Induction of oestrus in prepubertal heifers

Sravan Kumar Achary

Adm. No. 18192C07

DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS
COLLEGE OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY
ODISHA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
BHUBANESWAR-751003

2020

Achary SK, MVSc. (Animal Reproduction, Gynaecology and Obstetrics) Thesis, 2020.
Induction of oestrus in prepubertal heifers

Induction of oestrus in prepubertal heifers

A THESIS SUBMITTED TO
THE ODISHA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF

MASTER OF VETERINARY SCIENCE

IN

ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

By

Sravan Kumar Achary

Adm. No. 18192C07

DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS
COLLEGE OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY
ODISHA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
BHUBANESWAR-751003

2020

ODISHA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS
COLLEGE OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY

Dr. Purna Chandra Mishra, Ph.D
Associate Professor and Head
Department of Animal Reproduction,
Gynaecology and Obstetrics
College of Veterinary Science and Animal Husbandry
Odisha University of Agriculture and Technology
Bhubaneswar-751003, Odisha.

Bhubaneswar
Date:

CERTIFICATE-I

This is to certify that the thesis entitled **"Induction of oestrus in prepubertal heifers"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Science (Animal Reproduction, Gynaecology and Obstetrics)** to the Odisha University of Agriculture and Technology is a faithful record of bonafide and original research work carried out by **Sravan Kumar Achary**, **Adm. No. 18192C07** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by him from various sources during the course of investigation has been duly acknowledged.

CHAIRMAN
ADVISORY COMMITTEE

CERTIFICATE-II

This is to certify that the thesis entitled **"Induction of oestrus in prepubertal heifers"** submitted by **Sravan Kumar Achary**, **Adm. No. 18192C07** to the Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **Master of Veterinary Science (Animal Reproduction, Gynaecology and Obstetrics)** has been approved/ disapproved by the students' advisory committee and the external examiner.

Advisory Committee

Chairman

Dr. P.C. Mishra, Ph.D
Associate Professor and Head
Department of Animal Reproduction,
Gynaecology and Obstetrics
C.V.Sc. and A.H, O.U.A.T., Bhubaneswar _____

Members

1. **Dr. S.K. Mishra, Ph.D**
   Professor
   Department of Animal Nutrition
   C.V.Sc. and A.H, O.U.A.T., Bhubaneswar _____
2. **Dr. B.K. Patra, Ph.D**
   Associate Professor
   Department of Animal Reproduction,
   Gynaecology and Obstetrics
   C.V.Sc. and A.H, O.U.A.T., Bhubaneswar _____
3. **Dr. S.R. Mishra, Ph.D**
   Assistant Professor
   Department of Veterinary Physiology
   C.V.Sc. and A.H, O.U.A.T., Bhubaneswar _____

External Examiner _____
(Name & Designation)

ACKNOWLEDGEMENT

I express my profound sense of gratitude and indebtedness to my guide and esteemed **Dr. Purna Chandra Mishra**, Associate Professor and Head, Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Bhubaneswar for his able guidance, constant inspiration and supervision, relentless efforts, constructive counsel and affectionate attitude during the entire course of my investigation and preparation of this manuscript.

Words fail to express my deep sense of reverence to my co-guide cum member of advisory committee **Dr. S.K. Mishra,** Professor, Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Bhubaneswar for his conceptualization of this experiment, scholastic guidance, prudent suggestions, painstaking efforts throughout the period of investigation, preparation of this draft and providing crucial facilities right through the period of research.

I am thankful to **Dr. B.K. Patra**, Associate Professor, Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, for co-operation during my research work.

I express my hearty regards and deep sense of gratitude to **Dr. S.R. Mishra**, Assistant Professor, Department of Veterinary Physiology, College of Veterinary Science and Animal Husbandry, Bhubaneswar and advisory committee member for constant inspiration, sustained interest, constructive criticism and good effort throughout my course of my study.

I am grateful to **Dr. D.K. Karna**, Associate Professor, Department of Animal Breeding and Genetics, for his sincere, advice and statistical analysis of the data.

I express my sincere sense of reverence to **Dr. S.K.H. Ray**, Ex-Professor and Head, Department of Gynaecology and Obstetrics, Orissa College of Veterinary Science and Animal Husbandry, Bhubaneswar for his innovative idea, supreme supervision and affectionate attitude, scholastic insight and guidance, and above all imprinting the sense of sincerity and creating thirst of knowledge during my course and oblation of obsolete idea during preparation of this manuscript.

I express my hearty regards to **Dr. D.N. Mohanty**, Ex-Professor and Head, Department of Animal Reproduction, Gynaecology and Obstetrics, Faculty of Veterinary Science and Animal Husbandry, Bhubaneswar for his able guidance during the experiment.

I express my hearty regards, deep sense of gratitude to **Prof. S. Das**, Ph.D., Ex-Director, Teaching Veterinary Clinical complex, College of Veterinary Science and Animal Husbandry, Bhubaneswar for conceptualization of this experiment, scholastic guidance, constant inspiration and supervision, sustained interest, constructive criticism and good effort throughout my course of study.

I express my hearty regards to **Dr. A.K. Barik**, Ex-Professor and Head, Department of Animal Reproduction, Gynaecology and Obstetrics, Faculty of Veterinary Science and Animal Husbandry, Bhubaneswar for his able guidance during the experiment.

My special obligations are due to **Dr. R.M. Moharatha**, Rtd JD-I Directorate of Animal Husbandry & Veterinary Services, Odisha, Cuttack now guest faculty Department of Animal Reproduction Gynaecology and Obstetrics, Faculty of Veterinary Science and Animal Husbandry, Bhubaneswar for his moral support, prudent suggestions, timely encouragement and providing necessary facilities during the course of investigation.

I acknowledge gladly the assistance rendered by **Dr. (Mrs) Basanti Jena**, Assistant Professor, Department of Gynaecology and Obstetrics, for her sincere and painstaking efforts, moral, technical support and good motivation throughout the course of my investigation.

I express my hearty regards and deep sense of gratitude to **Dr. Anil Kumar Nahak**, Assistant Professor, Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Bhubaneswar and advisory committee member for constant inspiration, sustained interest, constructive criticism and good effort throughout my course of my study.

I acknowledge gladly the assistance rendered by **Dr. S.S. Biswal**, Assistant Professor, Department of Gynaecology and Obstetrics, for his sincere efforts, moral, technical support and good motivation throughout the course of my investigation.

I am extremely obliged to the **Commissioner-cum-Secretary**, Department of Fishery and Animal Resources Development (FARD), Govt. of Odisha for allowing me to undergo postgraduate study as government sponsored candidate for the interest of livestock owners of my state.

I am obliged to the **Director** of Animal Husbandry & Veterinary Services, Government of Orissa, Cuttack for his dynamic far sight to allow me to undergo this higher study for the interest of public service and develop my sphere of knowledge for

rendering better services to the livestock owners of our state. I am also grateful to him for giving me permission to conduct experiment in collaboration with the Frozen Semen Bank, Khapuria, Cuttack.

I am grateful to acknowledge the honest co-operation and help of the **S.D.V.O., Chhatrapur, S.D.V.O, Rairakhol** and his staff for drawing my monthly salary and other allowances during my entire course of study.

I am privileged to express my sincere thanks to my post graduate colleagues **Dr. S.K. Singh**, **Dr. S.K. Beuria, Dr. J.J. Nayak, Dr. (Miss) S.S. Dash, Dr. A. Kumar** and others from different departments for their assistance and encouragement during my study.

I am very much thankful to **Mr. G.C. Achari,** Technician, **Akuli and Jagdish,** of the department of Animal Reproduction Gynaecology and Obstetrics and all staff members of Teaching Veterinary clinical Complex, C.V.Sc & A.H for their kind cooperation and help during the course of work.

I acknowledge gladly the assistance rendered by **Mr. N.C Panda,** L.I., Othaka, Kakatpur for his sincere efforts, moral, technical support and good motivation throughout the course of my investigation.

I express my deepest sense of gratitude to my beloved parents, brother **(Sampat),** father-in law for their association and affection during my higher study.

I express my deepest sense of appreciations with love to my wife **(Swapna),** son **(Swastik)** and daughter **(Tanmayee)** for their help, co-operation and assistance who have suffered silently to my timely absence and untimely presence during the entire period of my M.V.Sc. study including research work.

I am thankful to Master **Kulu,** Siripur for preparing such a neat and beautiful manuscript.

Last but not the least, I surrender before **"LORD JAGANNATH'' & "MAA TARINI"** The Almighty for fulfillment of His/Her eternal desire.

Place : Bhubaneswar

Date : **(Sravan Kumar Achary)**

CONTENTS

LISTS OF FIGURES

LISTS OF TABLES

LIST OF ABBREVIATIONS

%	:	Per cent
&	:	And
^{0}C	:	Degree centigrade
AI	:	Artificial Insemination
Fig.	:	Figure
g	:	Gram
Hrs	:	Hours
i.e.	:	That is
ml	:	Milliliter
mm	:	Millimeter
GnRH	:	Gonadotropin Releasing Hormone
PG	:	Prostaglandin
$PGF_2\alpha$	:	Prostaglandin $F_2\alpha$
E_2	:	Estrogen
Expt.	:	Experimental
P_4	:	Progesterone
Dl	:	Deciliter
µl	:	Microliter
EB	:	Estradial Benzoate
FTAI	:	Fixed Time Artificial Insemination
CL	:	Corpus Luteum
PCL	:	Persistent Corpus Luteum
AICRP	:	All India Coordinated Research Project
ARD	:	Animal Resources Development
pg	:	Picogram
ng	:	Nanogram

ABSTRACT

The present investigation is carried out in the Dept. of Animal Reproduction Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar for a period from January 2020 to March 2020 with an objective to evaluate the efficacy of different treatment protocols and mineral supplementation on enhancing puberty in pre pubertal heifers of different villages of Kakatpur block in Puri district of Odisha. On the basis of survey and gyneco-clinical findings, 30 anovulatory heifers assigned to five different treatment groups including one control group. Group II heifers treated with Mineral supplementation (Area specific mineral mixture (ASMM) supplementation @ 50 g/day/ animal for 60 days), Group III heifers were treated with 3 doses of injectable progesterone followed by Inj. of Prostaglandin after 4 days along with Mineral supplementation Group IV heifers were treated with one dose of GnRH along with Mineral supplementation. Group V heifers were treated with GnRH (along with 1st dose of progesterone), 3 doses of Progesterone at 4 days interval and Inj. of Prostaglandin after 4 days along with Mineral supplementation. The selected animals were bred artificially by inseminating with frozen semen thawed semen from proven bulls and the conception rate is calculated after diagnosis of pregnancy through rectal palpation after 45-60 days. The control group did not show any induced estrus, where as Gr. V showed 100 percent result in estrus response. Gr. II and Gr. III and Gr. IV showed 16.6%, 50% and 33.3% induced estrus respectively. The survey finding revealed that the heifers of the first two groups i.e. Gr. I and Gr. II did not lead to conception while the heifers in the Gr. III, Gr. IV, Gr. V have conception percentage of 33.3, 16.6 and 66.6 respectively. An increased level of serum Calcium and Phosphorus was observed on post treatment day. An increased level of serum Glucose, Cholesterol and Total Protein for the treated groups were observed on post treatment sampling. A significant higher Progesterone level in post treatment Group V was observed while in other groups the change in the levels of Progesterone is not significant. Hence it can be concluded that, the use of Progesterone, GnRH and $PGF_2\alpha$ along with the mineral supplementation can effectively be used to induce estrus in pre pubertal heifers and the cost per conception is also viable for the farmers.

INTRODUCTION

India, inspite of having the largest population of cattle in the world, is found lacking when it comes to milk production per animal. During the last few decades, improving production with the genetic up gradation of indigenous cattle, has been in the fore front of animal husbandry. Due to this effort, India now has a sizeable population of crossbred animals. Consequently, milk production per animal has significantly improved. However, the life time milk production of an animal has not been satisfactory.

Indian agriculture being primarily a crop-livestock mixed production system, animal husbandry plays a vital role. It helped to generate employment, provide nutritional security and supplement to the family income (Srivastava, 2016).

Induction of estrus at pre pubertal stage would obviously lead to optimal use of reproductive life. As per Wiltbank *et al.* (2002), reproduction is five times more important than growth rate and milk production, when it comes to commercial cattle production.

Many factors like species, genetic potentiality, plane of nutrition, growth, body weight, role of different hormones, health and other managemental conditions are responsible for attainment of timely growth, puberty and sexual maturity in animals Age at puberty can be improved by different practices but the improvement in environment and adoption of optimum nutritional practices are more effective.

Adoption of modern reproductive technologies involving use of different hormonal protocols, proper management, housing and feeding system of the dairy animals, can also reduce the age of puberty and sexual maturity (Gupta *et al*., 2016). Energy balance and plane of nutrition influence reproductive performances in heifers (Short and Adams, 1988.; Butler and Smith, 1989). Early puberty is also important because pregnancy rate in heifers are found to be dependent upon the number of displaying estrus, prior to or early in the breeding season (Short and Bellows, 1971).

Heifers normally have their first estrus cycle between 12-15 months of age. The major factors delaying onset of puberty are poor nutrition or a genetic predisposition for late maturity. In cattle, maturation of the reproductive axis occurs

in a gradual fashion and the process requires endocrine and metabolic changes that happen in a harmonious fashion (Day and Anderson, 1998). From an endocrine mechanism standpoint, the occurrence of puberty is the result of a decrease in the estradiol negative feedback in the hypothalamus on secretion of gonadotropin releasing hormone which leads to an increase in the secretion of LH in response to an increment of GnRH release (Rodriguez and Wise, 1998) resulting in final growth and maturation of follicles leading to a successful ovulation (Day *et al*., 1984; Kinder *et al*., 1987). Heifer require increasing amount of luteinizing hormone and follicle stimulating hormone which are accordingly released from the pituitary. The increase in LH and FSH stimulates ovarian follicle development. The change in the frequency pattern of the GnRH release during the early pre pubertal development drives the increase in the secretion of gonadotropins that is critical for the activation and proper follicle growth and development (Madgwick *et al*., 2005; Whitlock *et al*., 2006).

Heifers that come to heat early in their first calving season have grater life time calf production and thus have more productivity. As the fertility at first estrus, is less compared to subsequent estrus cycles, the calves should come to estrus as early as possible leading to fertile estrus at an early age and thus improved productivity (Byerly *et al*., 1987).

As per Burfenning (1976), age of puberty is a heritable trait and Induction of puberty in heifers over several generations might result in situation in which puberty is difficult to attain without hormone treatment. This consideration cannot be overlooked and a need to explore treatments to induce puberty in cattle is highly essential in pre pubertal heifers.

Early maturity improves the production rate of female as they enter early into the breeding herd. So there is necessity of exogenous intervention to improve the ovarian activity in pre pubertal heifers. Different measures have been practiced for reducing the age at first calving. Hormonal therapy for induction of cyclicity in animals, hastens the onset of puberty and has been attempted using a variety of exogenous hormones coupled with managemental practices. The pubertal process can be hastened by administration of ovarian steroids, especially progesterone. Investigations into mechanism by which steroid, hastens puberty may advance understanding of the process. Protocols involving GnRH have been effective in inducing cyclicity in cattle. Progestin supplementation is another effective method

that has been used to induce estrus in pre pubertal heifers and seems to be more effective coupled with dietary supplementation. Primarily prostaglandins are used for luteolysis and GnRHin a protocol induces folliculogenesis and partial lutenization of follicle while estradiol preparations are used for formation of new follicular wave.

Keeping in view the above facts, the present experiment is designed to induce estrus in pre-pubertal heifers with the following objectives.

- To evaluate the efficacy of different hormonal protocols and mineral supplementation on enhancing puberty in pre-pubertal heifers.
- To study the ovulation status of the pre-pubertal heifers following hormonal protocol and mineral supplementation.
- To assess the conception rate in induced pre-pubertal heifers.

REVIEW OF LITERATURE

Heifers are future of a dairy farm, wherein, the pubertal period should be shortened for an economical dairy breeding unit. By delaying the age to first calving and lactation, pubertal anoestrous will significantly influence the life time productivity of buffaloes.

2.1 Puberty in heifers

Puberty is defined as the attainment of a developmental state that supports normal ovarian cyclicity (follicular development and ovulation) and the ability to become pregnant. Activation of central reproductive axis is a major event preceding onset of ovarian cycles in all mammalian females, including heifers.

Foster and Jackson (2006) observed that as puberty approaches, an increase in the release of a key hormone from the hypothalamus (lower brain) occurs. Gonadotropin - releasing hormone (GnRH), master regulator of reproductive function, secretion preceding puberty results in a concomitant increase in production and release of the pituitary hormone-luteinizing hormone (LH). Each biologically significant GnRH pulses results in a pulse of LH and in an overall increase in the concentration of LH in the general circulation. Elevation in LH is the signal that drives final maturation of ovarian follicles and the production of steroid hormones within the follicle (viz., estrogens).

According to Wildt *et al.* (1980) a limiting factor for the onset of puberty is the lack of high-frequency pulses of GnRH and LH. Relative inactivity of the central reproductive axis during prepuberty, is created primarily by a negative feedback system involving estradiol-1713 (E2), the most physiologically-relevant estrogen produced by the ovarian follicle.

Foster *et al.* (1979) and Day *et al.* (1984) found out that hypothalamus becomes less sensitive to the negative feedback effect of E2 as puberty approaches. As a result, GnRH release from the hypothalamus increases which in turn stimulates increased circulating LH. Development and maturation of a large, estrogen-active follicle that follows these events represents a "switch- from a negative to a positive feedback effect on both the hypothalamus and pituitary. Increased release of E2 by the maturing follicle also causes the expression of behavioral estrus (heat) and is responsible for triggering a surge release of LH.

This results in the first ovulation and the formation of a corpus luteum (CL). CL produces progesterone which regulates the length of the estrous cycle. Changes in expression of specific signalling peptides (i.e., neuropeptide Y; NPY; agouti-related peptide, AgRP) in a metabolic-sensing region of the hypothalamus, serve as developmental focal points for modifications that precede activation of the GnRH pulse generator (Allen *et al.,* 2009). In addition, the discovery of a new family of neuropeptides, the RF-amides, has revolutionized our understanding of the regulation of GnRH neurons.

Messager *et al.* (2005) opined that, the hormone, kisspeptin has been shown to stimulate GnRH secretion, may communicate both the positive and negative effects of estradiol on GnRH/LH release (Smith *et al.,* 2007) and is absolutely critical for pubertal development (Seminara *et al.,* 2003).

Schams *et al.* (1981) and Schillo *et al.* (1982) observe that components of the reproductive endocrine axis are functional long before onset of puberty. Pulses of LH are apparent in the peripheral circulation of heifers as early as one month of age. Hypothalamic-hypophysial portal system and LHRH-producing neurons are functioning and the anterior pituitary is producing LH and is responsive to LHRH.

Schams *et al.* (1981) and Schillo *et al.* (1982) indicated that Increase in LHRH induced LH release may be attributed partially to an age-related increase in pituitary LHRH receptors. Ovaries of female heifer calves respond to gonadotropins long before first ovulation. Antral follicles are present at birth and ovulation can be induced at 1 month of age (Marion and Geir, 1971, Seidel *et al.,* 1971). The number of induced ovulations was greater in 4 month old than in 1 month old dairy heifers, indicating that ovarian responsiveness to gonadotropins increases with age (Seidel *et al.,* 1971).

Odell *et al.* (1970) and Anderson (1981) reported post-castration rises in LH concentrations in heifers at 3, 6 and 9 month of age. Primary source of negative feedback effects in prepubertal heifers seems to be estradiol. Estradiol blocked the post-ovariectomy rise in circulating LH concentrations in heifers (Day *et al.,* 1984).

Schillo *et al.* (1982b) reported that acute i.v. injections of estradiol suppressed pulsatile LH release in ovariectomized heifers and the duration of suppression was dependent on dose of the steroid and age of heifers. Estradiol suppressed LH release for a longer duration in 4 month old animals than in 8- and 12-month old animals, suggesting that responsiveness to estradiol negative feedback decreased with age.

Day *et at.* (1984) demonstrated that the ability of estradiol-filled Silastic implants to suppress pulsatile LH secretion in ovariectomized heifers decreased with advancing age and that the decrease in responsiveness to estradiol coincided with onset of puberty in ovary-intact animals.

Kinder *et al.* (1987) observed that the decrease in responsiveness to estradiol negative feedback is associated with a decrease in the number of unoccupied estradiol receptors in both the hypothalamus and anterior pituitary gland. Thus the reduction in responsiveness to estradiol negative feedback allowed pulsatile LH to increase to a level that would stimulate development of ovarian follicles to the preovulatory stage. Prepubertal increase in pulsatile LH secretion therefore could be the rate-limiting step in sexual maturation.

Swanson (1972) reported an increase in serum concentrations of LH during the 10 day preceding first estrus in heifers. Gonzalez *et al.*(1975b) found LH concentrations to be highly variable during the 2 month preceding onset of puberty but failed to detect an increase in mean concentrations during this period. McLeod (1984) also failed to detect an increase in circulating concentrations of LH prior to onset of puberty. The variation in age related changes in LH among these studies is probably due to the fact that frequency and duration of blood sampling was insufficient to assess secretory profiles of LH accurately.

2.2 Effect of hormones on puberty and conception.

During last few decades, various hormones are extensively used to improve reproductive efficiency in dairy animals. However, these hormone preparations should be used judiciously to get optimum results.

2.2.1 Gonadotropin Releasing Hormone (GnRH)

Gonadotropin releasing hormone has been widely used as an integral part of oestrous synchronization protocol in cattle. It is a decapeptide hormone, synthesized and stored in the medial basal hypothalamus. In response to neural

signals, pulses of GnRH are released into the hypophyseal portal system for the release of FSH and LH from the anterior pituitary which in turn controls the functioning of ovaries (Hafez and Hafez, rh edition). Exogenous Injection of a GnRH agonist at any stage of the oestrous cycle in cattle increases the number of medium sized follicles within 3 days of treatment, eliminates the large follicles by ovulation or atresia and induces the emergence of a new follicular wave within 2 to 3 days of treatment (De Rensis and Peters., 1999). Similar effect of exogenous GnRH on follicular development was also reported by Dharani *et al.* (2010).

