

Animal Reproductive Health: Evaluating the Benefits of Mineral and Bypass Fat Supplements for Preventing Abnormalities

Shaikh Adil^{1*}, Dr. Sriom², M N Nachappa³

¹Assistant Professor, Department of Dairy Technology, Parul Institute of Technology, Parul University, Limda, Vadodara-391760, Gujarat, India. Email id: adilshaikh36@yahoo.com; shaikh.adil23773@paruluniversity.ac.in,

ORCID ID: <http://orcid.org/0000-0002-98700-6073>

²Assistant Professor, Department of Agriculture, Sanskriti University, Mathura, Uttar Pradesh, India, Email Id- sriomsoa@sanskriti.edu.in, Orcid Id- 0009-0008-5973-1162

³Professor, Department of Computer Sceince and Information Technology, Jain (Deemed to be University), Bangalore, India, Email Id- mn.nachappa@jainuniversity.ac.in, Orcid Id- 0000-0002-4007-5504

Abstract

A vital component of livestock management, animal reproductive health affects sustainability and production. This study looks into the possible advantages of mineral and bypass fat supplements in reducing animal reproductive problems. It is well known that a healthy diet, which includes vital minerals and energy sources, affects a woman animal's ability to reproduce. Over two years, 19,200 cows from 16,405 farmers participated in fertility improvement camps across seven states in India. The animals had a poor fertility history. A study was conducted to assess the nutritional status of 720 non-infectious, sterile animals in relation to their reproductive health. Give ivermectin 100 mg s/c or fenbendazole + ivermectin 3g orally to kill non-infectious clean animals. In Maharashtra, 230 estrus animals, 250 recurrent mating animals and 240 anestrus animals were chosen for the study. For one month, 240 anestrus animals were given nutritional supplements in the form of bypass fat, multi-mineral shakes, or chelated area-specific mineral mixture (ASMM). After 120 days, follow-up visits were conducted to assess reproductive health. The results indicated that anestrus cows differ from estrus along with recurrent mating cows in terms of average blood sugar levels ($p < 0.05$), total lipids, the amount of zinc and phosphorus ($p < 0.05$). Compared to anestrus cows, dairy cows that mated again had decreased plasma concentrations of iron, calcium, phosphorus, magnesium and total protein. The fatty acids such as omega-3, omega-6 and amino acids are lower. About 30% had insufficient calcium levels, suggesting calcium can play a role in dairy cow infertility. The phosphorus ratio differed among the animals studied in different fields, indicating that it plays a part in dairy cow infertility. In pubertal/postpartum anestrus animals, supplementing with ASMM, multi-mineral shake and bypass fat led to a 35-50% estrus induction response and a 30-55% conception rate; these outcomes were superior to those obtained from mineral supplementation alone.

Keywords: Animals Reproductive Health, Estrus, Anestrus, Recurrent Mating, Bypass fat, Minerals, area-specific mineral mixture (ASMM).

INTRODUCTION

Timely conception of cows is essential to the survival of a thriving dairy industry. One important characteristic that influences milk production and reproductive efficiency in dairy cows is the stage of pregnancy (1). Cows that have poor reproductive outcomes are less profitable to milk since they can continue to do so long after they should. Farmers must disperse 80 days following childbirth to sustain pregnancy and create a 365-day calving gap. Extended detection times have financial consequences. Cows diagnosed as pregnant later in the lactation process are sometimes culled due to extended dry spells or unsuitable calving patterns. Patients who are pregnant are diagnosed by veterinarians (2). COVID-19 has forced the livestock sector to reevaluate its dietary plans for the well-being and productivity of buffalo. The health and well-being of buffalo have improved due to developments in animal husbandry, nutrition, genetics and animal management (3). However, these tactics have been reexamined in light of the changing climate, shifting economic conditions and rising demand from developing nations. The sector needs to come up with alternate feeding plans that satisfy consumer demands for affordable, hygienic, halal, environmentally friendly and morally sound animal goods (4). With a frequency of

