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Research Article

EFFECT OF GnRH AND PHOSPHORUS IN DELAYED PUBERTAL SURTI BUFFALO HEIFERS

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ABSTRACT: The study was conducted on eighteen delayed pubertal Surti buffalo heifers, divided into three equal groups (6 in each) to evaluate the efficacy of GnRH alone and in combination of phosphorus. The buffalo heifers in Group-I and Group-II were treated with Buserelin acetate (5 ml, IM). Buffalo heifers in Group-II also received additional injection of Toldimphos sodium (10 ml, IM) at 3 day interval for 4 times, while buffalo heifers in Group-III served as control. The percentage of induced estrus was highest (83.33%) in each treated groups as compared to control group (50%). The mean estrus induction intervals were significantly (P<0.05) shorter in Group-I (20.20 ± 2.18 days) and Group-II (18.80 ± 2.32 days) as compared to control group (30.24 ± 0.81 days). The conception rate at induced estrus was highest in Group-II (50%) followed by Group-I (33.33%). The plasma progesterone levels being significantly lowest on the day of estrus (less than 0.5 ng/ml) as compared to pre-treatment days in all groups. The mean total protein and triglycerides levels were differed significantly between the groups on the day of estrus and being significantly higher in Group-II as compared to Group-I and III on that day. A significantly higher level of cholesterol in both treatment groups as compared to the control group during different intervals and also being higher on the day of estrus as compared to pre-treatment days. The mean plasma glucose levels were differed non-significantly between and within the treatment and control groups. It is concluded that estrus can be successfully induced in delayed pubertal heifers with the use of GnRH alone and in combination with phosphorus.

Key words: Buffalo heifers, GnRH, Phosphorus, Estrus, Hormonal, Metabolic profile.

INTRODUCTION

The ovarian activity in buffalo begins with puberty. Puberty is complex physiological phenomenon whose origins are the neuro-endocrine mechanisms that determine the first ovulation and changes in primary and secondary sexual characters in the female. Follicle stimulating hormone (FSH) and luteinizing

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hormone (LH) are the gonadotropic hormones, which are stimulated by hypothalamic neuro-hormone gonadotropin releasing hormone (GnRH) that promote the maturation of ovarian follicle and determine dehiscence of mature follicle as well as ovulation of pre-ovulatory follicle as initiation of puberty in dairy animals (Terzano *et al.,* 2012). Buffalo usually attain puberty, when they reach about 60 percent of their adult body weight (250 to 400 kg.), but the age at which they attain puberty can be highly variable, ranging from 18 to 46 months (Jainudeen and Hafez, 1993). The factors that influence this are genotype, nutrition, management and climate. It could be attained under optimized conditions at 15 to 18 months in river buffalo and 21 to 24 months in swamp buffalo (Borghese, 2005). The delay in puberty, consequently delays conception and results in low reproductive efficiency and lengthening of the non-productive life. A major cause of delayed puberty may be poor feeding and management under field conditions (Warriach *et al.,* 2015).

The application point of view, the role of GnRH is causing the physiological synthesis and release of gonadotropins (LH and FSH) and in turn controls the process of gametogenesis and steroidogenesis. The wide use of GnRH in bovine reproduction is due to its ability to promote follicular development and subsequent estrus and ovulation of dominant follicle and formation of corpus luteum leads to production and release of progesterone hormone from the luteal tissue (Soni, 2014). GnRH and its analogues are commonly used in the therapy and reproductive management in cattle and buffaloes, and have a wide application in female animal reproduction. Use of GnRH in delayed pubertal heifers for estrus induction and better conception rate was carried out by various researchers (Mwaanga *et al.,* 2004; Raj *et al.*, 2006; Ghuman *et al*., 2009; Ahmed *et al.,* 2010; Derar *et al.*, 2012) and found satisfactory estrus induction response (75 to 100%) with poor conception rate (less than 50%) at induced estrus following exogenous GnRH treatment. Giri and Yadav (2001) studied the role of phosphorus in improving conception rates in heifers and reported that the phosphorus treatment enhances reproductive efficiency in heifers. In the recent years considerable attention has been focused on adopting appropriate therapeutic measures to augment bovine fertility. The study was aimed to evaluate the effect of GnRH alone and in combination with phosphorus on fertility response and plasma hormonal and metabolic profile in delayed pubertal Surti buffalo heifers.

MATERIALS AND METHODS

Ethical approval

The study was approved by the University Animal Ethics Committee of Navsari Agricultural University, Gujarat, India constituted for research purpose.

