GnRH in Group I (AI + GnRH) was cheaper with higher conception rate than other Group II (AI + GnRH on day 12) and Group III (AI + GnRH on day 0 & day 12).

Table 7. Comparative analysis of serum alkaline phosphatase (IU/L) values pregnant and non-pregnant between treated and untreated control repeat breeder buffaloes

Groups	0th		12th		20th		40th	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant
Group I (n=08) AI + GnRH	65.60±5.10^{X}	71.33±15.01^{X}	51.00±6.04^{X}	48.00±5.78^{X}	67.60±2.77^{X}	62.33±6.38^{X}	220.40±13.10^{aY}	50.00±4.16^{bX}
Group II (n=08) AI + GnRH on day12	66.00±6.92^{Y}	60.20±6.48^{X}	39.67±2.03^{X}	57.40±7.07^{X}	64.67±6.44^{Y}	69.40±4.22^{X}	254.33±4.05^{aZ}	55.60±9.44^{bX}
Group III (n=08) AI + GnRH on day 0 and 12	65.00±8.19^{XY}	71.50±3.97^{X}	46.75±4.46^{X}	59.50±6.70^{XY}	81.25±5.22^{aY}	53.75±3.73^{bXY}	236.00±7.79^{aZ}	47.25±4.66^{bY}
Group IV (n=08) Control	56.50±7.50^{X}	61.83±6.01^{X}	56.50±5.50^{X}	51.00±2.46^{X}	71.00±7.00^{X}	60.16±6.97^{X}	249.00±37.00^{aY}	52.67±2.72^{bX}

Means bearing different superscripts differed significantly (P<0.05).
$^{a, b}$ Within the groups, within the days between the pregnant and non-pregnant animals.
^{XYZ} Within the groups, within pregnant or non-pregnant animals, between the days.

Figure 6. Comparative values of serum alkaline phosphatase (IU/L) in pregnant and non-pregnant between treated and untreated control repeat breeder buffaloes



Discussion

V DISCUSSION

The results of research work entitled “Efficacy of GnRH treatment in repeat breeder buffaloes” were discussed in detail as mentioned below.

5.1 White side test

In the present study, 56.17% of repeat breeder buffaloes showed uterine infections as indicated by the white side test. This incidence of uterine infection was similar to previous studies by Bhalerao (2014) and Venkateswarulu (2019) with 60.85% and 50.78% subclinical endometritis, respectively. However, Alwana *et al*. (2001) and Vala *et al*. (2019) had reported a higher incidence of endometritis in buffaloes which predisposed for repeat breeding but, Kavani *et al*. (1984), Kanuya *et al*. (2000), Raju *et al*. (2007) and Hema (2020) have reported lower incidence with 10.92%, 7.5%, 6.1% and 11.12%, respectively contrary to the above findings in repeat breeder buffaloes.

White side test was an effective, simple and easy to do animal side test which could be used to diagnose clinical or subclinical endometritis in repeat breeders. The higher incidence of uterine infection in the present study might be due to poor hygiene, unhygienic and faulty insemination and possibly, wallowing habit . The variation observed with other studies might be due to variation in regional climatic and managemental conditions.

5.2 Conception rate

5.2.1 Group I (AI + GnRH)

In the present study, out of 8 repeat breeder buffaloes, 3 were conceived in the first and 2 in the second estrous cycle with a 62.50% overall conception rate.

These results were in agreement with previous studies by Rao (2000), Batavani and Eliasi (2004), Ahmed *et al.* (2010), Chandra Prasad and Anand Rao (2014), Parmar *et al*. (2016), Abo-farw *et al*. (2016) and Gautam *et al*. (2017) with reported conception rates between 55 to 66.70%. Similarly, Samad *et al*. (2002), Rao (2012) and Pandey *et al*. (2016) were reported conception rates of 53.33, 50.00 and 51.30%, respectively in repeat breeder buffaloes which were slightly lower than present study findings. However, Sharma *et al*. (2008), Mandal *et al*. (2009), Dhami *et al*.(2009), Ramlal *et al*. (2017) and Tiwari *et al*. (2019) had reported considerably very high conception rates between 80-100%. Contrary to these, recent studies by Ramya *et al*. (2019) and Hema (2020) reported much lower conception rates of 25.00 and 33.33% in repeat breeding buffaloes.