5% to 15%, infertility is a global public health concern that affects 80 million individuals. Particularly when it comes to reproduction, environmental toxins, such as organic contaminants in drinking water, offer serious health hazards. According to a 2012 WHO survey, from 1990 to 2010, the prevalence of infertility among women from 190 countries did not change. According to recent research, several industrial and environmental toxins could have a negative effect on pregnancy and fertility. To enhance reproductive health and stop the spread of infertility, these problems must be addressed (5). Since they have a well-defined estrous cycle that can be handled safely, mice and rats are employed in studies on reproductive function. They are helpful in tracking estrous or timed pregnancy and their evaluation is essential for determining how well the female reproductive system and the hypothalamic-pituitary-ovarian axis are working. They can look at how pharmaceuticals and chemicals affect reproductive function, which leads to changes in the morphology, cytology and histology of reproductive organisms, as well as modifications during particular periods of the estrous cycle (6). Due to limited feed supplies, poor nutrition and a lack of high-quality fodder, milk output has remained relatively high in the tropics. The average production in the dry zone is three to five liters per cow per day (7).

Milk is a transparent, yellowish-white liquid that contains water, fats, proteins, carbohydrates, minerals, vitamins and other substances. It has a sweetish taste and a pleasant aroma (8). The rise in the buffalo herd worldwide has led experts to investigate (9) the possibility of supplementing ruminant diets to enhance production characteristics. Without affecting buffalo development, concentrate and rumen bypass fat supplements can boost vital nutrients and improve rumen metabolism. Studies show that these supplements improve blood factors, development hormones, physical score and organic substance consumption. Still, further research is needed to determine the long-term impacts on buffalo productivity and health. The purpose of the study (10) evaluated the effects of feeding 0.45 kg/d of organic rumen-protected lipid (RPL) to organic certified Holstein cows for one to one hundred fifty days of milking on their metabolic state, overall health and reproductive function. In Northern Colorado, USA, a certified organic dairy carried out a randomized controlled experiment. The results showed that throughout 90 days in milk, RPL supplementation avoided the decline of body condition and enhanced the daily milk supply. RPL supplementation, however, did not affect reproductive results, milk composition, serum metabolite concentrations, or overall health. The study emphasizes how better dietary supplements are required for organic dairy systems. The paper (11) analyzes the dairy animals supplement with bypass fat to satisfy the 4-6% fat need for high-producing animals and to give high energy density. This supplement boosts farmers' net income while improving milk production, composition, body characteristics and reproductive efficiency. Despite as a long-standing practice, bypass fat is studied and developed to increase its profitability and accessibility for farmers. The study (12) looks at how feedlot water buffaloes' the addition of concentrating and rumen bypass fat to their basic diet affects their mineral intake, development, blood compounds, hormonal fluctuations and nutrition expenses. 3 dietary experimental groups and thirty-six male Murrah crossbred and swamp buffaloes participated in the study. The results showed that diet C resulted in the highest average daily rise, higher levels of growth hormone, hormone called growth factor-I, which is similar to insulin, blood total protein and cholesterol. Because of the supplementation, the buffaloes gained more weight and they were able to reach market weight faster, which increased farmer productivity. The study (13) investigated into industrialized nations, fertility has decreased and a major risk factor is one's nutritional status. Vital vitamins and minerals are essential for many physiological functions, such as the quality, maturation, fertilization and implantation of acolytes. There have been reports of infertility in women with reduced levels of micronutrients. Multiple micronutrient supplements help lower oxidative stress and restore micronutrient status. Randomized controlled trials are required to validate the specific impacts on fertility despite the availability of modest research. The study (14) examined the effects of protected fat and full-fat soybeans fed to cows producing milk that are starting to lactate. Higher dry matter intake, feed efficiency, milk output, fat and energy-corrected milk was seen in the MEGALAC group. The Full-Fat Soy group had a higher milk fat/protein ratio but decreased capric acid and increased oleic acid. The MEGALAC group had higher serum albumin and albumin/globulin ratios, while the Full-Fat Soy group had lower levels of NEFA and BHB. The study concluded that protected fat supplementation positively impacts cows' productive and reproductive traits. The review (15) of current developments in bypass nutrition technology to improve animal production: Insufficient feed supply in India results in reduced output and ineffective crop selection. An increase in animal population puts further strain on fragile food supplies. The primary feed sources are oil seed cakes and