Experimental animals

The study was conducted on eighteen delayed pubertal Surti buffalo heifers (4 to 7 years old having average body weight of 250 kg.) maintained at University farm, Navsari, Gujarat. They were then randomly divided into 3 equal groups each of 6 buffalo heifers. The buffalo heifers in Group-I and Group-II were treated with 5 ml Buserelin acetate (Gynarich, Intas Pharmaceuticals Limited, India) given intramuscularly. Buffalo heifers in Group-II also received additional Injection of 10 ml Toldimphos sodium preparation (T-phos, Zydus

Table 1. Reproductive performance of delayed pubertal Surti buffalo heifers in different treatment and control groups.

Parameters	Group-I	Group-II	Group-III	Overall
Induction of estrus / Heifers responded (%)	5/6 (83.33)	5/6 (83.33)	3/6 (50.00)	13/18 (72.22)
Estrus Induction Intervals (Days) (Mean ± SEM)	20.20 ± 2.18^{x}	18.80 ± 2.32^{x}	30.24 ± 0.81^{y}	23.08 ± 1.77
Conception rate (%)	2/6 (33.33)	3/6 (50.00)	0/6 (0.0)	6/18 (33.33)

Group-I = GnRH alone, Group-II = GnRH + Phosphorus, Group-III = Control. Figures in parenthesis indicate percentage. Means bearing different superscripts within a row (x,y) differ significantly ($p < 0.05$).

Animal Health Limited, India) given intramuscularly at 3 day interval for 4 times. Buffalo heifers in Group-III served as control. Observations like time of estrus and duration (in days) for induction of estrus (from the day of treatment) were taken. The buffalo heifers exhibiting estrus were bred either naturally or by using artificial insemination. The buffalo heifers which did not return to estrus following breeding were confirmed for pregnancy per-rectum 60 days later.

Biochemical analysis

The blood samples were collected in EDTA vial from each animal on 0 day (before treatment) and on the day of estrus exhibition. Blood plasma was separated immediately by centrifugation for 15 minutes at 2000 rpm. Plasma samples were stored at -20°C till analyzed for biochemical parameters. Plasma progesterone concentrations were measured by using a commercially available Enzyme Immunoassay Kit (Randox Laboratories Ltd., UK). A standard curve was obtained by plotting the concentrations of the standard versus the absorbance. Estimation of total protein was done by Biuret method (Ryan and Chopra, 1976), total cholesterol as per Enzymatic Endpoint method (Allain *et al.*, 1978), Plasma glucose by Glucose Oxidase / Peroxidase (GOD - POD) method (Walker *et al.*, 1990) and triglycerides were measured by commercially available Kit (Randox Laboratories Ltd., UK). The absorbance and concentration was measured using an autoanalyzer (Merck's Micro-lab 300 analyzer, Vital Scientific, Netherlands).

Statistical analysis

The data collected were suitably tabulated and analyzed following standard statistical methods of ANOVA and Duncan's new multiple range tests for between the groups and *t*-test for within the groups as shown by Steel and Torrie (1981).

RESULTS AND DISCUSSION

Fertility Response

The estrus was induced in five out of six heifers (83.33%) in both Group-I and Group-II with mean estrus induction intervals of 20.20 ± 2.18 and 18.80 ± 2.32 days in Group-I and Group-II, respectively. While 50.0 per cent of buffalo heifers (3/6) came in estrus following

Table 2. Plasma Progesterone (ng/ml), Total Protein (g/dl), Total Cholesterol (mg/dl), Triglycerides (mg/dl) and Glucose (mg/dl) in delayed pubertal Surti buffalo heifers before treatment and at the time of estrus in different groups (Mean ± SEM).