The administration of GnRH analogue might provide the necessary threshold stimulus to initiate the estrous cycle by the release of gonadotropins, which causes growth of medium sized follicles and atresia of large follicles or induces ovulation and the formation of new corpus luteum (Pursley *et al*., 1995).

Nautiyal *et al.* (1997) reported 85.71 and 80 per cent estrus rate and pregnancy rate, respectively in anoestrus pubertal buffalo heifers when treated with 1.5 ml GnRH analogue Tamuli *et al.* (2000) recorded an overall fertility rate of 87.55 per cent in delayed pubertal heifers treated with either 1.5 ml or 2.5 ml of GnRH (Fertagyl) along with 500 IU PMSG on 7th day for 2 or 3 consecutive days. Thakur *et al.* (2001) reported a conception rate of 66.66 per cent in delayed pubertal cross bred heifers when treated with treated with 5 ml receptal intramuscularly.

Sirmour *et al.* (2006) recorded an oestrus response and concentrate rate as 83.33 and 40 per cent, respectively in delayed pubertal crossbred heifers when treated with GnRH analogue (Buserelin acetate, 200 pg) intramuscularly. The interval for oestrus induction was 12.60±3.00 days. Buffalo heifers when treated with a single intramuscular injection of 200 pg GnRH Arab *et al* (2013) observed 16 and 37.5 per cent estrus induction and conception rate, respectively in anoestrus cows treated with GnRH. Thus, variable response has been observed by many workers in terms of oestrus induction and conception rate in delayed pubertal heifers.

2.2.2 Prostaglandins ($PGF_2\alpha$)

Synchronization of estrus with prostaglandins is one of the oldest ways used in cattle reproduction. $PGF_2\alpha$, a causes regression of the corpus luteum due to its luteolytic effect (Lauderdale, 1972; Louis *et al*., 1972; Roche, 1977). It is

effective only if used between days 8 to 17 of the estrus cycle when corpus luteum is available in one of the ovary.

Randel *et al*. (1996) stated that the $PGF_2\alpha$ have direct effects on follicular growth prior to luteinizing hormone (LH) surge to initiate ovulation but the reason is unknown. $PGF_2\alpha$ increases pituitary responsiveness to GnRH to release LH in the post partum cows. High fertility has been achieved with prostaglandin treatment. Synchrony of estrus and fertility with this product is good with cyclic females, such as virgin heifers, but cannot induce estrous in non-cycling cows (Islam, 2011).

Stevenson *et al*. (1997) reported 50.00 per cent pregnancy rate in heifers when administered with two doses of 25 mg $PGF_2\alpha$ at 14 days interval. Lopes *et al.* (2013) reported 58.80 per cent pregnancy rate in Holstein heifers treated with 25 mg $PGF_2\alpha$ every 11 days till exhibition of estrus.

2.2.3 Progesterone

Progesterone is considered as fertility hormone and treatment with proge sterone causes maintenance of high level of progesterone in the female's body even after regression of corpus luteum and synchronization of estrus occurs 2 to 5 days following progestin removal (Islam, 2011). It is widely used in the treatment of anoestrus with prime consideration of its safety. The withdrawal effect of the progesterone is essential for priming of hypothalamic pituitary axis for induction of next estrous cycle.

Exogenous progesterone suppresses LH release, alters ovarian function, suppresses estrus and prevents ovulation. Both progesterone and progestins have been used in the estrus synchronization protocols in cattle by oral source (melengesterol acetate), ear implant (Synco-mate-B, Crestar) and intravaginal (CIDR, PRID).

Imwalle *et al*. (1998) reported that pre-pubertal heifers exhibited corpus luteum and progesterone concentration similar to luteal phase within 10 days of their treatment with MGA feeding @ 0.5mg/animal/day. Higher estrus and pregnancy rates in prepubertal heifers were also reported by Rodrigues *et al*. (2013) with administration of intravaginal progesterone releasing device (CIDR) for 12 days followed by heat detection and insemination.

Progesterone or progestogens is used in acyclic animals to stimulate estrus by artificially inducing the luteal phase. Induced luteal phase then enables the accumulation of gonadotropins. When this progesterone source is suddenly stopped, there is release of gonadotropins followed by an LH peak and thus ovulation (Belloso *et al*., 2002).

Gonadotropins like PMSG and GnRH have been used in combination with progesterone in order to induce accessory corpus luteum by stimulating ovulation of dominant follicle of the first follicular wave, as well as the production of progesterone by primary CL (Schmitt *et al.*, 1996). Prostaglandins in combination with progesterone had also been used in synchronization protocol with variable results.

Zulu *et al*. (2000) reported that progesterone may be the treatment of choice for smooth ovaries. It mimic the ovarian cycle with favouring storage to release of gonadotropins, which results in LH surge and ovulation on the day of its withdrawal. It also primes the brain for exhibiting behavioral oestrus.

2.2.4 Progesterone supplemented protocol

Various protocols have been evolved for inducing cyclicity in delayed pubertal heifers. Progesterone supplementation to standard protocols at appropriate stage is also giving promising results in heifers. The literature related to this topic is given as under.

Jaeger *et al.* (1992) recorded an estrus induction rate of 71.4 per cent when the yearling heifers were fed MGA @ 0.5mg/animal/day for 14 days followed by an injection of 25mg $PGF_2\alpha$ 17 days after last MGA feeding.

Imwalle *et al*. (1995) reported that when pre pubertal heifers were fed with MGA @ 0.5mg/animal/day for 8 days, there was an induction of puberty in 100 per cent treated heifers compared to only 44 per cent in control group.

Anderson *et al.* (1996) observed that when prepubertal heifers were given either a single norgestomet implant or 3 implants placed for 10 days, induction of puberty was observed in 85.71 and 81.25 per cent of heifers in the single or 3 norgestomet implant groups, respectively.

Hall *et al.* (1997) reported that 82 per cent heifers attended puberty at the age of 12.5 months when an ear implant containing 6 mg Norgestomet was administered for 10 days.

Solorzano *et al.* (2004) reported an estrus induction rate of 78 per cent in virgin heifers when reused CIDR combined with 1 mg estradiol benzoate was administered and kept for 8 days and Al done 12 h after heat detection.

Kuroiwa *et al*. (2005) reported an oestrus induction rate of 83.3 and 80.0 per cent in heifers in early luteal phase treated with PRID with or without a capsule including 10mg estradiol benzoate left in situ for 12 days.

Cetin *et al.* (2007) observed 86.2 per cent fertile estrus in acyclic post pubertal Holstein heifers when implant containing 6 mg norgestomet was implanted for 11 days plus an injection of 3 mg norgestomet and 5 mg oestradiol valerate given intramuscularly at the time of implant insertion. Al was carried out twice at 48 and 72 h later.

Ozyurtlu *et al.* (2009) reported induced estrus rate of 73.08 per cent in acyclic Holstein heifers treated with PRID for 11 days and an additional injection GnRH (0.0042 mg bruserelin acetate) on the day of PRID withdrawal.

Polat *et al.* (2009) recorded 93.90 per cent oestrus induction rate in delayed oestrus heifers with inactive ovaries after treating with PRID for 12 days followed by FTAI performed at 48 and 72 h.

An estrus induction rate of 85.00 per cent with mean interval response of 57.4 ± 2.5 h was recorded in cycling and prepubertal heifers after MGA feeding for 13 days and an injection of $PGF_2\alpha$ 19 days later (Mallory *et al.* 2010).

Chaudhari *et al.* (2012) treated delayed pubertal anoestrus Kankrej heifers with Crestar implant for 9 days followed by insemination at detected estrus. All the animals exhibited estrus within treatment response interval of 25±0.94 h with mean duration of 18.88±1.45 h.

Ghallab *et al*. (1984) reported improved conception rate of 61.00 per cent in crossbred heifers after treatment with norgestomet ear implant. Norgestomet was given in two occasions, 16 days apart for 8 days each. Injection of 3 mg norgestomet and 5 mg estradiol valerate were given on the day of first implant administration.

Jaeger *et al.* (1992) recorded conception and pregnancy rate of 64.20 and 48.70 per cent in crossbred yearling heifers receiving MGA feeding @5mg/animal/day for 14 days followed by an injection of 25 mg $PGF_2\alpha$ 17 days after the last MGA feeding. Heifers were inseminated 12 h post estrus detection.

Cavalieri *et al.* (1998) recorded pregnancy rate of 50.35 per cent in heifers receiving Norgestomet ear implant for 17 days and CIDR inserted on day 14 which was then removed 23.5±0.07 h later, with insemination done at detected estrus.

Solorzano *et al.* (2004) reported pregnancy rate of 69.00 per cent in virgin heifers when reused CIDR combined with 1mg estradiol benzoate was administered and kept for 8 days and Al done 12 h after heat detection.

Kuroiwa *et al*. (2005) suggested that a PRID treatment from 2 days after ovulation for 12 days in the presence or absence of EB has an effect on the synchronization of estrus and produces a beneficial conception rate in heifers. 80.00 and 100 per cent conception rates were reported in progesterone with and without estradiol benzoate groups, respectively.

Cetin *et al*. (2007) observed pregnancy rate of 48.20 per cent in acyclic post pubertal Holstein heifers when treated with implant containing 6 mg norgestomet for 11 days plus an injection of 3 mg norgestomet and 5 mg oestradiol valerate intramuscularly at the time of implant insertion. Double insemination was carried out at 48 and 72 h later.

Ozyurtlu *et al*. (2009) reported pregnancy rate of 53.85 per cent in acyclic Holstein heifers treated with PRID for 11 days and an additional injection GnRH (0.0042mg bruserelin acetate) on the day of withdrawal of PRID.

Polat *et al*. (2009) recorded 54.60 per cent pregnancy rate in delayed oestrus heifers with inactive ovaries after insertion of PRID for 12 days followed by FTAI performed at 48 and 72 h.

Gottschall *et al*. (2011) obtained a total Pregnancy rate of 50.00 and 71.40 per cent from 221 numbers of 2 (n=144) and 3 (n=77) years old heifers when given the following three treatments: 5 ml (5 mg) injectable progesterone, 8 ml (80 mg) and third used intravaginal progesterone device for 8 days

Chaudhari *et al*. (2012) recorded an overall conception rate of 33.33 per cent in Delayed pubertal anoestrus Kankrej heifers treated with Crestar implant for 9 days followed by insemination at detected estrus.

2.2.5 Progesterone supplementation with GnRH

Hittinger *et al.* (2004) reported 94.00 per cent ovulation rate following $PGF_2\alpha$. induced luteolysis in post pubertal Holstein heifers treated with CIDR on day 0 along with 100pg GnRH and then divided into three groups: $PGF_2\alpha$ on day 7 and CIDR removal on day 8; $PGF_2\alpha$ concurrent with CIDR removal on day 7; or $PGF_2\alpha$ along with CIDR removal on day 8.

Leitman *et al*. (2009) recorded an estrus induction rate of 94.00 per cent in prepubertal or cycling heifers administered with CIDR insert for 14 days, followed by GnRH on day 23 and $PGF_2\alpha$ on day 30. Artificial Insemination was done at detected estrus.

Lima *et al.* (2011) recorded an estrus induction rate of 61.40 per cent in heifers synchronized for insemination with CIDR insert from day 0 up to day 5, followed by $PGF_2\alpha$ on day 5. GnRH was given at the time of Al done 72 h after $PGF_2\alpha$.

Lamb *et al*. (2006) obtained conception rate of 49.30 per cent in heifers after treatment with CIDR Implant for 7 days, followed by $PGF_2\alpha$ on 7th day, GnRH 60 h later and insemination done 84 h after $PGF_2\alpha$ injection.

Leitman *et al.* (2009) recorded conception rate of 58.00 per cent followed by pregnancy rate of 81.00 per cent in prepubertal or cycling heifers administered with CIDR insert for 14 days, followed by GnRH on day 23 and $PGF_2\alpha$, on day 30. Insemination was done at detected estrus.

Lima *et al.* (2011) carried out synchronization for first insemination in heifers with CIDR insert from day 0 up to day 5, followed by $PGF_2\alpha$ on the day of insert removal. Second GnRH was given concurrent with Al 72 h after $PGF_2\alpha$ Pregnancy rate of 55.00 per cent was observed.

2.2.6 Progesterone plus gonadotropin protocol

Okazaki *et al.* (2007) observed increased in CL number, progesterone concentration and improved pregnancy rates in Holstein heifers receiving CIDR (7-9 days) along with an injection of 2 mg estradiol benzoate (IM). Injection $PGF_2\alpha$ (15 mg, IM) was administered on the day of CIDR removal, followed by 100 mg GnRH (IM) 2 days later. The heifers then either received 1000 I.U. eCG at the time or

48h before $PGF_2\alpha$ injection/CIDR removal. The pregnancy rate was found to be greater in eCG supplemented groups than control.

Chaudhari *et al*. (2012) treated delayed pubertal anoestrus Kankrej heifers with Crestar for 9 days plus 500 IU of PMSG on implant removal day and an addition of injection receptal (@2m1) at the time of insemination. Estrus induction response of 100 percent within mean interval of 22.6831.46 h and mean estrus duration of 13.4811.92 h and an overall conception rate of 50.00 per cent was obtained.

Bridges *et al.* (2009) successfully treated beef heifers with CIDR for fourteen days followed by ovsynch protocol and reported an estrus induction rate of 63.05 per cent.

Khade *et al*. (2011) reported an estrus induction rate of 100 per cent in anoestrus Gir heifers when treated with CIDR along with ovsynch protocol.

Martinez *et al*. (2012) reported that the size of the dominant follicle in beef heifers prior to ovulation was significantly increased (12.830.4 vs 11.430.4) in CIDR plus ovsynch protocol than ovsynch protocol.

Lamb *et al*. (2006) recorded a conception rate of 53.00 percent with an overall conception rate of 87.00 percent in replacement beef heifers when treated with CIDR insert kept for 7 days along with injection GnRH, followed by $PGF_2\alpha$, injection at the time of removal and second GnRH injection 60 h after $PGF_2\alpha$ injection. Insemination was done 84 h after $PGF_2\alpha$ injection.

Bridges *et al*. (2009) obtained 75.02 and 70.04 per cent conception and pregnancy rate in beef heifers treated with CIDR for fourteen days followed by ovsynch protocol.

Khade *et al*. (2011) studied combination of Ovsynch-CIDR protocol in pubertal anoestrus heifers, where animals were bred by FTAI and ultrasonography was carried out on day 26 post-insemination. The first service, second service and overall conception rates were 50.00, 33.33 and 66.66 per cent, respectively. Nak *et al*. (2011) reported higher pregnancy rate of 53.30 per cent in acyclic heifers treated with ovsynch plus norgestomet ear implant and double TAI performed at the time of second GnRH injection and 18 to 20 h later. Ghuman *et al.* (2012) treated prepubertal buffalo heifers with ovsynch plus CIDR insert for 7 days. The

first service conception rate and overall conception rate was 35.70 and 50.00 per cent, respectively.

In ovsynch protocol, an injection of first gonadotropin-releasing hormone (GnRH) given randomly during the estrous cycle causes ovulation or luteinization of large follicles present in the ovary and synchronizes the recruitment of a new follicular wave (Thatcher *et al*., 1989), followed by an injection of prostaglandins 2α ($PGF_2\alpha$) on 7^{th} day which induces regression of the corpus luteum and allows for final maturation of the synchronized dominant follicle. Second injection of gonadotropin-releasing hormone (GnRH) 48 h after $PGF_2\alpha$ injection synchronizes ovulation of the dominant follicle, occuring approximately 28 h later (Pursley *et al.*, 1995).

The Ovsynch protocol was influenced by the number of follicular waves or length of the follicular wave (Pursley *et al.*, 1997b) as well as the stage of oestrous cycle when the first GnRH dose was administered (Vasconcelos *et al*., 1997; Vasconcelos *et al.*, 1999). Cows were inseminated once at fixed time between 0 to 32 h after second GnRH injection resulting in maximum conception at 16 h (Pursley *et al.*, 1998).

Pursley *et al.* (1995) observed a synchronization rate in 87.00 per cent dairy heifers, when treated with GnRH- $PGF_2\alpha$ respectively. 100 per cent synchronization rate was also observed for dairy heifers treated with Ovsynch protocol.

Moriera *et al*. (2000) reported an overall ovulation rate of 90.40 per cent in cycling Holstein heifers treated with ovsynch/TAI protocol where first GnRH was given at different days of estrous cycle.

Naik *et al*. (2005) reported an oestrus induction rate of 29.41 per cent in both cyclic and non-cyclic dairy heifers treated with standard Ovsynch protocol. Ghuman *et al.* (2009) reported an oestrus response of 82.00 per cent in anoestrus buffalo heifers treated with ovsynch protocol. Vijayaranjan *et al.* (2009) recorded oestrus induction rate of 100 per cent in crossbred heifers at random stages of estrous cycle when treated with ovsynch protocol. Karen and Darwish (2010) reported that 33.00 per cent acyclic buffalo heifers ovulated after 2^{nd} GnRH injection in 12 h treatment with ovsynch protocol. Derar *et al.* (2012) obtained an ovulation rate of 87.50 per cent on first and 100 per cent on second GnRH injection when cyclic buffalo heifers were treated with standard ovsynch protocol.

Castilho *et al.* (2000) treated Girolando heifers (Gir × Holstein) aged 20 to 30 months with modified Ovsynch protocol where the second GnRH injection was replaced by hCG. The heifers were administered with 8 mg GnRH on day 0, 25 mg $PGF_2\alpha$ injection 7 days later and an injection of hCG in split dose of 1000 IU intravenously, and 2000 IU was given intramuscularly 248 later. Insemination was done 20h post hCG injection without estrus detection. Pregnancy rate of 50.00 per cent was recorded.

Moriera *et al.* (2000) conducted an experiment in cycling Holstein heifers with Ovsynch/TAI protocol at different stages of the estrous cycle. They started with an injection of first GnRH agonist (8pg,I/M) at day 2, 5, 10, 15, or 18 of the cycle, followed by $PGF_2\alpha$ injection (25mg, I/M) on day 7 and second GnRH injection at 36 h after $PGF_2\alpha$. Heifers were then inseminated 16 hrs after second GnRH injection. An overall pregnancy rate diagnosed by ultrasonography at day 28 following insemination was found to be 37.50 per cent.

Naik *et al*. (2005) reported a first service pregnancy rate of 58.28 per cent in both cyclic and non-cyclic dairy heifers treated with standard Ovsynch (n=34) protocol.

Rose *et al*. (2008) reported conception rate of 27.00 per cent with standard ovsynch protocol when used in mature, nulliparous Holstein heifers. Roy and Prakash (2008) stated that ovsynch treatment to repeat breeder buffalo heifers yielded a conception rate of 45.50 per cent, and it can also be used to induce estrus during harsh climate of summer season.

Ghuman *et al*. (2009) recorded conception rate of 18.00 per cent when anoestrus buffalo heifers were treated with ovsynch protocol. Vijayaranjan *et al* (2009) observed first and second service conception rate of 20.00 and 37.50 with an overall of 50.00 per cent in crossbred heifers treated with ovsynch protocol at random stages of estrous cycle.

Naik *et al*. (2011) recorded conception rate of 26.30 per cent in acyclic heifers treated with ovsynch protocol. Derar *et al.* (2012) treated cyclic buffalo heifers with standard ovsynch protocol, and a conception rate of 62.50 per cent was obtained The review of literature cited above available through various resource may help in discussing the results of the experimentation and will help to interpret the observations.

2.3 Micro mineral profile

Number of experiments were conducted on the importance of different minerals on the reproductive status of the animal. Low mineral levels in blood results in reproductive disorders and infertility because these are constituents and activators of many metabolic and hormone related enzymes. Minerals provide the essential nutrients for metabolic functions such as growth, development, immunity and reproduction.

Hidiroglouin (1979) reported that calcium (Ca), phosphorus (P), zinc (Zn) and manganese (Mn) affected post-partum reproduction in cattle. Moreover, Campbell *et al.* (1999) examined several cows with reproductive disorder and found that deficiency of mineral elements like P, Zn, Cu and Mn are associated with subnormal fertility and anestrus conditions.

Along with different minerals, different forms of the minerals also put their impact on the reproduction of CB animals. Boland *et al*. (1996) examined different forms of Cu, Zn, Mn and Se (inorganic versus organic) and found that there is increase in the conception rates and days to first service by replacing inorganic minerals with their organic form. Ballantine *et al.* (2002) also reported similar improvements from replacing inorganic sulphate salts of Cu, Mn, Zn and Co with organic forms.

Puvarajan *et al.* (2013) reported that crossbred heifers in rural hamlets of Madurai District after supplementation 92.16% heifers (153/ 166) exhibited estrus. Of these 149(97.38%) conceived with 1 to 4 insemination. The first conception rate was 28.18 per cent and second insemination have maximum conception rate (65.77%). However, progressive decrease in conception rate was observed from third to fourth insemination. The overall conception rate was 27.17 per cent.

Amin (2014) opined that cessation of estrus with suppression of ovulation or ovulation without estrus result from sub maintenance of feeding. Another important effect of under-nutrition is more number of services per conception which reduces the production level of animal. Moreover he provided some of the available information regarding relation of nutrition with reproduction of animals.

2.3.1 Calcium

Calcium deficiency can upset the normal reproduction possibly due to lack of tone of uterine muscle. About half of the calcium present in the plasma is bound to proteins in a nondiffusible form (45-50 %). A small amount (5 %) of the remainder is complexed with citrate or phosphate and the ionized calcium (Ca++), which accounts for nearly half of the blood calcium, is of clinical importance. Only the ionized calcium is physiologically active.