The Buserelin injection on the day of insemination showed 37.5% higher overall conception rate compared to untreated control group animals (62.5% *vs.* 25%). This improved conception rate could attributed to timed ovulation by LH surge resulting in progesterone secretion by the corpus luteum (Singh *et al*., 2017), due to its beneficial effect on embryo survival by enhancing luteal function (Attoo *et al*., 2013), or by stimulating the transformation of follicular cells to luteal cells, which required at least 2 to 3 days for optimum P_4 production (Stevenson *et al*., 1993). GnRH at estrus might potentiate conversion of small luteal cells to large luteal cells resulting in the development of large-sized functional CL which improves embryo survival through enhanced P_4 secretion (Attoo *et al*., 2013).

5.2.2 Group II (AI + GnRH on day 12)

In the present study, out of 8 repeat breeder buffaloes, 2 were conceived in first and 1 in third estrous cycle with 37.50% overall conception rate.

These findings were in line with previous studies by Vijayarajan *et al.* (2007) and Rao (2012) with a reported overall conception rate of 40.00%. However, Rao (2000), Batavani and Eliasi (2004) and Pandey *et al*. (2013) were reported higher conception rates of 50.00, 58.00 and 52.90 per cent, respectively. Further, GnRH administration on day 12 estrus/mating is reported to had a positive effect on the pregnancy rate (80 per cent) of repeat breeder buffalo heifers (Abo-Farw *et al.,* 2016).

The Buserelin injection on Day 12 showed 12.5% higher overall conception rate compared to untreated control group animals (37.5% *vs.* 25%), which was in agreement with several previous studies ((Macmillan *et al*., 1986; Peters *et al*., 1992; Singh 1997).

The estrogen secretion from a large follicle of second follicular wave present on 14 to 17 days of pregnancy might negatively affect embryo survival (Komar *et al*.,2001; Inskeep, 2004). However, a single injection of GnRH analogue, buserelin, given on Day 12 post-insemination promoted the ovulation of second-wave dominant follicle or induces luteinization and/or atresia of predominant follicles (Thatcher *et al*., 1993) along with delaying luteolysis and their by increases the life span of CL which gets more time to secrete progesterone (Mac-Millan and Thatcher, 1991) which was reported to reduce embryo mortality in cattle and sheep (Beck *et al*.,1994; Peters *et al*., 2000). This delaying of luteolysis might improve pregnancy rate by allowing embryos more time to produce sufficient quantities of interferon-τ (IFN- τ).

5.2.3 Group III (AI + GnRH on day 0 and 12)

In the present study, out of 8 repeat breeder buffaloes, 3 were conceived in first and 1 in the second estrous cycle with a 50.00% overall conception rate.

These results agreed with Ramya *et al*. (2019) and Hema (2020) with a reported conception rate of 50.00 per cent in repeat breeder buffaloes by both studies. Further, Zakiuddin (2013) had also reported positive effects of GnRH analogue on conception rate in non-infectious repeat breeder buffaloes with considerably higher pregnancy (90.00%) compared to present study findings.

Venkateswarulu (2019) reported a slightly lower conception rate (46.67 per cent) whereas, Rao (2000), Anbule (2018) and Hema (2020) were reported higher conception rates of 70.00, 66.66 and 55.55 per cent, respectively in repeat breeder buffaloes compared to present findings.

The GnRH injection on days 0 and 12 following estrus had dual advantages, the first being assured ovulation and the other being increased chance for forming the accessory CL and also delaying luteolysis, which could enhance progesterone production along with allowing embryos more time to produce sufficient quantities of interferon-τ (IFN- τ) resulting in reducing the chance for early embryonic mortality (Lo´pez-Gatius *et al*., 2006).

5.2.4 Group IV (Control)

The overall conception rate of control repeat breeder buffaloes in the present study was 25.00 per cent which is in agreement with studies by Rao (2012) and Hema (2020)

with reported pregnancies of 30.00 and 20.00 per cent, respectively. A lesser conception rate of 14.3 and 0.0 percent was reported by Ahmad *et al.* (2002) and Ahmad *et al.* (2010), respectively in untreated repeat breeder buffaloes.

However, Samad *et al*. (2002), Dhami *et al*.(2009), Chandraprasad and Anand Rao (2014) and Butani *et al.* (2016[a]) were reported slightly higher conception rates in repeat breeder buffaloes with 33.33, 39.13, 33.33 and 38.46 percent, respectively. This variation might be due to age, breed, climate and management of the animals.

