COMPARATIVE EFFICACY OF DIFFERENT DRUGS PROMOTED TO INCREASE CONCEPTION RATE IN NON-INFECTIOUS REPEAT BREEDER BUFFALOES

T H E S I S

Submitted
In partial fulfillment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE
IN
ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

BY
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2023

DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"COMPARATIVE EFFICACY OF DIFFERENT DRUGS PROMOTED TO INCREASE CONCEPTION RATE IN NON-INFECTIOUS REPEAT BREEDER BUFFALOES"** or part there of has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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Shri. **CHOUTMAL VAIBHAV VIJAY** has satisfactorily prosecuted his course of research for a period of not less than one semester and that the thesis entitled **"COMPARATIVE EFFICACY OF DIFFERENT DRUGS PROMOTED TO INCREASE CONCEPTION RATE IN NON-INFECTIOUS REPEAT BREEDER BUFFALOES"** submitted by him is the result of original research work and is of sufficiently high standards to warrant its presentation to the examination in the subject of **ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS** for the award of **MASTER OF VETERINARY SCIENCE** Degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part there of has not been previously submitted by him for a degree of any other University.

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This is to certify that the thesis entitled **"COMPARATIVE EFFICACY OF DIFFERENT DRUGS PROMOTED TO INCREASE CONCEPTION RATE IN NON-INFECTIOUS REPEAT BREEDER BUFFALOES"** submitted by Shri. **CHOUTMAL VAIBHAV VIJAY** to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE** in the subject of **ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS** has been approved by the Student's Advisory Committee after oral examination in collaboration with the External Examiner.

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Acknowledgement

ACKNOWLEDGEMENT

प्रथमवयसि दत्तं तोयमल्पं स्मरन्तः
शिरसि निहितभारा नारिकेला नराणाम् ।
सलिलममृतकल्पं दद्युराजीवनान्तं
न हि कृतमुपकारं साधवो विस्मरन्ति ।।

Remembering the small amount of water which it was given in its early age, the coconut trees carry nectar like water on their head throughout their life. [In the same manner] good and noble people do not forget a favour done to them. Likewise, I take immense pleasure in expressing my appreciativeness for every person who has guided, encouraged, helped and supported me in life.

Gratitude is a powerful trait, they say, which I express primely to my Advisor and Research Guide, Dr. M. G. Patil, Asst. Professor, Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Parbhani. No words can justify her contribution not only to this thesis but my personality as a whole. I sincerely thank her for believing in me when I was doubtful of my choices. She gave me all the freedom necessary in OPD which enriched me as a Gynaecologist.

I am intensely grateful to the Krupachyarya of Veterinary Gynaecology, Dr. N. M. Markandeya, Professor and Head of the Department of Animal Reproduction, Gynaecology and Obstetrics, as well as a member of my advisory committee whose role in my life at this institute is inexplicable. He helped me through a very crucial phase and made me wonder his pragmatic personality. This thesis would have remained just a dream had it not been for him.

My words fall short in expressing gratitude for Dr. A. G. Sawale, Asst. Professor, Department of Animal Reproduction, Gynecology and Obstetrics, Parbhani. Calling me "Dada" all the time in his entrancing voice made me aware of his forever cheerful charisma which seldom failed to make my milieu lively.

Heartfelt benediction towards the members of my advisory committee Dr. G. R. Gangane, Associate Professor, Department of Veterinary Pathology, and Dr. N. D. Jadhav, Asst. Professor, Department of Veterinary Pharmacology and Toxicology, COVAS, Parbhani, for their permanent concord during my research period.

I owe my special gratitude to Dr. Pankaj Hase AP,MVC, Mumbai, Dr. S D. Chepte, Dr. Sandeep Rindhe and Dr. Mane, APs, COVAS, Parbhani, for being the source of valuable suggestions, corrective criticism and positive reinforcement.

I shall forever be indebted to respected seniors Dr. Shubham Bibekar, Dr. Ajay Shelke, Dr. Ravi, Dr. Ankush Dhabale, Dr. Kapil Pandit & Dr. Ujwal Bawanthade for cradling through my initial days in COVAS. Special thanks to dear colleague Dr. Shubhangi and my departmental juniors cum friends Dr. Joytsna Guwhade and Dr. Lalit Misalwar for their valuable help in the completion of research work and making Manuskich department.

Friends have always been an inseparable part of my life. I'd not thank them but rather express an abiding affection towards Dr. Akshay Adkine, Dr. Balaji Telange, Dr. Anand Jeughale, Dr. Harshwardhan Patil, Dr. Saurab Naik, Dr. Dipali Pathade, Dr. Pooja Chavan, Dr. Nikhil Malik, Dr. Sneha Swami, Dr. Sujay Chaphekar, Dr. Apurv Nanavare & Dr. Komal Dalal for making this two-year journey a memorable one. Without them there would have been no joy in this 'City of Dust'.

Dear juniors cum comrades Avinash Patil , Ganesh Karad , Sachitanand sakhre, Hrushikesh Patil , V. Ambhore, Poonam Padole, Pratiksha Bhagvat, Nana, Deva Bawaskar, Prashant, Rajendra, Saicharan and Rohit proved to be very obliging in reducing my coursework stress.

I extend special thanks to Shri. Bhopale Mama Shri. Vasant Mama and Rajratan for their valuable cooperation in the department.

I am deeply obliged to Dr. Omkar and Dr. Pranav for listening to me crib about everything in COVAS, and being a part of my Master's journey even without their physical presence.

My eternal love goes for Dr. Piyush, Dr. Jayant, Dr. Rajkumar, Dr. Sudrik (Patil), Dr. Pratik (Qazi), Dr. Shubham (Mau), Dr. Kailas (Bapu), Dr. Roshan (Salim). Having called as the 'Diamonds' owing to our camaraderie and strictness in UG, it won't be objectionable to call them a family beyond home. This acknowledgment would be incomplete without a mention of my Alma mater Nagpur Veterinary College, Nagpur. I feel graciously blessed to have spent the most blissful 5 years of my life there. Apart from igniting a spark in my personality, it sculpted me into the best version of myself and made me confident of achieving toughest of the feats.

I would specifically like to thank my music teacher Mr. Pandit Patil and dear junior Sakshi Kashikar for introducing art in my monochromatic academic life. I had never imagined myself playing Harmonium and Tabla in my wildest dreams.

And last but not the least, I wish to acknowledge the four people having supreme paramountcy in my life. My parents Vijay & Kusum Choutmal, for contributing my genotype and my guardians Tukaram and Sangita Choutmal for shaping my phenotype. Younger sister Vaishali and younger brothers Abhay - Omkar are the basis of my very existence.

It is difficult for me to mention the name of everyone in this piece of paper. I would like to take this opportunity to extend my deepest sense of gratitude and words of appreciation towards those, who have dedicated their today for my tomorrow.

You cannot teach a man anything; you can only help him discover it in himself.

- Galileo

Place: Parbhani

Date: / / ***(CHOUTMAL VAIBHAV VIJAY)***

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LIST OF ABBREVIATIONS

Abbreviation	Full Form
et al.	Et alia (and others)
i.e.	That is
viz.	Namely
IM	Intramuscular
%	Per cent
A.I	Artificial insemination
Inj.	Injection
Mg	Milligram
Kg	Kilo gram
@	at the rate of
<	Less then
>	Grater then
CVM	Cervico vaginal mucus
PMNs	Polymorphonuclear cells
UDM	Uterine defense mechanism
WST	White side test
R	Recovery cases
NR	Non-recovery cases
HM	Harbominvit
GM	Glycine chelated minerals
IP	Inorganic Phosphorus
CON	Control group
mcg	Micro-gram
$P< 0.01$	Significance less than 1%
$P< 0.05$	Significance less than 5%
*	Significant at 5%
**	Significant at 5%

Introduction

CHAPTER - I

INTRODUCTION

The successful key to animal production is reproduction. For successful animal production, each animal in the herd must have a proper and optimal reproductive cycle (Sastry, 2017). The dairy sector relies heavily on reproduction since it makes it possible to produce both the calf and milk for the following generation. Effective reproduction is the fundamental factor that determines the economics of animal husbandry.

Reproduction of domestic animals helps to play vital role even in times of lockdown, natural catastrophes, and harsh weather, dairy generates consistent revenue (Bhandari and Ravishankar, 2020). The term "animal husbandry" has been replaced in the era of contemporary dairy farming by the terms "reproductive management" and "reproductive nutrition," which denote a farmer focused on making a profit. Each of the year, every farmer should prepare for 80 percent of lactating and 80 percent of pregnant dairy animals on their farms, which calls for the use of cutting-edge technologies like the "calf a year" program, ration balancing, stress-free environments, preventive healthcare, and managed reproduction. Farmers are now more cautious of the need to raise dairy animals to early maturity, regular pregnancies, and higher lifetime calving rates.

Infertility due to failure of fertilization is the main cause for worry in non-infectious repeat breeding, but the factors that contribute to smooth pregnancy failure cannot be disregarded (Khosa, 2020). In the reproductive physiology of dairy cattle, key processes such as luteal development, structural construction of corpus luteum, progesterone availability, and continual progesterone level growth are anticipated. According to (Moore and Thatcher, 2006) in buffalo embryonic mortality is caused by the corpus luteum (CL) not working properly. Luteal dysfunction, also known as insufficient corpus luteum function, is often characterized by an estrous cycle that lasts a normal amount of time and a low concentration of peripheral progesterone (p4) because CL synthesis during the luteal phase is delayed. Lowered embryonic survival is caused by insufficient luteinizing hormone secretion during estrus, which is caused by a delay in cl development (M Fields and P Fields, 1996). It is well knowledge that 60–65 percent of animals have settled pregnancies during normal reproduction, compared to 85–95 percent of animals who have smooth fertilization processes. By increasing conception rates, Krishnan *et al*., (2017) claimed that the gap between the number of animals settling and those being fertilized must be reduced by 25 to 30 percent.

Reproduction which is luxuries process of animal's life for that the main entry point for trace elements into the food chain, soil also serves as a sink for elements derived from environmental sources. Due to their biological functions in development, maintenance, and metabolism, minerals are necessary for reproduction. According to Dring *et al*., (2021), minerals play certain functions and are necessary for certain reproductive tissues. These roles and requirements can also alter depending on the physiological state, such as cyclicity or pregnancy. Infertility is hypothesized to be related to mineral imbalances.

Glycine-chelated mineral mixture includes Calcium, Phosphorus, Potassium, Sodium, Chlorine, Magnesium, and Sulfur. They are also known as inorganic or mineral elements. Iron, Iodine, Zinc, Copper, Manganese, Cobalt, Molybdenum, Selenium, Boron, Lithium, Bromine, and Vanadium are among the microminerals or trace elements. It is yet unknown how Fluorine, Chromium, Tin, Silicon, Nickel, and Arsenic play a part. Multiple mineral deficiencies are often the cause of decreased fertility in most instances (McDowell, 1996). Ruminants needs for minerals are too low, and there aren't many of them to be found in feed. Chemical fertilizers have been used indiscriminately and often in agriculture for the past 50 years, which has led to an imbalance in the mineral status of the soil, plants, and blood. Mineral deficiencies are challenging to diagnose because they seldom exhibit overt symptoms and because estimates are expensive.

Injection of inorganic phosphorus is commonly used to repair the most common link between phosphorus and defects in reproduction. The million-dollar question is, "do we work hard for diagnostic techniques in repeat breeder cases?" for that serum phosphorus estimation help to diagnose the problem of repeat breeding which is due to hypophosphatemia helps us.

The distinction between infectious and non-infectious cases must be made on field conditions with the help of white side test, Cervical pH, and PMNs cell count. (Saraswat and Purohit, 2016)

GnRH at the time of AI is a diamond solution in non-infectious repeat breeding which is due to such factors like delayed ovulation, ovulatory abnormalities, premature insemination, and disruptions in the normal physiology of cycles like short/prolonged oestrus, split oestrus, induction/initiative oestrus, super ovulatory oestrus, irregular oestrus, false oestrus, gestational oestrus, etc. Where estrual management varies from type to type for conception.

The Powder Herbominvit combination is ideal for reproductive proficiency in modern dairy buffaloes and carefully re-evaluation based on well-designed scientific research. Supplementation includes Indian-origin herbs, extra vitamins and minerals that have frequently been proposed as a "golden bullet" solution to reduce declines in buffaloes fertility by various commercial interests. (Vlad *et al*. 2021)

Repeat breeding is responsible for huge loss in dairy economy. Due to less diagnostic approach for segregating infectious repeat breeding animals from non-infectious animals. As in field condition buffaloes are more sustainable for non-infectious etiologies. As there is no role of antibiotics in non-infectious condition so after conform diagnosis, we can reduce the use of antibiotics and also help to stop antimicrobial resistance.

Non-infectious cases of repeat breeding can be treated with GnRH support, mineral support, and herbal support at the time of artificial insemination, day after insemination to manage timely ovulation, luteinization of ruptured graafian follicle, better development of corpus luteum and high level of progesterone for preparation of uterine bed for implantation, the optimum concentration of minerals and energy. Hence this research work is undertaken with the following objectives.

1. **To study the effect of single dose of GnRH in repeat breeder buffaloes.**
2. **To study effect of Inorganic Phosphorus by parental route in non-infectious repeat breeder buffaloes.**
3. **To study efficacy of supplement Herbo-min-vit and Glycine chelated mineral mixture in non-infectious cause in Repeat Breeding buffaloes in two separate groups.**
4. **To study conception and pregnancy rate in buffaloes treated with Hormonal, Inorganic phosphorus, Herbo-min-vit and Glycine chelated trace minerals.**

Review of Literature

CHAPTER - II

REVIEW OF LITERATURE

The present experiment was planned on "Comparative efficacy of different drugs promoted to increase conception rate in non-infectious repeat breeder buffaloes". One of the most important reproductive issues has been identified as buffaloes being bred repeatedly. The primary cause of infertility has plagued dairy farms all over the world. The decreased conception rates in dairy buffaloes, cause time-consuming and expensive losses. Repeat breeder buffaloes were categorized generically as contagious and non-contagious. Veterinarians have been employing various therapies on non-infectious repeat breeder buffaloes under field conditions for a very long time.

This chapter refers to and reviews a variety of reports, interpretations, field cases, scientific publications, reference books, textbooks, compendia, websites, research journals, and online publications regarding non-infectious repeat breeder buffaloes due to mineral deficiency, hormonal imbalance, and delayed ovulation.

2.1 Non-Infectious repeat breeding

Singh *et al*. (2008) have found that repeat breeding occurs from (5.5%) to (33.33%) of the time in cattle and from (6%) to (30%) of the time in buffaloes in India. This is less than buffaloes, in comparison with cattle.

Ahmed *et al*. (2010) concealed that repeat breeding is one of the most significant and frequently seen subfertile situations in buffalo that is essential to the economics of the dairy industry. The disorder may be brought on by gamete defects, failure of gametic contacts, endocrine dysfunctions, infection, nutritional flaws, etc., which finally results in either failed fertilization or early embryonic mortality.

Wm *et al*. (2010) on a field assessment of 1358 female buffaloes found that (4.34%) of the buffaloes under examination had clinical repeat breeding (RB). Regular repeat breeders made up (7.25 %) of all female buffalo reproductive problems.

Kumar *et al*. (2011) on research (8.82%) of repeat-breeding buffaloes. The rate of recurrent breeding was highest in the second parity (27.77%) and lowest in the fourth and subsequent partum (11.11%). And also, they stated in their research that the failure of gametic contacts, endocrine dysfunction, infection, nutritional abnormalities, and other factors may be the cause of non-infectious repeat breeding. These factors ultimately result in either failed fertilization or early embryonic mortality. According to earlier studies, (39.7%) of implantation failures result from non-fertilization and (39.2%) from early embryonic fatality.