Jain and Pandita (1995) studied mean plasma calcium levels in normal cyclic and $PGF_2\alpha$ treated cows (before estrus/treatment and at estrus). The values of calcium were 7.86 ± 0.78, 8.79 ± 0.92 and 7.19 ± 0.94, 7.53 ± 0.86 mg/dl, respectively.

Lodhi *et al.* (1998) reported significantly higher calcium concentration in cycling buffaloes as compared to non-cycling, repeat breeder and endometritic buffaloes, whereas the inorganic phosphorus was significantly higher in both repeat breeder and cycling group of buffaloes ($P<0.05$) as compared to non-cycling and endometritic buffaloes.

Kalita *et al.* (1999) reported higher level of calcium in normal cycling cows than in repeat breeder and anestrus cows, and opined that ration Ca:P has adverse effect on fertility of cows.

Dutta *et al.* (2001) observed that the mean circulatory level of serum calcium was significantly higher ($P<0.01$) in normal cyclic (10.73 ± 0.08 mg %) than in anoestrus (9.54 ± 0.22 mg %) or repeat breeding cows of Assam (9.95 ± 0.18 mg %).

Chandrakar *et al.* (2003) reported significantly lower ($P<0.01$) level of serum calcium in normal fertile crossbred cows (6.17 ± 0.17 mg %) as compared to repeat breeding cows (9.63 ± 0.36 mg %).

Ahlawat (2003) reported that the mean calcium levels in conceiving and non-conceiving crossbred cows were 9.09 ± 0.25 and 8.59 ± 0.38 mg per cent, respectively.

The ratio of calcium to phosphorus may affect ovarian function through its blocking action on pituitary gland. This results in prolongation of first estrus and ovulation after parturition (Kumar, 2003).

Patel and Dhami (2005) recorded overall mean values of calcium for conceived and non-conceived animals as 7.73 ± 0.11 and 8.03 ± 0.07 mg/dl, respectively.

Patel *et al*. (2005) reported the mean plasma calcium level to be significantly lower and phosphorus to be higher ($P<0.05$) in GnRH and $PGF_2\alpha$ treated repeat breeder HF cows as compared to untreated control group.

Mishra *et al.* (2007) recorded serum calcium concentration to be 8.77 ± 0.36 mg per cent in anoestrus cows, which was much lower than in case of repeat breeder cows (11.27 ± 0.59 mg %).

Singh *et al.* (2007) reported significantly lower concentration of serum calcium in cyclic as compared to anoestrus Hariana cows.

Rajesh Kumar *et al*. (2009) reported serum calcium in normal cyclic and repeat breeding crossbred cows as 9.66 ± 0.27 and 8.90 ± 0.21 mg/dl, respectively, which differed significantly ($P<0.05$).

Butani *et al*. (2011) observed that the serum calcium and phosphorus levels were non-significantly lower in repeat breeding than normal cycling buffaloes (8.75 ± 0.22 vs. 9.17 ± 0.47 and 9.09 ± 0.28 vs. 9.60 ± 0.39 mg/dl).

Parmar *et al.* (2013) reported overall mean plasma calcium concentration in repeat breeding Gir cows as 11.918 ± 0.159 mg/dl. The levels were lower in GnRH treated as compared to untreated control, without significant differences between weeks or between conceived and non-conceived groups.

2.3.2 Phosphorus

The total phosphorus is distributed chiefly as inorganic phosphorus, organic acid soluble phosphate ester and lipid phosphorus. Infertility due to nutritional deficiency is usually characterized by a failure of estrus or a cessation

of estrous cycle, where mineral deficiency mainly includes phosphorus than any other trace minerals.

Bhaskaran and Abdulla Khan (1981) documented that the marginal deficiency of phosphorus is enough to cause disturbances in pituitary-ovarian axis, without manifesting specific systemic deficiency symptoms.

Jain and Pandita (1995) studied the plasma phosphorus levels in normal cyclic cows and $PGF_2\alpha$ treated cows (before oestrus/treatment and at oestrus). The values were 5.10 ± 0.20, 5.47 ± 0.34 and 4.02 ± 0.20, 5.26 ± 0.21 mg/dl, respectively. The phosphorus levels varied significantly ($P<0.05$) between control and $PGF_2\alpha$ treated groups.

Lodhi *et al*. (1998) reported significantly higher inorganic phosphorus concentration in both repeat breeder and cycling group of buffaloes ($P<0.05$) as compared to non-cycling and endometritic buffaloes

Srivastava and Sahni (2000) recorded non-significantly higher plasma inorganic phosphorus at the time of AI in both village cows and buffaloes that conceived (4.16 ± 0.15 and 4.96 ± 0.14 mg/100 ml) than those that did not conceive (3.64 ± 0.12 and 3.38 ± 0.10 mg/100 ml).

Dutta *et al*. (2001) observed that the mean circulatory level of serum inorganic phosphorus was significantly higher ($P<0.01$) in normal cyclic (4.22 ± 0.07 mg %) than in anoestrus (3.48 ± 0.12 mg %) or repeat breeding cows of Assam (3.62 ± 0.14 mg %).

Cetin *et al*. (2002) reported the mean phosphorus levels to be 5.00 ± 0.23 and 5.69 ± 0.12 mg/dl in repeat breeding and fertile cows, respectively.

Chandrakar *et al*. (2003) reported significantly higher ($P<0.01$) level of serum phosphorus in normal fertile (4.60 ± 0.04 mg %) than the repeat breeding crossbred cows (3.98 ± 0.05 mg %).

Patel and Dhami (2005) recorded the overall mean values of phosphorus for conceived and non-conceived cows as 6.58 ± 0.10 and 6.75 ± 0.07 mg/dl, respectively.

Mishra *et al.* (2007) recorded values of scrum phosphorus as 5.99 ± 0.28 and 6.49 ± 0.38 mg per cent in anoestrus and repeat breeding cow, respectively.

Kumar *et al*. (2009) reported serum phosphorus in normal cyclic and repeat breeding crossbred cows as 8.76 ± 0.25 and 7.35 ± 0.17 mg/dl, respectively, which differed significantly ($P<0.05$). The level was also significantly higher ($P<0.05$) in GnRH treated group than normal cyclic and untreated control groups.

Parmar *et al*. (2013) reported overall mean plasma inorganic phosphorus concentrations as 8.193 ± 0.123 mg/dl. The levels were insignificantly lower in GnRH treated repeat breeding Gir cows as compared to untreated control, without significant differences between weeks or between conceived and non-conceived groups.

Patel (2013) reported plasma inorganic phosphorus in conceived and non-conceived repeat breeding crossbred cows in mid cycle PG protocol as 5.99±0.10 and 6.15±0.18 mg/d1. The levels were higher in non-conceived groups than conceived groups, without significant difference.

2.4 Bio Chemical profile

2.4.1 Glucose

Manowar and Singh (2001) reported the blood glucose in non-cyclic heifers, cyclic heifers and cyclic lactating cows as 50.70 ± 2.80, 58.70 ± 2.99 and 64.06 ± 3.59mg/dl ($P < 0.01$), respectively.

Cetin *et al.* (2002) found relatively higher blood glucose level at oestrus in repeat breeder than the fertile cows (52.33 ± 2.20 vs. 46.26 ± 1.68 mg %), but the serum total protein levels were almost identical (8.26 ± 0.13 vs. 8.50 ± 0.13 g %).

Ahlawat (2003) reported the mean total protein levels in cyclic and non-cyclic crossbred cows to be 6.94 ± 0.11 and 7.74 ± 0.13 g/dl ($P < 0.05$), respectively. The values were 6.80 ± 0.17 and 7.12 ± 0.14 g/dl in conceiving and non-conceiving cows, respectively. He also reported that the mean blood glucose levels in cyclic and non-cyclic cattle were 93.67 ± 3.78 and 49.15 ± 2.14 mg per cent, respectively, which differed significantly ($P < 0.01$).

Chandrakar *et al.* (2003) reported significantly ($P < 0.01$) higher blood glucose (70.41 ± 0.87 vs 60.43 ± 0.73 mg%) and serum total protein (8.02 ± 0.30 vs 6.94 ± 0.08 g%) in normal fertile cows as compared to repeat breeders.

Dhoble *et al.* (2004) compared the variation in blood biochemical constituents during post-partum period in cross bred cows and found increased blood glucose at the time of parturition (59.17 mg/dl) in all experimental cows but the level declined (48.96mg/d1) up to one month in all experimental cows that become pregnant, but it was 57.05 and 44.31 in the cows that were not conceived.

Lakum (2004) found significantly higher plasma total protein in normal HF cows as compared to repeat breeders (7.87 ± 0.18 vs 6.50 ± 0.07 g/dl). No significant difference was observed between normal fertile cows and treated conceived cows suffering from repeat breeding condition.

Patel (2004) reported that mean glucose level was higher in f1F cows of PGF2α group than the GnRH group from 10th week post-partum with significant difference at 16th week postpartum and in the overall pooled means (68.44 ± 0.83 vs 63.99 ± 0.84mg/dl). Moreover, the trend of higher levels observed in GnRH control than the treatment group was reverse between $PGF_2\alpha$ control and treatment groups. He also reported that weekly mean plasma triglycerides concentrations of GnRH and PGF2α groups did not differ significantly between weeks or even between groups at any of the intervals postpartum, including the pooled means (6.51 ± 0.09 vs 6.64 ± 0.08 g/dl).

Singh *et al.* (2007) reported that the normal cyclic animals had comparatively higher blood glucose concentration compared to anoestrus animals. Glucose (mg/dl) concentration was 51.67 ± 2.24 and 58.33 ± 6.22 in anoestrus and normal cyclic animals, respectively. He also reported that the normal cyclic animals (7.08 ± 0.0C g/dl) had a significantly lower concentration of serum total protein as compared tc anoestrus cows (6.55 ± 0.21 g/dl) and there was no relation of total protein-concentration with the exhibition of estrus symptoms.

Pattanayak *et al.* (2010) investigated into the effect of some fertility improvement traits, had observed value of serum glucose as 66.02±5.44 mg/dl repeat breeder animal on the day of estrus.

Krupakaran (2013) studied about blood biochemical constituents in cross breed (Jersey x ND) heifers during estrus or anestrous conditions and found glucose 57.32±3.11, 40.01±1.88 mg/dl, respectively.

Agrawal *et al*. (2015) conducted a study and pointed out that high incidence of repeat breeding and anoestrous in Sahiwal cows has been attributed to a decrease in circulation of glucose, total protein, calcium and phosphorus.

Veena *et al*. (2015) studied to investigate certain blood parameters indices of the post-partum reproductive performance in cattle. The blood samples were collected from 25 lactating animals on 10th day of calving and thereafter at weekly intervals up to 12 weeks post-partum. Animals with more amount of blood glucose (55.49mg/d1) came to heat within 2 months compared to those with lesser blood glucose (50.20mg/d1), which came to heat after 2 months post-partum.

Qureshi *et al*. (2016) studied to find relationship of blood metabolites with reproductive cyclicity in dairy cows and found that the serum glucose level was lower (39.93 ± 3.14 mg/dl) two months of lactation and showed an increasing trend (49.63 ± 2.47 mg/di) towards commencement of estrus as well as during estrus (48.20 ± 2.42 mg/dl) and then a declining trend was observed.

2.4.2 Total protein

Cetin *et al*. (2002) compared the blood biochemical parameters of 15 repeatbreeder cows and 30 fertile cows on the day of estrus and the levels of glucose, total protein, phosphorus were found to be 46.26±1.68 mg%, 8.26±0.13 gm%, 5.69±0.12mg% in fertile cows and 52.33±2.20 mg%, 8.50±0.14 mg%, 5.00±0.23 mg% in repeat breeder cows.

Ahlawat (2003) reported the mean total protein levels in cyclic and non-cyclic crossbred cows to be 6.94 ± 0.11 and 7.74 ± 0.13 g/dl ($P < 0.05$), respectively. The values were 6.80 ± 0.17 and 7.12 ± 0.14 g/dl in conceiving and non-conceiving cows, respectively. He also reported that the mean blood glucose levels in cyclic and non-cyclic cattle were 93.67 ± 3.78 and 49.15 ± 2.14 mg per cent, respectively, which differed significantly ($P < 0.01$).

Jayanthi *et al.* (2003) found a significantly lower total serum protein (6.40±0.05 gm%), calcium (10.03±0.03 mg%) and inorganic phosphorus (4.12±0.03 mg%) in repeat breeders than the normal cyclic cows.

Ahmed *et al*. (2004) reported that the total protein level was significantly higher (P<0.05) in endometritic (19.16 :F 1.0001) cows as compared to cyclic (9.19 ± 0.45 g/d1) and noncyclic 15.23 ± 0.89 g/dL) cows. The level in non-cyclic cows was significantly (P<0.05)) higher than that of cyclic animals. Higher level of total scrum protein was associated with low fertility particularly if the animals were fed over protein diet.

Dogan and Cetin (2004) observed lower level of serum protein 6.83±0.71 gm/d1 in repeat breeder and 7.51, 10.86 gm/dl in normal cycling cows.

Das and Bisoi (2005) compared the level of total protein in blood samples of six normal oestrus and six repeat breeder cows which showed a concentration of 7.12±0.25 gm/dl in normal cows and 7.03±0.2 gm/dl in repeat breeder cows.

Patel *et al.* (2006) studied the blood profile on day of estrus of 08 fertile Holstein Friesian cows with 16 infertile Holstein Friesian cows in well managed farm condition. They found the value of glucose, total protein, calcium and phosphorus to be 68.31±4.25 mg%, 6.92±0.45 gm%, 7.13±0.39 mg%, 6.56±0.34 mg% in fertile cows those conceived and 68.93±2.65 mg%, 6.42±0.42 gm%, 7.91±0.20 mg%, 6.14±0.25 mg%, respectively cows which fails.

Patel and Dhami (2006) monitored recently calved HF cows (24) through clinical diagnosis and weekly plasma profile of total protein from the day of calving till 21st week postpartum. Study included GnRH (Receptal) and $PGF_2\alpha$ (Lutalyse) treatment at day 48-49 postpartum in anoestrus and sub oestrus cows (6 each), respectively. The total protein concentrations of anoestrus (GnRH) and sub oestrus ($PGF_2\alpha$) groups did not differ significantly between weeks or even between groups at any of the intervals postpartum, including the pooled means (6.510.09 vs 6.64±0.08 g/dl). The mean levels in both GnRH and $PGF_2\alpha$ treatment, control and their pooled groups also did not vary between weeks, except in GnRH control group, where it was significantly low at first week (5.61±0.25 g/dl) and high at 12th (7.40±0.37 g/dl) and 18th (7.250.30 g/dl) week postpartum. Moreover, there was no significant difference between PGF2α treatment and control groups at any of the intervals

postpartum. The weekly variation in plasma total protein levels of conceived, non-conceived and pooled groups was not significant and so also between the groups, except at calving.

Aman Kumar *et al.* (2007) reported that the concentration of serum total protein in anoestrus crossbred cows was 5.19 ± 0.03 and in normal cycling cows it was 6.72±0.08 g%. The concentration of scrum total protein in blood of anoestrus cross bred cows was found significantly lower in comparison of normal cycling crossbred cows.

Singh (2007) reported that the normal cyclic animals had comparatively higher blood glucose concentration compared to anoestrus animals. Glucose (mg/di) concentration was 51.67±2.24 and 58.33±6.22 in anoestrus and normal cyclic animals, respectively. He also reported that the normal cyclic animals (7.08 ±0.09 g /dl) had a significantly lower concentration of serum total protein as compared to anoestrous cows (6.55±0.21 g/dl) and there was no relation of total protein concentration with the exhibition of estrus symptoms.

Kaneko *et al*. (2008) reported that normal range of serum total protein in cattle as 6.7 to 7.4 gm/dl, albumin3.03 to 3.55 gm/dl and globulin 3.00 to 3.48gm/d1.

Onita and Colibar (2009) made research on 20 (HF) cows and got total protein ing/dl on days 5-6, 20-21 and 40-41 postpartum as 7.23±0.08, 7.45±0.11 and 8.09±1.02, respectively. Total proteins had normal values (6.4-8.7 mg/dl) followed by an increase from 7.23 ± 0.08 (at 5-6 days postpartum) to 8.09 ± 1.02 (40-41 days Postpartum).

Rajesh *et al*. (2009) conducted a comparative field study on serum profile of metabolites of 21 anestrous, 10 sub-estrus, 31 repeat breeding and 8 normal cyclic crossbred cows revealed that the serum total protein was significantly ($P<0.01$) lower in repeat breeders and normal cyclic cows than in anestrous and sub-estrus cows.

Khan *et al*. (2010) undertook a study in six repeat breeding and six normally cycling Jersey-Sindhi crossbred cows. In repeat breeding cows, the highest and the lowest concentrations of plasma proteins were recorded on day 5 and 20 of the cycle, respectively. The repeat breeding cows had significantly lower ($P < 0.01$) concentration of protein when compared to normally cycling cows irrespective of the days of the cycle.

Knipakaran (2013) studied about blood biochemical constituents in cross bred (Jersey x ND) heifers during estrus or anestrous conditions and found total protein, albumin, globulin and A:G ratio 6.23 ± 0.011, 2.82 ± 0.08, 3.36 ± 0.12, 0.833 ± 0.25 g/dl, respectively) for estrus and 5.8310.12, 2.114-0.05, 3.72 ± 0.10, 0.567 ± 0.014 01 for anestrus, respectively.

Arnie *et al.* (2014) conducted a study on 28 repeat breeding cross bred cows during their postpartum period in their second to seventh lactation from different villages of Satara district. There was no significant difference between NC and RB crossbred cows in present study with respect to the levels of BUN, Globulin and Albumin/Globulin ratio. However, the reported values of HUN and AIG ratio were lower in NC than R.B crossbred cows. Significantly lower ($P<0.01$) concentration of scrum total protein in the R13 crossbred cows in comparison with the NC crossbred cows. The RI crossbred cows showed significantly lower ($P<0.01$) concentration of albumin when compared to NC crossbred cows. Similar finding is reported. This high level of albumin in normally cycling cows revealed increased demand for amino acids and protein for the biosynthesis of GnRH and LH to initiate ovulation.

2.4.3 Cholesterol

Sivaraman *et al*. (2002) did not find significant difference in serum total cholesterol levels of lactating and lactating pregnant Jersey crossbred cows (227.91±6.46 vs. 218.47±3.96 mg/100m1), but it was significantly lower in dry non-pregnant cows (176.17 ± 2.66 mg/100m1).

Ahlawat (2003) reported that the mean cholesterol levels in cyclic and non-cyclic crossbred cows were 195.43±4.56 and 109.80 ± 5.25 mg per cent, respectively, which differed significantly ($P<0.01$). The values were 164.35 ± 7.55 and 166.94±2.98 mg percent in conceiving and non-conceiving cows, respectively. He also reported that the mean triglyceride levels in cyclic, non-cyclic, conceiving and non-conceiving crossbred cows were 96.01±4.20, 102.51±4.52, 97.13±6.43 and 94.45±4.64 mg per cent, respectively.

Patel and Dhami (2004) conducted an investigation on postpartum HF cows to access the post-parttun plasma profile of cholesterol with or without GnRH and $PGF_2\alpha$ treatment. The mean cholesterol concentration of CmRI-1 and $PGF_2\alpha$ group

did not differ significantly (93.98±1.87 Vs 99.89±1.90 mg/d1) but significantly higher than control group. The animals that conceived had relatively low cholesterol during early postpartum and it increased significantly by 4th week and further by 19thweek postpartum compared to non-conceived group.

Prasad and Singh (2004a) conducted an experiment on anestrus crossbred cows to record the serum cholesterol concentration and its relation in resumption of estrous cycle after treatment with UM injection of $PGF_2\alpha$ and progesterone. Significant ($P<0.05$) increase in total serum cholesterol concentration was recorded on day 15 (208.0±0.93 mg/dl) in the animal of group who received 25 mg $PGF_2\alpha$ on day '0' and 1 ml Duraprogen at day 5 and 15 and 209.33±0.95 mg/dl in the animal of group who received $PGF_2\alpha$ at day '0', 2 ml. Duraprogen at day 5 and day 15. Also they observed that serum cholesterol concentration on the day of estrous was significantly higher (239.50 ±1.08 to 240.00 ± 8.00 mg/di).

Patel *et al*. (2005) got mean cholesterol level in conceived and non-conceived groups of repeat breeding cows as 218.34±1.15 and 223.79±0.77 mg/dl ($P<0.01$). The triglyceride level in conceived cows were significantly ($P<0.05$) higher than in non-conceived cows (63.19 ± 0.70 vs 60.45 ± 0.99 mg/dl).

Mishra and Mohanty (2007) reported that the lowest value of serum cholesterol was in repeat breeder as compared to normal cyclic cow and the value recorded was158.07 ± 12.26, and 171.23 ± 9.65, respectively.

Ahlawat and Derashri (2009) reported that the plasma cholesterol (165.43 ± 4.56 vs. 109.80 ± 5.25 mg %) levels were significantly ($P<0.01$) higher in cyclic when compared with anoestrus crossbred cows. The levels of plasma triglycerides, however, did not show significant variation between cyclic and anoestrus (96.01± 4.20 and 102.51± 4.52 mg %) cows or even conceiving and non-conceiving cows.

Kumar *et al*. (2009) reported serum total cholesterol in normal cyclic and repeat breeding crossbred cows as 279.94±26.33 and 215.31±10.66 mg/dl ($P>0.05$), respectively, without significant variation between conceived and non-conceived groups in either of the categories.

Bhoraniya *et al.* (2010) recorded the plasma total protein and total cholesterol profile in Ovsynch and ClDR treated anestrous Kankrej cows on day 0, 9(Al) and on day20 post-Al. Findings revealed that levels of protein and cholesterol were within normal physiological limits with overall means of 5.9310.09 g/dl and 162.69 ± 3.56 mg/d1, respectively. The levels neither varied significantly between sampling days of different protocols nor between protocols on any of the days/periods studied.