5.3 Overall conception rate/Comparison between the groups

The GnRH injection at the time of artificial insemination, was more effective in treating repeat breeding buffaloes than AI + GnRH on day 0 & 12 and AI + GnRH on day 12 protocols. These findings were in agreement with Ahmad *et al.* (2010) and Abo-Farw *et al*. (2016).

The improvement in CR of repeat breeder buffaloes treated with GnRH on day of estrus observed in the present study might possibly related to better synchrony of preovulatory LH surge and timed ovulation (Tanabe *et al*., 1994; Singh *et al*., 2017).

5.4 Progesterone levels in treated and control repeat breeder buffaloes

5.4.1 Group I (AI + GnRH)

The progesterone levels (ng/mL) were lower (0.60 ± 0.08) on day 0 and then increased to 5.19 ± 0.58 on day 12 and reduced to 4.95 ±1.05 on day 20 and again increased to 6.44 ± 1.45 on day 40 in repeat breeder buffaloes. The mean serum progesterone levels on 12, 20 and 40 days of post AI were non significantly different from each other.

The mean serum progesterone level on day 0 were comparable with Parmar *et al*. (2016) with similar basal levels. However, higher levels of 0.95 ± 0.01 ng/ml and 1.04 ± 0.57 ng/ml of serum progesterone were reported by Ahmad *et al.* (2002) and Venkatesan *et al*. (2005), respectively. Further, compared the findings of present study lower levels of 0.35 ± 0.05, 0.25 ± 0.81, 0.45 ± 0.05, 0.45 ± 0.01 and 0.49 ± 0.01 ng/ml serum progesterone were reported by Savalia *et al.* (2014), Butani *et al.* (2011), Abo-Farw *et al*. (2016) and Lattoo *et al.* (2013), respectively.

The serum progesterone levels on day 12 were in agreement with Lattoo *et al.* (2013) and Abo-Farw *et al*. (2016). However, lower levels of progesterone were reported by Ahmad *et al.* (2002) on day 10.

The mean serum progesterone levels on day 20 were in line with Abo-Farw *et al*. (2016) and Savalia *et al.* (2014), with reported values of 4.29 ± 0.92 and 4.40 ± 1.17 ng/ml respectively in repeat breeder buffaloes. However, Lattoo *et al.* (2013) reported slightly higher progesterone levels of 5.97 ± 0.08 ng/ml on day 18.

The higher mean serum progesterone levels on day 40 in the present study could be attributed to the pregnant buffloes producing higher progesterone which is required for the maintenance of pregnancy.

5.4.2 Group II (AI + GnRH on day 12)

The progesterone levels (ng/mL) were higher (5.17 ± 0.86) on day 20 followed by 4.96 ± 0.24 on day 12, then 4.18 ± 1.52 on day 40 and 0.59 ± 0.08 on day 0 in repeat breeder

buffaloes. The progesterone levels were significantly low on day 0 compared to 12, 20 or 40 days post-AI.

Lattoo *et al.* (2013) reported similar progesterone concentration (0.63 ± 0.01 ng/ml) on day 0. In contrary, Abo-Farw *et al*. (2020) reported lower progesterone levels of 0.37 ± 0.03 ng/ml in repeat breeder buffaloes.

The mean serum progesterone levels on day 12 was 4.96 ± 0.24 ng/ml, which were closely in agreement with Abo-Farw *et al*. (2016) with reported values of 4.63 ± 0.33 ng/ml. In contrary, Abo-Farw *et al*. (2020) reported lower progesterone levels of 3.66 ±0.24 ng/ml on day 15 in repeat breeder buffaloes.

The mean serum progesterone levels on day 20 was in line with Abo-Farw *et al*. (2016) with reported mean of 5.04 ± 0.65 ng/ml. However, higher levels were reported by Lattoo *et al.* (2013) on 18th day post AI and Abo-Farw *et al*. (2020) reported lower progesterone levels of 4.00 ±0.07 ng/ml compared to present study findings in repeat breeder buffaloes.

The mean serum progesterone levels on day 40 in the present study were 4.18 ± 1.52 ng/ml in repeat breeder buffaloes could be attributed to the pregnant buffaloes producing higher progesterone which is required for the maintenance of pregnancy.