Saraswat and Purohit (2016) stated that ineffective fertilization and early embryonic mortality can be the causes of repetitive breeding in buffaloes. Only a few reasons why fertilization in buffaloes fails have been found. In buffaloes, ovarian cysts and ovulatory abnormalities are infrequent and cysts seldom produce noticeable clinical symptoms.

Butani *et al*. (2016) stated that repeat breeding occurs less frequently in small-holding animals housed individually rather than in big herds, with a range of incidences between (15%) and (32%). Repeat breeding in dairy cows is mostly brought on by hormonal imbalance, poor nutrition, poor breeding management, early embryonic death, and infectious pathogens that induce clinical and sub-clinical endometritis.

Shahzad *et al*. (2018) identified the primary cause of a buffalo's declining reproductive efficiency as non-infectious repeat breeding. Undernutrition is the main cause of anoestrus and repeated breeding among many other reasons.

Mohyuddin *et al.* (2019) found that repetition of breeding has an economic influence on buffalo production, which in turn affects the production of local meat and milk.

Arun and Amarna (2020) carried out research on pluriparous Jaffarabadi buffaloes. They found that compared to cattle the reproduction efficiency of buffalo is lower. because of anoestrus, repeat breeding, and abortion, protracted calving is caused by both infectious and non-infectious causes.

Khosa (2020) reported that repeat breeding continues to be the biggest danger to a successful farm's profitability, claims. The primary factors that turn high milk-yielding dairy cattle and buffaloes into repeaters are largely conception failure and early fetal mortality, which may also have a variety of infectious and non-infectious etiologies significantly upsetting the revenue.

Kumbhar *et al*. (2020) found that the prevalence of infected and non-infectious repeat-breeding buffaloes was (44.66%) and (53.33%), respectively, in 60 repeat-breeding buffaloes of the Aarey colony Mumbai.

2.2 Diagnosis of Non-infectious repeat breeding buffaloes

One of the most important factors affecting an animal's lifetime performance is its fertility. In the Marathwada area of Maharashtra, gynaecological issues such as non-infectious repeat breeding have a negative impact on the reproductive efficiency of buffalo. With the use of field diagnostic tests like cervical pH, white side test, PMNs cell count, Fern Pattern, etc., accurate and prompt separation from infectious causes lower a farmer's expense of antibiotics and also helps to stop antimicrobial resistance.

Kumbhar *et al.* (2020) found that repeat-breeding non-infected buffaloes with a characteristic fern pattern, thin mucus consistency, alkaline pH, and translucent colour can be predicted to have a higher conception rate.

Alai (2021) examined that for confirmatory diagnosis of non-infectious repeat breeding buffaloes white side test, cervical pH, fern pattern are best diagnostic tools at field level.

2.2.1 White side test

Krishnakumar *et al*. (2003) found that the effectiveness of the white side test for the identification of subclinical endometritis in crossbred cows investigated. They recorded the percentages of cows in the natural service group having normal (0.0%), mild (46.15%), moderate (38.46%), and deep yellow (15.38%) colour alterations. Additionally, the percentage of cows in the artificial insemination group showing normal (44.12%), mild (32.35%), moderate (14.70%), and deep yellow (8.82%) colour changes. They came to the conclusion that the white side test was an affordable and practical method for detecting non-infectious subclinical (occult) endometritis in cows and buffaloes in the field.

Bhat *et al.* (2014) found that the white side test is predicated on the presence of leucocytes in cervicovaginal mucus. The quantity of leukocytes present in uterine discharge exhibits a variety of colour in infectious animals but does not colour non-infectious animals after boiling the discharge.

Sarkar *et al*. (2015) conducted that bovine endometritis was treated with leucocyte-enriched autologous plasma, and the type of cervical vaginal mucus was reported as purulent (50–60%) or mucopurulent (40–50%) in the pre-treatment. All samples also showed a moderately to slightly positive response to the white side test. In (90%) and (80%) of the animals infused with autologous plasma together with leucocytes and autologous plasma post-treatment estrus, the cervical vaginal mucus went clear and became negative to the white side test, respectively. Only (16.7%) of control group animals had an additional estrus.

Thombare (2017) concluded that the white side test is often favoured to distinguish the physical characteristics of cervical mucus of infected and non-infectious repeat breeder animals before and after treatment.

Kumbhar *et al*. (2020) studied the white slide test he collected cervical mucus from infected repeat breeder buffaloes as well as non-infected repeat breeder buffaloes. Infected repeat breeder buffaloes had a dark yellow colour, whereas non-infected repeat breeder buffaloes had no colour.

2.2.2 pH of Cervico Vaginal Mucus:

Wani *et al*., (1982) examined the physical properties of cervical mucus in the majority of the repeat breeding cows and showed that the color was turbid (64.44%), consistency was thick (84.44 %), elasticity was elastic (62.22%) and fern pattern was typical (60.00%). The pH value of cervical mucus in repeat breeding (7.95 ± 0.096) cows.

Modi *et al.* (2011) resulted that the cervical pH is the earliest sign of a change in the reproductive environment in reproductive animals. As a result, cervical pH would be altered in infectious repeat breeder buffaloes but not in non-infectious repeat breeder buffaloes.

Kumar *et al.* (2011) found that the hydrogen ion concentration (pH) of the cervical mucus of non-infectious buffalos was measured using a narrow range pH paper (range 6.5 to 9.00) with a difference of just 0.5, and the overall mean pH of cervical mucous prior to and after treatment was 8.027 ± 0.11 and 7.458 ± 0.11, respectively.

Ramsingh *et al.* (2013) reported that uterine infection is also brought on by infertility during insemination and unclean parturition. Increased vaginal secretions with a pH of 7.5 to 8.0. This may be caused by bacterial contamination of the uterine fluids, and the elevated pH makes it impossible for spermatozoa and embryos to survive in the uterus. So normal pH of cervical/vaginal mucosa is indicative of no infection.

Varma *et al.* (2014) reported that the pH of their cervical mucosa 7.0 to 7.5 pH in (12.76%), 7.5 to 8.0 pH in (68.09%), and more than 8 pH in (19.15)% divided the repeat breeding Murrah buffalo into three groups. It is greater than repeat non-infectious animal breading.

Puro (2016) in his investigation came to the conclusion that in buffaloes and cows, cervical pH clearly distinguishes between infectious and non-infectious causes. Higher above the typical discharge range of 6.5 to 7.4 suggests to an infection.

Kumbhar *et al.* (2020) in his study reviewed that the cervical mucus of all 32 non-infectious repeat-breeding buffaloes was alkaline, which is known to make the animals pH more alkaline in response. In conceived buffaloes, the mean pH of cervical mucus was 7.540 ±09, but in non-conceived buffaloes, it was 7.440 ±06.

2.2.3 Fern pattern of cervical mucosa

Tsiligianni *et al*. (2001) demonstrated that in repeat-breeding buffaloes cervical mucus crystallization is distinctly influenced by the action of estrogen, which increases its incidence, and progesterone, which decreases it.

Samad *et al*. (2002) showed that fern pattern was typical in (55%), atypical in (45.0%), and nil in buffaloes that were repeatedly bred. The best evidence of successful artificial insemination is the fern pattern.

Sharma *et al.* (2011) clearly demonstrated that the fern pattern of estrual mucus and the plasma progesterone and estrogen profile are directly correlated and that the favorable rheological characteristics of estrual cervical mucus support sperm survival and transport in the cervical/uterine lumen to help improve conception rates in dairy animals. He also resulted that buffaloes at the time of insemination had a frequency of the typical, atypical, and nil types of fern pattern in (39.34%), (42.63%), and (18.03%) of the mucus samples, respectively, with corresponding plasma Progesterone values of 0.28± 0.03, 0.52±0.11, and 0.73±0.09 ng/ml.

Gohel *et al*. (2012) found that in the Summary of findings, (80%) of the buffaloes showed characteristic fern arrangements, which are favourable for conception in repeat breeders.

Kumar *et al.* (2013) recorded that as the pattern appears 84 hours before oestrus and starts diminishing before ovulation. The fern pattern demonstrates increased branching and the ideal moment for fertilization. The viscosity of mucus decreases at the time of oestrus.

Kumbhar *et al*. (2020) studied 32 non-infectious buffaloes. Out of 32 non-infectious repeat breeder buffaloes, 21 (65.63%), 10 (31.25%), and 1 (3.12%) buffaloes respectively had typical, atypical, and nil fern patterns of cervical mucus.

Zakiuddin *et al*. (2022) resulted in repeat breeder buffaloes, there were (32.00%) typical, (54.00%) atypical and (14.00%) nil type arborization patterns. In all experimental buffaloes, (15%) of infection cases and (45.83%) of non-infectious cases showed typical arborization, respectively. Atypical arborization was seen in the estrual mucus of infected and non-infected buffaloes in (55%) and (54.16%) respectively.

2.2.4 <u>PMN cell count</u>

To identify the existence of exfoliated cells from the vaginal wall, which would change its morphological features, vaginal cytology was performed. During vaginal cytology, PMN cells, epithelial cells, and lymphocytes are typically seen.

Azawi *et al.* (2008) when the discharge had a fetid smell, was purulent, or was clear and odorless, the PMNs were substantially ($P<0.01$) linked with $r = 0.894$ with the nature of the vaginal discharge, as they were (48.10%), (40.38%), and (05.00%), respectively.

Barlund *et al.* (2008) in their study came to the conclusion that a cytological examination of eight percent of PMN cells indicates the presence of an endometric condition. After treatment, the percentage decreased to below five percent, and the reduced count was comparable to that of non-infectious animals.

Babu *et al*. (2013) affirmed that inflammation in the genital tract causes an increase in PMN cells, according to the polymorphonuclear leucocyte cells (PMN, PML, or PMNL) are white blood cells that are distinguished by having granules in their cytoplasm. Granulocytes are also known as these cells because of the various forms of their multilobed nuclei. When the bacterial burden rises, this sort of cell grows. However, there is no inflammation to the uterine parenchymal in non-infectious buffalos, which gives a smaller count in such a state. He found that all 100 cases of endometitis in infectious repeat breeder buffaloes were positive. The PMN cell count ranged from 05 to 15 cells. The principal immunological defense mechanism in the uterus, PMN cells are known to increase when infection is present and can range from mild to severe.

Dutt *et al.* (2017) were done research on 10 repeat-breeding murrah buffaloes that were examined for the presence of PMN cells, AST, and bacterial identification. They concluded that the PMN cell count in infected and non-infected buffalo was (10%) and (5%), respectively, by utilizing the cytobrush approach.

Singh *et al.* (2018) non-infectious buffalo have PMN cells (5%), which are most comparable to regular buffaloes (4%). This demonstrates that the non-infectious buffalo's PMN cell count is within the normal range.

Bibekar (2022) found that all 100 cases of endometitis in infectious repeat breeder buffaloes were positive. The PMN cell count ranged from 05 to 15 cells. The principal immunological defense mechanism in the uterus, PMN cells are known to increase when infection is present and can range from mild to severe.

2.2.5 Serum phosphorous

Kumbhar *et al*. (2012) the average haemato-biochemical values for serum phosphorus in the treatment group were 3.610.20, 5.750.39, and 5.210.31 correspondingly for the before and after measurements. In the treatment group, out of 10 buffalo heifers treated, 9 (90%) exhibited oestrus following the treatment, and altogether, 7 (70%) buffalo heifers were conceived, whereas in the control group, out of 6 buffalo heifers, only 1 (16.66%) exhibited oestrus with an overall conception rate of 1 (16.66%). In the Marathwada region, mineral supplementation and good management practices could increase the fertility in delayed pubertal buffalo heifers under village management conditions.

Akhtar *et al*. (2014) in the study found that repeat-breeding buffaloes had substantially lower serum calcium, inorganic phosphorus, magnesium, copper, iron, and zinc contents than non-cyclic buffaloes. Mineral combinations should be included in the feed to increase the reproductivity of buffaloes that are non-infectious repeat breeders.

Maurya and Singh (2015) the serum phosphorus levels (mg/dl) in the buffaloes of the landless, marginal, small, and large categories of farmers were estimated to be 5.01±0.120, 5.08±0.088, 5.15±0.139, and 5.35±0.178, respectively. In the entire population of buffaloes, the serum phosphorus level was 5.15±0.068.

Shahzad *et al*. (2018) studied the impact of the minerals calcium (Ca), magnesium (Mg), and inorganic phosphorus (Pi) on the cyclicity of Nili Ravi buffaloes revealing that a high phosphorus-calcium ratio may reduce a buffaloes ability to reproduce.

Kumbhar *et al*. (2020) found that the average mean blood phosphorus content was 4.29±13 mg/dL when 32 non-infectious repeat breeder buffaloes were tested in the Mumbai area.

Kumar *et al.* (2020) infertility related to hypophosphatemia is thought to be characterized by anestrus, sub-estrus, irregular cycles, a low incidence of conception, and recurrent breeding syndrome in the absence of clinical symptoms of phosphorus shortage in farm animals.

2.3 Therapeutics

2.3.1 GnRH at the time of AI :

The use of artificial insemination, which is crucial for genetic advancement and for managing the buffalo's reproductive cycle, has considerably risen during the past 20 years. Due to erratic estrous cycles, diminished estrous behaviour and seasonal reproduction. AI in buffalo is more challenging than in cattle. During the non-breeding season, the latter is linked to an increased anestrum incidence and embryonic death. The management of the estrous cycle stage has recently undergone development in buffalo.

Pandey (1983) found that when 20 mcg/animal of GnRH was administered to Murrah buffaloes on the day of AI. There was a (51.30%) conception rate which is more than the control group.

Niswender *et al*. (1985) studied that in order to increase the active luteinization of granulose and theca cells and ensure adequate progesterone production in developing CL, the GnRH induces a second surge of LH. Second GnRH may have stimulated the conversion of tiny luteal cells into large luteal cells, which are in charge of around (85%) of basal progesterone production at the luteal phase by acting on the growing CL.

Rangnekar *et al*., (2002) used Buserelin acetate and Gonadorelin were injected through intra muscular route for the treatment as GnRH analogues. Depending upon the groups, either single or double inseminations were performed in these cows. In double insemination group, the cows were re inseminated 24 hours after the first AI. Among the Jersey cows 59.6 and 53.8% animals conceived, following GnRH administration and 40.0 and 45.0% conceived in control group (without GnRH) following single or double insemination, respectively. Similarly, among crossbred cows, 58.2 and 55.5% animals conceived when inseminated along with GnRH and 45 .0 and 60.0% conceived in control groups following single and double insemination, respectively.

Batavani and Eliasi (2004) examined river buffalo conception rates were examined in field experiments to see how gonadorelin affected them. 84 lactating cyclic buffalos were divided into three treatment groups for this study: an untreated control group, an I.M. At the time of artificial insemination gonadorelin 0.25 mg was injected and I.M. On days 11 through 13 of estrous, gonadorelin 0.25 mg is injected. Rectal palpation of the uterus was used to detect pregnancy between days 42 and 55 following insemination. The corresponding pregnancy rates were (25%), (55%), and (58%). The outcomes showed that whether given at the time of AI or during the mid-luteal phase following AI, gonadorelin dramatically increased pregnancy rates in buffalos.