Khan *et al.* (2010) undertook a study in six repeat breeding and six normally cycling Jersey- Sindhi crossbred cows. The serum cholesterol concentration in the repeat breeding cows revealed the highest level on 10th day of the cycle, whereas the lowest concentration was observed on day 5 of the cycle. However, the difference was not significant between days of the cycle. The cholesterol levels were significantly lower (P= 0.01) in all the five days of the cycle in repeat breeding cows in comparison with the normally cycling cows.

Mahour *et al.* (2011) conducted a study on 40 postpartum anestrous and induced estrus crossbred cows. They got a mean total cholesterol level of 125.01 ± 9.65 mg/dl in the cows during anestrous before treatment and at induced estrus the level was 118.30 ± 10.21 mg/dl.

Nath (2014) conducted a study in normal and prolonged oestrus crossbred cows and found the mean level of serum cholesterol to be 109.33 ± 1.87 to 119.09 ± 2.30 mg/dl in control group, 105.12±3.85 to 115.56±4.05 mg/dl in 2 days duration group, 105.90±2.31 to 115.64±4.64 mg /dl and 105.24 ± 5.21 to 115.85 ± 3.74 mg/dl in 3 days and 4days duration group, respectively. Level of serum cholesterol varies significantly between days of oestrus; highest level being recorded on 10th day.

MATERIALS AND METHODS

3.1 Place and period of study

The present study was undertaken in Department of Animal Reproduction Gynaecology and obstetrics, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar, in collaboration with AICRP on Nutritional and Physiological Approaches for Enhancement of Reproductive Efficacy in Animals (NPAERPA) project. The field work was carried out in some villages of Kakatpur Block of Puri District.

The Kakatpur block is located between 20° 00' North latitude and 86° 19' East longitudes at an altitude of 19.3 meters. The trial was conducted during month of January 2020 to March 2020.

3.2 Source of animals

Crossbred pre pubertal heifers owned by farmers of different villages in the Block- Kakatpur, in district of Puri were considered for present investigation. The animals belonging to rural farmers were maintained by traditional animal husbandry practices. The animals were subjected to standard feeding and managemental practices with provision of quality mineral supplement provided by the project.

3.3 Selection of animals

Pre pubertal heifers of different villages in the block of Kakatpur were surveyed, from where total 30 heifers were selected for carrying out the investigation. Heifers of around one year age and having body weight of 150 kgs were considered as pre pubertal. Certain preliminary observation of experimental heifers pertaining to age, body weight habitat, behaviour of the animals were ascertained from the owner and from the clinical records to serve as guidelines.

3.4 Gynaeco-clinical examination

The animals were properly restrained in a trevis, the hind quarters cleaned thoroughly with soap water and wiped with clean towel and per-rectal examination was done. Internal reproductive organs were examined by inserting lubricated cleaned

hand. After back racking, the cervix was palpated through the rectal wall on the ventro-medial aspect of the pelvic brim. Body of the uterus and uterine horns were palpated by using cervix as a guideline on either side of the pelvic brim or in pelvic cavity. The contour, consistency, tonicity and patency of the cervix and uterine horns were assessed by careful manipulation and handling. The ovaries were palpated gently after retracting the cervix and uterus towards cranio-ventral and slightly lateral to the bifurcation of uterine horns. It was palpated as distinct rounded mass on either side of the body of the uterus. Presence or absence of CL was assessed. The dimensions of the ovary and the dynamic structure (follicle/CL at different stages) present on the ovary were palpated. Heifers with subactive ovaries, which are soft, round and have follicles or soft flocculating areas were considered as pre pubertal.

3.5 Treatment groups

The selected animals were equally divided into 5 groups (n=6) and subjected to different therapeutic protocols.

Group I : Containing 6 pre pubertal heifers with no treatment considering as control.

Group II : Containing 6 pre pubertal heifers to be treated with Mineral supplementation (Area specific mineral mixture (ASMM) supplementation @ 50 g/day/ animal for 60 days)

Group III : Containing 6 pre pubertal heifers to be treated with 3 doses of injectable progesterone followed by Inj. of Prostaglandin* after 4 days along with Mineral supplementation

Group IV : Containing 6 pre pubertal heifers to be treated with one dose of GnRH** along with Mineral supplementation.

Group V : Containing 6 pre pubertal heifers to be treated with GnRH (along with 1st dose of progesterone), 3 doses of Progesterone*** at 4 days interval and Inj. of Prostaglandin after 4 days along with Mineral supplementation.

---

*CLOSTENOL, Zydus Animal Health Limited

**RECEPTAL, MSD Pharmaceutical Limited

***P-DEPOT, Zydus Animal Health Limited

3.6 Preparation of mineral mixture

The area specific mineral mixture was prepared as per the reported formulation of Mohapatra *et al*. (2012). The ingredient composition of the area specific mineral mixture is given below. Individual packets of 1.0 kg mineral mixture prepared and distributed among the farmers for feeding of animals in groups II, III, IV and V.

Composition of area specific mineral mixture

Ingredients	Amount/1000 g
Dicalcium phosphate	800 g
Cupric sulphate	200 mg
Potassium iodide	1.63 mg
Manganous sulphate	400 mg
Zinc sulphate	500 mg
Wheat flour	To add up to 1000 g

3.7 Blood collection and serum preparation

The animals after being properly restrained in a trevis, blood was collected from pre (on the day of examination) and post (day 10 post AI) treatment for estimation of Serum biochemical and hormonal profile. Seven ml blood was collected from each of the animal from jugular vein adopting proper collection procedure with clean and sterilized syringe and needle. The collected blood sample was put in a sterilized clot activator.

After collection of blood in sterilized clot activator, it was kept in a slanting position for 30 to 60 min without any disturbances. When serum oozed out, it was collected by the help of a micro pipette and kept in a vial without contamination and centrifuged at 2500 rpm for 10 minutes. Four ml of serum was collected and transferred to a dry sterile cryovial and finally kept at -20°C in deep freeze for future experiment.

Blood was collected twice for estimation of Bio chemical parameters (pre treatment – on 0day and post treatment – on the day of estrus/ day 60 for heifers that did not come to estrus) and thrice (Pre treatment-0 day, Post treatment day-Day of

estrus /Day 60 and on 23rd day post AI/ day 83 following treatment)for estimation of serum Progesterone levels.

3.8 Estimation of serum biochemicals

3.8.1 Serum calcium

Calcium was estimated with OCPC method with the help of an estimation kit*

Principle

Calcium in an alkaline medium combines with O-Cresolphthalein Complexone to form a purple coloured complex. Intensity of the colour formed is directly proportional to the amount of calcium present in the sample.

Calcium + OCPC → Purple Coloured Complex

Materials required

Photometer analyzer with standard thermostatic cuvette holder, Micropipette and appropriate laboratory equipment.

Contents

L1: Buffer Reagent 150ml L2: Colour Reagent 150ml

S: Calcium Standard (10 mg/dl) 5 ml Composition

DEA Buffer 500 mM; OCPC 0.6 mM; 8 Hydroxyquinilone, Detergent

Procedure

Distilled water, Reagent (L1&L2), calcium standard and test sample were pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T) in following sequence.

Additional sequence	B (ml)	S (ml)	T (ml)
Buffer Reagent (L1)	0.5	0.5	0.5
Colour reagent (L2)	0.5	0.5	0. 5
Distilled water	0.02	-	-
Sample	-	-	0.02

*CREST BIOSYSTEMS, a division of CORAL clinical systems, Gitanjali, Dr. Antonio Do RegoBagh, Alto SantacruzBambolim Complex P.O., Goa-403202, INDIA.

All were mixed well and incubated at Room temperature for 5 minutes. Observations were noted for the absorbance of the Standard (Abs. S) and Test Sample (Abs. T) against Blank, within 60 minutes using wave length/filter of 570 nm (Hg 578 nm), Temperature: R.T Light path: 1 cm.

Calculations

Calcium (mg/dl) = Absorbance of T/Absorbance of S x100

3.8.2 Serum phosphorous

Serum phosphorus level was estimated using Molybdate U.V method with the help of estimation kit*.

Principle

Phosphate ions in an acidic medium react with ammonium molybdate to form a phosphomolybdate complex. This complex has an absorbance in the ultraviolet range and is measured at 340 nm. Intensity of the complex formed is directly proportional to the amount of inorganic phosphorus present in the sample.

Phosphorus + Ammonium Molybdate → Phosphomolybdate Complex.

Contents

L1: Acid Reagent 60 ml

L2: Molybdate Reagent 15 ml

S: Phosphorus Standard (5 mg/dl) 5 ml

Procedure

Reagents (L1&L2), Standard and test sample were pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T) in following sequence.

---

*CREST BIOSYSTEMS, a division of CORAL clinical systems, Gitanjali, Dr. Antonio Do RegoBagh, Alto SantacruzBambolim Complex P.O., Goa-403202, INDIA.

Addition sequence	B (ml)	S (ml)	T (ml)
Working reagent (L1+ L2)	1.0	1.0	1.0
Distilled water	0.01	-	-
Phosphorus Standard	-	0.01	-
Sample	-	-	0.01

All were mixed well and incubated at R.T for 5 minutes. Observations were noted for the absorbance of the Standard (Abs. S) and Test Sample (Abs. T) against Blank within 60 minutes using wavelength/filter of 340 nm / Yellow, Temperature: R.T. Light path: 1 cm.

Calculations

Phosphorous (mg/dl) = Absorbance of T/Absorbance of S X 5

3.8.3 Serum glucose

Glucose was estimated by GOD/POD method with the help of estimation kit *

Principle

Glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4–aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of glucose present in the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\textit{Glucose oxidase}} \text{Gluconate} + H_2O_2$$

$$H_2O_2 + 4\ \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\textit{Peroxidase}} \text{Red Quinoneimine dye} + H_2O$$

Contents

L1: Glucose Reagent 150 ml

S: Glucose Standard (100 mg/dl) 5 ml

---

*CREST BIOSYSTEMS, a division of CORAL clinical systems, Gitanjali, Dr. Antonio Do RegoBagh, Alto SantacruzBambolim Complex P.O., Goa-403202, INDIA.

Procedure

Reagents (L1), Standard and test sample were pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T) in following sequence.

Addition sequence	B (ml)	S (ml)	T (ml)
Glucose reagent (L1)	1.0	1.0	1.0
Distilled water	0.01	-	-
Glucose Standard	-	0.01	-
Sample	-	-	0.01

All were mixed well and incubated at R.T for 30 minutes. Observations were noted for the absorbance of the Standard (Abs. S) and Test Sample (Abs. T) against Blank within 60 minutes using wavelength/filter of 505 nm / green, Temperature: R.T. Light path: 1 cm.

Calculations

Total glucose in mg/dl = (Abs. T/ Abs. S) × 100

3.8.4 Serum total protein

Glucose was estimated by Biuret method with the help of estimation kit*

Contents

L1: Biuret Reagent 150 ml

S: Protein Standard (8g/dl) 5 ml

Principle

Proteins, in an alkaline medium, bind with the cupric ions present in the biuret reagent to form a blue–violet coloured complex. The intensity of the colour formed is directly proportional to the amount of proteins present in the sample.

Protein + $Cu^{++} \rightarrow$ Blue violet coloured complex

---

*CREST BIOSYSTEMS, a division of CORAL clinical systems, Gitanjali, Dr. Antonio Do RegoBagh, Alto SantacruzBambolim Complex P.O., Goa-403202, INDIA.

Procedure

Reagents (L1), Standard and test sample were pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T) in following sequence.

Addition sequence	B (ml)	S(ml)	T (ml)
Biuret reagent (L1)	1.0	1.0	1.0
Distilled water	0.02	-	-
Protein Standard	-	0.02	-
Sample	-	-	0.02

All were mixed well and incubated at R.T for 30 minutes. Observations were noted for the absorbance of the Standard (Abs. S) and Test Sample (Abs. T) against Blank within 60 minutes using wavelength/ filter of 550nm /Yellow-Green, Temperature: R.T. Light path: 1 cm.

Calculation

Total protein (g/dl) = Absorbance of T/Absorbance of S X 8

3.8.5 Serum cholesterol

Serum cholesterol level was estimated by cholesterol estimation kit*

Principle

Cholesterol esterase hydrolyses esterifies cholesterols to free cholesterol. The free cholesterol is oxidised to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidise to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\textit{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\textit{Cholesterol oxidase}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{4 Aminoantipyrine} + \text{Phenol} \xrightarrow{\textit{Peroxidase}} \text{Red Quinoneimine dye} + H_2O$$

*CREST BIOSYSTEMS, a division of CORAL clinical systems, Gitanjali, Dr. Antonio Do RegoBagh, Alto SantacruzBambolim Complex P.O., Goa-403202, INDIA.

Contents

L1: Enzyme Reagent 60 ml

L2: Enzyme Reagent 15 ml

S: Cholesterol standard (200mg/dl) 5 ml

Procedure

Reagents (L1), Standard and test sample were pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T) in following sequence.

Addition sequence	B (ml)	S (ml)	T (ml)
Working reagent (L1+L2)	1.0	1.0	1.0
Distilled water	0.01	-	-
Cholesterol Standard	-	0.01	-
Sample	-	-	0.01

All were mixed well and incubated at R.T for 15 minutes. Observations were noted for the absorbance of the Standard (Abs. S) and Test Sample (Abs. T) against Blank within 60 minutes using wavelength/filter of 505 nm / Green, Temperature: R.T. Light path: 1 cm.

Calculation

Cholesterol (mg/dl) = Absorbance of T/Absorbance of S × 200

3.8.6 Serum progesterone estimation

Assay for plasma progesterone was done by solid phase enzyme immuno-assay using commercially available progesterone kit*. Each kit was having microplates for 96 Wells. The results were read on Wallac 1420 Multilabel Plate Reader.

---

*XEMA MEDICA, Muscow, Russia

Principle of assay

Solid phase enzyme-linked immunosorbent assay (ELISA) is based on the competition principle. Competition occurs between an unlabeled antigen (present in standards, controls and samples) and an enzyme-labelled antigen (conjugate- horse radish peroxidase 17-α- OH progesterone) for a limited number of antibody (17-α-OH progesterone coated) binding sites on the microwell plate (solid phase). After incubation, washing and decanting procedures the unbound materials were removed. After the washing step, the enzyme substrate (H_2O_2) and TMB substrate were added. The enzymatic reaction was terminated by addition of the stopping solution. The absorbance was measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of 17α-OHP in the sample. Progesterone concentration in the sample was calculated based on a series by a set calibration. Absorbances were measured at 450 nm using ELISA plate reader. A standard curve was obtained by plotting the concentration of the standard versus the absorbance. The progesterone concentration of the specimens and controls run concurrently with the standards were calculated from the standard curve by the plate.

Reagents

1. **Calibrators (C1, C2, C3, C4, C5, C6, C7):** 6 x 1ml each vial before use, mixed for 5 min, with rotating mixer. The calibrators have the following concentration of 17-α- OH progesterone.

	C1	C2	C3	C4	C5	C6	C7
Concentration (nmol/l)	0	1	3	10	30	100	300

2. **Enzyme conjugate:** The vial contains 6ml of HRP- progesterone in buffer supplemented by bovine serum albumin (0.5%) and progesterone binding protein displacers.
3. **Micro well plate:** The bag contains a microplate of 12 strips x 8 wells. Each well is coated with anti- 17 -α – OH progesterone (rabbit).
4. **Substrate solution:** The vial contains 12 ml of stabilized mixture of TMB (3,3', 5,5'-Tetramethylbenzidine) and H_2O_2 (hydrogen peroxide).
5. **Stop solution:** The vial contain 12 ml of 0.15 mol/L sulphuric acid.

Preparation of assay

1. All reagents were brought to room temperature.
2. Sufficient strips were left in the strip holder to enable the running of calibrators, controls, and samples in duplicate, plus one well for chromogen blank.
3. For a photometer blank 100 µl of substrate and 100 µl of stop solution were pipetted in one well.
4. The washing solution was prepared by mixing of the bottle with 450 ml of distilled water.

Procedure of assay

i. 25 µL of each Standard (calibrators), Control and samples were pipetted into the respective wells of the Microtiter Plate.
ii. 200 µL of Enzyme Conjugate (HRP-Progesterone) was added into each well.
iii. Plate was shaken carefully and incubated for 60 min at room temperature (18-25°C).
iv. Incubation solution was discarded. Plate was washed 3 times with 250 µL of diluted Wash Buffer. The excess solution was removed by tapping the inverted plate on a paper towel.
v. Pipetting was carefully carried out in the same time intervals for Substrate and Stop Solution.
vi. 100 µL of TMB Substrate Solution was pipetted into each well and incubated for 30 min at room temperature (18-25°C).
vii. Substrate reaction was stopped by adding 100 µL of TMB Stop Solution into each well and contents were mixed by gently shaking the plate.
viii. Optical density was measured with a photometer at 450 ± 10 nm within 30 min after pipetting of the Stop Solution.

Calculation

The standard curve was constructed as follows:

1. Progesterone standard value was checked on each standard vial.
2. To construct the standard curve, the absorbance for progesterone standards (vertical axis) versus progesterone standard concentrations (horizontal axis) was plotted on a linear graph paper.

3. The absorbance for controls and each unknown sample was read from the curve. The value for each control or unknown sample was recorded.
4. By substituting the absorbance in the equation derived from the plotted standard curve the concentration of progesterone was estimated.

3.9 Insemination

The animals were inseminated in the induced estrus with good quality frozen semen supplied by ARD Dept. The control animals were inseminated twice at an interval of 12 hours at observed estrus following AM-PM rule.

3.10 Pregnancy diagnosis

The efficacy of the different therapeutic protocols was evaluated in terms of conception, when the first two inseminations after treatment were taken in consideration. All the non return animals from all five groups were subjected to pregnancy diagnosis after 45-60 days of last insemination by rectal palpation method. The conception rates were calculated in percent. The efficacy of the individual treatment was assessed by comparing with that of count.

3.11 Analysis of data

All the data generated in the above experiments were statistically analyzed using SPSS computer package. Charts were done with the help of Data analysis tool of MS-Excel 2016.

RESULTS

Puberty and age of sexual maturity are among the most prominent factors that determine the profitability and economic viability of dairy sector in rural India. But they have been ignored for a long time and were not given the due importance. They reduce the time of entry of heifers into the milk producer group from a consumer group and thus increase the life time production of the animal. In the long run they have the potential to improve the condition of dairy industry from the present day difficulty to maintain profitability to a thriving and viable sector.

The present study deals with the Pre pubertal crossbred heifers and the reduction of age of puberty with intervention of exogenous hormones in the form of a protocol(s) and necessary mineral supplementation, to address the condition quickly with high success rate of fertile estrus and succeeding conception. The treatment and supplementation helps in the improvement of the hypothalamo-hypophyseal-gonadal axis and its secretor hormones necessary for attainment of early puberty/sexual maturity in heifers, and maintenance of the conceptus.

As far as the age and body weight are concerned a crossbred heifer attains sexual maturity at around 18 months of age (Hafez & Hafez, 2000) with an approximate body weight of 250 kgs. Under sub-optimal managemental condition it has been encountered in the present experimental locality, animals of 12 month of age and having a body weight of 150 kgs were considered as pre pubertal heifers and are selected for the experiment.

In the present study 30 pre pubertal heifers were taken into consideration dividing them into five separate groups, the first group (n=6) were maintained with normal feed and management without any supplementation and treatment protocols and are considered as control group. Second group (n=6) were supplemented with area specific mineral mixture at the rate of 50 gms per day for 60 days. The third (n=6) were provided with three doses of injectable Progesterone @ 500mg at four days interval, $PGF_2\alpha$ four days later alongwith the mineral mixture supplementation. While the fourth group is injected with one dose of GnRH at the beginning of the

experiment along with the mineral mixture supplementation for 60 days and the fifth group of animals were given all the treatment and supplement protocols i.e. Progesterone, GnRH protocols alongwith mineral mixture supplementation for 60 days.

4.1 Estimation of serum biochemical assay

4.1.1 Calcium

The mean (±S.E) pre and post treatment serum, calcium (mg/dl) in pre pubertal heifers is depicted in table 1. The pretreatment values of serum Ca (mg/dl) during the current study ranged between 7.23± 0.05 and 7.35±±0.10.On post treatment Gr. I animals registered a serum Ca level of 7.49 ±0.10 (mg/dl). The corresponding values of Gr. II, Gr. III, Gr. IV and Gr. V animals on the day of estrus/day 60 were 10.46+0.30, 10.62±0.26, 11.05±0.09 and11.07±0.18 respectively.

The analysis of Variance (table 2) and test of Significance (Table-1) indicated a significant (P<0.05) difference between the treated groups and the control on post treatment day. The Gr. II, Gr. III, Gr. IV and Gr. V did not differ significantly in their serum Ca level on post treatment day. The groups did not show any significant difference on the pre treatment day.

Table 1: Mean (±S.E) pre and post treatment serum calcium (mg/dl) in pre-pubertal heifers

Expt. groups	Pre treatment	Post treatment
Group I (n=6)	7.23^{Aa}± 0.05	7.49^{Bb} ±0.10
Group II (n=6)	$7.35^{A\ a}$±0.10	10.46^{Bb}±.30
Group III (n=6)	7.28^{Aa}±.06	10.62^{Bb}±.26
Group IV (n=6)	7.25^{Aa}±0.02	11.05^{Bb}±.09
Group V (n=6)	7.25^{Aa}±0.05	11.07^{Bb}±.18

Means bearing different superscripts differ significantly (P<0.05) within rows (A, B) and coloumns (a, b)

Table 2: Analysis of variance of serum calcium concentration on pre-treatment and post treatment days

Protocol / Source of variation	Pre treatment				Post treatment			
	ss	df	MS	F	ss	df	MS	F
Between groups	0.04	4	0.01	0.45^{NS}	54.09	4	13.52	54.34^{**}
Within groups	0.66	25	0.02		6.459	25	0.25	

NS Not significant. **P< 0.01

4.1.2 Phosphorous

The mean (±S.E) pre and post treatment serum, phosphorous (mg/dl) pre pubertal heifers is depicted in Table-3. The pre-treatment value of phosphorous (mg/dl) during the current study ranged between 4.31±0.04 to 4.39±0.07.On the day of induced estrus/ day 60, Gr. I animals registered a serum phosphorous level of 5.413± 0.085 (mg/dl). The corresponding values of Gr. II, Gr. III, Gr. IV and Gr. V on the day of estrus were 7.16±0.06 and 7.36±0.06,7.34±0.05 and 7.36±0.03 respectively.