5.4.3 Group III (AI + GnRH on day 0 and 12)

The progesterone levels (ng/mL) in repeat breeder buffaloes were 0.62 ± 0.07 on day 0, 5.49 ± 0.68 on day 12, 6.20 ± 1.24 on day 20 and 5.63 ± 1.69 on day 40, respectively. The progesterone levels on day 0 were significantly lower compared to 12, 20, and 40 days

of post AI in this group. The GnRH injection on days 0 and 12 following estrus had dual advantages, the first being assured ovulation and the other being increased chance for forming the accessory CL and also delaying luteolysis, which could enhance progesterone production along with allowing embryos more time to produce sufficient quantities of interferon-τ (IFN- τ) resulting in reducing the chance for early embryonic mortality (Lo´pez-Gatius *et al*., 2006). Increasing mean progesterone levels on 0 to 12day and 12 to 20 day it was attributing more pregnant animals but compared to group- I lesser pregnant animal was observed in our study.

5.4.4 Group IV (Control)

The repeat breeder buffaloes in control group, showed significantly low serum progesterone concentration on day 12 and 20 post-AI when compared to three treatment groups. Further, the progesterone levels were also significantly higher on days 12^{th}, 20^{th} and 40^{th} post-AI compared to day 0 within the groups.

The mean progesterone levels on day 0 was 0.51 ± 0.08 ng/ml, which were in agreement with Abo-Farw *et al*. (2016) with reported mean of 0.48 ± 0.04 ng/ml. However, higher levels were reported by Lattoo *et al.* (2013) and lower levels by Abo-Farw *et al*. (2020) in repeat breeding buffaloes.

The mean progesterone levels on day 12 was in agreement with Abo-Farw *et al*. (2020) with reported concentration of 2.75 ± 0.24 ng/ml on day 15. However, Ahmed *et al*. (2002) reported lower progesterone concentration of 1.07 ± 0.03 ng/ml and Abo-Farw *et al*. (2016) and Lattoo *et al.* (2013) reported higher levels compared to the present findings in repeat breeder buffaloes.

5.5 Progesterone levels (ng/mL) on different days in pregnant and non-pregnant repeat breeder buffaloes

The serum progesterone concentration on day 12, 20 or 40 days following treatment with GnRH on day 0 or day 12 or on both days along with insemination, was higher in pregnant ($P < 0.05$) and non-pregnant ($P > 0.05$) animals compared to control animals. Further, progesterone was also higher in pregnant animals compared to non-pregnant animals in all the groups except on day 0 and on the day 12 in the control group.

The progesterone concentration in pregnant buffaloes was in agreement with Carvalho *et al.* (2007) with higher plasma P_4 concentrations than the non-pregnant buffaloes. The low level of progesterone (luteal deficiency) was one of the predisposing factors causing low fertility/early embryonic mortality (Grimard *et al.*, 2006; Santos *et al*., 2004). Further, the non-pregnant animals in all the groups had non-significantly higher progesterone values of estrus. This higher progesterone, referred as suprabasal progesterone was reported to cause peri-ovulatory disturbances (Singh *et al*, 2005), resulting in delayed ovulation, extended follicular phase and reduced signs of oestrus (Sood *et al*., 2015).

5.6 Alkaline phosphatase values in treated and control repeat breeder buffaloes

5.6.1 Group - I (AI + GnRH)

The serum alkaline phosphatase concentration on day 0 was (67.75 ± 5.88) (IU/L), similar with findings of Dhabale and Sharma (2002) and Acar *et al.* (2013) with reported levels of 71.09 ± 6.42 (IU/L) and 77.73 ± 17.26 (IU/L), respectively. However, Eissa

(1996) and Yogesh (2015) have reported higher values, of 142.62 ± 8.31 IU/L and 210 ± 13.77 IU/L, respectively.

The serum alkaline phosphatase concentration on day 12 and 20th day were 49.87 ± 4.11 and 65.62 ± 2.83 IU/L respectively. Yogesh (2015) reported higher values of 209.70 ± 20.85 and 208.50 ± 9.54 IU/L on day 11 and 16 of estrus cycle, respectively.

The serum alkaline phosphatase concentration on day 40 post-AI was significantly higher compared to other days in repeat breeder buffaloes. Similar results were reported by Talvelkar *et al.* (2008) with recorded concentration of 200.45 ± 40.23 IU/L. This increased alkaline phosphatase during gestation might be due to liberation of enzymes of placenta during pregnancy and the uterine events related to pregnancy (Visha *et al*., 2002).

