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B Reddamma
PG Scholar, Department of Veterinary Gynaecology and Obstetrics, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

B Chandra Prasad
Assistant Professor, Department of Veterinary Clinical Complex, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

M Srinivas
Professor and Head, Department of Veterinary Gynaecology and Obstetrics, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

M Sreenu
Associate Dean, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Corresponding Author:
B Reddamma
PG Scholar, Department of Veterinary Gynaecology and Obstetrics, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Effect of gonadotropin releasing hormone and flunixin meglumine in repeat breeder buffaloes

B Reddamma, B Chandra Prasad, M Srinivas and M Sreenu

Abstract
The present study was conducted to assess the efficacy of Flunixin and GnRH on conception rate among repeat breeder buffaloes which were presented to Large Animal Gynaecology Unit of Department of Veterinary Gynaecology and Obstetrics, NTR College of Veterinary Science, Gannavaram, Krishna District, Andhra Pradesh. Postpartum Graded Murrah buffaloes were screened based on history, clinico-gynaecological examination, pH of cervico-vaginal mucus, leucocyte esterase test, white side test and endometrial cytology. respectively. Based on endometrial cytology, the animals showing oestrus without subclinical endometritis (n=30) were selected and divided into three groups which were administered with normal saline (Group I, n=10), Flunixin (Group II, n=10) and Gonadotropin releasing hormone injection (Group III, n=10) between 11-13 days post AI. In the present study, higher conception rate was recorded in buffaloes injected with Gonadotropin releasing hormone – 40.00% compared to buffaloes injected with Flunixin Meglumine – 30.00%. Hence, the injection of GnRH could be recommended for field practice to improve conception rate in repeat breeder buffaloes.

Keywords: GnRH, flunixin, repeat breeder buffaloes, conception rate

1. Introduction
India is a predominantly agrarian society where lactating animals are the backbone of the national economy. Buffaloes (*Bubalus bubalis*) are premier milk-producing animals that contribute more than fifty percent of total milk production in the country (Chandra Prasad *et al.*, 2020) [8]. The term "repeat breeder" or "cyclic non-breeder" is used to characterize an animal that has been unsuccessful in conceiving after undergoing 3 or 4 services either by a fertile bull or artificial insemination (Butani *et al.*, 2016b) [5]. Despite displaying regular heat cycles and evident signs of oestrus, these animals doesn't show any clinically detectable reproductive disorders (Yusuf *et al*., 2010) [36]. Two of the most consistent causes of repeat breeding were reduced rates of fertilization and embryonic survival (Chandra Prasad and Rao, 2014) [7]. 70-80% of embryonic mortality occurs between days 8 and 16 after insemination (Santos *et al.*, 2004) [28]. GnRH injection leads to LH secretion which causes luteinization as well as progesterone secretion. Consequently, GnRH treatments have proven successful in preventing embryonic death due to luteal deficiency (Sheldon and Dobson, 1993) [30]. An alternative and latest method to regulate maternal and fetal relation, to retard or inhibit luteolysis, is to maintain high progesterone levels in the critical days of pregnancy by application of Non-Steroid Anti-inflammatory Drugs (NSAID), leading to enhanced pregnancy rate. Non-Steroidal Anti-inflammatory Drugs (NSAID) inhibit the synthesis of cyclooxygenase (COX) enzyme resulting in the inhibition of prostaglandin production, which will protect the corpus luteum (Singh *et al.*, 2021) [31].

2. Materials and Methods
The present study was carried out on Graded Murrah buffaloes, which were presented to the Department of Animal Reproduction Gynaecology and Obstetrics, NTR College of Veterinary Science, Gannavaram from November 2022 to October 2023. Postpartum Graded Murrah buffaloes with different parities were screened (n=71) based on the following criteria *viz*., history, clinico-gynaecological examination, pH of cervico-vaginal mucus, LEST, white side test and uterine discharge cytology.