Parameters	Group-I	Group-II	Group-III	Overall
Progesterone				
Pre-treatment	0.53 ± 0.45^{b}	0.86 ± 0.28^{b}	0.70 ± 0.53^{b}	0.69 ± 0.23^{b}
At estrus	0.31 ± 0.03^{a}	0.33 ± 0.02^{a}	0.38 ± 0.04^{a}	0.33 ± 0.02^{a}
Total Protein				
Pre-treatment	7.88 ± 0.13	8.07 ± 0.25	8.00 ± 0.43	7.98 ± 0.16^{a}
At estrus	7.19 ± 0.11^{x}	7.48 ± 0.22^{y}	7.18 ± 0.15^{x}	7.30 ± 0.10^{a}
Total Cholesterol				
Pre-treatment	86.00 ± 6.43^{a}	83.00 ± 7.03^{a}	85.83 ± 7.29^{a}	84.94 ± 3.77^{a}
At estrus	153.20 ± 10.68^{by}	131.40 ± 5.79^{by}	123.0 ± 19.34^{bx}	137.84 ± 6.8^{b}
Triglycerides				
Pre-treatment	40.00 ± 5.81	34.66 ± 4.34	34.00 ± 4.98	36.22 ± 2.83^{a}
At estrus	33.60 ± 4.41^{y}	41.20 ± 6.97^{z}	19.33 ± 4.09^{x}	33.23 ± 3.88^{a}
Glucose				
Pre-treatment	56.25 ± 3.04	57.96 ± 3.05	59.94 ± 3.59	58.05 ± 1.79^{a}
At estrus	59.94 ± 0.83	61.17 ± 1.32	56.22 ± 3.86	59.56 ± 1.09^{a}

Group-I = GnRH alone, Group-II = GnRH + Phosphorus, Group-III = Control. Figures in parenthesis indicate percentage. Means bearing different superscripts within a column (a,b) and row (x,y) differ significantly ($p < 0.05$).

mean estrus induction intervals of 30.24 ± 0.81 days in control group. These intervals were significantly ($p<0.05$) shorter in treatment groups as compared to control group. The earlier researchers have also used GnRH alone or in combination with phosphorus with similar results in delayed pubertal buffalo heifers (Ahmed *et al.*, 2010; Parmar *et al.*, 2012; Derar *et al.*, 2012). The anterior lobe of the pituitary of the pre-pubertal animal will produce FSH and LH if stimulated by exogenous GnRH. Also, the ovaries of pre-pubertal females will respond by producing follicles and estradiol when stimulated with FSH and LH (Senger, 2005). Madgwick *et al.* (2005) reported that administration of exogenous GnRH to stimulate the secretion of gonadotropic hormones in the adenohypophysis that promotes the maturation of ovarian follicles and early resumption of ovarian activity in buffalo. Further Bhandari *et al.* (1975) opined that the administration of Toldimphos sodium might have corrected the deficiency arising from intake of phosphorus to promote gonadal and genital activity.

The conception rate at induced estrus in Group-I, II and III were 33.33 per cent (2/6),

50.0 per cent (3/6) and 0.0 per cent (0/6), respectively. The present findings were in agreement with Verma *et al.* (2010). Whereas Ghuman *et al*. (2009) reported lower conception rate in heifers than the present study and stated that the ovarian response to exogenous GnRH in heifers is poorer than that of cows. In the present study, lower conception rate might be due to failure of the hypothalamus to produce sufficient quantities of GnRH to cause gonadotropin release is known to be the major factor limiting delayed pubertal heifers. Singh and Madan (1998) reported that the increased release of pituitary gonadotrophs towards advancing age during prepubertal period considered acting as primer sensitizer to modulate hypothalamic hypophyseal system and intrinsic rhythm to bring about onset of puberty in animals. The spontaneous releases of gonadotrophin hormone from prepubertal to pubertal age determine the release pattern of hypothalamic hormone (GnRH) and sensitivity of hypophyseal gonadotrophs to GnRH.

Hormonal profile

The mean plasma progesterone levels were differed significantly within the each group but not between the treatment and control groups. The present findings were in close agreement with those of Parmar *et al.* (2012) in Surti buffaloes. The mean plasma progesterone levels of heifers during pre-treatment days were nearly at basal level (less than 1 ng/ml) which is revealed that the ovaries were acyclic and confirming the anoestrus status of Surti buffalo heifers. These findings corroborated with the Soni (2014) in anoestrus buffaloes. The progesterone values found significantly ($p<0.05$) lowest on the day of estrus (less than 0.5 ng/ml) as compared to pre-treatment days in both treatment and control groups. The lower concentration of progesterone on the day of estrus in present study was also corroborated with Mondal (2007), who reported peripheral progesterone concentrations were minimal on the day of estrus (0.1 ng/ml), rise to peak concentrations of 1.6 to 3.6 ng/ml on days 13 to 15 of the cycle or even on day 17 before declining to basal levels at the onset of next estrus. Honparkhe *et al.* (2008) reported the need for measurement of progesterone as an aid to rectal screening of genitalia for differential diagnosis of true anestrous and sub-estrus. Progesterone in cyclic animals acts as a regulator of di-estrus period, because as soon as the corpus luteum fails to secrete progesterone, development of follicles begins leading to pro-estrus phase (Hafez and Hafez, 2013).