Giordano *et al.* (2013) animal that ovulates in response to the Ovsynch protocol's first GnRH treatment are more fertile than cows that do not ovulate in response to the second GnRH treatment. The creation of a new CL during ovulation to the single dose of GnRH therapy of Ovsynch (GnRH) resulted in a rise in circulating progesterone (P4) throughout the Ovsynch regimen.

Sahu *et al.* (2014) affirmed that in order to ensure successful fertilization at AI ovulation is induced using a GnRH analogue. GnRH was administered right before AI to time the LH surge and ovulation.

Shephard *et al.* (2014) stated that rather than shortening the time between AI and ovulation, GnRH's beneficial effects at AI may be caused by increased egg maturation and luteal function.

Neglia *et al.* (2020) resulted that the GnRH at the time of artificial insemination currently reach equivalent levels of efficacy as procedures in beef and dairy animals. Utilizing buffalo that have undergone genetic improvement in

Europe, the Americas, and Asia has allowed for a larger dissemination of germplasm.

2.3.2 <u>Treatment of Inorganic Phosphorus :</u>

Parmar *et al.* (2012) resulted in three village cooperative societies in the Panchmahal district, fifty-two postpartum anoestrus Surti buffaloes were chosen by gynaeco-clinical examinations, and they were then randomly divided into four groups. Group-A (n=14) received oral supplementation of chelated mineral mixture at 30 g/day/animal for one month; Group-B (n=14) received intramuscular injections of inorganic phosphorus and vitamin AD3E (10 ml each) at weekly intervals for three consecutive weeks; Group-C (n=12) received an intramuscular injection of GnRH (5.0 ml), once; and Group-D (n=12) received no treatment. At various stages, the mean plasma levels of calcium and inorganic phosphorus in buffaloes from other groups indicated no significant differences.

Ali *et al*. (2014) found that however, there was no statistically significant difference in these values between repeat breeders and estrus-phased calves. Serum phosphorus levels in normal estrual (4.990.08 mg/dl), repeat breeder (3.900.06 mg/dl), and anestrous (3.820.04 mg/dl) Cholistani cattle were considerably higher (P0.01) than those in those two groups.

Khan *et al.* (2015) designed an experiment on buffalo providing a higher level of nutrition, vitamin E, and mineral supplementation for the purpose of enhancing the improvement in reproductive performance. On day 15 before delivery it was discovered that the concentrations of plasma Ca and plasma inorganic P were significantly different ($P<0.01$).

Kalasariya *et al*. (2016) in non-pregnant buffalos, the mean plasma inorganic phosphorus levels decreased considerably ($p<0.05$) and changed non-significantly during gestations.

Shahzad *et al.* (2018) studied the impact of the minerals calcium (Ca), magnesium (Mg), and inorganic phosphorus (Pi) on the cyclicity of Nili Ravi buffaloes. In repeat and non-cyclic breeders, they observed that the Phosphorus was out of balance.

Dhami *et al.* (2019) reported that a biochemical analysis of the serum was around (20%) of animals found to be deficient in calcium and phosphorus and the calcium: phosphorus ratio ranged from 1:1 to 1.8:1 in animals under various studies, suggesting that it may play a role in the infertility of dairy animals. A lack of phosphorus results in irregular oestrus, anoestrus, decreased ovarian activity, and a lower rate of conception. Epiphyseal growth during embryogenesis was demonstrated to be impacted by dietary manganese deficit, resulting in the birth of calves with congenital skeletal abnormalities.

2.3.3 Herbominvit

Verma *et al.* (2014) conciliated that animals stress is reduced by L-Dopa, which is found in *Mucuna pruriens*. It is abundant in several necessary amino acids, vital fatty acids, carbohydrates, and crude protein which enhance the milk production of dairy-feeding animals. Follicle-stimulating hormone and luteinizing hormone levels rise in a dose-dependent manner, increasing the number of oocytes released during ovulation.

Kumar *et al.* (2016) conducted an experiment to investigate the impact of Aegle marmelos and Murraya koenigii on the follicular development in delayed pubertal Sahiwal heifers. He investigated 14 Sahiwal heifers split into Group 1 and Group 2 for that purpose. Following dose extrapolation from the effective 50% ethanolic extract dose in rats (i.e., 1000 mg/Kg b. wt.) indicating optimal ovarian function, heifers in Group 1 were fed with shade-dried ground leaves in combination in a concentrated mixture for 9 days. In G-2, no therapy was provided to the heifers. Every other day for ten times, a trans-rectal USG scanner was used to check on all the heifers. they discovered that Aegle marmelos and Murraya Koenigii supplements can influence the growth processes of LF by achieving dominance, accelerating growth rate, preovulatory size, and the ovulation process.

Das *et al.* (2016) stated that due to the synergistic or cumulative effects of their active principles, herbal mixtures can have notable positive benefits. In their natural condition, plants offer a wide range of positive benefits, and they hold enormous promise as a source of novel treatments. In lab rats, anoestrus caprine, and cattle, the traditional treatments Murraya koenigii (curry leaves) and Aegle marmelos (bael) have been proven to improve reproductive performance and also claimed that Murraya koenigii boosted ovarian glucose-6 dehydrogenase and 3 beta HSD enzyme expression as well as the quantity of big surface and embedded follicles. This further activates steroidogenic activity, especially oestradiol 17- in the ovaries, which activates the mitosis of granulosa cells in growing follicles, hence enhancing follicular growth. Murraya leaves are a great source of minerals, including calcium, phosphorus, iron, and other elements. They help to induce estrus and increase these minerals serum concentrations.

Dutt *et al*. (2018) studied two herbal plants, Murraya koenigii and A. marmelos, have undergone individual and combined scientific testing for ovarian dynamic research in rats, goats, cattle, and buffaloes. Anoestrous dysfunction, repeated pregnancy and endometritis have all been treated successfully with these botanicals.

Sarswat & Purohit (2020) said that the immunomodulatory ability of *Aristolochia indica* (Isharmur) helped to avoid uterine infection by boosting the local immune system.

Suryawanshi *et al*. (2020) as agreed with Mucuna is a valuable plant that has been utilized for medical and biochemical purposes since ancient times, and has a high market value since it contains several bioactive chemicals. It has a lot of phenolics, flavonoids, and antioxidants, which help to relieve oxidative stress.

Satheshkumar *et al*. (2021) claim that in cattle, Aloe barbadensis has been shown to enhance follicular growth and steroidogenic activity. Thymoquinone (TQ), one of Nigella sativa's most potent constituents, has several advantageous traits. This plant is useful because of its anti-inflammatory, immunomodulatory, and antibacterial characteristics.

Trigo *et al.* (2021) found that Moringa oleifera, which has a nutritional profile rich in high biological value proteins, vitamins A and C, antioxidants, omega-3 fatty acids, and minerals such as calcium, iron, potassium, and phosphorus, may be utilized in animal feed.

Dutta *et al.* (2022) found that out of 108 cattle, 84 (77.7%) were conceived as a result of the creation of the ethnoveterinary medication for the management of repeat breeding in accordance with the feeding regimen. The manufacture of ethnoveterinary medicines includes 100 grams of white radish (Raphanus sativus rhizomes), 100 grams of entire sliced aloe vera leaf, 50 grams of drumstick leaves (Moringa oleifera), and 100 grams of hadjod stem (Cisuss quadrangularis) 50 grams of curry leaves (Murraya koenigii) and 10 grams of turmeric (Curcuma longa). In terms of species, 56 (79.5%) buffaloes and 26 (74.3%) cows gave birth. In comparison to cattle, buffaloes had a somewhat greater conception rate.

2.3.4 Therapies of Glycine-chelated mineral mixture

Boland and Lonergan (2003) were undeniable that minerals have the ability to have a big impact on herd fertility. The minerals that are particularly important are divided into major and trace elements, like iron, iodine, copper, manganese, zinc, cobalt, molybdenum, and selenium.

Ahmed *et al.* (2010) concluded that the treatments with chelated mineral mixture, GnRH, and Lugol's solution were effective for repeat breeder buffalo-cows, with recovery rates of 63.64, 61.54, and 60.00%, respectively. According to the study's findings, particular attention should be given to food additives to manage this syndrome.

Méplan (2011) Some of the bioelements, like Cu, Zn, Mn, Fe, and Se, function as co-factors for numerous antioxidant enzymes and are involved in a variety of metabolic processes in living things. They are crucial for cell metabolism and a number of other bodily functions, such as energy production, growth, reproduction, and nervous system function.

Tiwari *et al*., (2012) experiment was conducted to study the incidence of cyclicity, biochemical profile (blood glucose, non-esterified fatty acid) (NEFA), macro minerals viz Ca, Pi, Mg, micro minerals viz Cu, Co, I, Zn, Mn, hormones, viz. progesterone and insulin in acyclic and cyclic Murrah buffaloes during summer (March to June 2009) in Chhattisgah. Out of 108 Murrah buffaloes 49 (45.37%) were found without palpable follicles and corpus luteum at per rectal examinations performed twice at 12 days apart, indicating true anestrus. The biochemical and hormonal profile were estimated in 6 cyclic and 24 acyclic Murrah buffaloes during summer. There was no significant difference in blood glucose and NEFA level in cyclic and acyclic buffaloes. The level of inorganic phosphorus and Mg were significantly lower in acyclic buffaloes than cyclic buffaloes. There was no significant difference in the level of calcium, however there was significant difference in Ca:P in cyclic and acyclic buffaloes. The micro minerals Zn, Fe, Cu, Co were significantly lower in acyclic buffaloes, however, there was no significant difference in the level of Mn and iodine. The serum progesterone level was significantly higher in cyclic than acyclic buffaloes. There was no significant difference in the level of insulin.

Patil *et al.* (2014) stated that anoestrus and repeat breeding conditions in nondescript cattle may be caused by deficiencies of Copper, Iron, Zinc, and Manganese alone or in combination. By enhancing the nutritional state, female animals' fertility can be increased.

Bhanderi *et al.* (2016) became clear that the Jharkhand state feeds and forages might not contain enough calcium, phosphorus, magnesium, copper, zinc, and cobalt to support a daily milk production of fewer than ten kilograms. To improve productivity and reproduction efficiency, it is therefore required to replace these lacking minerals by the use of an area-specific mineral mixture in dairy cows' diets.

Gayathri and Panda (2018) compared other feeding innovations, with chelated minerals and found chelated minerals as a superior solution. By enhancing their absorption and preventing any interaction with other minerals, organic chelates are used to boost the bioavailability of minerals. Chelates are absorbed post-ruminally in the intestine in a way that is distinct from how inorganic minerals are taken. Chelates are stable in the rumen. The chelated minerals that produce plasma are unaltered, but at the point of use, separation occurs.

Joshi *et al*. (2019) in order to evaluate the impact of chelated mineral mixture (CMM) supplementation on blood biochemistry, hormonal and mineral status, nutritional intake, and reproductive performance of the repeat breeder buffaloes (n = 24), an on-farm trial lasting 90 days was carried out at four tribal communities. The chosen animals were divided into two groups of 12 each at random. The T2 treatment group (T1 + CMM @ 50 g/animal/day) was compared to the T1 control group (the farmer's feeding schedule). The research showed that supplementing with fake minerals helped repeat breeding buffaloes' health, nutrition, and reproductive status.

Kantwa *et al*. (2021) carried out studies to determine how supplementing buffalo with chelated mineral mixtures affected their ability to reproduce. Until 90 days into the early lactation phase, the treatment group received 40 g of chelated mineral combination daily whereas the control group did not get any supplements. The farmer every day in the morning and evening, as well as the researcher every two weeks, collected the data. At the same time between both groups, other reproductive performance characteristics, such as the start of the first estrum after calving, the number of AI needed for conception, and the service period, were also noted. These results may indicate that chelated mineral combination supplementation improved the productive and reproductive performance for a greater return and long-term profit from buffalo farming.

George *et al*. (2022) categorized the minerals into major and trace elements, and the minerals that are of particular interest include iodine, iron, copper, manganese, cobalt, zinc, molybdenum, and selenium. The minerals undoubtedly play significant roles in the fertility of the herd. Complexes of trace minerals with amino acids are more bioavailable and, consequently, better absorbed by the body than those obtained from inorganic sources.

Materials and Methods

CHAPTER-III

MATERIAL AND METHODS

The present research entitle "Comparative efficacy of different drugs promoted to increase conception rate in non-infectious repeat breeder buffaloes" from March 2022 to November 2022 in non-infectious repeat breeder buffaloes from various private dairy farms, through an infertility camp organized by NDDB in Nanded district, Veterinary Clinical Complex of College of Veterinary and Animal Sciences (COVAS) Parbhani, animals reported to Government Animal Hospital and Government Mini Polyclinics from Parbhani district and field level. Examination of the samples were carried out in a laboratory at Veterinary Biotechnology, and Veterinary Pharmacology and Toxicology in College of Veterinary and Animal Sciences (COVAS), Parbhani.

3.1 Selection of animals

In present research, a total of 75 repeat breeder buffaloes were examined. Buffaloes were selected for study from various breeds, age, and parity. Post-partum buffaloes in late lactation and in the dry stage were examined. Buffaloes heifers having repeated more than 3 times were also involved in study. Preventive health care plans were continued during the period of experimentation. The repeat breeder buffaloes were per rectally and per vaginaly examined for tubular genitalia (palpabral Utero-cervical pathology) and ovaries of animal with genital abnormalities, ill and deprived health condition (BCS < 2.5), low pH of cervical mucus, PMN cell count (>5) and positive for white side test (WST) were eliminated from this study. Table 3.1 shows details of the selection and rejection of animals.

Table 3.1. Details of clinical cases of repeat breeder buffaloes for selection of clinical experimentation.

Criteria to select or reject cases for trials	Repeat breeder cases		
	Heifers	**Post-partum**	**Total**
Total number of repeat breeder animals attended	18	57	75
Cases rejected due to palpable genital pathology	04	01	05
Number of animals rejected due to low pH of cervical mucus (> 10)	04	05	09
Number of animals rejected on the basis of PMN cell count (>5)	00	01	01
Number of animals selected on negative Whiteside test and PMN cell count (< 4).	10	50	60
Animal kept for control group	05	07	12
Animal with Body score condition Score <2.5 or 3	16	44	60
Total cases selected for experimentation	**10**	**50**	**60**

3.2 Clinical examination and history of animals

Buffaloes with a history of normal estrus repeating every 18-21 days but were unable to conceive after 3 or more consecutive A.I or natural services were subjected for per rectal and per vaginal examination. Body conditions greater then BCS > 2.5 visual a copious, clear and watery discharge similar to egg white in nature were considered for the present study.

3.3 Diagnosis of Non-infectious repeat breeding cases

3.3.1 <u>Collection of cervical mucus</u>

With the help of A.I sheath and 20ml syringe cervical mucus of buffalo has collected aseptically on the day of estrus from the cervix or by per rectally

Plate 1. Screening of repeat breeder buffaloes for the research study

giving upward direction to the cervix and collecting the vaginal discharge in the sterile test tube.

3.3.2 Clinical evaluation of cervical mucus

I. <u>Fern Pattern</u>

A thin smear of cervical mucus was made on the clean grease-free slide. The smear was dried in air and examined under a microscope and classified as under:

a) Typical fern pattern

The arborization was visible throughout the smear. The branches were well-marked and had bright and thick boundaries. The primary, secondary and tertiary branching were visible in the smear.

b) Atypical fern pattern

The fern pattern scattered in the smear with coarse branching without subventions and of irregular type was categorized as atypical.