insufficient agricultural wastes. By supplying starch, cheated minerals and vitamins together with shielding fat as well as protein from degradation in the rumen, bypass nutrition technologies are gaining traction as a possible means of boosting animal output. The research (16) examined the impact of adding calcium salts of omega-3 and omega-6 fatty acids (FA) to the diet on the composition of colostrums and the reproductive abilities of parous and nulliparous Holstein cows. Three treatments were given to the cattle: a Ca-salt supplement enriched in C18:2n-6 (CSO), no fat supplement (CON) and a Ca-salt supplement high in docosahexaenoic and eicosapentaenoic acid FA (CFO). According to the findings, fat created greater IgG concentrations, whereas CSO or CFO produced higher FA levels. The study (17) gives the importance of micronutrients for fetal development, embryogenesis and mother health; pregnancy is a crucial period for appropriate nutrition. Severe deficiency can have an adverse effect on the development of the fetus during pregnancy. Treatments such as oral iron, potassium iodide and folic acid can improve neurodevelopment, lessen maternal anemia and avoid neural tube abnormalities. Nonetheless, pre-eclampsia, gestational diabetes and nausea have been connected to certain micronutrients. To ascertain the advantages of various supplements and modify the suggested daily dosages, more investigation is required. The study (18) analyzes dairy animal production. It depends on energy and poor rationing during the early stages of lactation can cause anemia, weight loss, metabolic and reproductive problems. Increasing the diet's energy density is a sensible feeding technique to optimize energy intake. The productivity of thirty healthy breastfeeding Murrah buffaloes was studied for ninety days after giving birth to determine the impact of extra energy supplements. While there was no appreciable change in the groups' percentages of total solids, lactose, or protein, the average quantity of fat in the milk jumped noticeably.

Animal Reproductive Health

For cow productivity and health in livestock management, the reproductive cycle is crucial. This encompasses the estrous process, a roughly 21-day period marked by alterations in behavior and physiology. Cows undergo 280 days of gestation after fertilization before becoming pregnant. For appropriate treatment and hygiene, pregnancy monitoring is crucial. For cows to have healthy reproductive systems, the postnatal phase is critical, requiring proper diet and care for the following cycle (19). Understanding and controlling cyclical hormonal changes is crucial for improved reproductive success in cow herds, as lactation and the reproductive cycle are linked. Female mammals go through several stages in their reproductive process, which include proestrus (readying for mating), Estrus (sexual receptivity), metestrus (post-estrus period) and diestrus (after the period of Estrus). Anestrous (the animal doesn't experience the cycle) (Figure (2)) (20).

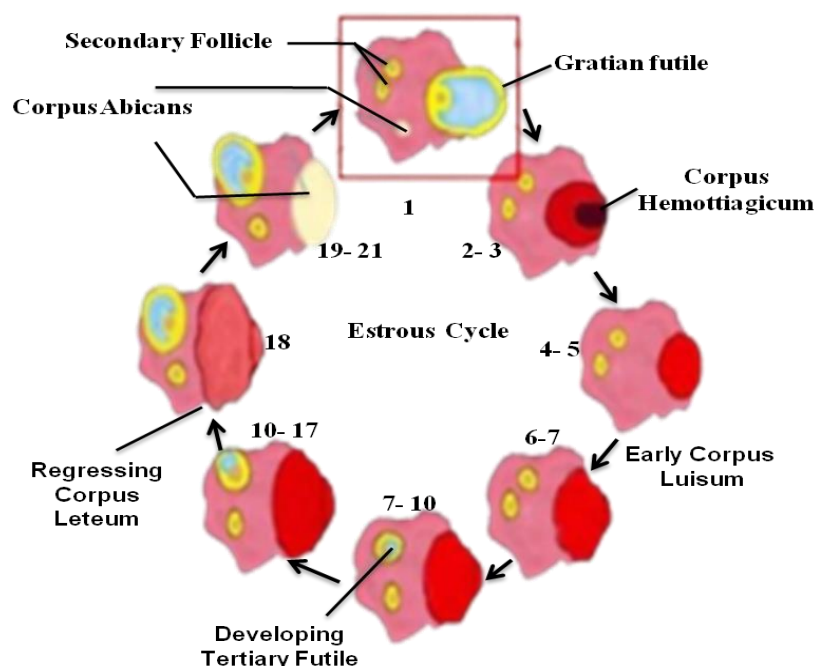


Figure (1). Estrous cycle

(Source: <https://www.slideserve.com/kylee-copeland/siklus-estrus>)