A test of significance and variance indicated a significant (P<0.05) difference between treatment groups (II, III, IV, V) and the control groups on post treatment day . The Gr. II, vary significantly with respect to Gr. III, Gr. IV and Gr. V in their serum phosphorous levels on the day of estrus/Day 60

Test of significance (Tab 4) and analysis of variance (Tab 5) indicated a no significant difference in the serum phosphorous level in Gr. I, Gr. II, Gr. III, Gr. IV, Gr. V on pre treatment day .

Table 3: Mean (±S.E) serum phosphorus (mg/dl) in pre pubertal cross breed heifers along with test of significance

Day of sampling / Expt. groups	Pre treatment	Day of estrus
Group I (n=6)	4.39^{Aa}± .072	5.41^{Ba} ±0.08
Group II (n=6)	4.38^{Aa}± 0.08	7.16^{Bb}± 0.06
Group III (n=6)	4.37^{Aa}± 0.06	7.36^{Bc}±0.06
Group IV (n=6)	4.33^{Aa}±0.07	7.34^{Bc}±0.05
Group V (n=6)	4.31^{Aa}±0.04	7.36^{Bc}±0.03

Means bearing different superscripts differ significantly (P<0.05) within rows (A, B) and coloumns (a, b)

Table 4: Analysis of variance of serum phosphorus concentration on day of treatment, day of estrus

Protocol / Source of variation	Day of treatment				Day of estrus			
	ss	df	MS	F	Ss	df	MS	F
Between groups	0.02	4	0.01	0.24^{NS}	17.47	4	4.36	179.79^{**}
Within groups	0.70	25	0.02		0.60	25	0.02	

NS: Non-significant **p<0.01

4.1.3 Glucose

The mean (±S.E) pre and post treatment serum, glucose (mg/dl) in pre pubertal heifers is depicted in Table-5. The pre treatment value of serum glucose (mg/dl) during the current study ranged between 43.91± 0.477and 44.93± 0.51. On the day of estrus/ day 60, Gr. I animals registered a serum glucose level of 46.55± 0.29. The corresponding value of Gr. II, Gr. III, Gr. IV, Gr. V animals on day of estrus were 56.54±0.84, 56.55±1.27, 54.82±0.41 and 57.39±0.73.

Gr. II, Gr. III, Gr. IV and Gr. V registered a significantly higher value from Gr. I control on post treatment day. All the treated groups had significant (P<0.05) variations in serum glucose level (mg/dl) within days of collection except Gr. I. In all these above cases the serum glucose level referred to be higher on day of estrus except Gr. I.

Table 5: Mean (±S.E) serum glucose (mg/dl) in pre pubertal cross breed heifers along with test of significance

Day of sampling / Expt. groups	Pre treatment	Day of estrus
Group I (n=6)	43.91^{Aa}±0.47	46.55^{Ba}±0.29
Group II (n=6)	44.23^{Aa}±0.60	56.54^{Bb}±0.84
Group III (n=6)	44.50^{Aa}±0.57	56.55^{Bbc}±1.27
Group IV (n=6)	44.47^{Aa}±0.51	54.82^{Bbc}±.41
Group V (n=6)	44.93^{Aa}±0.51	57.39^{Bc}±0.73

Means bearing different superscripts differ significantly (P<0.05) within rows (A, B) and coloumns (a, b)

Table 6: Analysis of variance of serum glucose concentration on day of treatment, day of estrus and 23 day post AI

Protocol / Source of variation	Day of treatment				Day of estrus			
	ss	df	MS	F	ss	df	MS	F
Between groups	3.38	4	0.84	0.484^{NS}	479.75	4	119.93	31.08^{**}
Within groups	43.77	25	1.75		94.28	25	3.77	

NS: Non-significant **p<0.01

4.1.4 Cholesterol

The mean (±S.E) pre and post treatment serum, Cholesterol (mg/dl) in pre pubertal heifers is depicted in table 8. The pre treatment serum cholesterol (mg/dl) registered values within the range of 111.81± 1.60 and 120.53± 2.02. Similarly, the corresponding values on day of estrus/day 60 were recorded to be 121.465± 1.85, 136.99± 1.75; 130.03± 2.84, 127.87± 2.65 and 137.62± 2.74 in Gr. I and Gr. II, Gr. III, Gr. IV and Gr. V respectively.

There is a significant difference between Gr. V and other groups on the pre treatment day. The 2nd sampling taken on day of estrus registered a significantly different value of serum cholesterol between Gr (I, II) and Gr. V. The Gr. I, Gr. II, Gr. III, Gr. IV and Gr. V had significantly higher (P<0.01) value on the 2nd sampling than the 1st sampling.

Table 7: Mean (±S.E) serum cholesterol (mg/dl) of pre pubertal heifers in different days of sampling along with test of significance

Day of sampling / Expt. groups	Pre treatment	Day of estrus
Group I (n=6)	117.04^{Aa}±1.76	121.46^{Ba}±1.85
Group II (n=6)	117.32^{Aab}±1.31	136.99^{Bab}±1.75
Group III (n=6)	111.81^{Aab}±1.60	130.03^{Bbc}±2.84
Group IV (n=6)	115.69^{Aab}±1.95	127.87^{Bcd}±2.65
Group V (n=6)	120.53^{Ab}±2.02	137.62^{Bd}±2.74

Means bearing different superscripts differ significantly (P<0.05) within rows (A, B) and coloumns (a, b)

Table 8: Analysis of variance of serum cholesterol concentration on day of treatment, day of estrus and 23 day post AI

Protocol / Source of variation	Day of treatment				Day of estrus			
	ss	df	MS	F	ss	df	MS	F
Between groups	239.29	4	59.82	3.24*	1088.16	4	272.04	7.75**
Within groups	460.53	25	18.42		877.07	25	35.08	

*p<0.05 **p<0.01

4.1.5 Serum total protein

The mean (±S.E) pre and post treatment serum total protein (mg/dl) in pre pubertal heifers is depicted in Table-6. The pre treatment value of serum total protein (mg/dl) during the current study ranged between 5.03±0.09 and 5.08± 0.10. On the day of estrus/ day 60, Gr. I animals registered a serum protein level of 5.83± 0.14. The corresponding values of Gr. II, Gr. III, Gr. IV and Gr.- V animals on day of estrus were 5.856±0.11, 5.566±0.092,5.553±0.149 and 5.911±0.13.

The groups showed significant variation in serum Total Protein levels within the different days of collection. Groups did not vary in the protein concentration between themselves irrespective day of sampling.

Table 9: Mean (±S.E) serum Total Protein (mg/ml) in pre pubertal cross breed heifers along with test of significance

Day of sampling / Expt. groups	Pre treatment	Day of estrus
Group I (n=6)	5.06^{Aa}±0.07	5.83^{Bb}±0.14
Group II (n=6)	5.08^{Aa}±0.10	5.85^{Bb}±0.11
Group III (n=6)	5.04^{Aa}±0.08	5.56^{Bb}±0.09
Group IV (n=6)	5.03^{Aa}±0.09	5.55^{Bb}±0.14
Group V (n=6)	5.17^{Aa}±0.11	5.91^{Bb}±0.13

Means bearing different superscripts differ significantly (P<0.05) within rows (A, B) and coloumns (a, b)

Table 10: Analysis of variance of serum total protein concentration on day of treatment, day of estrus and 23 day post AI

Protocol / Source of variation	Day of treatment				Day of estrus			
	ss	df	MS	F	ss	df	MS	F
Between groups	0.07	4	0.01	0.35^{NS}	0.70	4	0.17	1.73^{**}
Within groups	1.34	25	0.10		2.53	25	0.10	

NS-Not Significant **p<0.01

4.1.6 Progesterone

The mean (±S.E) values of pre treatment and post treatment serum progesterone (ng/ml) level of the pre pubertal heifers under different groups is presented in Table-10. The pre treatment values of different groups registered a value within the range of 0.37 ± 0.14 to 0.45 ± 0.01. The second sample taken on the day of estrus/day 60 also registered nearly similar values in Gr. I (0.0.49± 0.01) Gr. II (0.46±0.01), Gr. III (0.50±0.02) Gr. IV (0.43±0.01) and Gr. V (0.49 ± 0.02) .The third sampling on 23 rd day post AI have serum conc. Of 0.49 ±0.01, 0.49±0.03, 1.6±0.71, 1.11±0.65 and 3.13±0.86 for Gr. I, Gr. II, Gr. III Gr. IV Gr. V respectively.

There existed also a significant (P<0.05) difference between Gr. II and Gr. III, Gr. IV, Gr. V animals. With respect to variation in the levels of progesterone (mg/dl) on different days of sampling, all the groups followed a similar trend showing significantly (P<0.05) different values on days of sampling.

There exists a significant difference between the serum progesterone values on pre treatment and on the day of estrus in all groups. Also a significant difference is found between groups on the pre treatment day and on the 23 days post AI (table 9). The serum progesterone values differ significantly between Gr. V and all other groups on the 3rd sampling.

Table 11: Mean (±S.E) serum progesterone (ng/ml) in pre pubertal cross breed heifers along with test of significance

Day of sampling / Expt. groups	Pre treatment	Post treatment	
		Day of estrus	23 days post AI
Group I (n=06)	0.451^{Aa}±0.01	0.49^{Ba}±0.00	0.49^{BCa}±0.01
Group II (n=06)	0.41^{Aa}±0.02	0.46^{Bab}±0.01	0.49^{BCa}±0.03
Group III (n=06)	0.45^{Aab}±0.01	0.50^{Bab}±0.02	1.60^{BCa}±0.715
Group IV (n=6)	0.73^{Aa}b±0.014	0.43^{Bb}±0.01	1.11^{BCab}±0.65
Group V (n=6)	0.38^{Ab}±0.019	0.49^{Bb}±0.02	3.13^{Cb}±0.860

Means bearing different superscripts differ significantly (P<0.05) within rows (A, B) and coloumns (a,b)

Table 12: Analysis of variance of serum progesterone concentration on day of treatment, day of estrus and 23 day post AI

Protocol / Source of variation	Day of treatment				Day of estrus				23 days post AI			
	ss	df	MS	F	ss	df	MS	F	ss	df	MS	F
Between groups	0.02	4	0.01	3.72^{*}	0.01	4	0.01	2.07^{NS}	28.73	4	7.18	3.55^{*}
Within groups	0.04	25	0.01		0.05	25	0.01		50.51	25	2.02	

NS-= Not Significant *p<0.05

4.2 Estrus response and conception rate

The response to induction protocols in different groups and their subsequent conception rate is presented in table 13. The control group did not show any induced estrus, where as Gr. V showed 100 percent result in in estrus response. Gr. II and Gr. III and Gr. IV showed 16.6%, 50% and 33.3% induced estrus respectively.

The heifers of the first two groups i.e. Gr. I and Gr. II did not lead to conception while the heifers in the Gr. III, Gr. IV, GR. V have conception percentage of 33.3, 16.6 and 66.6 respectively.

Table 13: Induced estrus response and conception in the experimental groups

Expt. groups	Induced estrus response	Conception
Group I (n=6)	0 (00.00)	0 (00.00)
Group II (n=6)	1 (16.60)	0 (00.00)
Group III (n=6)	3 (50.00)	2 (33.30)
Group IV (n=6)	2 (33.30)	1 (16.60)
Group V (n=6)	6 (100.00)	4 (66.6)

Figures in the parenthesis indicate the percentage.

4.3 Estrus induction interval

The estrus induction interval (days) recorded during the study has is presented in table 14. The control animals did not record estrus during 60 days of observation. The average estrus induction interval (days) for Gr. II, II, IV and V animals are recorded to be 56.00, 49.00, 51.00 and 48.33 days respectively.

Table 14: Mean Estrus induction interval (days) in different experimental groups

Expt. groups	Induction interval (in days)
Group I (n=0)	-
Group II (n=1)	56.00
Group III (n=3)	49.00
Group IV (n=2)	51.00
Group V (n=6)	48.33

4.4 Cost analysis of treatment

To evaluate the economics of treatment a calculation has been made (table 15) to evaluate the cost per conception (Rs.). Since the animals in Gr. I and II did nor

register any pregnancy, cost of pregnancy could not be calculated. The cost per conception for the animals in Gr. II, IV and V came to Rs. 1890/-, 3000/- and 1425/- respectively.

Table 15: Cost analysis of treatment adopted

Expt. groups	Cost of medicine in Rs.					No. of Conceptions	Total cost in (Rs.)	Cost per conception (Rs.)
	MM	GnRH	P4	PG	Total			
Group I (n=0)	-	-	-	-	-	0/6	0	-
Group II (n=1)	300	-	-	-	300	0/6	1800	-
Group III (n=3)	300	-	330	-	630	2/6	3780	1890
Group IV (n=2)	300	200	-	-	500	1/6	3000	3000
Group V (n=6)	300	200	330	150	950	4/6	5700	1425

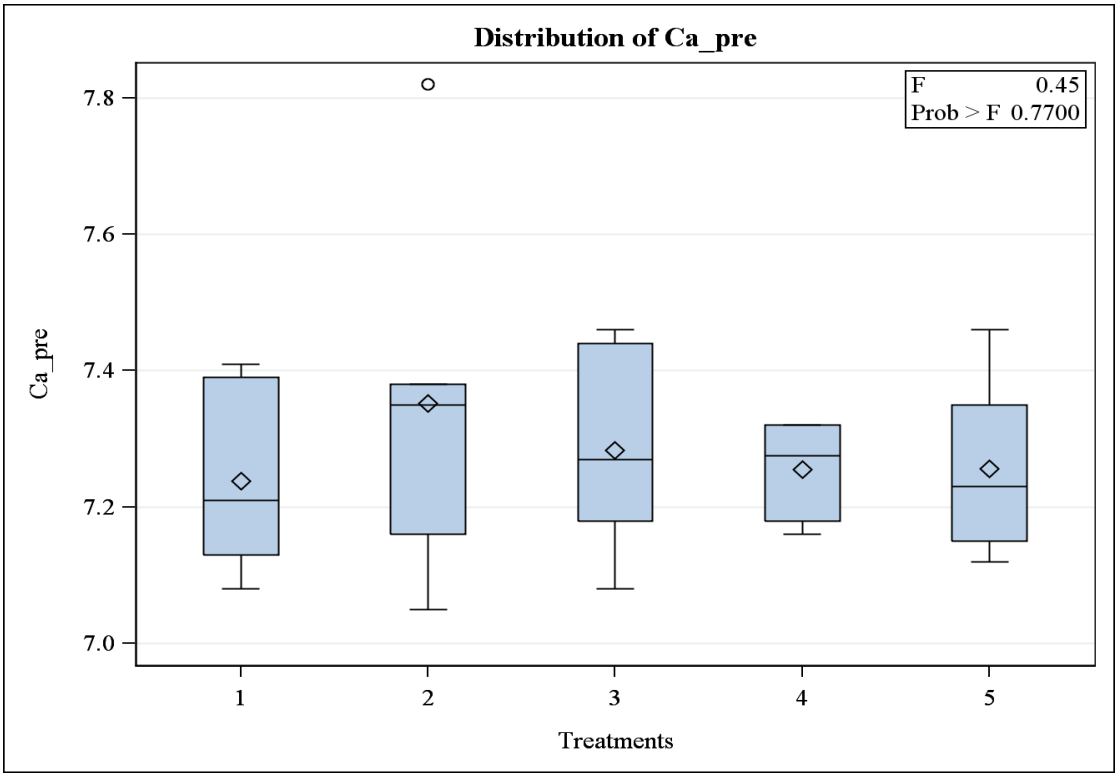

Fig. 1: Box and whisker plot of pre-treatment serum calcium concentration in experimental groups

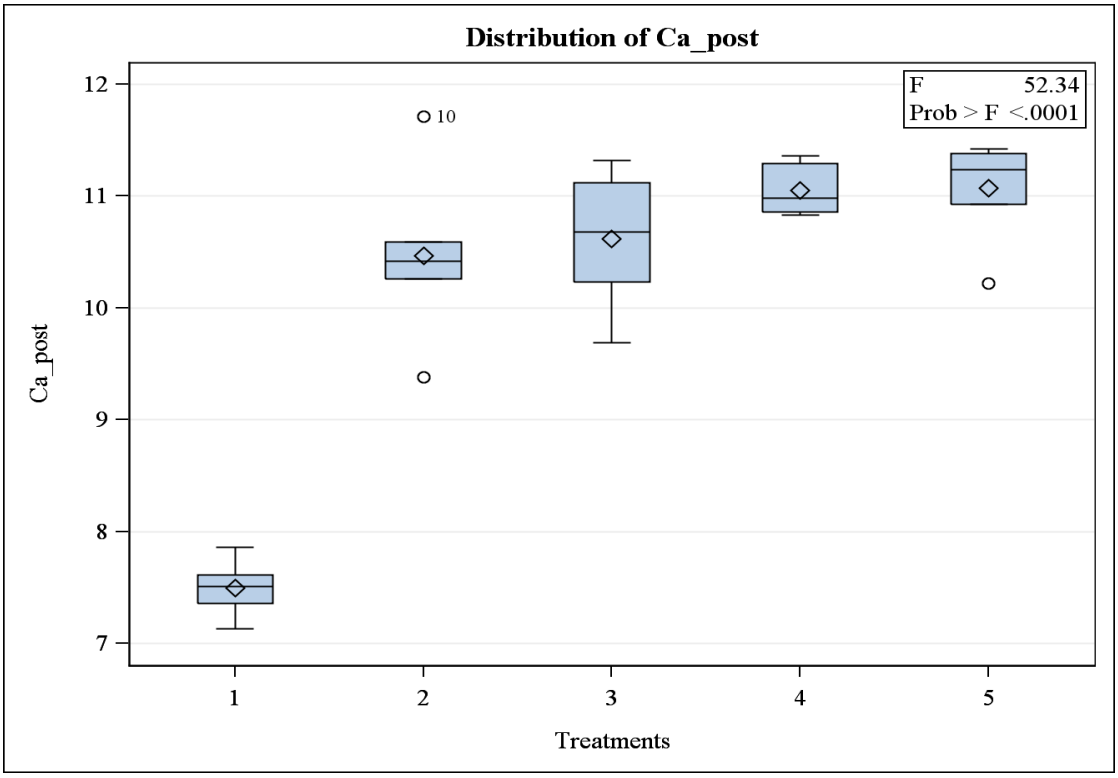

Fig. 2: Box and whisker plot of post-treatment serum calcium concentration in experimental groups

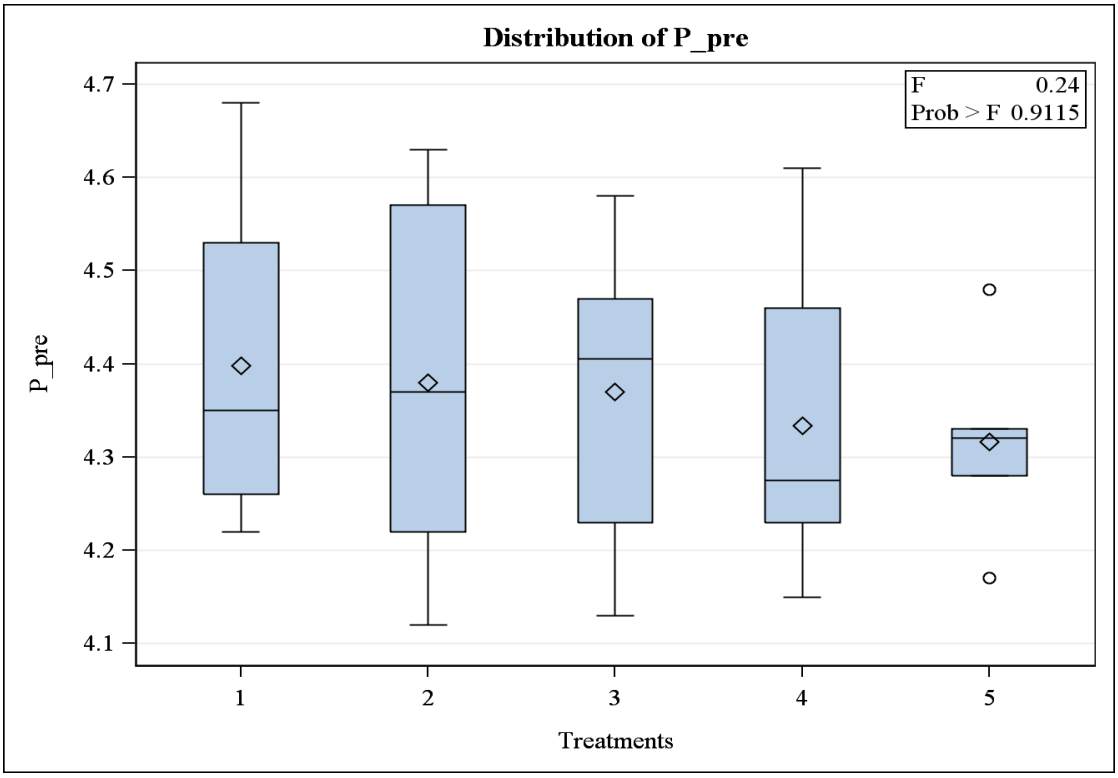

Fig. 3: Box and whisker plot of pre-treatment serum phosphorus concentration in experimental groups