5.6.2 Group - II (AI + GnRH on day 12)

The serum alkaline phosphatase concentration on day 40 post-AI was significantly higher compared to other days in repeat breeder buffaloes. On day 40 post-AI, 3 buffaloes were pregnant which was reason for recording higher mean values on this day. This higher alkaline phosphatase during gestation could be due to higher concentration of circulating estrogen and hepatic lipidosis along with liberation of enzymes of placenta during pregnancy and the uterine events related to pregnancy (Visha *et al*., 2002). Further, parathyroid overactivity might also play some part in the higher values of alkaline phosphatase activity during pregnancy, which was related to maternal mobilization of calcium from bones to provide material for mineralization of skeleton of growing foetus (Singh *et al*., 1992).

5.6.3 Group - III (AI + GnRH on day 0 and 12)

The serum alkaline phosphatase concentration on day 40 post-AI was significantly higher compared to other days in repeat breeder buffaloes. Similar to other groups, the increased alkaline was attributed to the number of pregnant buffaloes in the group, which would have higher enzymatic activity due to liberation of enzyme from placenta along with over activity of parathyroid glands for mineralization of fetal bones foetus (Singh *et al*., 1992).

5.6.4 Group IV (Control)

The serum alkaline phosphatase concentration on day 0, 12, 20 and 40 post-AI were statistically not significant in repeat breeder buffaloes. As number pregnant animals were less in this group which could be the reason for recording non-significant values on day 40 also.

5.7 Serum alkaline phosphatase (IU/L) values on various days in pregnant and non-pregnant repeat breeder buffaloes

The serum alkaline phosphatase values did not showed any significant changes between the pregnant and non-pregnant animals on corresponding days or between the groups within pregnant or non-pregnant animals on day 0, 12 and 20. However, on day 40, the alkaline phosphatase values were significantly increased in pregnant animals compared to non-pregnant animals in all the groups. This increased alkaline phosphatase was attributed to the pregnancy due to increased enzyme liberation from the placenta and the uterine events related to pregnancy (Visha *et al*., 2002).

5.8 Economy of GnRH treatment

A single injection of GnRH (10 mcg) costing about Rs. 165 was much cost effective and achieved good conception rates. Similar cost was reported by Zakiuddin (2013) and lower cost was reported by Biradar (2018). Variation of single injection cost might be due to different manufacters.

Summary

VI SUMMARY

The present study was focused on efficacy of GnRH treatment in repeat breeder buffaloes on different days and its effect on serum progesterone and alkaline phosphatase concentration on different days and economy of the GnRH treatment.

In the present study, repeat breeder buffaloes in the age group of 6-8 years with history of 2 to 3 calving presented to Veterinary Clinical Complex of Veterinary College Nandinagar, Veterinary Hospital, APMC Bidar, Veterinary Dispensaries and from different dairy farms of Bidar district, Karnataka state were examined. First, all the animals were screened for white side test, among the 73 repeat breeder buffaloes, 41 were found positive and excluded from the study. The remaining, 32 repeat breeder buffaloes which were negative for white side test were divided randomly into four groups with 8 animals in each group. Among these, group I animals were injected with buserelin acetate (Gynarich, Intas Pharmaceuticals, Ahmedabad, India), 2.5 mL, intramuscularly immediately after artificial insemination (AI), for group II inj. Buserelin acetate, 2.5 mL was injected intramuscularly on 12^{th} day post-AI, for group III inj. Buserelin acetate was injected twice, one at the time of AI and the other on 12^{th} day post-AI @ 2.5 ml and group IV (Control group) animals were inseminated without any hormonal injections at observed estrus. Further, all the buffaloes were inseminated with subsequent two estrus cycles if repeated.

Blood was collected through jugular venipuncture from all the buffaloes on 0^{th} (day of insemination), 12^{th}, 20^{th} and 40^{th} day of post insemination in a vacutainer with clot activator. The serum was separated by centrifugation (3000 rpm for 20 min) and collected in sterile vials and preserved at -20°C until analysis.

Serum progesterone was estimated by using CALBIOTECH progesterone ELISA kit and serum alkaline phosphatase measured by the IFCC method using the commercially available kit from Transasia Bio-medicals Ltd. duly following the procedure as directed by the suppliers with a semi-automatic biochemical analyzer (Microlab-300, Elite Tech Ltd).