Female calves have standing estrus, sometimes referred to as "standing heat," during adolescence. This phase lasts for nine to fifteen months. During this 21-day cycle, which can last anywhere from 8 to 30 hours at a time, male bulls are open to mating. Other behaviors that can occur during estrus include vulva enlargement, trepidation, or attempts to mount other animals (21). Although they are invisible to the unaided eye, estrous levels can be deduced from the corpora lutea or follicular composition. Estrous activity is the term used to describe the times when a female animal, such as a cow, exhibits estrous activities and gets ready for mating. The success of conception and breeding depends on this window of time. Estrous inactivity occurs when a female does not exhibit any signs of Estrus and it is not open to sexual activity, which is called anestrus (22). Many species, including cows, go through a recurring process called the estrous cycle (23), which has four distinct phases: proestrus, Estrus, metestrus (diestrus) and anestrus. Animals differ in the length and frequency of these phases, which are impacted by age, environment, diet and overall health. In livestock, controlling estrous activity and inactivity is crucial to successful reproduction.

MATERIAL AND METHOD

This study investigates the effects of bypass fat and mineral supplements on the reproductive health of animals, with particular attention to their function in immune system support, bone development and metabolic processes. Additionally, it investigates how bypassing fats might improve reproductive outcomes by meeting energy needs at crucial times such as Estrus, conception and gestation. To monitor changes over time and examine biochemical parameters, the research employs longitudinal studies involving a wide range of animal subjects. The findings of this study can lead to an overall improvement in reproductive health, which would raise productivity and sustainability in the livestock sector. In this journal, we analyze the performance of the animal reproductive system in various phases, such as the estrus period, recurrent mating period and anestrus period. Then, calculating the clinical experiment, the performance improvement parameters such as minerals, amino acids, ions, etc.; the workflow model (figure (2)) is given below.

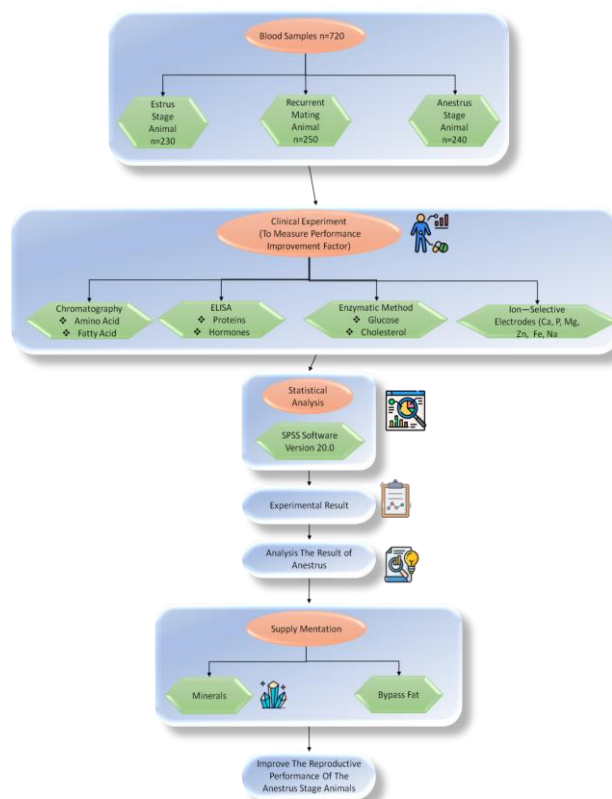


Figure (2). Work Flow Model (Source: Author)

Place of the Research

The study was carried out at 940 BAIF Development Research Foundation Artificial Insemination (AI) centers spread over 147 districts in seven states: *Uttarakhand, Uttar Pradesh, Rajasthan, Madhya Pradesh, Gujarat, Maharashtra and Odisha* (24). In more than 100,000 communities, the NGO offers door-to-door AI services for cattle to 5 million small farmers. Over two years, 19,200 cows from 16,405 farmers. This study prefers 720 animals of estrus period ($n = 230$), pregnant animals ($n = 250$) and anestrus ($n = 240$) in the region of Maharashtra ($19^{\circ} 45' 5.327''$ N and latitude $75^{\circ} 42' 49.998$ E). The data storage as well as management pipeline provided by BAIF was used to gather and analyze cow reproduction data.