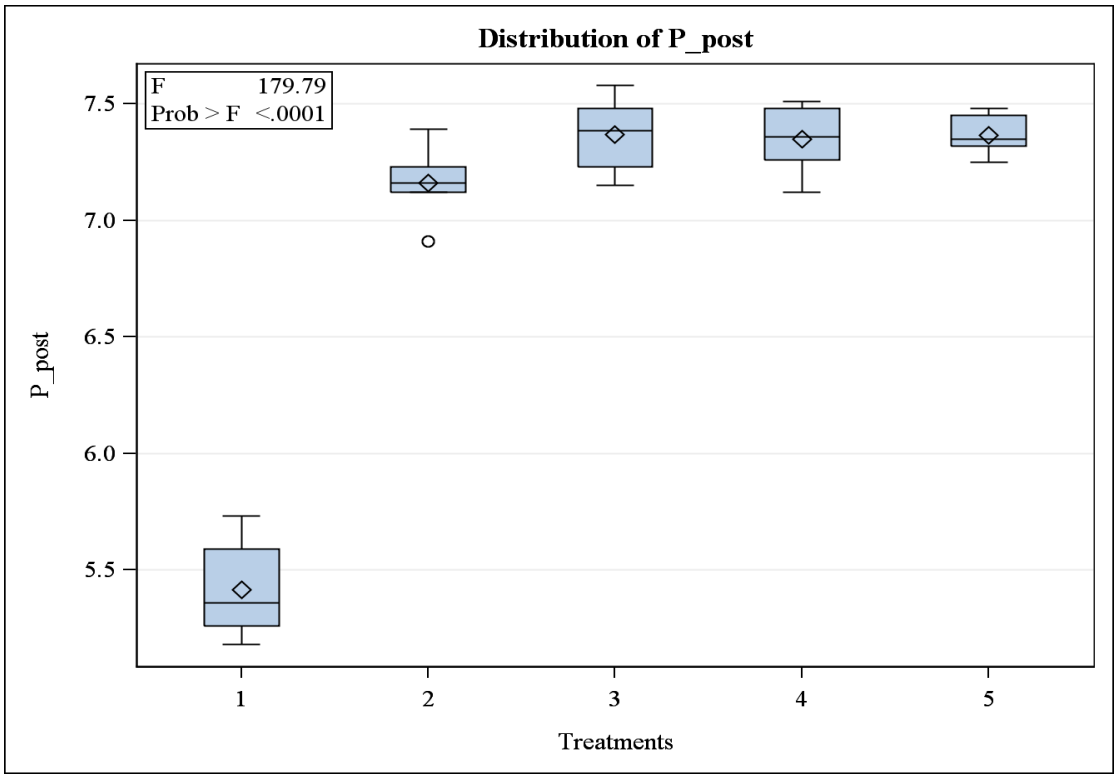

Fig. 4: Box and whisker plot of post-treatment serum phosphorus concentration in experimental groups

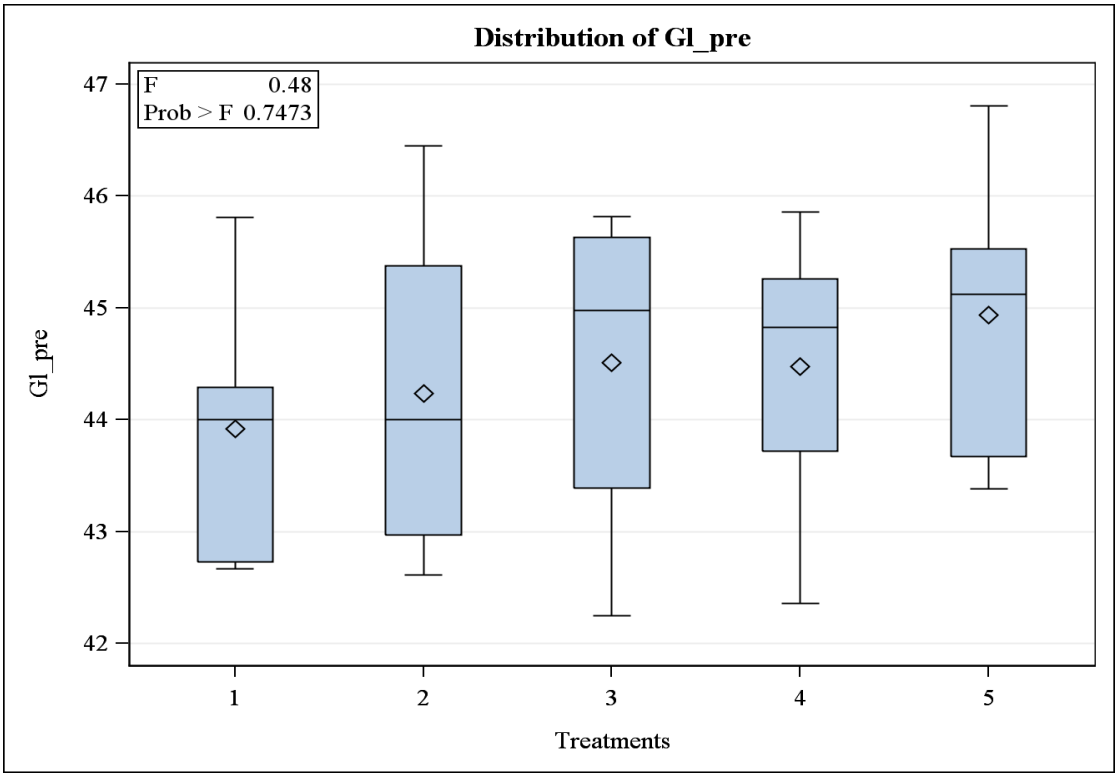

Fig. 5: Box and whisker plot of pre-treatment serum glucose concentration in experimental groups

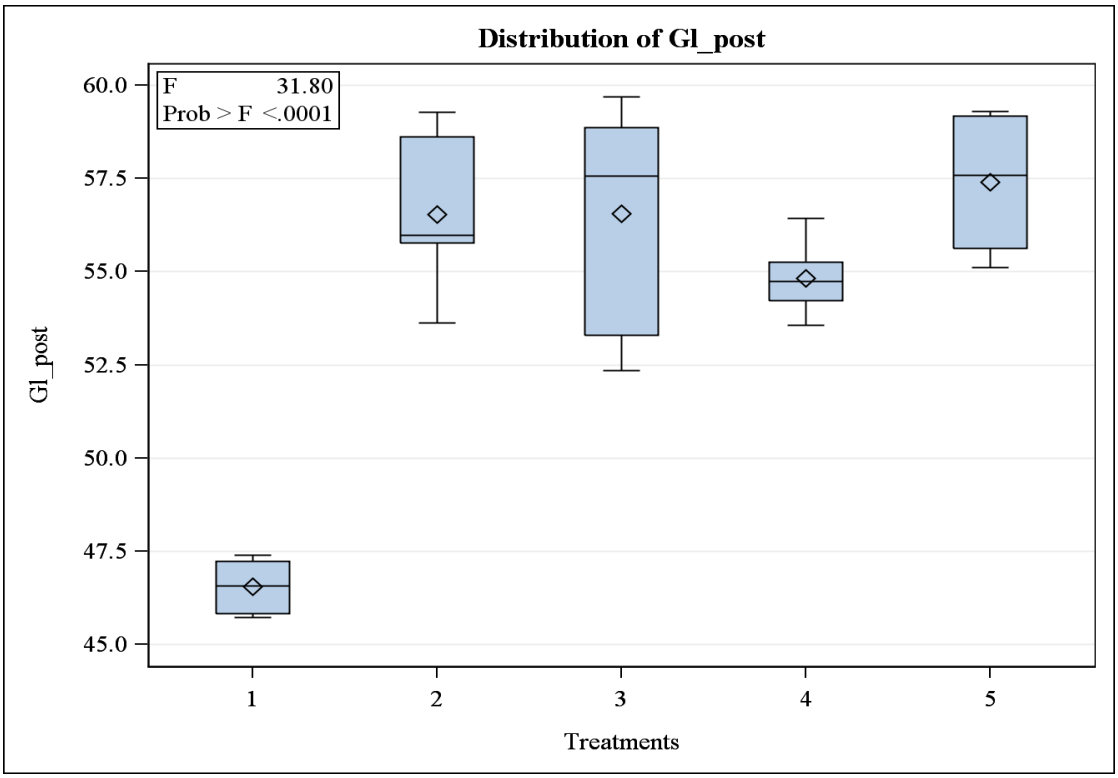

Fig. 6: Box and whisker plot of post-treatment serum glucose concentration in experimental groups

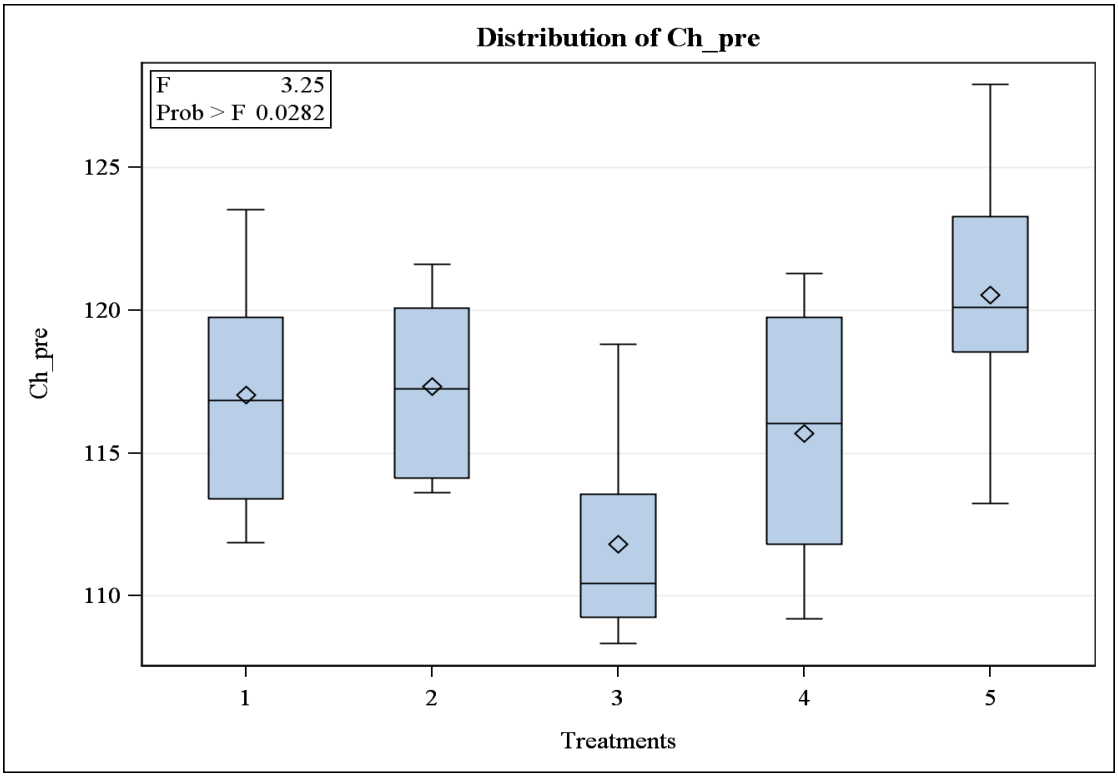

Fig. 7: Box and whisker plot of pre-treatment serum cholesterol concentration in experimental groups

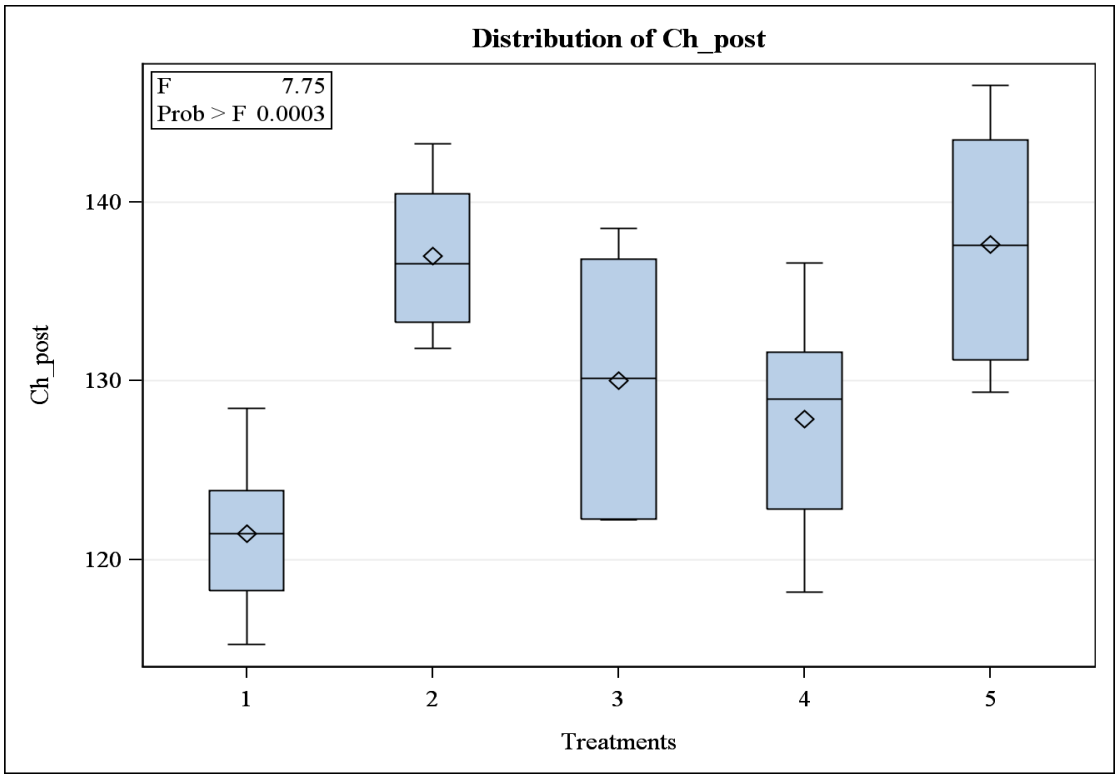

Fig. 8: Box and whisker plot of post-treatment serum cholesterol concentration in experimental groups

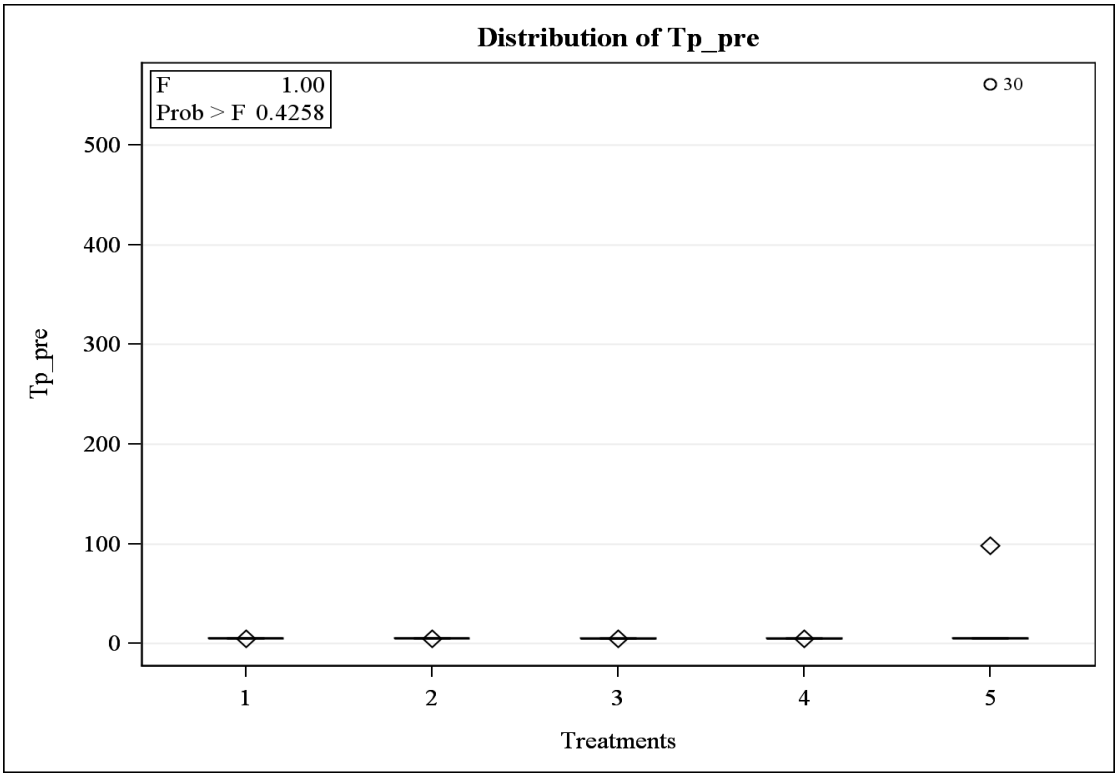

Fig. 9: Box and whisker plot of pre-treatment serum total protein concentration in experimental groups

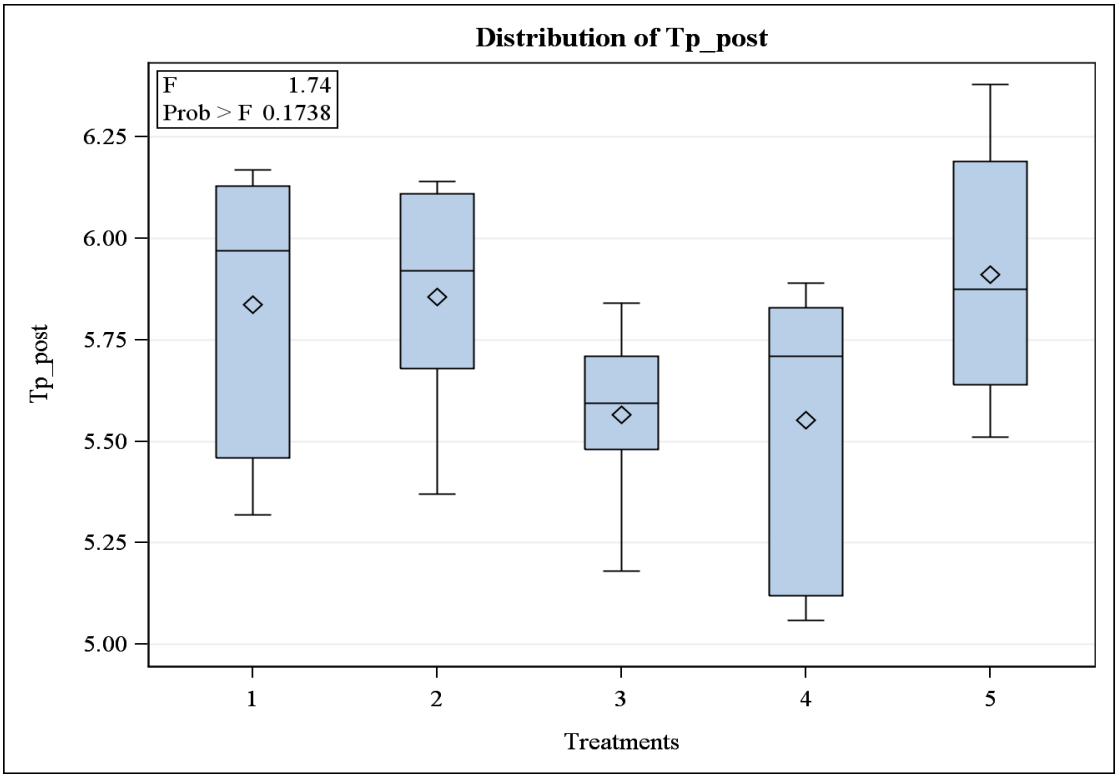

Fig. 10: Box and whisker plot of post-treatment serum total protein concentration in experimental groups

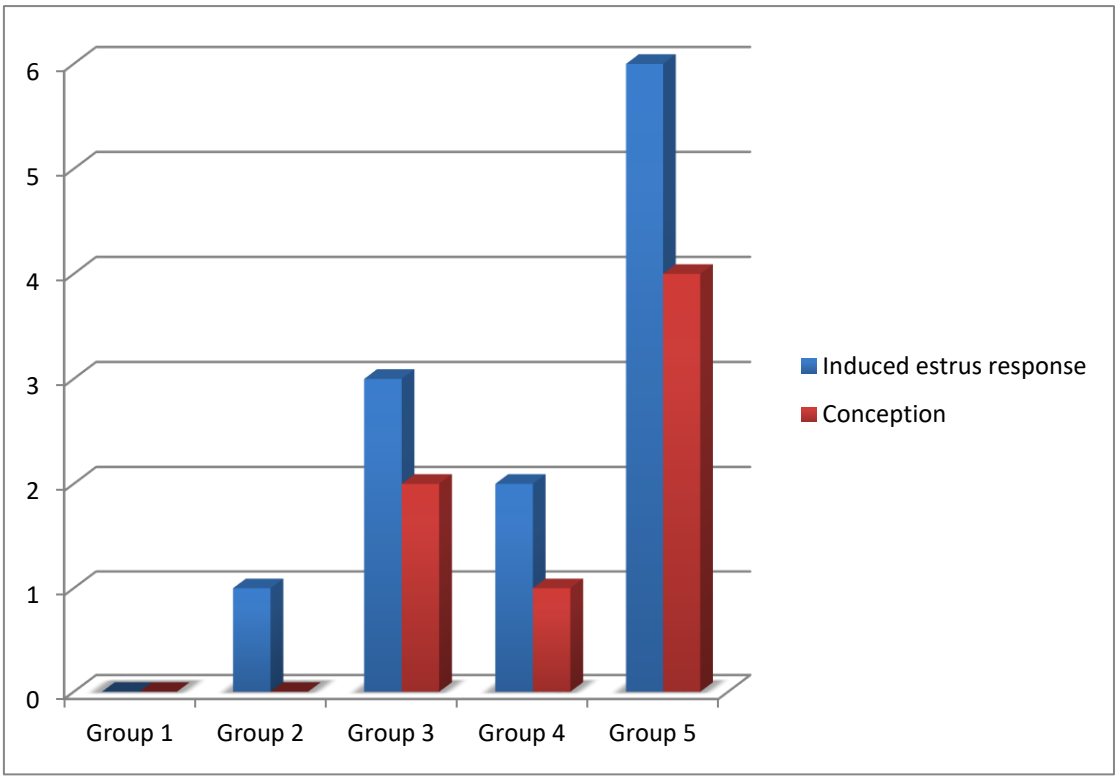

Fig. 11: Graphical representation of induced estrus response and conception in experimental animals