Blood samples were collected in EDTA vacutainers to assess the haematological parameters, while blood collected in clot-activating vacutainers was utilized to obtain the necessary serum for assessing biochemical parameters. Haemoglobin levels were determined and expressed in grams per deciliter (g/dL) using the Sahli's haemoglobinometer technique, while packed cell volume (PCV) was assessed using a microhematocrit scale and reported as a percentage. Biochemical parameters, such as Total protein (Biuret and end point assay method) and Cholesterol (CHOD-PAP enzymatic end point assay method) and hormones such as Cortisol and Progesterone evaluated by Calbiotech ELISA kit. Selected repeat breeder buffaloes which were devoid of palpable reproductive abnormalities and negative for subclinical endometritis were divided into three groups with each Group 10. Group I were administered with normal saline, Group II were injected with 1.1 mg/Kg b.wt. of flunixin meglumine and Group III were injected with 10 µg of GnRH (Receptal) intramuscularly, respectively. All groups were administered once between days11-13 post AI. The statistical analysis of the data was done as per the procedures outlined by Snedecor and Cochran (1994) [32]. The haematological and serum biochemical parameters within and between the groups were compared by Independent Sample T Test and One Way ANOVA. The conception rate was analysed by Fisher exact test.

3. Results and Discussion

3.1 Haematological parameters

In the present study the mean haemoglobin (gm/dL) and PCV (%) concentration recorded was non-significant (p>0.05) in between conceived and non-conceived repeat breeding buffaloes. The mean haemoglobin (gm/dL) concentration recorded was non-significantly (p>0.05) higher in conceived compared to non-conceived repeat breeding buffaloes of Group I (8.59±0.03 vs 8.52±0.04), Group II (8.63±0.10 vs 8.51±0.04) and Group III (8.64±0.05 vs 8.54±0.06), respectively. The mean PCV (%) level recorded was non-significantly (p>0.05) higher in conceived compared to non-conceived repeat breeding buffaloes of Group I (32.50±0.65 vs 31.88±0.80), Group II (34.30±0.41 vs 32.02±0.60) and Group III (34.50±0.62 vs 33.01±0.93), respectively, which were similar to reports of Patel *et al.* (2016) [24], who also found a non-significantly higher values of Hb (gm/dL) and PCV (%) in conceived compared to non-conceived buffaloes (12.36±0.85 vs 11.41±0.82) (34.05±1.39 vs 32.41±1.63) (Table 1, Plate 1). The higher concentrations of HB and PCV were desirable physiological characteristics for efficient transport of oxygen and carbon-di-oxide which is essential for maintaining the health of animals. Consequently, buffaloes exhibiting higher concentrations of HB and PCV were more economical due to enhanced reproductive efficiency.

3.2 Biochemical parameters

In the present study, no significant (p>0.05) difference was recorded in the mean concentration of total protein (gm/dL) between conceived and non-conceived buffaloes. The mean serum total protein (gm/dL) in conceived buffaloes of Group I, II and III was 7.28±0.08,7.57±0.20 and 7.68±0.08**,** meanwhile the same in non-conceived buffaloes was 7.16±0.09, 7.33±0.08 and 7.43±0.05**,** respectively, which were in accordance to Savalia *et al*. (2014) [29] and Chirag *et al.* (2016) [9]. The results might be attributed to better feeding of animals which increased the concentration of amino acids required for biosynthesis of gonadotropins and other gonadal hormones (Table 2, Plate 2).

The serum total cholesterol levels were non-significant (p>0.05) between conceived and non-conceived buffaloes of the Group I and II with higher values in conceived buffaloes. The mean cholesterol concentration (mg/dL) was recorded as 84.72±0.07, 82.93±0.66 and 82.71±0.62 in conceived buffaloes of the Group I, II and III, while the same in non-conceived buffaloes was 83.70±0.89, 82.24±0.65 and 81.70±0.57, respectively. (Table 2, Plate 2). The present observations were also very much similar to that documented by Chirag *et al*. (2016) [9] who observed insignificantly higher mean cholesterol concentration in conceived than non-conceived repeat breeder buffaloes (142.32±18.84 vs. 140.73±26.96). In the present study, the non-significantly higher levels of cholesterol in conceived compared to non-conceived repeat breeding buffaloes across all groups might be due to extraction of cholesterol stored in the tissues and variations in quality of feed, energy status of the animal and the level of milk production (Ashwani *et al*. 2015) [3].