Blood Sample Collection

Using blood samples ($n = 720$) of estrus stage animals ($n = 230$), recurrent mating animals ($n = 250$) and anestrus animals ($n = 240$) from representative animals of groups of non-infectious infertility under each region, the profile of blood glucose, plasma total protein, total cholesterol and macro minerals like calcium, phosphorus and magnesium was determined using the following biochemical procedure:

1. Chromatography.
2. Enzyme-Linked Immunosorbent Assay (ELISA).
3. Ion-selective electrodes.
4. Enzymatic method.

Design of Experiment

Different analysis techniques are used in veterinarian research to measure biochemical parameters in blood samples. These methods include chromatography (25), which separates mixtures based on the differential partitioning of compounds between a stationary phase, a mobile phase and spectrophotometer, which quantifies the amount of light absorbed by a substance at various wavelengths. Chemicals like amino acids, fatty acids and hormones are separated and quantified using High-Performance Liquid Chromatography (HPLC) (27) and Gas Chromatography (GC). The ELISA (26) is a commonly used method for measuring antibodies, proteins and hormones. Ion-selective electrodes are used to measure ions such as calcium, potassium and sodium directly in blood samples by measuring the electrical potential of a particular ion in a solution. Flame photometry, which is used to quantify specific metals like sodium and potassium in blood samples, measures the intensity of light emitted when atoms are excited in a flame. Here, Enzymatic (28) methods are used to test the amounts of glucose and cholesterol levels in animal blood.

Statistical Analysis

The *statistical package for the social sciences, or SPSS*, version 20.00 was used to analyze the blood profile data, to determine the ANOVA and Mean \pm STD Err (Standard Error) group-wise. The estrus/post-hoc test was used to examine the mean differences between the groups.

RESULT AND DISCUSSION

Experimentation Result

The biochemical test result found the variance supply balance in the plasma of the animals during three distinct reproductive stages: Estrus, recurrent mating and anestrus (Table (1)). In comparison to Estrus, the mean glucose levels are higher during recurrent mating and lower during anestrus. Between-group differences in protein levels are marginal and standard deviations imply variability. Compared to Estrus, cholesterol is lower during recurrent mating and higher during anestrus. These results suggest that differences in blood biochemical components are related to reproductive conditions. Recurrent mating raises glucose levels, which can be a sign of increased metabolic demands or hormonal influences. There are slight variations in protein levels, with a

slight decrease during anestrus. Recurrent Mating is associated with lower cholesterol levels than Estrus and Anestrus.

Table (1). Infertile Cattle's Blood Plasma Biochemical Profile
(Source: Author)

Plasma's biochemical components	Productive Condition		
	Estrus (n=230)	Recurrent Mating (n=250)	Anestrus (n=240)
The glucose level in Blood (mmol/L)	49.83 ^{xy} ±4.09	59.36 ^x ±5.34	36.18 ^y ±3.81
Protein level in the blood (g/dl)	8.96±7.54	9.02 ^x ±8.45	6.95 ^y ±5.45
Cholesterol level in the Blood (mg/dl)	185 ^{xy} ±9.3	159 ^x ±6.85	190 ^y ±6.85

This shows that there are significant differences ($p < 0.05$) in the chance of a species having unusual superscripts as designation across all groups.