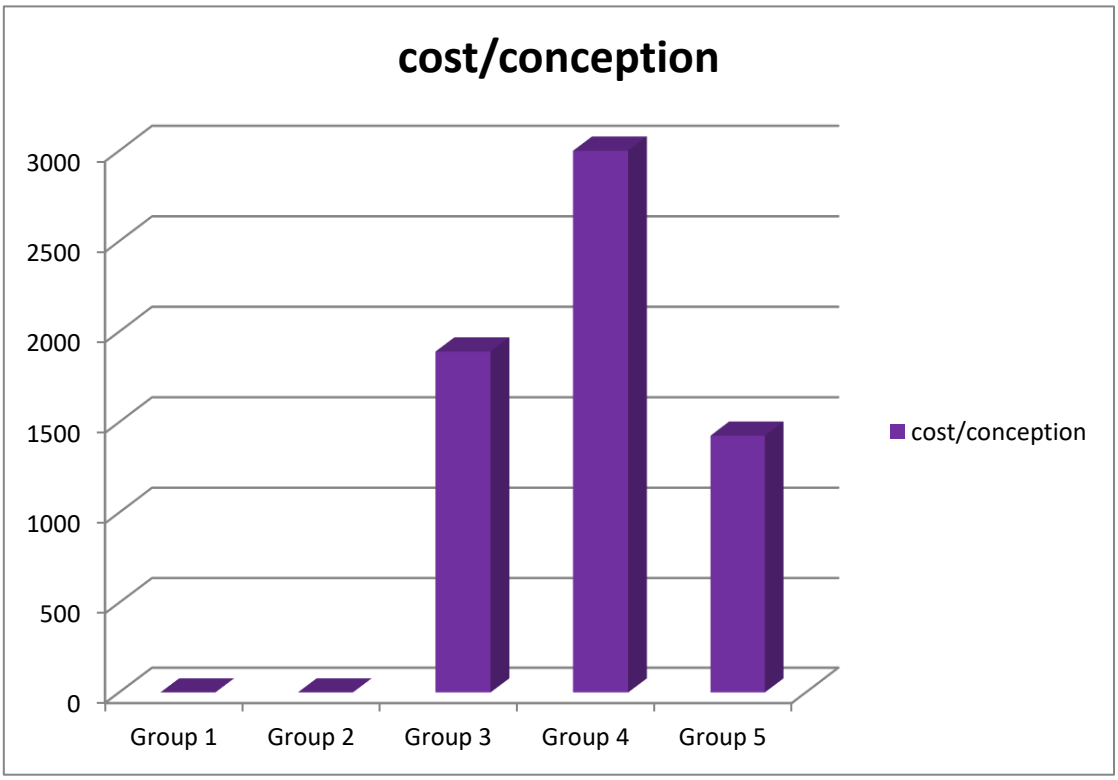

Fig. 12: Graphical representation of cost per conception (Rs.) under different protocols in experimental animals

DISCUSSION

The Early Attainment of sexual maturity and observation of fertile estrus are imperative for high reproductive efficiency in growing heifers. Anoestrum is a broad term that indicates lack of expression of estrus or absence of estrus signs despite efficient detection (Lucy *et al.,* 2007).

Pre pubertal is a term denoted in the present study to those heifers who had not come to estrus at around 12 months of age. Since most of the etiological factors responsible for this condition are directed towards nutrition and management after ruling out the genetic factor. Insufficiency of endocrine secretion which is governed by HPG - axis is frequently disturbed due to managemental and nutritional deficiencies. This ultimately affects the normal reproductive physiology for attainment of puberty and sexually Maturity.

Hence modulation of the nutrition through supplementation of minerals as well as improvement in feeding and management along exogenous hormones supplementation has been tried all over the globe to minimize the time required for attainment of puberty. In the present study four such protocols are utilized as induction protocols in pre-pubertal heifers. In this light ovulatory estrus are being successfully induced in anoestrum cows (why not in pre-pubertal heifer) by using GnRH, prostaglandin and other combinations in presence of a dominant follicle, in absence of which the treatment is not effective (Rhodes *et al*., 2003). Still under optimum growth and age farmer is always interested to breed the heifer at an early age. Hence a trial has been to experiment different treatment protocols.

5.1 Serum biochemical and hormonal profile

It is an established fact that bioavailability of different, biochemical, minerals and hormones in serum are important indicates for assessment of proper reproductive health of the animal. This also is variable and can be affected by the nutritional status of animal to a large extent.

5.1.1 Calcium

The pre-treatment values of serum calcium (mg/dl) in Pre pubertal heifers along with its post treatment values are depicted in Table 1. The serum calcium level

of the experimental animals range between 7.23 ± 0.05 to 7.49 ± 0.10, which is well within the normal range (Kaneko and Cornelius, 1989). Higher level of calcium (10.58±0.14) has been reported by (Mishra, 2011), while marginally lower level 6.88±0.17 has also been reported in anestrus buffaloes (Parida, 2015).

5.1.2 Phosphorus

The present finding of serum phosphorus (Table 3) corroborates the finding of Krupakaran (2013) but higher values 6.47 ± 0.11 have also been reported (Mishra 2011). Similar lower values 4.14 ± 0.26 has also been reported in anoestrus buffaloes (Parida, 2015).

The lower level of phosphorus might be due to metabolic stress and discrepancy in different feeding and managemental conditions (Roberts, 1971). A significant increase the of phosphorus on day of estrus encountered might be due to the resultant release of metabolic stress and anabolic action of hormone.

5.1.3 Glucose

The serum Glucose encounterdin the Pre pubertal heifer (Table 5) is comparable to the reports of Mishra (2011) and (Parida, 2015). There are also reports of lower glucose level Krupakaran (2013). Similarly reported of higher level of serum glucose are not uncommon (Kaneko and Cornelius 1970). The mean plasma glucose concentration has been reported to be higher in estrus animals (Singh and Singh 2006) and Krupakaran (2016). The increased the concentration plasma glucose level is responsible for elevating progesterone production through LH increase (Kaneko, 1989). Significantly higher level of glucose on day of estrus in hormone treated group might be due to positive energy balance created due to intentional incremental feeding by the farmer.

5.1.4 Cholesterol

The Cholesterol values observed in the present study (Table 7) are corroborating with the observations of Mishra (2011), Krupakaran (2013) and (Parida, 2015) in buffaloes. However, higher (240 ± 265) and lower (70 ± 118) values have also been reported by Paul *et al.* (1991), the lower Cholesterol values of the serum cholesterol in hormone treated groups finds the

support of Krupakaran (2013) however the control animals showing a still higher values (100.55 ± 4.21) could not be justified and might be due to power to expression of estrus.

5.1.5 Total protein

The pre-pubertal heifers are in their active phase of growth and usually are protein deficient under field conditions. However since the selected heifers are in their best health and physical condition, the protein level is well within the physiological limit (Kaneko and Cornelius 1970). There has been a nonsignificant increase in the post treatment sampling during the study, which seems obvious after nutritional and hormonal supplement (McDonald, 1989).

5.1.6 Progesterone

The present finding of serum progesterone at the pre treatment stage (Table 11) is in agreement with other reports (Deka *et al.,* 2015, Mishra, 2013). However higher values comparable to the present finding have also been reported (Kumar *et al.,* 2009, Parida *et al*., 2015). Several reports tell about suprabasal (>1ng /ml) level of progesterone to be the cause of repeat breeding (Bage *et al.,* 2003). However, Mac Donald (1989) felt the variation in progesterone concentration to be highly dependent on the season. The lower level of serum Progesterone on the day of estruses finds the supports of most of the workers (Naik *et al.,* 2013; Parida *et al.,* 2015). The sharp increase in Progesterone level 23 days post AI obliviously indicates towards pregnancy. The group with the highest conception rate (group V) obliviously shows the highest mean of concentration of progesterone with the lowest shown by control group animals.

5.2 Estrus response

The estrus response (100%) after successful induction encountered during studies in the group V) is much higher than the earlier reports (Mishra, 2013 and Parida, 2015). Since these protocols are comparatively recent and not used by many of the workers earlier, to add to this were tried presently in treatment of Pre pubertal heifers such a higher rate of response could be achieved. It points towards effective drug delivery coordinating endocrine events (Gordon, 1996). Similarly a moderate

response has been recorded with Gr. II, III and IV animals, while the control group did not respond. Similar trials have been done by Parida, 2017 and Mahanta, 2019 which corroborate to the present finding. Enumerable workers worldwide have used implant form of P4 and has got best result. Morotti *et al.* (2018) have experimented injectible P4 but along with inj. of estradial, which obviously yielded higher response. Due to non-availability of estradial preparations it was not experimented during the study.

5.3 Conception rate

The conception rate achieved in present study were found to be 33.33, 16.66 and 66.66 in Group III, Group IV and group V respectively, which were treated with hormonal protocols. The overall conception rate was 23 % which is lower than the values reported by Mishra, 2011, Vameozami, 1999). Highest conception rates (overall) have also been reported (Abdul-Khalek *et al.,* 2012). Evidence supported GnRH and P4 combination showed efficient ovulation response. However, the discrepancy in induced ovulation and conception rate might be due to individual response and presence of dominant follicle at the time ovulation. The chi- square analysis employed have to test the significance of conception rate, did not provide solid support by way of proving a non significant value whichis purely due to lesser number of experimental animals. Similar trial has been made in delayed mature heifers (KPS Dora, 2016) with comparable result.

Several studies have reported various strategies to induce puberty in heifers. Nutritional management (Gasser *et al.,* 2006), biostimulation (Quadros *et al.*, 2004) and use of exogenous hormones (Claro *et al.,* 2010). Day and Anderson (1996) suggested that use of progestin decreases the estrogen receptors in hypothalamus which in turn decrease the negative feedback effect of estradial on GnRH release. This trial is a humble attempt to induce puberty in the country heifers. The higher rate of response of estrus and conception in group V animals is suggestive of correct triggering of HPG axis in achieving the result.

5.4 Estrus induction interval

During the study all the group of animals except the control showed a moderate induction interval with the minimum with Gr. V animals. Due to paucity of literature the result achieved could not be compared.

5.5 Cost analysis

To attract farmers, a novel attempt has been made to analyze the cost effectiveness of the treatment. Similar attempts usually are less reported, that looks to the prosperity of farming community on most occasions. The minimum cost (Rs. 1425) per pregnancy was achieved with Gr. V animals treated, which seems to be economical hence can be recommended to field after a large trial.

SUMMARY AND CONCLUSION

The present study deals with the pre-pubertal crossbred heifers and its amelioration with intervention of a group of exogenous hormones in the form of a protocol(s) to address the condition quickly with high success rate of fertile estrus and succeeding conception. The experiment was conducted in 4 villages of Kakatpur block during the period from January to March 2020.

Thirty pre-pubertal heifers over the age group of 12 months with 150 kg body wt. or more were randomly selected into five groups. The Gr. I animals acted as untreated control. Gr. II heifers were treated with mineral supplement alone for 60 days, while Gr. III animals were treated with inj. progesterone 500 mg I/M thrice at 4 days interval followed by Inj. of prostaglandin after 4 days along with mineral supplement. Another 6 animals (Gr. IV) were treated with 10 mcg of Buserelin acetate (GnRH) once along with mineral supplement, while the fifth group was treated with all the above treatments. Blood samples were collected for estimation of calcium, phosphorous, glucose, cholesterol, total protein and progesterone at pretreatment, day of estrus and 23 day post AI(for progesterone). Estrus induction rate (%) and interval (day) was recorded within 60 days of treatment. The animals were inseminated at observed estrus and conception rate was calculated after pregnancy diagnosis at 45-60 days post AI. The data were analysed statistically by SPSS.

The pretreatment values of serum Ca (mg/dl) during the current study ranged between 7.23 ± 0.05 and 7.35± 0.10. On post treatment Gr. I animals registered a serum Ca level of 7.49 ±0.10 (mg/dl). The corresponding values of Gr. II, Gr. III, Gr. IV and Gr. V animals on the day of estrus/60day post treatment were 10.46+0.30, 10.62±0.26, 11.05±0.09 and 11.07±0.18 respectively.

The analysis of variance and test of significance indicated a significant ($P<0.05$) difference between the treated groups and the control on post treatment day. The Gr. II, Gr. III, Gr. IV and Gr. V did not differ significantly in their serum Ca level on post treatment day. The groups did not show any significant difference on the pre treatment day.

The pre-treatment value of phosphorous (mg/dl) during the current study ranged between 4.31±0.04 to 4.39 ± 0.07. On the day of induced estrus/ day 60, Gr. I animals registered a serum phosphorous level of 5.413 ± 0.085 (mg/dl). The corresponding values of Gr. II, Gr. III, Gr. IV and Gr. V on the day of estrus were 7.16 ± 0.06 and 7.36 ± 0.06,7.34 ± 0.05 and 7.36 ± 0.03, respectively.

A test of significance and variance indicated a significant ($P<0.05$) difference between treatment groups (II, III, IV, V) and the control groups on post treatment day . The Gr. II, vary significantly with respect to Gr. III, Gr. IV and Gr. V in their serum phosphorous levels on the day of estrus/Day 60

Test of significance (Tab 4) and analysis of variance (Tab 5) indicated a no significant difference in the serum phosphorous level in Gr. I, Gr. II, Gr. III, Gr. IV, Gr. V on pre treatment day .

The pre treatment value of serum glucose (mg/dl) during the current study ranged between 43.91 ± 0.477and 44.93 ± 0.51. On the day of estrus/ day 60, Gr. I animals registered a serum glucose level of 46.55 ± 0.29. The corresponding value of Gr. II,Gr. III, Gr. IV, Gr. V animals on day of estrus were 56.54±0.84, 56.55±1.27, 54.82±0.41 and 57.39±0.73.

Gr. II, Gr.III, Gr. IV and Gr. V registered a significantly higher value from Gr. I control on post treatment day. All the treated groups had significant ($P<0.05$) variations in serum glucose level (mg/dl) within days of collection except Gr. I. In all these above cases the serum glucose level referred to be higher on day of estrus except Gr. I.

The pre treatment serum cholesterol (mg/dl) registered values within the range of 111.81 ± 1.60 and 120.53 ± 2.02. Similarly, the corresponding values on day of estrus/day 60 were recorded to be 121.465 ± 1.85, 136.99 ± 1.75; 130.03 ± 2.84, 127.87 ± 2.65 and 137.62± 2.74 in Gr. I and Gr. II, Gr. III, Gr. IV and Gr. V respectively.

There is a significant difference between Gr. V and other groups on the pre treatment day. The 2nd sampling taken on day of estrus registered a significantly

different value of serum cholesterol between Gr (I, II) and Gr. V. The Gr. I, Gr. II, Gr. III, Gr. IV and Gr. V had significantly higher ($P < 0.01$) value on the 2nd sampling than the 1st sampling.

The pre treatment value of serum total protein (mg/dl) during the current study ranged between 5.03±0.09 and 5.08 ± 0.10. On the day of estrus / day 60, Gr. I animals registered a serum protein level of 5.83 ± 0.14. The corresponding values of Gr. II, Gr. III, Gr. IV and Gr. V animals on day of estrus were 5.856± 0.11, 5.566±0.092, 5.553±0.149 and 5.911±0.13.

The groups showed significant variation in serum Total Protein levels within the different days of collection. Groups did not vary in the protein concentration between themselves irrespective day of sampling.

The pre treatment values of different groups registered a value within the range of 0.37 ± 0.14 to 0.45 ± 0.01. The second sample taken on the day of estrus/ day 60 also registered nearly similar values in Gr. I (0.0.49 ± 0.01) Gr. II (0.46 ± 0.01), Gr. III (0.50± 0.02) Gr. IV (0.43 ± 0.01) and Gr. V (0.49 ± 0.02) . The third sampling on 23 rd day post AI have serum conc. Of 0.49 ±0.01, 0.49±0.03, 1.6± 0.71, 1.11±0.65 and 3.13 ± 0.86 for Gr. I, Gr. II, Gr. III Gr. IV Gr. V, respectively.

There exists a significant difference in the serum progesterone values on pre treatment and on the day of estrus in all groups. Also a significant difference is found between groups on the pre treatment day and on the 23 days post AI . The serum progesterone values differ significantly between Gr. V and all other groups on the 3rd sampling.

The control group did not show any induced estrus, where as Gr. V showed 100 percent result in estrus response. Gr. II and Gr. III and Gr. IV showed 16.6%, 50% and 33.3% induced estrus respectively.

The heifers of the first two groups i.e. Gr. I and Gr. II did not lead to conception while the heifers in the Gr. III, Gr. IV, Gr. V have conception percentage of 33.3, 16.6 and 66.6 respectively. The control animals did not record estrus during 60 days of observation. The average estrus induction interval (days) for

Gr. II, II, IV and V animals are recorded to be 56.00, 49.00, 51.00 and 48.33 days respectively.

To evaluate the economics of treatment a calculation has been made to calculate the cost per conception (Rs.). Since the animals in Gr. I and II did not register any pregnancy, cost of pregnancy could not be calculated. The cost per conception for the animals in Gr. II, IV and V came to Rs. 1890/-, 3000/- and 1425/- respectively.

CONCLUSION

The following conclusions were made at the end of the experiment

1. The animals showed improvement in levels of serum calcium, phosphorus, glucose, total protein and cholesterolin post treatment sampling.
2. Hormonal protocols involving multiple hormone like GnRH, PG and inj. progesterone could be used as estrus induction protocols in pre-pubertal heifers to bring fertile estrus and successful conception with high success rate.
3. There was minimization of estrus induction interval and improved induction and conception rate after the hormonal intervention, which can increase the life time production of calf and milk.

REFERENCES

Agrawal JK, Saxena A and Singh V. 2015. Study on metabolic profile of repeat breeder, post-partum anoestrus and normal cyclic sahiwal cows, *Indian Journal of Animal Reproduction*, **36**(1).

Ahlawat A and Derashri HJ. 2009. Metabolic profile in cyclic and noncyclic vis-à-vis conceiving and non-conceiving cows, *Indian journal of Field veterinarians*, **4**(4): 13-16.

Ahlawat AR. 2003. Clinical, biochemical and endocrinological diagnosis of patho physiological conditions in cattle, M.V.Sc. Thesis, Gujarat Agricultural Universities, Anand Campus, Anand, India.

Ahmad I, Lodhi LA, Qureshi ZI and Younis M. 2004. Studies on blood glucose, total proteins, urea and cholesterol levels in cyclic, non-cyclic and endometritic crossbred cows, *Pakistan Veterinary Journal*, **24** (2): 92-94.

Aman Kumar, Singh AP, Pandey RP, Akhtar MH and Roy GP. 2007. Influence of some Haemato-Biochemical profile on incidence of anoestrum in cross-bred cows, XXIII Annual convention of ISSAR and National Symposium, OUAT, Bhubaneswar. 56.

Amle M, Patodkar V, Shelar R and Birade. 2014. Serum Biochemical Levels of Repeat Breeder Crossbred Cows under Rural Condition of Satara District of Maharashtra, *International Journal of Advanced Veterinary Science and Technology*, **3** (1): 109-113.

Anderson, Investigations on delayed puberty in Egyptian buffalo heifers with emphasis on clinic pathological changes and treatment using GnRH., Global Veterinaria 4 (1) : 78-85, 2010. *Indian Journal of field Veterinarians: 7 issue, p4*.

Bage R. 2003. Conception rates after AI in Swedish red and white dairy heifers in relationship with progesterone concentrations at AI, *Reproduction of Domestic Animals*, **38**(3):199-203.

Belloso ES, Martinez GP, Ondiz A, Rojas N, Castillo G S, Iglesia LR, Ganchu F P (2002). Improvement of reproductive performance in crossbred zebu anoestrus primiparous cows by treatment with norgestomet implants or 96 h calf removal, *Theriogenology*, **57**: 1503-1510.

Bhaskaran B and Abdullakhan CK. 1981. Role of blood serum inorganic phosphorus in postparturient anestrous cows, *Livestock Advisor,* **9:** 33-36.

Bhoraniya HL, Dhami AJ, Killedar A and Damor AM. 2010. Influence of estrus synchronization protocols on fertility and plasma progesterone and biochemical constituents in Kankrej cows, Proccedings of International Symposium. "Biotechnologies for optimization of reproductive efficiency of farm & companion animals to improve global food security & human health" and 26th Annual Convention of ISSAR, GBPUAT, Pantnagar, Nov. 10-12, pp. 62.

Bridges AS, Lake R, Lemenger M, Claeys, P and Gunn and Culp K. 2009. Choosing an oestrus synchronization program for replacement beef heifers, *Purdue Extension AS-592-W.*

Burfening PJ. 1979. Induction of puberty and subsequent reproductive performance, *Theriogenology*, **12** (4): 215-221

Butani MG, Dhami AJ and Kumar R. 2011. Comparative blood profile of progesterone, metabolites and minerals in anestrous, sub estrous, repeat breeding and normal cyclic buffaloes, *Indian J. Field Vets.,* **7(2):** 20-24.

Butler WR and Smith RD 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle, *Journal of Dairy Science*, **72**: 767-783.