II. <u>pH measurement</u>

The pH of samples of cervical discharges collected in buffaloes was measured with a pH meter (Eco Testr pH 1, Precision Scientific Instruments Corporation, Delhi, India). The hydrogen ions concentration (pH) of cervical discharge was studied immediately after the collection of the sample with a digital pH meter. The cervical pH of non-infectious buffalos lies between 7 to 8

III. <u>White SideTest</u>

Aseptically collected cervical mucus sample was mixed with an equal volume of 5% NaOH in a test tube and mixed thoroughly and heated up to boiling. The change in color of the sample shows the degree of endometrial infection. Table 3.2. Shows change in color of the sample with respect to the degree of infection were rejected. Negative for this test were selected.

Table 3.2. Comparison of cervical mucus sample for white side test.

Color	Test results	Degree of Infection
No color	Negative	Nil
Light yellow	Positive	Mild
Yellow	Positive	Moderate
Dark Yellow	Positive	Severe

IV. <u>PMN cell count</u>

With the help of cytobrush uterine cytology was carried out on day of estrus. Cytobrush was fixed with a stylet and this was pulled in an intrauterine catheter, then catheter along with cytobrush was introduced into uterus through vagina and cervix. When the cranial end of the catheter was at the anterior part of the cervix, the stylet was moved in such a way that only the brush touched the uterine wall. The caudal portion of the stylet was slowly screwed in both directions together uterine material on the cytobrush and the same was then slowly removed.

After the removal of the cytobrush from the genital tract, a smear was prepared by rolling a brush on a glass slide. The slide was fixed with methanol solution and stained with Geimsa (1/10) for 30 minutes. The slide was viewed under a microscope at 40X and 100X. Samples containing less than five percent polymorphonuclear cells were considered negative cases of infectious repeat-breeding buffaloes.

V. <u>Serum Phosphorus</u>

Serum inorganic phosphate is measured colorimetrically or isotopically (^{32}P); is used to estimate serum phosphorus in non-infectious repeat breeding buffalos.

3.3.3 Diagnosis of infectious cases

For the present research work repeat breeder animals are selected on the basis of clear vaginal discharge and those cases were diagnosed by digital pH meter, white side test, PMN cell count and Fern pattern.

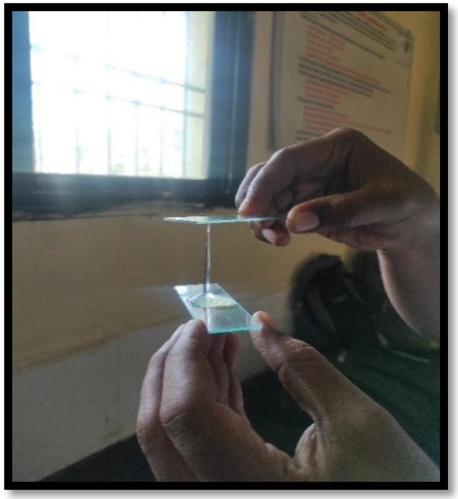


Plate 3. Slide for Fern Pattern

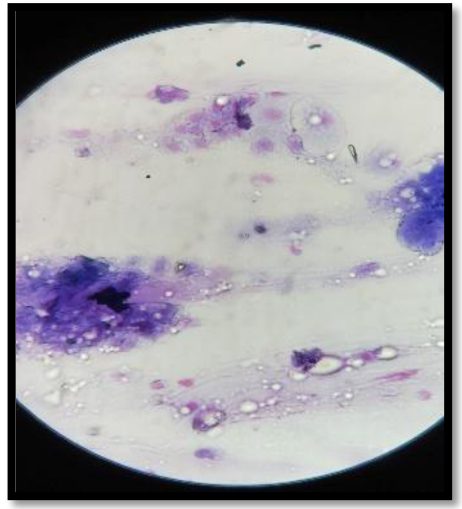


Plate 4. PMNs Cell Count

Plate 5. Smear for PMNs cell count

Plate 6. Cervical mucosal pH

Plate 7. Negative White Slide test

Plate 8. Reagents used for WST

After evaluation of cervical mucus sample by digital pH meter, White side test, PMN cell count and fern pattern were recorded was explain in detail in table 3.3.

Table 3.3. Details of clinical investigations were studied through different laboratory tests for confirmation of non-infectious repeat-breeding buffaloes before treatment.

Sr. no.	Type of test	Total No. of cases	Diagnostic Remark
1	pH estimation	60	Cervical mucus pH towards acidic and alkaline side indicates infertility
2	Whiteside test (confirmation for No infection)	60	Normal (Turbid or no color/transparent) indicates no infection.
3	PMNs cell count	60	Less count of PMN cells indicates there is no uterine defence mechanism active
4	Fern pattern	60	Aeborization or crystallization is visible throughout the smear. Tertiary to quaternary or branching is referred to as the typical good stage for insemination.
5	Serum Phosphorus	60	fertility of the Non-infectious buffaloes tended to be reduced if the serum inorganic phosphorus level falls.

3.4 Grouping of Animals and Treatment Protocols

A total of 60 buffaloes suffering from non-infectious repeat breeding syndrome were selected for the present study and their grouping was done as shown in table 3.4.

Table 3.4. Grouping of Animals and Treatment protocol.

Sr. no.	Group	Treatment	No. of animals	Dose	Route	Schedule
I.	**GN**	GnRH	12	5ml /Animal	IM	At the time of AI
II.	**IP**	Inj. Inorganic Phosphorus	12	10 ml per animal	IM	0^{th}, 3^{rd},5^{th} days
III.	**HM**	Powder Herbominvit	12	40 gm/animal/ day	PO	Daily For 7 days
IV.	**GM**	Glycine Chelated Mineral Mixture	12	1 Bolus/day	PO	Daily For 7 Days
V.	**CON**	Control	12	No Treatment	-	-

3.4.1 Drugs used for trials

Non-infectious repeat breeder buffaloes treated with different treatment protocols. Various herbal medicinal plants with a combination of vitamins and minerals, hormone GnRH, Glycine chelated minerals and inorganic minerals like phosphorus were used.

I. <u>GnRH.</u>

GnRH is a natural hormone originating from the hypothalamus, which is responsible for estrus activity in buffalos. GnRH analogues which are available in field conditions were used.

Each ml of GnRH analogues used contains Buserelin I.P. 4 mcg, Benzyl Alcohol I.P. 10mg (used as preservatives).

II. <u>Inj. Inorganic Phosphorus.</u>

Inorganic phosphorus is vital for maintenance of reproductive activity in buffaloes.

Each ml contains sodium acid phosphate IP 40.3% w/v (equivalent to elemental phosphorous), Methylparaben IP and Propylparaben IP (used as preservatives).

III. <u>Powder Herbominvit.</u>

Harbominvit name its self indicates that it is a combination of herbs of Indian origin having medicinal property with fortifying vitamins and minerals.

Table 3.4.1: Active ingredients found in extracts of Harbominvit Powder

Activity	Herbs	Extracts from
Reproductive function	*Aloes barbadensis*	Leaves
	Murraya koenigii (Curry Patta)	Leaves
Stress relieving	*Mucuna pruriens* (Velvet bean)	Seeds
Managing Uterine Infections	*Aristolochia indica*	Root
	Nigella Sativa (Kalonji)	Fruits
	Tinospora cordifolia (Giloy)	Leaves
Nutrition Support	*Moringa oleifera* (Drumstick)	Leaves & Seeds
For Epithelial regeneration	Vitamin - A	
Immune Defenses	Zinc	
	Copper	

<u>Glycine Chelated Mineral mixture.</u>

Glycine chelated trace minerals are used as ayurvedic feed supplement which contain amino acid (glycine) chelated minerals used for increase ovarian activity in farm animals.

Mineral like Iodine, Selenium, Manganese, Chromium, Copper, Cobalt, Zinc, Iron and Magnesium is chelated with glycine.

Table 3.4.1: Content and concentration of glycine chelated minerals mixture.

Content	Concentration	Content	Concentration
Iodine	150 mg	Manganese	600mg
Selenium	8mg	Zinc	600mg
chromium	10mg	Iron	1500mg
copper	1500mg	Magnesium	350 mg
cobalt	120mg		

IV. <u>Control</u>

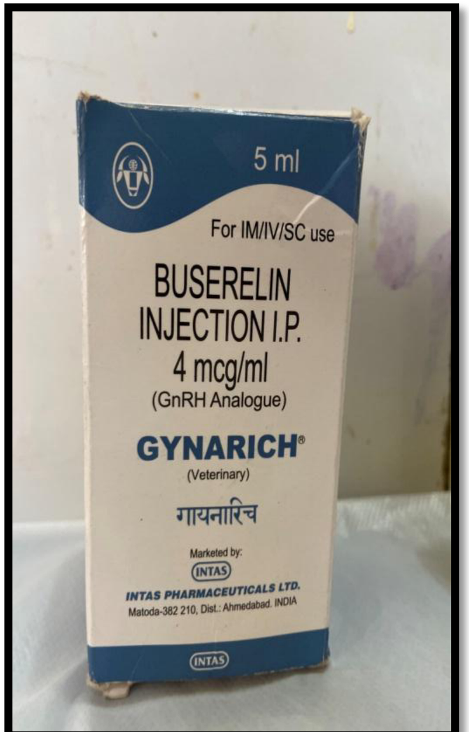
Under this group cases were given no treatment

3.4.2 Treatment protocols

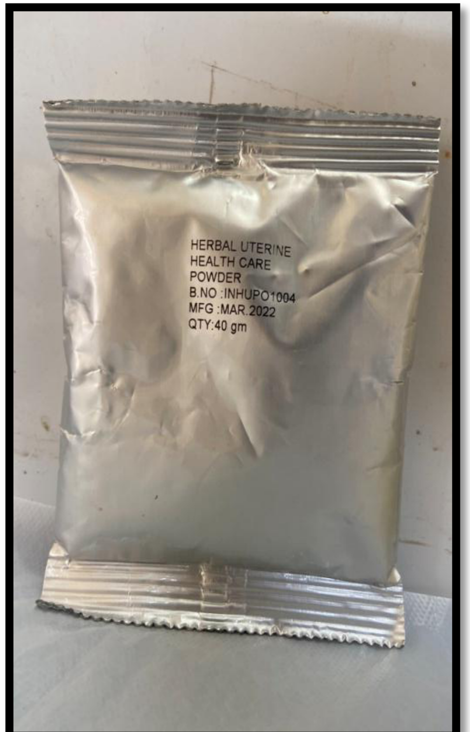
All 60 repeat breeder buffaloes were categorized in four different treatment protocols group and one control group. All animals were equally divided in each group.

I. Group I (GN):

Under this group, noninfectious repeat breeder buffaloes were given infusion of 5 ml GnRH analog by intramuscular route on day of estrus at the time of AI.

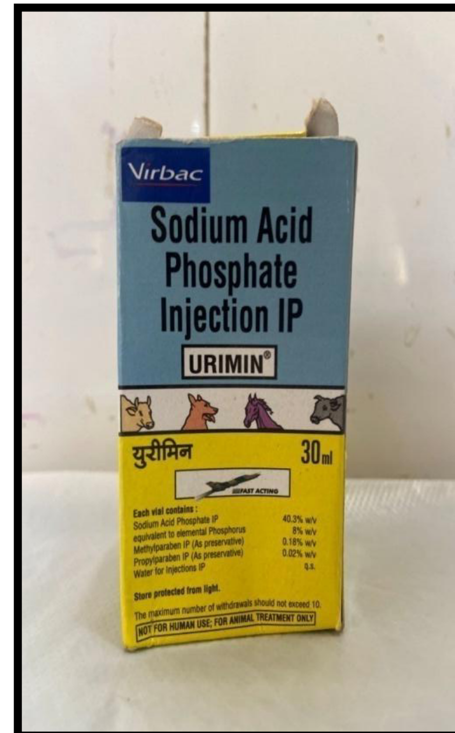


GnRH



HarbominVit

Glycine chelated minmix



Inorganic Phosphorus

Plate 2. Drug used for treatment of non-infectious repeat breeder Buffaloes

II. Group II (IP):

In this group, animals were treated with the injection of inorganic phosphorus 10 ml/animal given to non-infectious repeat breeding buffalos on the 0^{th}, 3^{rd} and 5^{th} day of estrus after AI.

III. Group III (HM):

Under this group, cases were given powder of Herbominvit 40 gm/animal/day onwards Insemination for 7 days.

IV. Group IV (GM):

Under this group, cases were given one bolus of glycine chelated mineral mixture to each animal for the next 07 days onwards Insemination.

V. Control Group (CON):

Under this group, cases were given no specific treatment

3.5 Follow up:

After attempting clinical experimentation, follow-up of treated cases was as under

1. Recovery rate (not return to estrus, 21st days)
2. Conception rate (ultrasonographic examination, 30^{th} days)
3. Pregnancy rate (per rectal examination, 60-70^{th} days)

3.6 Conception rate

On the basis of the non-return of oestrus after 21-30 days animal was considered as conceived**.**

The conception rate was assessed by using the following formulae

$$\text{Conception rate (\%)} = \frac{\text{Number of animals conceived}}{\text{Number of animals inseminated}} \times 100$$

3.7 Pregnancy rate

On per-rectal examination for pregnancy membrane slip, asymmetry in uterine horns, the dorsal bulge of uterine horn along with the palpation of corpus luteum on one of the ovaries after two months of insemination but its difficult to differentiate between pregnancy corpus luteum and cyclic corpus luteum on rectal palpation hence ultrasonography is used at 30th day to confirm the pregnancy.

$$\text{Pregnancy rate (\%)} = \frac{\text{Number of animals pregnant}}{\text{Total number of animals inseminated}} \times 100$$

3.7 Data evaluation

All clinical observations and parameters were correctly reported and tabulated. The data was evaluated using the Completely Randomized Design Test provided by Snedecor and Cochran (1980). The statistical significance of the results was interpreted and discussed with the available references.

Plate 9. Pregnancy diagnosis by per rectal examination

Plate 10. Embryo along with embryonic vessel

Plate 11. Pregnancy diagnosis by USG

Results and Discussion

CHAPTER - IV

RESULTS AND DISCUSSION

The present work on "Comparative efficacy of different drugs promoted to increase conception rate in non-infectious repeat breeder buffaloes" was carried out in various private dairy farms, Veterinary Clinical Complex of College of Veterinary and Animal sciences (COVAS) Parbhani, animal reported to government animal hospital and government mini polyclinics from Parbhani district and field level and examination of sample was carried on laboratory at department of Veterinary Biochemistry, and Veterinary Pharmacology and Toxicology in College of Veterinary and Animal Sciences (COVAS), Parbhani.

The selected animals were assigned to five groups on the basis of history, screening by Body score condition, white side test, PMNs cell count, Cervical pH, Fern pattern and Serum phosphorus. Sixty buffaloes were selected and equally divided in four treatment groups and one control group i.e., each group having 12 animals, Group I (GnRH at the time of AI), Group II (Inj. Inorganic Phosphorus), Group III (Powder Herbominvit), Group IV (Glycine Chelated Mineral mixture), and Group V (Control). During the study conception rate and pregnancy rate estimated in above five groups. The observations recorded during the study are presented and discussed as below.

4.1 Investigative Parameters

On the basis of the animal's history, the veterinarian's observations and the results of laboratory tests, non-infectious repeat breeder cases were identified and confirmed. Ferning pattern, the White side test, the pH of the cervicovaginal discharge, the PMNLs cell count of endometrial content and serum phosphorus were all noted as observations. These parameters overall observations have been explored as follows.