3.3 Hormonal Profiles

The mean serum cortisol levels in conceived buffaloes of Group I, II and III was 6.02±0.06, 6.04±0.18 and 6.02±0.11 ng/mL whereas in non-conceived buffaloes, the levels were 6.15±0.04, 6.14±0.09 and 6.12±0.10, respectively (Table 2, Plate 2). Rajamanickam *et al.* (2022) [25] observed a significant increase in cortisol levels in non-pregnant animals than pregnant animals suggesting that systemic cortisol levels may affect embryo implantation and hormonal balance. The variations in the serum cortisol levels of the present study might be due to environmental stress (Agarwal and Sharma 2002) [2], like hot temperature and less stable ventilation management which interferes with the release of hormones there by effecting fertility (Da Costa *et al.*, 2017) [10].

Among the Group I buffaloes, the mean serum progesterone concentration (ng/mL) on 0, 11th and 42nd day of insemination was recorded as 0.13±0.01, 3.29±0.15 and 4.68±0.16 respectively in conceived buffaloes, whereas in non-conceived buffaloes it was 0.12±0.02, 2.31±0.08 and 1.39±0.07. The mean serum progesterone levels in Group I conceived buffaloes were in close agreement with Pandey *et al.* (2016) [23] as 4.76±0.38, whereas lower value was recorded by Vijayarajan *et al*. (2007) [34] as 3.85±0.05 and higher value by Mandal *et al.* (2009) [20] as 5.34±0.82 (ng/mL). The mean serum progesterone (ng/mL) in Group II on the day of estrus was 0.17±0.43 and 0.14±0.01 in conceived and non-conceived buffaloes, while the same on 11th day was 3.63±0.14 and 2.64±0.08, whereas on 42nd day of post breeding was 5.49±0.10 and 1.64±0.07, correspondingly. The mean serum progesterone levels in conceived buffaloes of Group II was in consonance with Rokade (2015) [26] and Damarany and Ghanem (2020) [11] who recorded the values as 6.61 and 6.72, correspondingly, whereas on contrary, lower values were recorded by Neto *et al.* (2008) [22] as 0.44 ng/mL. On the other hand, Rossetti *et al*. (2011) [27] and Barkhori *et al*. (2018) [4] reported higher value as 9.78 and 9.21 ng/mL, respectively. The mean serum progesterone concentration was recorded in conceived buffaloes of Group III on the day of oestrus, 11th and 42nd day of post breeding was 0.31±0.06, 4.12±0.17 and 6.82±0.31 ng/mL, while the corresponding levels in non-conceived buffaloes was 0.19±0.02, 3.45±0.10 and 1.93±0.17 ng/mL, respectively (Table 3, Plate 3). The mean serum progesterone levels on 0th day shows non-significant difference (p>0.05) between conceived and non-conceived buffaloes between three groups, whereas on 11th

and 42nd day the serum progesterone level is significantly higher (p<0.05) in conceived buffaloes of Group III than Group II and Group I. Campanile *et al*. (2008) [6], Kishorekumar (2010) [18] and Deshpande (2017) [13] observed increased progesterone levels in conceived buffaloes compared to non-conceived buffaloes following GnRH injection. The values in Group III conceived buffaloes were in agreement with Mandal *et al.* (2009) [20], Lattoo *et al*. (2013) [19] and Hemadeepthi (2020) [17] as 7.33±1.10, 6.66, and 6.21±0.09 (ng/mL). The increased progesterone levels in Group III might be due the effect of GnRH injection which might have increased the life span of CL due to its luteotropic and luteoprotective properties thereby contributing to pregnancy maintainance (Lattoo *et al*., 2013) [19].