Byerley DJ, Staigmiller RB, Berardinelli JG, and Short RE. 1987. Pregnancy rates of beef heifers bred either on pubertal or third estrus, *Journal of Animal Science,* **65**:645-650.

Cavalieri J, Kinder J. E, Driath G, Flitzpabick L. A. (1998). Effects of short term treatment with progesterone super imposed on 11 or 17 days of norgestomet treatment on the interval to oestrus and fertility in Bos indicus heifers, *Anim Reprod Sci.,* **51**(3):169-83.

Cetin M, Dogan I, Polat U, Yalcin A and Turkyilmaz O. 2002. Blood biochemical parameters in fertile and repeat breeder cows. *Indian J. Anim. Sci.,* **72:** 865-866.

Cetin, Yunus, Baris A. Uslu, orsan Gungor, and Sad Sendag 2007. Induction of oestrus with norgestomet in acyclic post-pubertal Holstein heifers. *Bull Vet Inst Pulawy,* **51**: 247-251.

Chandrakar D, Tiwary RP, Awasthi MK and Hirpurkr SD. (2003). Serum biochemical profile of repeat breeder crossbred cows, *Indian J. Anim. Reprod.,* **4**(2): 125-127.

Chaudhari CF, BN Suthar , VK Sharma, VS Dabas, NF Chaudhari, HH Panchasara 2012. Estrus induction and fertility response in delayed pubertal Kankrej heifers treated with norgestomet ear implant, *Vet. World,* **5**(8): 453-458.

Das PB and Bisoi PC. 2005. Clinical importance of some serum constituents in repeat breeder cow, *Indian Veterinary Journal*, **82**: 905.

De Rensis F and AR. Peters 1999. The control of follicular dynamics by PGF2α, GnRH, hCG, and estrous synchronization in cattle, *A review: Reprod. Domest. Anim.* **57**: 23-33

Deka KC, Borgohain BN, Nath KC, Goswami J, Biswas RK and Das PK. 2005. A comparative appraisal of serum progesterone level during estrus cycle in repeat breeder crossbred cows with ovulatory problem and normal breeders, XXI annual convention and nationalsymposium on recent trends and innovations in animal reproduction, I.S.S.A.R., November, 23-25, Jammu.pp-242.

Derar R, HA Hussein, S Fahmy, TM EI-Sherry and Megahed G. 2012. Ovarian response and progesterone profile during the ovsynch protocol in buffalo heifers and post partum buffalo cows (*Bubalis bubalis), Buffalo Bulletein,* **31(**3) . 136-147.

Dharani S, Kathiresan D, Devanathan TG, Balachandaran C and Satheeshkumar S. 2010. Ovulatory response of the first follicular wave growing and regressing phase follicle for GnRH administration in cyclic buffaloes, *Buffalo Bull:* **29**: 199-205.

Dhoble RL, Bhoite DP, Kumbhar UB, Sawale AG, Gaikwad SM and Deokate KR. 2004. Therapeutic application of progesterone for estrus induction in postpartum anestrus crossbred cows, XX Annual convention and National Symposium on Advanced Reproductive Technologies for management of fertility in livestock (Dec-14-15, 2004). Compendium P-68.

Dogan I and Cetin M. 2004. Biochemical investigation of blood plasma during estrus and diestrus in repeat breeder cows, *Indian Veterinary Journal*, **81**: 1232-1234.

Dora KPS. 2016. Induction of fertile estrus by application of different synch protocols in delayed mature heifers, Thesis submitted to Odisha University of Agriculture and Technology, Bhubaneswar, Pg- 63.

Dutta A, Sarmah BC and Baruah KK. 2001. Concentration of serum trace elements in cyclic and anestrous heifer in lower Brahmaputra valley of Assam, *Indian Vet.J.*, **78:** 300-302.

Foster DL and Jackson LM 2006. Puberty in the sheep. In knobil and neillls physiology of reproduction, NEIL Ed, *Elsevier,* 5127-2176.

Ghallab, AM, On RS, Cmarik GF, Kesler DJ, Faulknern DB, Nixon JE. 1984. Effects of repetitive norgestomet treatments on pregnancy rates in cyclic and anoestrus beef heifers, *Therioaenoloay*, **22**(1):67-74.

Ghuman SPS, Jagir Singh, M Honparkhe and D Dadarwal. 2009. Induction of ovulatory estrus using ovsynch protocol and subsequent fertility in true anoestrus buffalo heifers. *Indian Journal of Animal Reproduction,* **30**(2).

Gordon I. 1996. Controlled reproduction in cattle and buffaloes. CAB International, London.

Gottschall CS, Aguiar PRL, Almeida MR de, Magero J, Tolotti F, Brittencourt HH and Sune YBP. 2011. Use of progesterone, injectable or impregnated in intravaginal device, in the induction of cyclicity in prepubertal heifers before of the breeding season, Veterinariaemfoco 2011 Vol. 8 No. 2 pp. 108-120 ISSN 1679-5237.

Gupta S, Gupta HK and Soni J. 2005. Effect of vitamin E and selenium supplementation on concentrations of plasma cortisol and erythrocyte lipid peroxides and the incidence of retained foetal membranes in crossbred dairy cattle, *Theriogenology*, **64**: 1273-1286.

Hafez ESE, Hafez B (7th edition) Reproduction in Farm Animals, Seventh Edition, Blackwell Publishing Ltd, 9600 Garsington Road, Oxford OX42DQ,UK pp. 38.

Hall JB, Staigmiller RB, Short RE, Bellows RA, MacNeil MD, and Bellows SE. 1997. Effect of Age and Pattern of gain on induction of puberty with a progestin in beef heifers, *J. Anim. Sci.,* **75**: 1606-1611.

Imwalle DB, Patterson DJ, and Schillo KK. 1998. Effects of melengestrol acetate on onset of puberty, follicular growth, and patterns of luteinizing hormone secretion in beef heifers, *Biology of Reproduction,* **58**: 1432-1436.

Islam R. 2011. Synchronization of estrus in cattle, *A Review. Veterinary World,* **4**(3) 136-141.

Jaeger JR, Whittier JC, Corah LR, Meiske JC, Olson KC and Patterson DJ. 1992. Reproductive response of yearling beef heifer to a melengestrol acetate-prostaglandin F2α estrus synchronization system, *J. Anim. Sci.,* **70**: 2622-2627.

Jain A and Pandita NN. 1995. Biochemical blood profile of normally cycling and prostaglandin F2α treated subestrus crossbred cows, *Indian J. Anim. Reprod,* **16:** 88-90.

Jayanthi N, Chellapandian M and Balachandran S. 2003. Blood biochemical profile of repeat breeding cows in Tirunelveli region of Tamil Nadu, *Indian Veterinary Journal*, **80**: 939-940.

Kalita DJ, Sarmah BC and Bhattacharya BN. 1999. Mineral profile and fertility of cows, *Indian Vet. J.,* **76**(11): 971-972.

Kaneko JJ and Cornelius CE. 1970. Clinical biochemistry of domestic animals. 1st Edn., *Academic press*, Inc., New York.

Khade NB, Patel DM, Naikoo, Mehrajuddin, Dhami KJ, Sarvaiya NP and Gohel M M 2011. Estrus induction in pubertal anoestrus Gir heifers using different hormone protocol, *Indian Journal of Field Veterinarians.*

Khan S, Thangavel A and Selvasubramaniyan S. 2010. Blood Biochemical profile in repeat Breeding cows, *Tamilnadu Journal of Veterinary and Animal Sciences*, **6**: 75-80.

Kinder JE, Roberson MS, Wolfe MW and Stumpf TT. 1987. Management factors affecting puberty in the heifer. In: Proc. 39th Ann. Beef Cattle Short Course. pp 1636. University of Florida, Gainesville.

Krupakaran RP. 2013. Serum biochemical profile of jersey cross bred heifers, *International Journal of Food, Agriculture and Veterinary Sciences*, **3**(1): 9-11.

Kumar R, Butani MG, Dhami AJ, Kavani FS and Shah RG. 2009. Effect of different therapies on fertility and serum progesterone, metabolites and minerals profile in repeat breeding crossbred cows, *Indian journal of Field veterinarians*, **5**(2): 1-8.

Kumar R. 2009. Genetic polymorphism of HSP-70, LHR, LEPR genes and selective mineral status in postpartum anestrous buffaloes. Ph.D thesis submitted to College of Veterinary Science, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hissar.

Lakum PD. 2004. Management of reproductive problems in Holstein Friesian cows, M.V.Sc. Thesis, Anand Agricultural University, Anand, India.

Lamb GC, Larson JE, Geary TW, Stevenson JS, Johnson SK, Day ML, Ansotegui RP, Kesler DJ, DeJernette JM and Landblom DG. 2006. Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin Fa, and progesterone, *J. Anim. Sci.*, **84**: 3000-3009.

Lauderdale, Patterson DJ and Perry GA. 2013. Protocols for synchronization of estrus and ovulation. Beef Reproduction TaskForce- January 2013.

Leitman NR, Busch DC, Wilson DJ, Mallory DA, Ellersieck MR, Smith MF and Patterson DJ. 2009. Comparison of controlled internal drug release insert-based protocols to synchronize estrus in prepubertal and estrous cycling beef heifers, *Journal of Animal Science,* **87**(12).

Lima FS, Ayres H, Favoreto MG, Bisinotto RS, LF Greco, ES Ribeiro, PS Baruselli, CA Risco, WW Thatcher, Santos JEP. 2011. Effects of gonadotropin-releasing hormone at initiation of the 5-d timed artificial insemination (Al) program and timing if induction of ovulation relative to AI on ovarian dynamics and fertility of dairy heifer, *Journal of Dairy Science*, **94**(10): 4997-5004.

Lodhi LA, Qureshi ZI, Khan A and Hayat S. 1998. Comparative study of blood glucose, total proteins, calcium and phosphorus in cycling, non-cycling, repeat breeding and endometritic buffaloes, *Pakistan J. Biol. Sci.,* **1**(2): 66-68.

Lucy MC, Billings H, Butler WR, Ehnis LR, Fields MJ and Kesler DJ. 2001. Efficacy of an intravaginal progesterone insert and an injection of PGF_2alpha for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers, *Journal of Animal Science*, **79**: 982–995.

Madgwicka S, Evansb ACO and Beard AP. 2005. Treating heifers with GnRH from 4 to 8 weeks of age advanced growth and the age at puberty, *Theriogenology,* **63**: 2323-2333.

Mahanta J. 2019. Investigation into anovulation in cattle, *Thesis submitted to Odisha University of Agriculture and Technology, Bhubaneswar*, Pg-88.

Mahour SS, Nema SP, Shukla SP, Shrivastava N and Mehta HK. 2011. Biochemical profile of postpartum anoestrus and induced estrus crossbred cows, *Indian journal of Field veterinarians*, **6**(3): 53-55.

Mallory DA, Wilson DJ, Busch DC, Ellersieck MR, Smith MF and Patterson DJ. 2010. Comparison of long term progestin based estrus synchronization protocols in beef heifers, *Journal of Animal Science,* **88**(11) 3568-35781.

Manowar SAL and Singh C. 2001. Blood glucose concentration in crossbred (Friesian x Hariana) heifers and cows, *Indian Journal of Animal Sciences*, **71**(9): 848-849.

Martinez MF, Kastelic JP, Gregg P, Adams GP, Olson WO and Mapleloft RJ. 2002. The use of progestin in regimens for fixed time artificial insemination in beef cattle. *Theriogenology*, **50**: 350.

Mc Donald LE. 1989. Veterinart endocrinology and Reproduction, *3rd Edition. Lea and Febiger, Philadelphia.*

Messager S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J, Thresher I. Malinge RR, Lomet D, Carlton MB, Colledge WH, Caraty A and Aparicio SA. 2005. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54.

Mishra PC and Mohanty DN. 2007. Studies on Biochemical and heamatological status of infertile cattle in costal Orissa. XXIII Annual convention of ISSAR and National Symposium, 7th-9th December, 2007, OUAT, Bhubaneswar.P-49

Mishra PC. 2011. Investgation into certain hormonal causes of infertility in cattle, Ph.D thesis submitted to Orissa University of Agriculture and Technology, Bhubaneswar, pp. 232.

Nak V, Nak D, Incas KS, tek HB, Keskin A, Tuna B and Kumru IH. 2005. Effects on reproductive performance in cyclic and non-cyclic vii dairy heifers of ovsynch or PRID+ PGFja+PMSG administrations. Veteriner Fak Ultesi Dergisi, Uludag Universitesi, **24**(4): 21-26.

Nath M. 2014. Addressing prolonged oestrus in crossbred cows, M.V.Sc. Thesis, Assam Agricultural University, Khanapara, Guwahati-22, Assam.

Nautiyal, Hemant, Uma Shankar and Agarwal SK. 1997. Effect of gonadotrophin releasing hormone (00141-1) on induction of ovarian cyclicity in pubertal anoestrus buffalo heifers, *IJAR* , **18**(1): 13-14.

Okazaki T, Sasaki E, Hasegawa K, Takani T and Abe S. 2007. The effect of eCG treatment in an estrus synchronization protocol on pregnancy rates aher embryo transfer in Holstein heifers, *Reproduction, Fertility and Development*, **19**(1): 223-224.

Onita P and Colibar O. 2009. Energy, protein and mineral profile in peripartal period at dairy cows, *Lucrări ştiinłifice medicină veterinară,* **42**(2): 398-404.

Ozyurtlu, Nihat, Yunus Cetin, Ibrahim Kucukaslan and Mesih Kocamuftuoglu. 2009. Induction of oestrus with Norgestomet ear implant and PRID in acyclic Holstein heifers, *Journal of Anim. and Vet Advances,* **8**(5): 1035-1039.

Parida S. 2017. Fertility improvement in sub fertile cows by different hormonal protocols in special reference to HF crosses, *Thesis submitted to Odisha University of Agriculture and Technology, Bhubaneswar,* Pg-91.

Parida. 2015. Induction of estrus in postpartum anestrous buffaloes by different hormonal protocols, Thesis submitted to Odisha University of Agriculture and Technology, Bhubaneswar, Pg-59.

Parmar BN. 2013. Augmenting reproductive efficiency of infertile buffaloes using controlled breeding techniques in tribal areas, M.V.Sc. Thesis, submitted to Anand Agricultural University, Anand, Gujarat, India.

Patel and Dhami. 2006. Postpartum plasma profile of glucose and total protein in Holstein Friesian cows with and without hormone therapy under tropical climate, *Indian Journal of Animal Sciences*, **76**(2):118-123.

Patel JA, Dhami AJ, Kavani FS and Jani RG. 2005. Effect of hormonal therapies at breeding on conception rates and plasma biochemical profiles in repeat breeding Holstein Fresian cows, *International J. of cow Sci.,* **1**(2): 47-52.

Patel JA, Dhami AJ, Kavani FS and Jani RG. 2005c. Modulation of conception rate and plasma biochemical profile in repeat breeding Holstein Friesian cows by hormonal therapy, *International Journal of Cow Science*, **1**(2): 47-52.

Patel PM and Dhami AJ 2004. Post-partum plasma profile of cholesterol and triglycerides in Holstein Friesian cows with and without hormone therapy under tropical climate, XX Annual convention and National Symposium on Advanced Reproductive Technologies for management of fertility in livestock (Dec-14-15, 2004), Compendium P-43.

Patel PM, Dhami J, Hinsu TV, Ramani VP, Sarvaiya NP and Kavani FS. 2006. Comparative evaluation of blood biochemical and progesterone profile of fertile and infertile oestrus cycles in post-partum Holstein Friesian cows, *Indian Journal of Animal Science*, **76** (3): 191-195.

Pattanayak JB, Mohanty DN, Das S, Barik AK and Mishra PC. 2010. Modulation of conception rate by exogenous application of agriculture of PGF2α in dairy cattle, *Indian Journal of Field Veterinarian*, **10**(2): 11-14.

Prasad RN and Singh C. 2004a. Serum cholesterol concentration of PGF2α and progesterone treated post-partum anestrus crossbred rural cows. XX Annual convention and National Symposium on Advanced Reproductive Technologies for management of fertility in livestock (Dec-14-15, 2004). Compendium P-54.

Pursley JR, Wiltbank MC, Stevenson JS, Ottobre JS, Garverick HA and Anderson LL. 1997b. Pregnancy rates per artificial insemination for cows and heifers inseminated at synchronized ovulation or synchronized oestrus, *J. Dairy Sci.*, **80**: 295 - 300.

Qureshi MU, Qureshi MS, Khan R, Rahman A, Sohail MS, Ijaz A, Ahmad I, Ahmad S, Shoaib M and basit A. 2016. Relationship of blood metabolites with reproductive cyclicity in dairy cows, *Indian Journal of Animal Research*, **50**(3): 338-348.

Rajesh Kumar, Butani MG. Patel MD and Shah RG. 2009. Progesterone, metabolites and minerals in anestrus, subestrus, repeat breeding and cyclic cows, *Indian Journal of Animal Reproduction*, **30**(2): 19-22.

Randel RD, Lammoglia MA, Lewis AW, Neuendorff DA, Guthrie MJ. 1998. Exogenous PGF2a enhanced GnRH induced LH release in postpartum cows. *Theriogenology*, **45**: 643-654.

Roberts SJ. 1971. Veterinary Obstetrics and Genital Diseases, 2nd *Edition, CBS Publication, New Delhi, India.*

Rose C, Tucker WB, Willard ST, Williams A, Fuquay J, Ryan PL and Whisnant CS 2008. Evaluation of hormone treatments in modified ovulation

synchronization protocol in dairy herds, *Journal of Animal and Veterinary Advances,* **7**(2): 154-159.

Roy KS and Prakash B. 2008. Plasma luteinizing hormone profiles during ovsynch treatment in murrah buffalo heifers, *Indian Vet. J.,* **85** : 678-679.

Schams D, Schallenberger E, Gombe S and Karg H. 1981. Endocrine patterns associated with puberty in male and female cattle, *Journal of Reproduction Fertility. (Suppl.)* **30**:103.

Schillo KK, Dierschke DJ and Hauser ER. 1982b. Regulation of luteinizing hormone secretion in prepubertal heifers Increased threshold to negative feedback action of estradiol, *Journal of Animal Science,* **54**: 325.

Schmitt EJ-P, Diaz T, Barros CM, de In Sota RL, Drost M and Fredriksson EW. 1996. Differential responses of luteal phase and fertility in cattle following ovulation of the first wave follicle with human chorionic gonadotropin or an agonist of gonadotropin releasing hormone, *Journal of Animal Science,* **74**: 1074-83.

Short RE and Adams DC. 1988. Nutritional and hormonal interrelationships in beef cattle reproduction, *Canadian Journal of Animal Science,* **68**: 29-39.

Short RE and Bellows RA. 1971. Relationship among Weight Gains, Age at Puberty and Reproductive Performance in Heifers, *Journal of Animal Science,* **32**: 127-131.

Singh AS and Singh ONK. 2006. Blood biochemical and enzyme profile in estrus and anestrous heifers, *Indian Journal Animal Reproduction*, **28**: 42-45.

Singh CP, Saxena A, Prasad S and Prasad JK. 2007. Comperative studies on metabolic profile of anoestrus and normal cyclic Haryana cows, *Indian Journal of Animal Reproduction*, **28**(1); 42-45.

Singh RB, Sharma RD and Singh GB. 1983. Incidence of repeat breeding in cows and Buffaloes, *Indian J. Dairy Sci.,* **36:** 314-315.

Sivaraman T, Shanmugasundaram S, Arunachalam S and Sivakumar T. 2002. Blood profile of Jersey crossbred cows under different physiological stages, *Indian Journal of Animal Research,* **36**(2): 114-117.

Solorzano CW, Mendoza JH, Oder J and Romo S. 2004. Pregnancy rates after estrus synchronization treatment with new and reused CIDR-B devices, *Reproduction, Fertility and Development* **16**: (1 & 2).

Srivastava SK and Sahni KL. 2000. Blood mineral level affecting pregnancy rates in cows and buffaloes, *Indian J. Anim. Sci.,* **70**(1): 33-34.

Stevenson, Ottobre JS, Garverick HA and Anderson LL. 1997b. Pregnancy rates per artificial insemination for cows and heifers inseminated at synchronized ovulation or synchronized oestrus, *J. Dairy Sci.,* **80**: 295 - 300.

Swanson LV, Hafs HD and Morrow DA. 1972. Ovarian characteristics and serum LH. prolactin, progesterone and glucocorticoids from first estrus to breeding size in Holstein heifers, *Journal of Animal Science,* **34**: 284.

Tamuli MK, Kulkami NR and Ahmed FA. 2000. Induction of oestrus in heifers using exogenous hormones, *Indian J. Anim. Reprod.,* **21**(2): 105-106.

Thakur MS and VK Bhatt 2001. Induction of estrus in delayed pubertal cross-bred heifers with Receptal and long acting steroid preparation, *Indian J. Anim. Reprod.,* **22**: 73-74.

Vasconcelos. 2013. Progesterone -based strategies to induce ovulation in prepubertal Nellore heifers, *Theriogenology,* **79**(1): 135-141.

Veena MP, Gowrakkal M, Girish Kumar V, Gupta PSP and Narayana Swamy MN. 2015. Relation of various physiological blood parameters with the postpartum reproductive efficiency in cattle, *International Journal of Biomedical Research*, **6**(10): 780-785.

Wildt L, Marshall G and Knobil E. 1980. Experimental induction of puberty in the infantile female rhesus monkey, *Science,* **207**: 1373-75.

Wiltbank JN, Kasson CW and Ingalis JE. 1969. Puberty in cross bred and straight bred beef heifers on two levels of feed, *Journal Animal Science,* **29**:602.

Zulu Victor Chisha, Toshihiko Nakao, Kyoji Yamada, Masaharu Moriyoshi, KenNakada, Yutaka Sawamukai. 2000. Clinical response of inactive ovaries in dairy cattle after PRID treatment, *The Journal of Reproduction and Development,* **46**(6): 415-422.