4.1.1 <u>White side test</u>

In the present study of repeat-breeding buffaloes. The screening and selection of the non-infectious buffaloes were made on the basis of the White side test (WST). WST was performed in buffaloes were at farms managed and maintained around the villages of Parbhani and Nanded (Maharashtra). Buffaloes negative to WST were considered as non-infectious repeat breeder and thus included for the study.

If no infection is detectable in the cervical mucus, the repeat breeding category is considered non-infectious. The white side test can be used to assess field cases for the presence or absence of infection in cervical mucus. All clinical cases cervical mucus was found by laboratory examination to be free of infection and the qualitative test failed to identify any color change in any of the instances (Table 4.1)

Table 4.1. Details of clinical investigations studied through White side tests for confirmation of Non-Infectious repeat breeding buffalos before treatment

Gro Sup	No. of animals	Change Color	Test results	Degree of infection
I	**12**	No color	Negative	Nil (100 %)
II	**12**	No color	Negative	Nil (100 %)
III	**12**	No color	Negative	Nil (100 %)
IV	**12**	No color	Negative	Nil (100 %)
V	**12**	No color	Negative	Nil (100 %)

The white side test response in non-infectious repeat breeding buffaloes is presented in Table 4.1. All buffaloes 60 Out of 60 buffaloes 100(%) buffaloes were negative to WST and were included in the study.

The color of the mucus sample changes when leukocytes and endometrial material are present in cervical mucus. Since WST is a qualitative test, it aids in separating low-grade endometritis buffaloes from non-infectious.

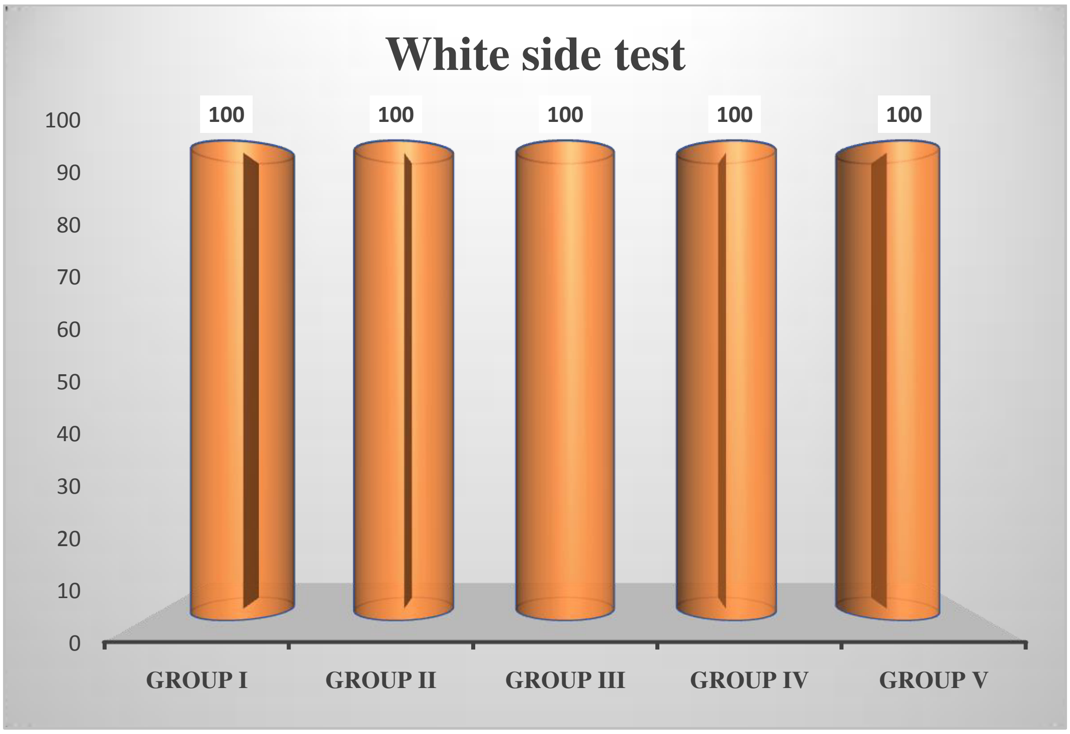


Fig 1: White slide test of all groups in non-infectious repeat breeder buffaloes before treatment

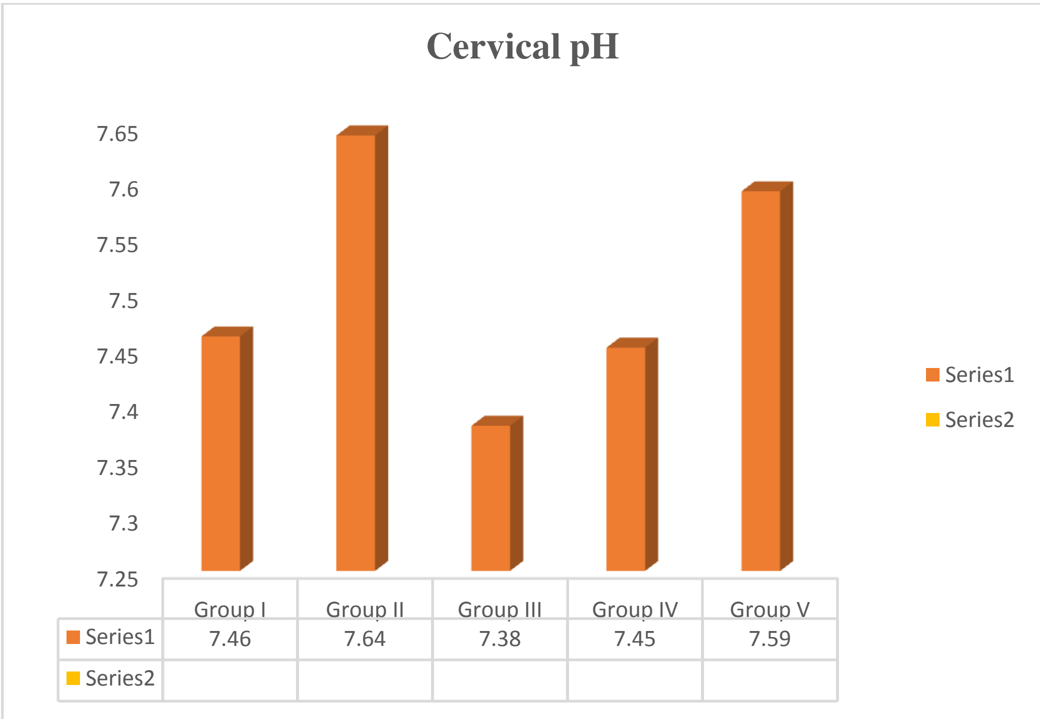


Fig 2: Cervical pH of non-infectious repeat breeder buffaloes before treatment

The result of white slide test for non-infectious repeat breeding buffaloes were negative as there is no change in colour of cervical mucous after treated with (5%) NaOH which is similar to Khumbhar *et al.* (2020) and Alai (2021).

Non-infectious repeat breeder buffaloes have been shown to test (100%) negative for the white side test, according to Bhalerao (2014) and Anbhule (2018).

These observations provide diagnostic support for the proper course of treatment in the corresponding indicated groups and confirm the non-infectious category of cases.

The white side test makes it simpler, more precise, rapid, dependable, and extremely beneficial to distinguish between healthy and infected genital tracts at the field level. Positive white side tests produce a range of colour patterns from light yellow to dark yellow, which indicates the degree of endometritis.

4.1.2 Cervical pH:

The overall mean of cervical pH was in non-infectious repeat breeding buffaloes 7.50±0.05. The 60 non-infectious repeat-breeding buffaloes were all alkaline, and this result is consistent with Khumbhar *et al*., (2020) who observed that the animals' pH was 7.48±0.05 which is significant for conception in response to repeat breeding.

In Non-infectious repeat breeder animal, there is less or absence of pathogen and so their metabolites absent in uterus which unalter the cervical mucus pH from normal, due to this it is possible to diagnose unification from endometritis. The shift of cervical pH from normal to acidic or alkaline reduces the conception rate.

Table 4.1.2 Cervical pH of non-Infectious repeat breeding buffalos before treatment

Group	No. of animals	Mean ± SE pH value
I	12	7.46±0.04
II	12	7.64±0.08
III	12	7.38±0.07
IV	12	7.45±0.08
V	12	7.59±0.08
Over All	60	7.50±0.05

Table 4.1.2.A. ANOVA for cervical pH of non-infectious repeat breeder buffaloes

Source of Variation	D.F.	Sum of Squares	Mean Squares	F-Calculated	P Values
Treatment	44	00.5420	0.136	2.196^{NS}	0.08133
Error	555	33.394	0.062		
Total	559	33.936			

: Significant (P<0.05)** *: Significant (P<0.01)**

NS: Non-significant **P Value is probable significance value**

Normal cervical pH is required for fertilization, however the oviductal fluid's capacity to buffer sperm ensures their safe travel to the site of fertilization without any negative consequences from pH changes in the tubular genitalia. Higher pH values and lower pH values outside of the usual range are signs of infected genitalia. As a result, the cases were grouped into the non-infectious category for the trial.

The current findings concur with those of Samad *et al*. (2002) who observed that repeat-breeding buffaloes had cervical mucus with a mean pH value of 7.49. Wani *et al*. (1982), and Rangnekar *et al*. (2002) showed slightly lower findings for repeat breeding cows, whereas Bishnoi *et al*. (1983) and Pandey *et al*. (1983) reported significantly higher findings.

Because the high levels of estrogen during oestrus are directly related to the conditions of cervical mucus and to a more alkaline pH, salt chloride levels and water content on the cervix also rise currently (Verma *et al*., 2014).

4.1.3 <u>Fern pattern:</u>

Clinical cases were assessed during the oestrus stage to identify the fern pattern of cervical mucus, and it was noted that in all cows and buffaloes reported for infertility treatment, the fern pattern of cervical mucus was of the normal type with tertiary branching. Only during the oestrus stage did all normal, healthy breedable animals exhibit the usual fernning pattern of cervical mucus; the pattern changed once oestrus stopped. indicates that the fern pattern's tertiary branching was present in all cases, confirming the usual oestrus stage in all samples.

Fern pattern of cervical mucus is defined as typical, atypical or nil and also defined for branching of fern pattern as primary, secondary and tertiary. Typical and tertiary branching of cervical mucus is characteristics of appropriate oestrual stage and the same was confirmed in all cases of present experiment. Fern pattern represents arrangement of chloride ions during oestrual stage.

Table 4.1.3 Fern pattern in non-infectious repeat breeder buffaloes

Sr. No.	Groups	No of animals showing estrus discharge	Fern pattern	
			Typical (%)	Atypical (%)
1	I	12	10 (83.33%)	2(16.67%)
2	II	12	12(100%)	0(o%)
3	III	12	11(91.67%)	1(8.33%)
4	IV	12	9(75%)	3(25%)
5	V	12	9(75%)	3(25%)

Table 4.1.3.A ANOVA for Fern Pattern in non-infectious repeat breeding buffaloes.

Source of Variation	D.F.	Sum of Squares	Mean Squares	F-Calculated	P Values
Treatment	4	0.167	0.042	0.207^{NS}	0.93360
Error	55	11.083	0.202		
Total	59	11.250			

: Significant (P<0.05)** *: Significant (P<0.01)**

NS: Non-significant **P Value is probable significance value**

The current study's higher percentage of normal fern production may be attributable to elevated peripheral estrogen concentrations during estrus, which is suggestive of the ideal period for artificial insemination.

According to earlier observations by Verma *et al*. (2014), Anbhule (2018) and Kumbhar *et al*. (2020), who described presence of fern as typical and a typical, the fern pattern of cervical mucus during estrus in treated buffaloes is consistent with those findings.

According to the findings of the current study's fern pattern of 60 buffaloes in separate groups typical fern 51 buffaloes ranged between 75 and 100(%) and atypical fern 09 buffalos varied between 0 and 25 (%) showed various percentages of animals (Table 4.1.3). Which is a comparison to Verma *et al*. (2014), Anbhule (2018), and Kumbhar *et al*. (2020) this is only marginally higher.

On comparison of present data on ferning pattern it is observed that statistically no significant difference was found in number of cases showing typical atypical and nil pattern (Table 4.1.3. A).

As agreed with Anbhule (2018) cases in the experiment were assessed for fern pattern during estrus to validate the normalcy of oestrus. This parameter was unrelated to the assessment of non-infectious stages of oestrus

As per Anbhule (2018) and Alai (2021), fern pattern and sperm penetrability have a positive linkage. A regular fern pattern has a better penetration advantage over an unusual fern pattern. Therefore, an abnormal or

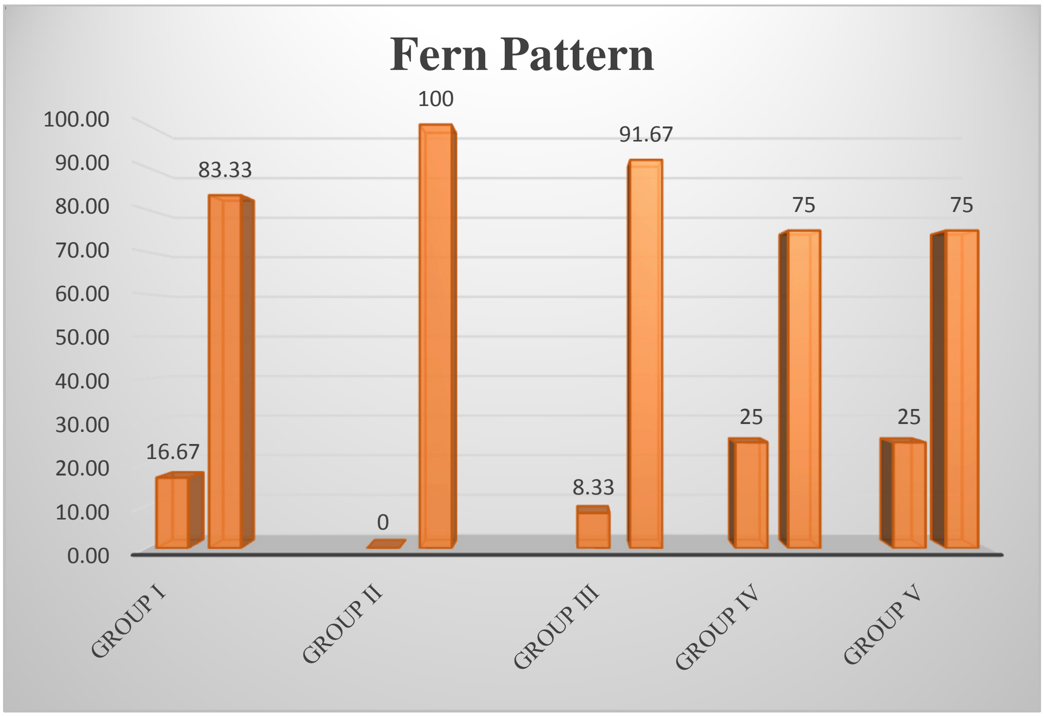


Fig 3: Fern pattern of non-infectious repeat breeder buffalo before and after treatment

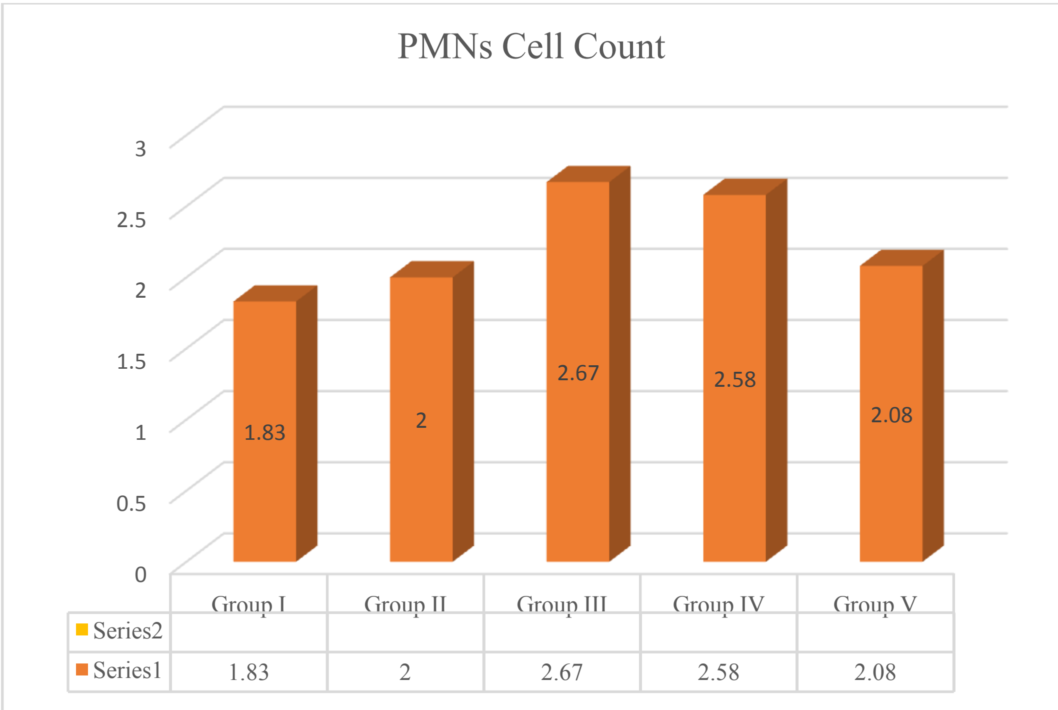


Fig 4: PMNs Cell count of non-infectious repeat breeder buffalo before treatment

absent fern pattern denotes an adverse uterine environment, which prevents conception by causing sperm to obstruct or be destroyed in the reproductive tract.