Table (2) shows the components of plasma minerals, measured in specific units, for animals under various reproductive situations. The findings indicate that during recurrent mating, calcium levels are more significant than during estrus and they are somewhat lower during anestrus. Higher phosphorus levels are seen during recurrent mating, suggesting that different dietary requirements can apply depending on the stage of reproduction. Magnesium levels are lower during anestrus and recurrent mating, respectively, indicating possible shifts in metabolic needs during Estrus. There are notable differences in zinc and phosphorus levels between the categories, with Estrus having a more significant concentration than Recurrent Mating and Anestrus. There are slight differences in iron levels throughout various reproductive disorders. The quantity of sodium varies greatly throughout populations, peaking during anestrus. According to the results, differences in plasma mineral components are linked to distinct reproductive circumstances. This highlights the need to tailor nutritional management to the individual requirements of animals at different phases of reproduction.

Table (2). Infertile Cattle's Blood Plasma Minerals Profile (Source: Author)

Units	plasma's mineral components	Productive Condition		
		Estrus (n=230)	Recurrent Mating (n=250)	Anestrus (n=240)
(mg/dl)	Calcium (Ca)	8.78 ±0.17	9.29 ±0.18	8.27 ±0.21
	Phosphorus (P)	4.39 ±0.32	5.96 ±0.45	3.99 ±0.27
	Magnesium (Mg)	2.98 ±0.15	2.46 ±0.19	2.17 ±0.35
(ppm)	Zinc (Zn)	1.89 ^{xy} ±0.01	0.79 ^x ±0.03	1.13 ^x ±0.02
	Iron (Fe)	2.07 ±0.05	2.25 ±0.05	2.18 ±0.04
	sodium (Na)	2.09±0.06	0.98±0.05	3.58±0.06

This indicates that the presence of uncommon superscripts for a species varies across the column ($p < 0.05$).

The findings show differences in the amounts of fatty acids (FA) and amino acids (AA), which can point to metabolic alterations related to the reproductive state. According to the study, capsaicin levels elevated during recurrent mating but fell during anestrus. Moreover, levels of lauric acid rose during repeated mating but fell during anestrus. Levels of myristoleic acid dropped during anestrus but emerged during recurrent mating. Under all reproductive situations, there was little difference in the ranks of omega-3 fatty acids, such as arachidonic acid, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). According to the research, Elevations in FA and AA concentrations in cow plasma could indicate changes in metabolism as shown in Table (3).

Table (3). Infertile Cattle's Blood Plasma Acids Profile

(Source: Author)

Plasma's biochemical Acid			Productive Condition		
			Estrus (n=230)	Recurrent Mating (n=250)	Anestrous (n=240)
Amino Acids	Caprylic acid (mg/dL)		7.09±0.03	8.03±0.08	7±0.01
	Lauric acid (mg/dL)		7.28±0.04	7.69±0.08	7.01±0.06
	Myristoleic acid (mg/dL)		7.89±0.07	7.95±0.02	7.03±0.02
Fatty Acids	Omega-3	Eicosapentaenoic Acid - EPA (mg/dL)	7.56±0.4	7.69±0.1	7.07 ^a ±0.1
		Docosahexaenoic Acid – DHA (mg/dL)	8.93 ±0.09	8.92±0.8	7.01±0.7
	Omega-6	Arachidonic Acid (mg/dL)	7.94±0.03	7.96±0.9	7.03±0.004
		Linoleic Acid (mg/dL)	7.84±0.4	7.98±0.7	7.15±0.08

Supplementation: A comprehensive strategy that considers genetics, management techniques, nutrition and health is needed to improve animal reproductive performance as well as to prevent animal abnormalities. Offering a balanced combination of vital nutrients, supplementation methods like ASMM (amino acid, mineral and vitamin Mix) and bypass fats can promote reproductive health. Bypass fats offer a concentrated energy source for reproductive processes such as Estrus, conception and gestation. They are derived from vegetable oils or fats treated to resist microbial degradation. Supplementing with proteins can increase the rate of birth and the development of the embryo. Proteins are necessary for hormones, enzymes and reproductive tissues. Supplementing with trace minerals, such as selenium, copper and zinc, is crucial for reproductive processes. It is essential to tailor supplementation strategies to the particular species, breed and stage of reproduction of each animal. In anestrous cattle, the estrus response occurs 120 days after the administration of minerals, ASM and bypass fat supplementation as shown in Table (4).