Metabolic profile

The overall mean plasma total protein concentrations before treatment were 7.98 ± 0.16 (g/dl) and at the time of estrus were 7.30 ± 0.10 (g/dl) and shown significant differences between the groups on the day of estrus and being found significantly higher values in Group-II. The present findings are in close agreement with Parmar *et al.* (2012) and Ghuman *et al.* (2009). The optimum protein level is necessary for the development of endocrine and sex organs. The ill-effect of low protein on reproduction is through pituitary and sex glands. Protein deficiency is associated with retardation of the development of sex organs and may affect subsequently the reproductive performance (Jain and Pandey, 1987).

The overall mean plasma cholesterol levels before treatment were 84.94 ± 3.77 (mg/dl) and at the time of estrus were 137.84 ± 6.81 (mg/

dl) and found significantly ($p<0.05$) higher levels of cholesterol on the day of estrus as compared to pre-treatment days in both treatment and control groups including overall means, and also a significantly higher ($p<0.05$) levels of cholesterol found in both treatment groups as compared to the control group during various time intervals. Similar results were found by Parmar *et al.* (2012), who reported higher mean plasma total cholesterol levels at induced estrus than that of pre-treatment level in GnRH treated buffaloes. The higher levels of cholesterol on the days of estrus in both treatment and control groups revealed increased production of cholesterol from the acetate for the synthesis of steroid hormones. The findings on higher cholesterol values in both the treatment groups were supported by Pal *et al.* (1991), who reported higher cholesterol concentrations is indicative of more secretion of steroids during estrus due to increased ovarian activity in the cycling dairy animals. In the opinion of Henricks *et al.* (1971), the highest adrenal cholesterol value occurred at estrus when female are under estrogen dominance eventually facing a decline later, when the progesterone phase sets in. Similarly Singh *et al*. (1983) observed that high level of cholesterol increased the estrogen synthesis resulting in manifestation of heat, because cholesterol is the precursor of steroid hormones.

The overall mean plasma triglycerides concentrations before treatment were 36.22 ± 2.83 (mg/dl) and at the time of estrus were 33.23 ± 3.88 (mg/dl) and shown significant ($p<0.05$) differences between treatment and control groups on the day of estrus and being significantly ($p<0.05$) highest value found in Group-II followed by Group-I and lowest in Group-III on the day estrus. The mean plasma triglycerides profile remained similar to the observations recorded by Khasatiya *et al*. (2005) and Ghuman *et al.* (2009) in buffalo heifers. The present findings on non-significant differences between the groups on the pre-treatment days are also corroborated with Ghuman *et al.* (2011), who reported that the mean plasma triglycerides levels were differed non-significantly ($P>0.05$) between treated and control groups of buffalo heifers. The significant ($p<0.05$) higher levels of triglycerides in treatment groups on the day of estrus in accordance to Mueller and Dabbert (2002), they reported triglycerides are directly affecting gonadal recrudescence, such as luteinizing hormone (LH). Patel (1988) reported that the level of triglycerides in the maternal circulation was positively associated with physiology of fertilization and implantation.

The plasma glucose concentration recorded in treatment and control groups were within normal physiological range and overall mean plasma glucose concentrations before treatment were 58.05 ± 1.79 (mg/dl) and at the time of estrus were 59.56 ± 1.09 (mg/dl). The mean plasma glucose levels were differed non-significantly between and within the treatment and control groups. The present findings are in close agreement with Ghuman *et al.* (2009) and Jayachandran *et al.* (2013), they reported that the non-significant variation in plasma glucose level between treated and control group of buffaloes. The blood glucose levels give an indication of the energy status of an animal. According to King (1971), the herds mean blood glucose concentration is valuable, since not all hypoglycemic herds suffer from clinical ketosis, but have few clinical problems and suffer from anoestrus.

CONCLUSION

The present study suggests that estimation of the plasma progesterone levels is helpful to detect the current reproductive / cyclical status while estimation of plasma total protein, triglycerides, glucose and cholesterol levels is also helpful in detect the metabolic status and reproductive steroid hormonal status of animal. It is concluded that the estrus can be successfully induced in delayed pubertal Surti buffalo heifers with the use of GnRH alone and in combination with Phosphorus. The pregnancy rate in heifers at induced estrus was quite low but the injection of GnRH may helps to improve the conception rate. Most of the heifers responded to treatment and those failed to conceive were able to continue to cycle normally.

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