4.1.4 PMNS Cell Count:

It is possible to assess the endometrial contents and count PMNLS cells to classify the endometrium's stage as infected or non-infectious. On the day that buffaloes were in oestrus, samples were collected and stained and an attempt was made to evaluate them.

Polymorphonuclear inflammatory cells (PMNs), whose phagocytic activity depends on bacterial opsonization by humoral antibodies, help in part to defend against microorganisms that contaminate the vagina and uterine lumen of buffalo. Significant numbers of lymphocytes are present in the infectious condition of repeat breeding buffalo while low counts are present in the non-infected buffaloes agreed with Dhaliwal *et al*. (2001).

Table 4.1.4. PMNs Cell count in non-infectious repeat breeder buffaloes

Group	No. of animals	Mean ± SE PMNs cell Count per 100
I	12	1.83±0.24
II	12	2.00±0.25
III	12	2.67±0.31
IV	12	2.58±0.26
V	12	2.08±0.23
Over All	60	2.21±0.17

Table 4.1.4.A ANOVA for PMN count in non-infectious buffaloes

Source of Variation	D.F.	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	6.567	1.642	2.044	0.10080
Error	55	44.167	0.803		
Total	59	50.733			

***: Significant (P<0.05) **: Significant (P<0.01)**

The principal immunological defense mechanism in the uterus, PMN cells are known to proliferate when infection is present in mild to severe forms which is agreed with Alai (2021) and Anbhule (2018).

The recent investigation found that the PMN cell count in non-infectious repeat breeder buffaloes ranged from 01 to 04 cells. The average number in each group is shown in Table 4.1.4. overall Mean ± SE PMNs cell Count per 100 is 2.21±0.17 which is lower than Anbhule (2018).

As Agreed with Anbhule (2018) In situations of infected repeat breeders and endometritis, PMNLs cell counts are typically tried; in non-infectious repeat breeder cases, the count evaluation is typically not attempted. Therefore, when searching the literature for a report on PMNLs cell count in non-infectious repeat breeder cases, nothing was found.

Finally, non-infectious repeat breeding was diagnosed using a PMNLs cell count of less than 04 and all instances fell into this group for the current investigation.

4.1.5 Serum Phosphorus

As agreed with Mahmood *et al*. (2013) due to the larger ratio of calcium and phosphorus in low-phosphorous diets with high calcium content, hypophosphatemia is caused by a reduction in phosphorous absorption from the gastrointestinal system.

The capacity of animals to use other microminerals is influenced by minerals like calcium and phosphorus. Lower plasma phosphorus levels may be a result of the influence of these minerals on the enzyme system, which may affect reproductive efficiency. According to Ali *et al*. (2014), phosphorus is necessary for the transfer of biological energy (ATP) and a lack of it can stop the process of fertilization and lead to early embryonic death, which makes livestock repeat breeders.

Table 4.1.5. Mean ± SE Value of serum Phosphorus values in non-infectious buffaloes

Group	No. of animals	Mean ± SE Value of serum Phosphorus
		Before Treatment
I	12	3.64 ±0.08[a]
II	12	3.26 ±0.10[b]
III	12	3.87±0.09[a]
IV	12	3.61±0.05[a]
V	12	3.69±0.09[a]
Over All	60	3.608±0.09

Table 4.1.5.A ANOVA for Serum Phosphorus Before in non-infectious repeat breeding buffaloes

Source of Variation	D.F.	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	2.015	0.504	5.302**	0.00110
Error	55	5.224	0.095		
Total	59	7.239			

: Significant (P<0.05)** *: Significant (P<0.01)**

Puberty and postpartum oestrus may be delayed by severe phosphorus insufficiency. On the other hand, a severe deficiency can cause repeat breeding problems and low conception rates. buffalos ability to use other microminerals is influenced by minerals like calcium and phosphorus. Lower plasma phosphorus levels could be an indication of decreased reproductive effectiveness due to these minerals' effects on the enzyme system Parmar *et al*. (2012).

This finding is comparable to that of Parmar *et al*. (2012) who found that all five treatment groups had higher plasma inorganic phosphorus concentrations than they had before treatment.

Serum inorganic phosphorus levels in group II have increased as a result of parental inorganic phosphorous treatment. The present findings on the levels of inorganic phosphorus before and after are comparable to the reports of Patel *et al*. (2009) and Tiwari *et al.* (2012).

Reduced reproductive function as a result of lower circulatory mineral concentration causes the cyclic activity to subside. Severe phosphorus deprivation may cause delays in postpartum oestrus and puberty. While a mild deficiency could result in repeated breeding and a low conception rate.

4.2 Evaluation of treatment protocols

Buffaloes from the non-infectious repeat breeding category were subjected to a total of four treatments with one control group. There were an equal number of control animals used in each treatment study. The therapies are intended to encourage cyclic animals regular reproductive processes in order to impregnate infertile cases. The study trial is discussed on a merit basis with available references.

4.2.1 GnRH at the time of AI (GN)

All animals were conformed clinically for negative WST and repeated oestrus more than three times cases are selected for present research work. On the day of estrus, twelve non-infectious buffaloes were given a GnRH treatment at the time of AI, and the results were compared to twelve non-infectious buffaloes that were not treated. All information of this trail group and clinical finding are given in detail in table 4.2.1

Two buffaloes from the treatment group and seven buffaloes from the control group were both found to be in estrus upon gyneco-clinical examination after twenty-one days these animals were re-inseminated. whereas five buffaloes from the control group and 10 buffaloes from the treatment group do not exhibit the estrus sign.

One buffalo from the treatment group and one buffalo from the control group both displayed estrus symptoms during gynecological assessment after 42 days. Whereas four buffaloes from the control group and nine buffaloes from the treatment group that showed no signs of estrus despite having been inseminated for forty-one days were deemed to be conceived.

After 21 days, a gynecological evaluation revealed that the treatment group's conception rate was 83.33 (%) and the control group was 41.66 (%). Following a 60-day gynecological assessment, it was discovered that the treatment and control groups respective pregnancy rates were 75(%) and 30(%).

In treatment group at day 0 before treatment repeat breeder buffaloes showed the Mean ± SE pH value was 7.46±0.04 while control group showed the Mean ± SE pH value was 7.59±0.08. White side test was 100% negative for both groups. Mean ± SE value for PMN cell count for treatment group is 1.98±0.24 while control group was 2.08±0.22. Mean ± SE value for serum phosphorus value for treatment group before treatment is 3.637^{a}±0.08 and after treatment is 5.14^{a}±0.13 while control group shows 3.68^{a}±0.09, 4.17^{a}±0.20 and Mean ± SE value for Fern pattern for treatment group and control group was 0.833±0.11 and 0.66±0.14 respectively. All the above test are conforming the non-infectious condition of the uterus.

It is expected that during the estrus stage exogenous GnRH acts on the anterior pituitary to cause the release of gonadotropins, particularly luteinizing hormone for LH surge during estrus, induction of ovulation, timely ovulation, synchronization of ovulation and insemination for increasing chances of conception, and luteinization of granulose cells and theca cells to form the follicle.

Zakiuddin *et al*. (2022) and Ahmed *et al*. (2010) reported 70(%) and 61.54 (%) conception in buffaloes which is marginally less than the current finding. They also stated that the mean pH of estrual mucus was 7.370±04. When cervicovaginal mucus is translucent, viscous, and has the typical fern pattern on the day of estrus, the chance of conception in buffaloes is higher. (Shephard *et al*., 2014) and (Neglia *et al.,* 2020) both found a similar conception rates to the present study.

When compared to the control group, the pregnancy rate in the "GnRH therapy regimen" was shown to be numerically higher.

Table 4.2.1. Gynaeco-clinical response to Inj.GnRH at the time of AI in non-infectious repeat breeder buffaloes

Days	Parameter	GnRH at the time of AI		Control	
	Time of AI/NS	Third Phase of Estrus		Third Phase of Estrus	
	No of animal given Treatment	12		12	
	Conception rate	NR	R	NR	R
		10(88.33%)	02(16.66%)	05(41.66%)	07(58.33%)
21	No. of animal exhibited signs of second estrus	04		08	
60	Pregnancy rate	(75%)		(33.33%)	

4.2.2 Inj. Inorganic phosphorus (IP)

In this treatment group 12 Non-infectious repeat breeder buffalos which are negative for white slide test, low PMNS cell count, normal cervical pH treated with inj. Inorganic Phosphorus on 0^{th}, 3^{rd} and 5^{th} days of estrus after AI.

Table 4.2.2 Serum Phosphorus values in non-infectious repeat breeding buffaloes

Group	No. of animals	Mean ± SE Value of serum Phosphorus	
		Before Treatment	After Treatment
I	12	3.64 ±0.08^{a}	5.14 ±0.13^{a}
II	12	3.26 ±0.10^{b}	5.11±0.17^{a}
III	12	3.87±0.09^{a}	4.94±0.18^{a}
IV	12	3.61±0.05^{a}	5.39±0.11^{a}
V	12	3.69±0.09^{a}	4.14±0.22^{b}
Over All	60	3.608±0.09	4.92±0.19

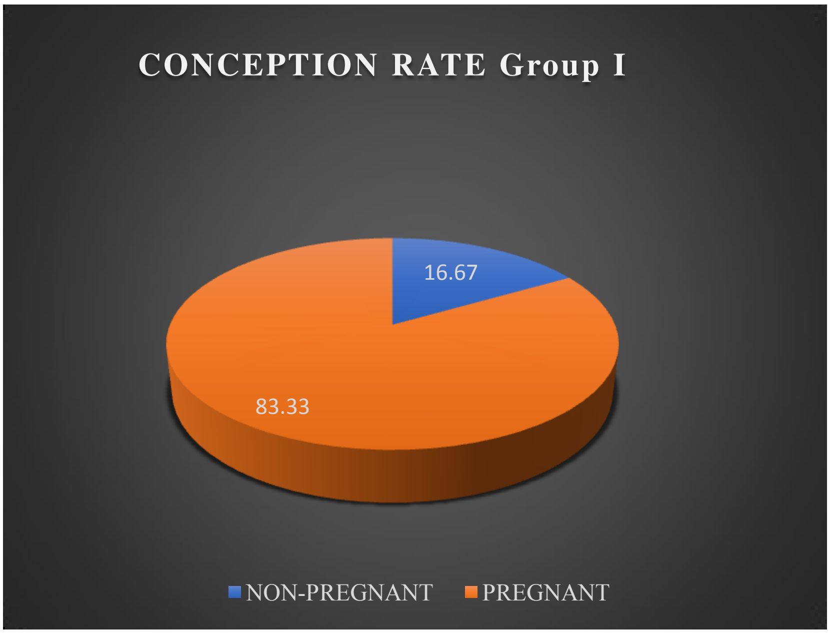


Fig 5: Conception rate in Group I(GnRH at the time of AI)

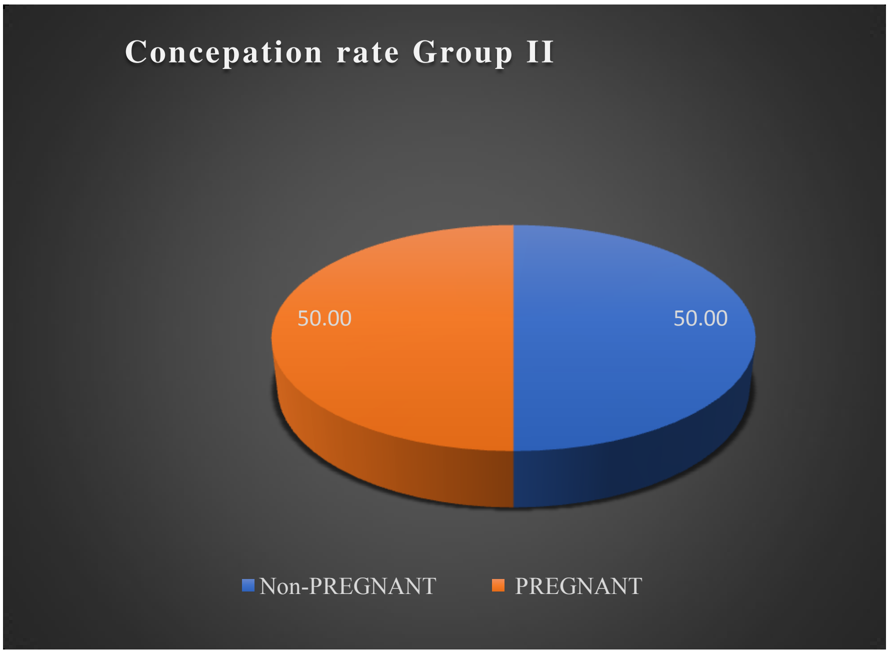


Fig 6: Conception rate in Group II(Injection inorganic phosphorus)

Table 4.2.2.A Gynaeco-clinical response to Inj. Inorganic Phosphorus in non-infectious repeat breeder buffaloes.

Days	Parameter	Inorganic phosphorus		Control	
	Time of AI/NS	Third Phase of Estrus		Third Phase of Estrus	
	No of animal given Treatment	12		12	
21	**Conception rate**	NR	R	NR	R
		06(50.00%)	02(50.00%)	05(41.66%)	07(58.33%)
	No. of animal exhibited signs of second estrus	07		08	
60	**Pregnancy rate**	(41%)		(33.33%)	

The difference in mean serum phosphorus concentrations before and after treatment were apparently higher in treatment group-II as compared to control group might be due to administration of phosphorus injection in Group-II resulted into apparently higher values after treatment.