3.4 Conception Rate

The conception rate in Group I buffaloes was 10.00% which is in close agreement with Kishorekumar (2010) [18] as 15.00 per cent. The conception rate was 30.00% in Group II buffaloes of the present study was in close agreement to the reports Damarany *et al.* (2017) [12] and Barkhori *et al*. (2018) [4] as 33.00 and 35.00 per cent, correspondingly. On contrary, higher conception rates was recorded by Geary *et al*. (2010) [14], Rossetti *et al*. (2011) [27], Navrange *et al*. (2012) [21] and Rokade (2015) [26] as 58.00, 63.00, 66.00 and 75.00 per cent respectively, which is due to different days and routes of administration. The present study, recorded a higher conception rate 40.00% (Table 4, Plate 4) in Group III buffaloes on 11th day of post breeding which was in close agreement to the findings of Vijayarajan *et al.* (2007), [34] Gumen *et al*. (2011) [15] and Venkateswarlu (2019) [33] who observed the conception rates as 40.00,44.30 and 46.67 per cent. On the contrary, Abo-Farw *et al*. (2016) [1] and Zakiuddin *et al.* (2022b) [37] recorded the conception rates as 80.00 and 90.00 per cent respectively in buffaloes administered with GnRH analogues. Research studies have demonstrated that administering GnRH on day 5 or 11(Willard *et al*., 2003) [35] and between days 11 to 14 (Hansen 2002) [16], following artificial insemination results in increased serum concentration of progesterone and a tendency towards higher pregnancy rates. Embryonic mortality is one of the cause of repeat breeding which may be due to luteal insufficiency in this group it was cared by supplementing the exogenous GnRH on days 11-13 of oestrus cycle, which might had reduced oestradiol-17β secretion during maternal recognition of pregnancy (Lattoo *et al*., 2013) [19] and it may be the possible reason for higher conception rate in Group II of the present study.

Table 1: Haematological parameters in conceived and non-conceived buffaloes in different groups

Haematological parameters		Groups		
		Group I	Group II	Group III
Haemoglobin (g/dL)	Conceived	8.59±0.03	8.63±0.10	8.64±0.05
	Non- conceived	8.52±0.04	8.51±0.04	8.54±0.06
PCV (%)	Conceived	32.50±0.65	34. 30±0.41	34.50±0.62
	Non-Conceived	31.88±0.80	32.02±0.60	33.01±0.93

Table 2: Total protein (g/dL), Cholesterol (mg/dL) and Cortisol (ng/mL) levels between conceived and non-conceived buffaloes of different groups.

Groups	Conceived /Non-conceived	Total protein (g/dL)	Cholesterol (mg/dL)	Cortisol (ng/mL)
Group I	Conceived	7.28±0.08	84.72±0.07	6.02±0.06
	Non- conceived	7.16±0.09	83.70±0.89	6.15±0.04
Group II	Conceived	7.57±0.20	82.93±0.66	6.04±0.18
	Non-Conceived	7.33±0.08	82.24±0.65	6.14±0.09
Group III	Conceived	7.68±0.08	82.71±0.62	6.02±0.11
	Non-Conceived	7.43±0.05	81.70±0.57	6.12±0.10

Table 3: Serum Progesterone (ng/mL) in conceived and non-conceived buffaloes in different groups

Group	Status	Days after AI		
		0	11	40-42
Group I	Conceived	0.13±0.01	3.29±0.15*	4.68±0.16*
	Non Conceived	0.12±0.02	2.31±0.08*	1.39±0.07*
Group II	Conceived	0.17±0.43	3.63±0.14*	5.49±0.10*
	Non Conceived	0.14±0.01	2.64±0.08*	1.64±0.07*
Group III	Conceived	0.31±0.06	4.12±0.17*	6.82±0.31*
	Non Conceived	0.19±0.02	3.45±0.10*	1.93±0.17*

* Indicates significance difference (p<0.05) in level of progesterone (ng/mL) between rows and columns of conceived and non- conceived buffaloes of different groups on 11th and 40-42ndday.

Table 4: Comparison of conception rate by Fisher exact test

Groups		No. of animals conceived	Fisher exact test statistic value
Group I	Control	1	0.58
Group II	Flunixin	3	
Group I	Control	1	0.30
Group III	GnRH	4	
Group II	Flunixin	3	1.0
Group III	GnRH	4	

Non-significant difference (p>0.05) was observed between the Groups I, II and III for conception rates.