Pregnancy of buffaloes were confirmed by serum progesterone assay on day 40 and per rectal examination on day 60.

Higher conception rate of 62.50% was recorded in repeat breeder buffaloes of group I (AI+GnRH) followed by 50.00% in group III (AI + GnRH on day 0 and 12), and 37.50% in group II (AI + GnRH on day 12). Lower conception rate of 25.00% was noticed in control group of repeat breeder buffaloes.

The repeat breeder buffaloes in control group, showed significantly low serum progesterone concentration on day 12 and 20 post-AI when compared to the three treatment groups. Further, the progesterone levels within the groups were significantly higher on days 12^{th}, 20^{th} and 40^{th} post AI compared to day 0.

The serum progesterone concentration on 12^{th}, 20^{th} or 40^{th} days following treatment with GnRH on day 0 or day 12 or on both days was higher in pregnant ($P < 0.05$) and non-pregnant ($P > 0.05$) animals compared to control animals. Further, progesterone was also higher in pregnant animals compared to non-pregnant animals in all the groups except on day 0 and 12 in the control group.

Serum alkaline phosphatase concentration showed non-significant (P>0.05) variation between the groups and also within the groups except on day 40 post-AI in treatment groups, which showed significant (P<0.05) increase compared to previous days.

The serum alkaline phosphatase values did not show any significant changes either between the pregnant and non-pregnant animals or between the groups within pregnant or non-pregnant animals on day 0, 12 and 20. However, on day 40, the alkaline phosphatase values were significantly increased in pregnant animals compared to non-pregnant animals in all the groups.

A single buserelin injection on the day of AI was more cost effective compared to other groups along with higher conception rates.

The following conclusions were drawn based on the present research findings.

1. Artificial insemination along with single injection of GnRH treatment in repeat breeder buffaloes was cheaper and more effective compared to other groups.
2. The repeat breeder buffaloes in control group, showed significantly low serum progesterone concentration on day 12 and 20 post-AI when compared to three treatment groups. Further, the progesterone levels were also significantly higher on days 12^{th}, 20^{th} and 40^{th} post-AI compared to day 0 within each group.
3. The serum progesterone concentration on 12^{th}, 20^{th} or 40^{th} days following treatment with GnRH on day 0 or day 12 or on both days was higher in pregnant (P <0.05) and non-pregnant (P>0.05) animals compared to control animals. Further,

progesterone was also higher in pregnant animals compared to non-pregnant animals in all the groups except on day 0 and 12 in the control group.

4. Serum alkaline phosphatase concentration showed non-significant ($P>0.05$) variation between the groups and within the groups, except on day 40 post-AI in treatment groups which showed significant ($P<0.05$) increase compared to previous days.

5. The serum alkaline phosphatase values did not show any significant changes either between the pregnant and non-pregnant animals or between the groups within pregnant or non-pregnant animals on day 0, 12 and 20. However, on day 40, the alkaline phosphatase values were significantly increased in pregnant animals compared to non-pregnant animals in all the groups.

6. A single buserelin injection on the day of AI was more cost effective compared to other groups along with higher conception rates.

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Abstract

VIII. ABSTRACT

EFFICACY OF GnRH TREATMENT IN REPEAT BREEDER BUFFALOES

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Efficacy of GnRH analogue (Busereline acetate) treatment on the day of AI or 12th day post-AI or on both the days in repeat breeder buffaloes was analysed. For the study, 32 white side negative, repeat breeder buffaloes were selected and divided into four groups with 8 animals each, group-I treated with 2.5mL(10mcg) GnRH analogue on the day of AI, group-II treated with GnRH on 12th day post-AI, group-III treated with GnRH on both the days and group-IV were inseminated without any treatment, considered as a control group. The blood samples collected on day 0, 12, 20 and 40th were analyzed for serum progesterone and alkaline phosphatase enzyme levels. There was a significant ($P<0.05$) increase in the progesterone concentration in the animals receiving GnRH treatment compared to control animals, suggesting a positive impact of GnRH treatment. The alkaline phosphatase concentration increased significantly ($p<0.05$) in pregnant animals only on day 40 post-AI. The conception rates were 62.5%, 37.50%, 50.00% and 25.00% for the group I, II, III and IV, respectively. From the study, it can be concluded that a single injection of buserelin on the day of AI was more cost effective compared to other groups along with better conception rates.