In treatment group at day 0 before treatment repeat breeder buffaloes showed the Mean ± SE pH value was 7.64±0.08 while control group showed the Mean ± SE pH value was 7.59±0.08. White side test was 100% negative for both groups. Mean ± SE value for PMN cell count for treatment group is 2.00±0.25 while control group was 2.08±0.22. Mean ± SE value for serum phosphorus value for treatment group before treatment is 3.260^{b} ±0.098 and after treatment is 5.11^{a}±0.17 while control group shows 3.68^{a}±0.09, 4.17^{a}±0.20 and mean ± SE value for Fern pattern for treatment group and control group was 0.750±0.13 and 0.66±0.14 respectively.

As expected, repeat-breeding buffaloes had much lower serum inorganic phosphorus concentrations than non-cyclic buffaloes. Numerous other investigations have similarly indicated that repeat breeder buffalo have lower inorganic phosphorus concentrations. Repeat breeder crossbred animals had significantly reduced inorganic phosphorus contents, according to Chaurasia *et al.* (2010) and Akhtar *et al*. (2014).

In the present study on gynecological examination on 21 days of estrus after treatment in a treatment group out of 12 buffaloes 06 shows signs of estrus and 06 not shows sign of estrus this resulted in the conception rate in this group being 50 (%) which is similar to Dhamsaniya *et al*. (2016) and higher than Raval *et al*. (2017).

As the fertility of the animals tended to be reduced if the inorganic phosphorus level falls, while increased blood phosphorus level was related to the improvement of ovarian activity. But a decrease in calcium-phosphorus ratio due to parental supplementation of only inorganic phosphorus is responsible for a slightly lower conception rate than other groups.

Table 4.2.2.B: ANOVA for Serum Phosphorus before in non-infectious buffaloes

Source of Variation	D.F.	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	2.015	0.504	5.302**	0.00110
Error	55	5.224	0.095		
Total	59	7.239			

Table 4.2.2.C: ANOVA for Serum Phosphorus after in non-infectious buffaloes

Source of Variation	D.F.	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	10.301	2.575	8.284**	0.00003
Error	55	17.098	0.311		
Total	59	27.399			

4.2.3 **Powder Herbominvit (HM)**

Aloe barbadensis and *Murraya koenigii* (Curry Patta) leaves have reproductive properties by influencing the growth processes of LF by achieving dominance, accelerating growth rate, preovulatory size, and the ovulation process. (Kumar *et al.,* 2016)

Repeat breeding in non-infectious buffalo is frequently caused by environmental stress, as is seen in the Marathwada region of Maharashtra. Due to the presence of many bioactive compounds such as flavonoids, antioxidants, and phenolics the seeds of the velvet bean Mucuna pruriens are highly valued on the market which helps to reduce oxidative stress (Suryawanshi *et al*., 2020).

Thymoquinone (TQ), one of the most potent components of *Aristolochia indica*, *Nigella sativa*, and *Tinospora*, has a range of beneficial properties. This plant's anti-inflammatory, immune-stimulating, and antibacterial properties make it beneficial (Satheshkumar *et al*., 2021).

Moringa oleifera (Drumstick) as a support for nutrition is a significant plant that is rich in specific micro and macronutrients. Its leaves stimulate the activity of the rumen microbiota by preserving particular micronutrients, vitamins, hormones, and enzymes that are necessary for effective digestion, absorption, and metabolism which facilitates luxurious processes like reproduction. El-Sanafawy *et al*. (2017)

Vitamin A and minerals like zinc and copper are used to activate immune defenses and promote epithelial regeneration. As no direct effect were found on conception rate in buffaloes (Patil *et al*., 2014).

Table 4.4. Details of Gynaeco-clinical response in non-infectious repeat breeder buffaloes to powder HarboMinVit

Days	Parameter	HarboMinVit Group		Control	
	Time of AI/NS	Third Phase of Estrus		Third Phase of Estrus	
	No of animal given Treatment	12		12	
21	**Conception rate**	NR	R	NR	R
		09(75%)	03(25%)	05(41.66%)	07(58.33%)
	No. of animal exhibited signs of second estrus	04		08	
60	**Pregnancy rate**	66.66%		33.33%	

In treatment group at day 0 before treatment repeat breeder buffaloes showed the Mean ± SE pH value was 7.38±0.07 while control group showed the Mean ± SE pH value was 7.59±0.08. White side test was 100% negative for both groups. Mean ± SE value for PMN cell count for treatment group is 2.67±0.31 while control group was 2.08±0.22. Mean ± SE value for serum phosphorus value for treatment group before treatment is 3.81^{a} ±0.11 and after treatment is 4.937^{a}±0.18 while control group shows 3.68^{a}±0.09, 4.17^{a}±0.20 and mean ± SE value for Fern pattern for treatment group and control group was 0.750±0.13 and 0.66±0.14 respectively.

A gynecological examination conducted after 21 days showed that the treatment groups conception rate was 75 percent, compared to 41.66 percent for the control group. a 60-day gynecological evaluation revealed that the pregnancy rates in the treatment and control groups were 66.66 and 30 percent, respectively.

In present study the conception rate after 21 days of insemination with powder Harbominvit which was in-between Dutta *et al*. (2022) 77.7 (%) and

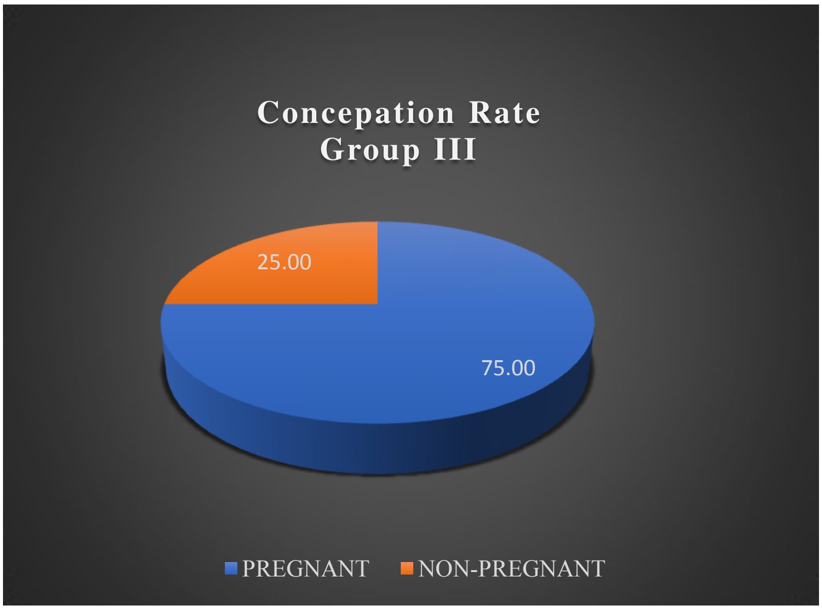


Fig 7: Conception rate in Group III(Powder Harbominvit)

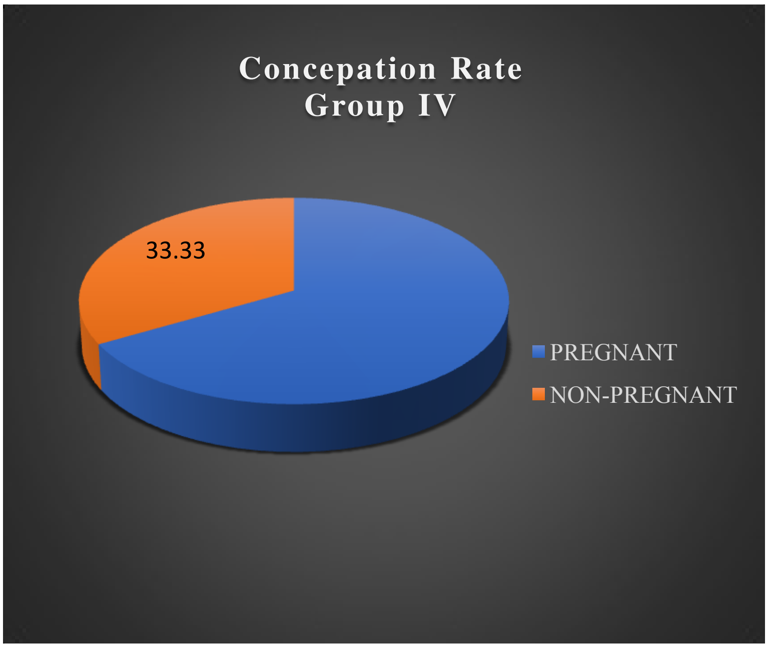


Fig 8: Conception rate in Group IV (Glycine chelated mineral mixture)

Verma *et al*. (2014) 73.1(%) while Liu *et al*. (2014) found higher conception rate than this study.

4.2.4. Glycine Chelated Mineral mixture (GCM)

An essential component of the biological system is nutrition. Any nutrient excess or shortage will reduce the availability of other nutrients and impair metabolic function. Minerals, protein, and energy are necessary for healthy reproduction.

Supplementing with inorganic mineral sources is recommended, by Gayathri and Panda (2018), since minerals that have been chelated with amino acids have a higher concentration in their uterine tissue. As a result, supplementing with minerals from organic sources is useful in easing ruminant reproductive issues.

According to Vlad *et al*. (2021) some bio elements, including Cu, Zn, Mn, Fe, and Se, function as co-factors for various antioxidant enzymes and are involved in a variety of metabolic processes in living things. They are crucial for cell metabolism as well as several other bodily functions, such as energy production, growth, reproduction and the functioning of the nervous system.

As agreed with Van Emon (2020) the development and functioning of luteal cells appear to be impacted by trace mineral supplementation, while follicular cells do not seem to be. This shows that after trace mineral treatment, the CL cells may generate progesterone more effectively. Animals receiving trace minerals also had higher levels of antioxidant enzymes. These findings show that supplementing with trace minerals enhances oocyte quality and CL function, which in turn increases reproductive performance.

In treatment group at day 0 before treatment repeat breeder buffaloes showed the Mean ± SE pH value was 7.45±0.08 while control group showed the Mean ± SE pH value was 7.59±0.08. White side test was 100% negative for both groups. Mean ± SE value for PMN cell count for treatment group is 2.58±0.26 while control group was 2.08±0.22. Mean ± SE value for serum phosphorus value for treatment group before treatment is 3.61^{a} ±0.05 and after treatment is 5.389^{a}±0.11 while control group shows 3.68^{a}±0.09, 4.17^{a}±0.20 and mean ± SE value for Fern pattern for treatment group and control group was 0.750±0.13 and 0.66±0.14 respectively.

Table 4.1.4 Details of Gynaeco-clinical response in non-infectious repeat breeder buffaloes to glycine chelated mineral mixture treatment.

Days	Parameter	Glycine chelated mineral mixture		Control	
	Time of AI/NS	Third Phase of Estrus		Third Phase of Estrus	
	No of animal given Treatment	12		12	
21	**Conception rate**	NR	R	NR	R
		08(66.66%)	04(33.33%)	05(41.66%)	07(58.33%)
	No. of animal exhibited signs of second estrus	05		08	
60	**Pregnancy rate**	58.33%		33.33%	

The conception rate in this group was 66.66 (%) in the current study on gynecological examination on 21 days of estrus after treatment in a treatment group out of 12 buffaloes, with 04 showing signs of estrus and 08 not showing signs of estrus; this is nearly similar to Ahmed *et al*. (2010) 63.64% and lower than Akhtar *et al*. (2014) 87(%).

As the pregnancy rate noted after 60 days was 58 (%) lower than the conception rate in the same group that is Glycine Chelated Mineral mixture. This may be due to a smaller number of days of supplementation of the glycine-chelated mineral mixture.

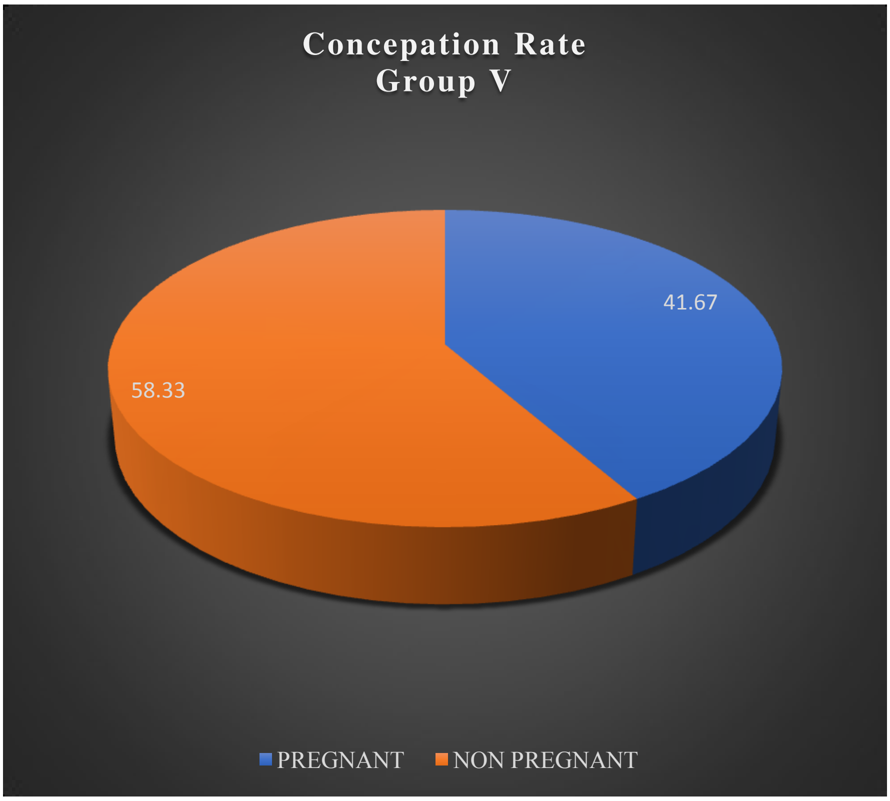


Fig 9: Conception rate in Group V (Control)

4.2 Comparison of treatment protocols

Present study was done in four different groups and one control group, each group is encoded above. Treatment protocol groups under this experimental study was made to compare with in group- Ⅰ, Ⅱ, Ⅲ, Ⅳ and Ⅴ in non-infectious repeat breeder buffaloes. The aim was to track recovery rate, conception rate and pregnancy rate. The comparison was given conclusive interpretations.

4.2.1 Conception rate and Pregnancy rate

On gynecological examination of all animals in respective groups after the twenty-first days of insemination conception rate and after sixty days pregnancy rate was in descending order where highest in Group I and followed by Group III, Group IV, Group II and Group V.

Table 4.2 Comparative details of efficiencies of different therapeutic protocols in non-Infectious repeat breeding buffaloes.

Sr. No.	Groups	Conception rate (%)	Pregnancy rate (%)
1	Group I	83.33	75
2	Group II	50	41
3	Group III	75	66.66
4	Group IV	66.66	58
5	Control	41.66	30

The treatment group GnRH at the time of AI shows the highest conception rate 83.88(%) as well as pregnancy rate 75(%) due to increased CL diameter, increased serum progesterone P4, and decreased serum estrogen E2, all of which are signs of a lower early-stage embryonic mortality. (Zakiuddin *et al*., 2022) and (Ahmed *et al*., 2010)

In Present study the second highest conception rate 75(%) and pregnancy rate 66.66(%) were seen in treatment in which powder Harbominvit is a mixture of *Aloe barbadensis*, *Murraya koenigii, Aristolochia indica*, *Nigella sativa*, *Mucuna pruriens, Tinospora cordifolia, Moringa oleifera*, vitamin A, zinc, and copper. Result of these may be due to improve the follicular maturation, ovulating capacity, triggering the follicular dynamics by enhancing the follicular recruitment, selection and rescuing the atresia of follicles Satheshkumar *et al.* (2021), Kumar *et al.* (2016).