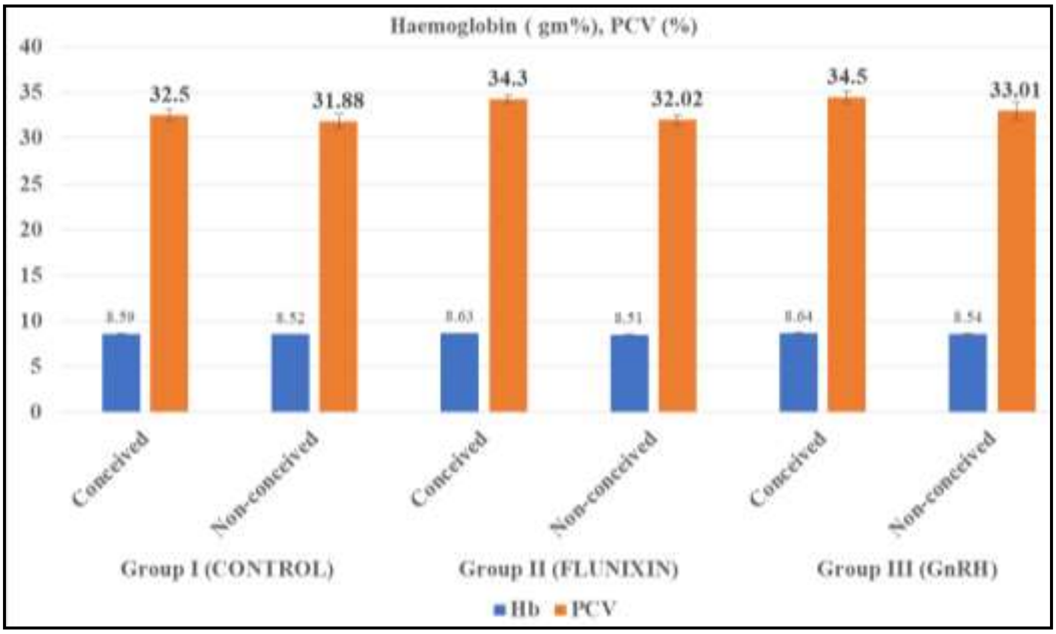

Plate 1: Haemoglobin (g/dL) and PCV (%) in conceived and non-conceived buffaloes of different groups