Table (4). Estrous reaction in anestrous cattle within 120 days of ASMM, mineral shake and bypass fat supplementation (Source: Author)

Supplements	Anestrous Cattles (n=240)		
	Quantity of Goods Supply	Quantity of Responses	Quantity of Conceived
ASMM	80	28	12
Bypass Fat	90	47	9
Minerals shake	70	32	6

The information shows a relationship between the addition of various items to anestrous animals and data on the number of calves that conceived the number of responses along with the number of commodities delivered. The information indicates that, in spite of the number of items delivered, the ASMM (Amino Acid, Mineral and Vitamin Mixture) has a greater conception rate than the Bypass Fat and Minerals Shake. Bypass Fat exhibits a greater response rate than ASMM but a lesser conversion to successful conception. Minerals Shake has a poor conception rate compared to a moderate response rate. Although more statistical research is required to make more reliable results and assess the importance of the changes identified, these data provide insights into the association between different types of supplements and reproductive outcomes. Results can be impacted by variables such as the length of supplementation, particular dietary requirements and individual differences between cattle. The supplementation produced a 35–50% estrus induction response and a 30–55% conception rate. It contains a particular formulation for reproductive assistance. Beyond the advantages of mineral

supplementation alone, the study discovered that the combination of supplements had a synergistic effect on reproductive function. Investigation and study of the particular formulation and processes behind this reaction are necessary. The study highlights how crucial it is to consider a thorough strategy for nutritional supplementation to support successful cow reproduction. To comprehend the precise formulation and underlying processes of this reaction, more investigation and analysis are required.

CONCLUSIONS

Supplements containing minerals and bypass fat are crucial for cattle reproductive health since they help with issues like abnormalities, including anestrus in cattle. The development of reproductive organs and hormone regulation are supported by the essential minerals found in mineral supplements, which supply a balanced diet with calcium, phosphorus, magnesium and trace minerals. Supplements containing bypass fat provide cows energy, especially those who are nursing or under stress. They promote hormone synthesis, which enhances estrous cycles and the likelihood of successful reproduction. They can improve a breastfeeding cow's physical state, fertility and milk production. However, it's crucial to speak with an animal nutritionist or veterinarian about how these supplements should be utilized in accordance with the particular nutritional requirements of the animals.

REFERENCES

1. Macmillan, K., Gobikrushanth, M., Behrouzi, A., López-Helguera, I., Cook, N., Hoff, B., & Colazo, M. G. (2020). The association of circulating prepartum metabolites, minerals, cytokines, and hormones with postpartum health status in dairy cattle. *Research in veterinary science*, 130, 126-132. <https://doi.org/10.1016/j.rvsc.2020.03.011>
2. Brand, W., Wells, A. T., Smith, S. L., Denholm, S. J., Wall, E., & Coffey, M. P. (2021). Predicting pregnancy status from mid-infrared spectroscopy in dairy cow milk using deep learning. *Journal of Dairy Science*, 104(4), 4980-4990. <https://doi.org/10.3168/jds.2020-18367>
3. Gobikrushanth, M., Macmillan, K., Behrouzi, A., López-Helguera, I., Hoff, B., & Colazo, M. G. (2020). Circulating Ca and its relationship with serum minerals, metabolic and nutritional profiles, health disorders, and productive and reproductive outcomes in dairy cows. *Livestock Science*, 233, 103946. <https://doi.org/10.1016/j.livsci.2020.103946>
4. Samad, M. (2020). A systematic review of research findings on buffalo health and production published during the last six decades in Bangladesh. *Journal of Veterinary Medical and One Health Research*, 2(1), 01-62. [https://doi.org/10.36111/jvmohr.2020.2\(1\).0016](https://doi.org/10.36111/jvmohr.2020.2(1).0016)
5. Ma, Y., He, X., Qi, K., Wang, T., Qi, Y., Cui, L., ... & Song, M. (2019). Effects of environmental contaminants on fertility and reproductive health. *Journal of Environmental Sciences*, 77, 210-217. <https://doi.org/10.1016/j.jes.2018.07.015>
6. Ajayi, A. F., & Akhigbe, R. E. (2020). Staging of the estrous cycle and induction of Estrus in experimental rodents: an update. *Fertility research and practice*, 6(1), 1-15. <https://doi.org/10.1186/s40738-020-00074-3>
7. Ranaweera, K. K. T. N., Mahipala, M. K., & Weerasinghe, W. M. P. B. (2020). Influence of rumen bypass fat supplementation during early lactation in tropical crossbred dairy cattle. *Tropical animal health and production*, 52, 1403-1411. <https://doi.org/10.1007/s11250-019-02140-5>
8. Marcu, A., Stef, L., Julean, C., Pet, I., Gherasim, V., Pacala, N., ... & Stef, D. S. (2022). Effect of the Supplementation with Protected Fats in the Diet of Dairy Cows on The Quantity and Quality of Milk. *SCIENTIFIC PAPERS ANIMAL SCIENCE AND BIOTECHNOLOGIES*, 55(2), 186-186.
9. Mohd Azmi, A. F., Ahmad, H., Mohd Nor, N., Goh, Y. M., Zamri-Saad, M., Abu Bakar, M. Z., ... & Abu Hassim, H. (2021). The impact of feed supplementations on Asian buffaloes: A review. *Animals*, 11(7), 2033. <https://www.mdpi.com/2076-2615/11/7/2033#>
10. Manriquez, D., Chen, L., Melendez, P., & Pinedo, P. (2019). The effect of an organic rumen-protected fat supplement on performance, metabolic status, and health of dairy cows. *BMC Veterinary Research*, 15, 1-14. <https://doi.org/10.1186/s12917-019-2199-8>
11. Rajneesh, S. J., Chauhan, P., & Kumar, N. (2020). Bypass fat as a feed supplement in ruminants: A review.