The active principles in the powder Herbominvit seem to operate either through mimicking gonadotrophins activity or stimulating the central mechanism for endogenous release of gonadotrophins along with possibility of local action, requiring further more detailed studies.

As third highest conception and pregnancy rate in treatment glycine chelated bolus was recorded due to stability of glycine chelates, electrically neutral complexes, which protect minerals from chemical reactions during digestion that would render the mineral unavailable to the animal (Gayathri and Panda, 2018).

Added minerals precomplexed with organic ligands thus are used to increase bioavailability and uptake. Glycine chelated mineral reaches to the plasma intact and separates at the site of action. Glycine Chelated minerals are suitable for use in non-infectious repeat breeding buffaloes as serum deficiency of such minerals noted Vlad *et al*. (2021).

Last but one conception rate (50%) and pregnancy rate 41(%) in group II were noted due to increased serum phosphorus level may be related to the improvement of ovarian activity but decreased calcium phosphorous ratio may be responsible for slightly poor result Chaurasia *et al.* (2010) and Akhtar *et al*. (2014).

As minimal conception rate 41.66(%) as well as pregnancy rate 30(%) in control group V animals in these group not given specific treatment.

Summary and Conclusions

CHAPTER – V

SUMMARY AND CONCLUSIONS

The present research work was carried under the title of “comparative efficacy of different intrauterine therapies in infectious repeat breeder buffaloes” between February 2021 to August 2021. It was conducted in private dairy farms, Teaching Veterinary Clinical Complex of College of Veterinary and Animal sciences (COVAS) Parbhani, animal reported to government animal hospital and government mini polyclinics from Parbhani district and field level and examination of sample was carried on laboratory at department of Veterinary Biochemistry, and Veterinary Pharmacology and Toxicology in College of Veterinary and Animal Sciences (COVAS), Parbhani.

The selected animals were assigned to four groups on the basis of history, screening by Body score condition, white side test, PMNs cell count, Cervical pH, Fern pattern and Serum phosphorus.During the study conception rate and pregnancy rate estimated in above five groups and all over study conclude below.

5.1 White side test

The white side test response in all 60 out of 60 non-infectious repeat-breeding buffaloes was negative for the white side test. White side test used to categorize infectious and non-infectious repeat breeding buffaloes.

5.2 Cervical pH:

The overall mean of cervical pH was in non-infectious repeat-breeding buffaloes 7.50±0.05. The 60 non-infectious repeat-breeding buffaloes were all alkaline. In conclusion, the majority of cases chosen under the category of non-infectious repeat breeding in the current conditions had cervical mucus pH that was normal.

5.3 Fern pattern:

Fern pattern of cervical mucus is defined as typical, atypical or nil and also defined for branching of fern pattern as primary, secondary and tertiary. Typical and tertiary branching of cervical mucus is characteristics of appropriate oestrual stage and the same was confirmed in all cases of present experiment. Fern pattern represents arrangement of chloride ions during oestrual stage.

5.4 PMNS Cell Count:

All over PMNS cell count values of all selected non-infectious repeat breeding buffaloes were before treatment 2.21±0.17. Finally non-infectious repeat breeding was diagnosed using a PMNLs cell count of less than 04 and all instances fell into this group for the current investigation.

5.5 Serum Phosphorus

All over serum phosphorus values of all selected non-infectious repeat breeding buffaloes were before and after treatment 3.61±0.09, 4.92±0.19.

5.6 Non-infectious treatment protocol

Sixty buffaloes were selected and equally divided in four treatment groups and one control group i.e., each group having 12 animals, Group I (GnRH at the time of AI), Group II (Inj. Inorganic Phosphorus), Group III (Powder Herbominvit), Group IV (Glycine Chelated Mineral mixture), and Group V (Control).

GnRH at the time of AI was given in the treatment group I on gynaecological assessment on 21st day found that out of 12 buffaloes 10 do not display signs of oestrus while display which revealed that a conception rate of 88.33 (%) was found. A similar assessment on 60 days found that from 10 buffaloes 01 shows signs of oestrus so the pregnancy rate is 75%.

Injection of inorganic phosphorus was given on 0, 3 and 5th day of oestrous in group II on gynaecological assessment on 21st day found that out of 12 buffaloes 06 do not display signs of oestrus while other 06 display which revealed that a conception rate of 50 (%) was found. A similar assessment on 60 days found that from 06 buffaloes 01 shows signs of oestrus so the pregnancy rate is 41(%).

Powder Harbominvit 40 gm is given orally to 12 buffaloes from day of oestrous for the next 7 days in group III. on gynaecological assessment on 21st day found that out of 12 buffaloes 09 do not display signs of oestrus while other 03 display which revealed that a conception rate of 75 (%) was found. A similar assessment on 60 days found that from 09 buffaloes 01 shows signs of oestrus so the pregnancy rate is 66.66 (%).

Glycine chelated mineral mixture one bolus per day per buffalo is given for 7 days from day of oestrous in group IV. on gynaecological assessment on 21st day found that out of 12 buffaloes 08 do not display signs of oestrus while other 04 display which revealed that a conception rate of 66.66 (%) was found. A similar assessment on 60 days found that from 09 buffaloes 01 shows signs of oestrus so the pregnancy rate is 58.33 (%).

Conclusions:

1) White side test, Cervical pH, PMNS cell Count are the best diagnostic field applicable test for differentiating infectious cases of repeat breeding from non- infectious cases.

2) Fern pattern is the gold standard test for successful AI. Which helps to make sure shot contact of both gametes leads to successful fertilization.

3) Serum phosphorus estimation help to know the exact cause of repeat breeding in the area of marathwada region where the soil is deficient for phosphorus.

4) As silent heat, delayed ovulation, split heat, prolonged estrous are common etiologies responsible for non-infectious repeat breeding buffaloes so GnRH treatment at the time of AI regimen gives the highest conception and pregnancy rate.

5) Repeat breeding in non-infectious buffalo is frequently caused by environmental stress. Herbominvit powder containing many bioactive compounds such as flavonoids, antioxidants, phenolics, nutrient supplementation, antioxidants shows highest conception and pregnancy rate among all groups

6) Supplementing with inorganic mineral sources is recommended since minerals that have been chelated with amino acids have a higher concentration in their uterine tissue. As a result, supplementing with

minerals from organic sources is useful in easing ruminant reproductive issues gives good conception and pregnancy rate for glycine chelated mineral mixture

7) In phosphorus deficient non-infectious repeat breeder buffaloes after parental injection of inorganic phosphorus elevated level of serum phosphorus is seen with moderate improvement of conception and pregnancy rate.

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Vitae

VITAE

The author Choutmal Vaibhav Vijay was born on 10th Septmber 1996 at Koli, Tq. Hadgaon Dist. Nanded in Maharashtra state. He has passed S.S.C examination in first division in the year 2012 from Mahatma Phule High School Baba Nagar Nanded and H.S.C in first division in the year 2014 from Shivshakti JR. College Kakandi, Tq. Nanded Dis Nanded.

Then he joined Nagpur Veterinary College, Nagpur in 2015 and successfully completed B. V. Sc. and A.H. degree course in first division from MAFSU, Nagpur in 2020. He later joined Masters Degree programme and completed coursework in Animal Reproduction, Gynaecology and Obstetrics, Parbhani under MAFSU, Nagpur.

He is registered member of Maharashtra State Veterinary Council, Nagpur. He was active member of National Service Scheme and participated voluntary in several camps during his graduation and post graduation studies. He also completed the B certificate examination of National Cadet Corps from Nagpur.

During graduation and post graduation he underwent various animal birth control programmes in different parts of Maharashtra.

He attended National and International seminars on different topics which were held at different places in India. He is having keen interest in Dairy farming, Goat farming and clinical practice and has worked sincerely during post- graduate studies at TVCC, COVAS, Parbhani. Till now he has published seven abstracts and one review in various Clinical case conference and publication.

Thesis Abstract

THESIS ABSTRACT

a)	Title of the thesis (in Capital letters)	:	**"COMPARATIVE EFFICACY OF DIFFERENT DRUGS PROMOTED TO INCREASE CONCEPTION RATE IN NON-INFECTIOUS REPEAT BREEDER BUFFALOES"**
b)	Full name of student	:	**CHOUTMAL VAIBHAV VIJAY**
c)	Name and address of Major Advisor	:	**Dr. M. G. Patil** Assistant Professor, Department of ARGO, College of Veterinary and Animal Sciences, Parbhani
d)	Degree to be awarded	:	M.V.Sc
e)	Year of award of degree	:	2023
f)	Major subject	:	Animal Reproduction, Gynaecology and Obstetrics
g)	Total number of pages in the thesis	:	53
h)	Number of words in the abstract	:	259
i)	Signature of Student	:	
j)	Signature,Name and address of forwarding authority (HOD/SH).	:	

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Professor and Head,
Department of ARGO,
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Thesis Title: "COMPARATIVE EFFICACY OF DIFFERENT DRUGS PROMOTED TO INCREASE CONCEPTION RATE IN NON-INFECTIOUS REPEAT BREEDER BUFFALOES"

ABSTRACT

In the present research, a total of 60 non-infectious repeat breeder buffaloes were examined. The selected non-infectious repeat breeder buffaloes were examined for genital abnormalities. The selected animals were assigned to four groups on the basis of history, screening by Body score condition, white side test, PMNs cell count, Cervical pH, Fern pattern, and Serum phosphorus. Sixty buffaloes were selected and equally divided into four treatment groups and one control group i.e., each group having 12 animals, Group I (GnRH at the time of AI), Group II (Inj. Inorganic Phosphorus), Group III (Powder Herbominvit), Group IV (Glycine Chelated Mineral mixture), and Group V (Control). During the study conception rate and pregnancy rate were estimated in the above five groups.

It was observed that the pH of cervical mucous in non-infectious buffaloes before treatment was in five groups 7.46±0.04, 7.64±0.08, 7.38±0.07, 7.45±0.08 and 7.59±0.08. WST value before treatment was negative for all groups. while PMN cells were revealed as 1.83±0.24, 2.00±0.25, 2.67±0.31, 2.58±0.26 and 2.08±0.23 before treatment and fern patterns typical in 85% and 25% in atypical animals under groups-I to V, respectively. Insemination was done in all groups at the time of treatment. On follow up the conception rate was found as 83.33, 50, 75, 66.66 and 41.66 per cent in buffaloes in group-I to V, respectively. Pregnancy rate was 75, 41, 66.66, 58 and 30 per cent in buffaloes from groups-I to V, respectively.

On conclusion, therapy in non-infectious repeat breeder buffaloes GnRH at the time of AI and Powder Harbominvit showed highest conception rate, and pregnancy rate.

प्रबंधाचे सारांश

१	**प्रबंधाचे शीर्षक**	:	" संसर्ग नसलेल्या आणि सतत माजावर येणाऱ्या म्हशींमध्ये गर्भधारणा वाढवण्यासाठी विविध औषधींचा तुलनात्मक अभ्यास"
२	विद्यार्थ्याचे पुर्ण नाव	:	वैभव विजय चौतमाल
३	मार्गदर्शक	:	डॉ. मंजूषा. ग. पाटील सहाय्यक प्राध्यापिका पशुप्रजनन व प्रसुती शास्त्र विभाग, पशुवैद्यक व पशुविज्ञान महाविद्यालय, परभणी.
४	प्रदान करण्यात येणारी पदवी	:	मास्टर ऑफ वेटेरिनरी सायन्स
५	पदवी प्रदान करण्यात येणारा वर्ष	:	२०२३
६	प्रमुख विषय	:	पशुप्रजनन व प्रसुती शास्त्र
७	प्रबंधातील ऐकून पान	:	५३
८	सारांशातील एकूण शब्द	:	२३०
९	विद्यार्थीची स्वाक्षरी	:	
१०	विभाग प्रमुखाची स्वाक्षरी	:	

डॉ. नितीन म. मार्कंडेय

प्राध्यापक आणि विभाग प्रमुख

पशुप्रजनन व प्रसुती शास्त्र विभाग, पशुवैद्यक व

पशुविज्ञान महाविद्यालय, परभणी.

प्रबंधाचे शीर्षक: संसर्ग नसलेल्या आणि सतत माजावर येणाऱ्या म्हशींमध्ये गर्भधारणा वाढवण्यासाठी विविध औषधींचा तुलनात्मक अभ्यास

प्रबंध सारांश

सदर संशोधनामध्ये एकूण ६० सतत माजावर येणाऱ्या म्हशींची तपासणी करण्यात आली. निवडण्यात आलेल्या जनावरांच्या प्रजनन अवयवांची तपासणी करण्यात आली. ही सर्व जनावरे त्यानंतर पार्श्वभूमी, शारिरीक अवस्था, व्हाईट साईड टेस्ट, पीएमएन सेल काउंटर, गर्भाशय मुख द्रवाचा सामू, फर्न पॅटर्न आणि रक्तातील फॉस्फरस ची पातळी या आधारावर ४ गटांत विभागले गेले.चार उपचार गट आणि एक नियमित गट अशा एकुण ५ गटांमध्ये जनावरांची विभागणी करण्यात आली म्हणजे प्रत्येक गटात १२ म्हशींची संख्या होती. गट १ (जीनआरएच - कृत्रीम रेतनाच्या वेळी), गट २ (इंजेकशन इनऑर्गनिक फॉस्फॉरस), गट ३ (हर्बोमिनविट पावडर) गट ४ (ग्लायसिन चिलेटेड मिनरल मिक्सर), गट ५ (कंट्रोल). सदर अभ्यासात वरील ५ गटांमध्ये गर्भधारणेचा दर पाहण्यात आला.

संसर्ग नसलेल्या म्हशींमध्ये उपचाराआधी ५ गटांमध्ये गर्भाशय मुख श्लेष्मा चे सामू ७.४६±०.०४, ७.६४±०.०८, ७.३८±०.०७, ७.४५±०.०८, ७.५९±०.०८ एवढा होता. डबलू एस टी चे मुल्य सर्व गटांमध्ये निगेटिव्ह होते. तसेच पमएन पेशी काऊंट १.८३०±० २४, २.००±०.२५, २.६७±०.३१, २.५८±०.२६, 2.०८±०.२३ उपचारांनी असा नोंदवण्यात आला आणि ८५ % जनावरांत वैशिष्ट्यपूर्ण तर २५ % जनावरांत अवैशिष्ट्यपूर्ण फर्न पॅटर्न क्रमशः 1 ते 4 गटांमध्ये नोंदवण्यात आला. सर्व गटांमध्ये उपचाराच्या वेळी कृत्रीम रेतन करण्यात आले आणि पुन्हा तपासणीमध्ये १ -५ गटांमध्ये क्रमशः गर्भधारणेचा दर ८३.३३%, ५०%,७५ %,६६.६६ %आणि ४१ % आढळून आला तसेच गर्भधारणेच्या प्रमाण ७५ %,४१ %,66.66%,५८ % आणि 30% आढळून आला.

सदर संशोधनानंतर, संसर्ग नसलेल्या सतत माजावर येणाऱ्या म्हशींमध्ये कृत्रीम रेतनाच्या वेळी जीनआरएच आणि हर्बोमिनविट चे पावडर यांचा वापर केलेल्या गटांमध्ये गर्भधारणा दर आणि अर्भक दर हा अधिक दिसून आला.