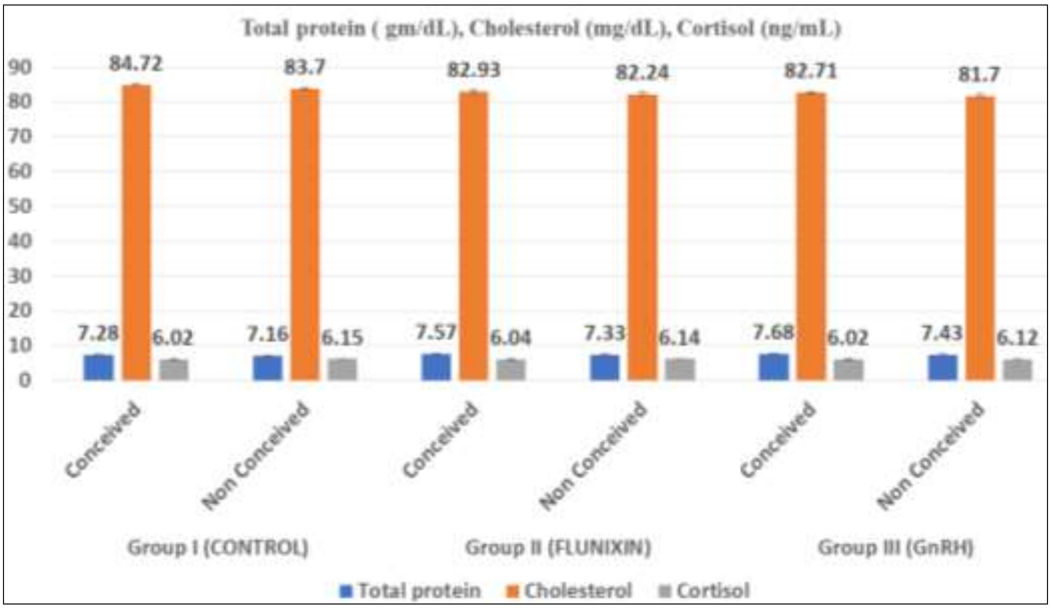

Plate 2: Total protein (gm/dL), Cholesterol (mg/dL), Cortisol (ng/mL) in conceived and non-conceived buffaloes of different groups

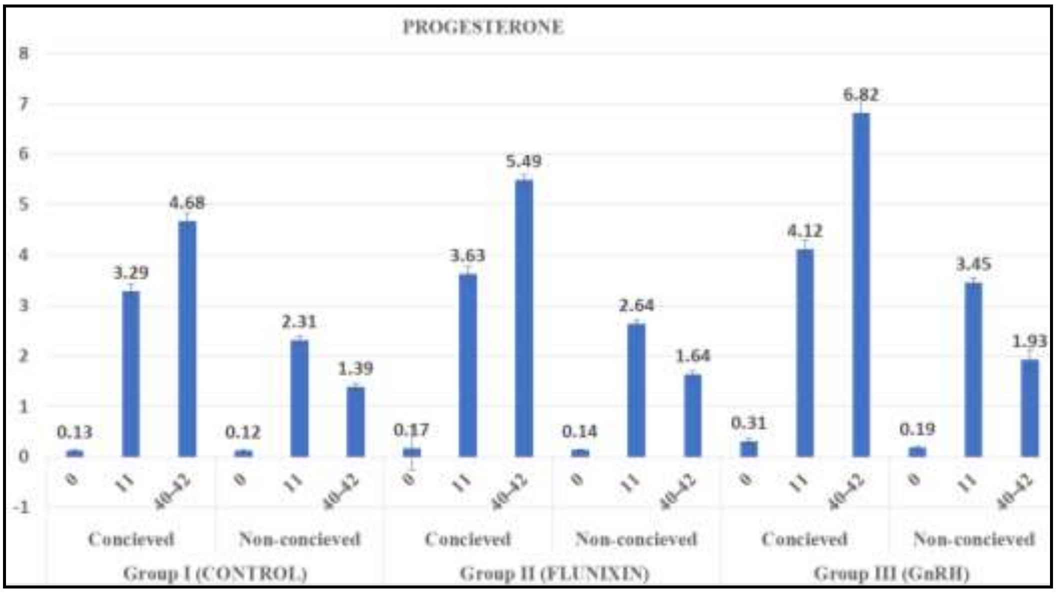

Plate 3: Levels of progesterone (ng/mL) in conceived and non-conceived buffaloes of different groups

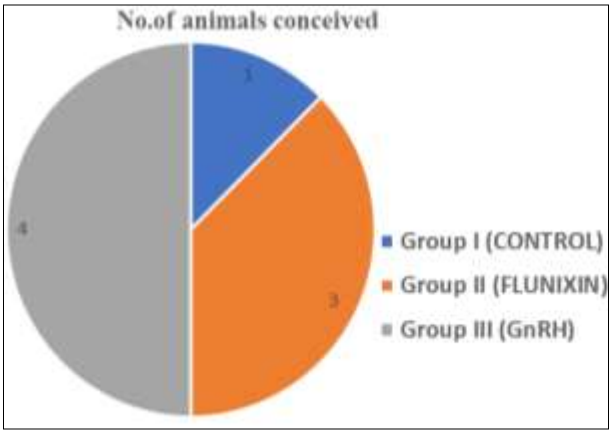

Plate 4: Conception rate in percentage in Group I, II and III

4. Conclusion

The current study concludes that GnRH injection could be a viable recommendation for practical application in the field to enhance conception rates in non-infectious repeat breeder buffaloes.

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