12. Manriquez, D., Chen, L., Melendez, P., & Pinedo, P. (2019). The effect of an organic rumen-protected fat supplement on performance, metabolic status, and health of dairy cows. *BMC Veterinary Research*, 15, 1-14. <https://doi.org/10.1186/s12917-019-2199-8>
13. Schaefer, E., & Nock, D. (2019). The impact of preconceptional multiple-micronutrient supplementation on female fertility. *Clinical Medicine Insights: Women's Health*, 12, 1179562X19843868. <https://doi.org/10.1177/1179562X19843868>
14. Sallam, S. M. A., Mahmoud, A. A. A., Hashem, N. M., Attia, M. F. A., Mesbah, M. M., & Zeitoun, M. M. (2021). Alterations of Productive and Reproductive Efficiencies in Dairy Cows by the Inclusion of Protected Fats and Full-Fat Soy in the Diet. *Animal Science*, MedDocs Publishers, 1, 18-30.
15. Ganai, A. M., Haq, Z., Beigh, Y. A., & Sheikh, G. G. (2019). Bypass nutrient technology with recent advances for enhancing animal production: A review. *Journal of Pharmacognosy and Phytochemistry*, 8(5S), 269-275.
16. Jolazadeh, A. R., Mohammadabadi, T., Dehghan-Banadaky, M., Chaji, M., & Garcia, M. (2019). Effect of supplementing calcium salts of n-3 and n-6 fatty acid to pregnant nonlactating cows on colostrum composition, milk yield, and reproductive performance of dairy cows. *Animal feed science and technology*, 247, 127-140. <https://doi.org/10.1016/j.anifeedsci.2018.11.010>
17. Santander Ballestín, S., Giménez Campos, M. I., Ballestín Ballestín, J., & Luesma Bartolomé, M. J. (2021). Is supplementation with micronutrients still necessary during pregnancy? A review. *Nutrients*, 13(9), 3134. <https://www.mdpi.com/2072-6643/13/9/3134#>
18. Yadav, D. S., Lakhani, G. P., Baghel, R. P. S., Roy, B., Mishra, A., & Nanavati, S. (2021). Effect of Additional Energy Supplement on the Productive Performance of Lactating Murrah Buffaloes. <https://doi.org/10.21203/rs.3.rs-881381/v1>
19. Ireland, J. J., Murphee, R. L., & Coulson, P. B. (1980). Accuracy of predicting stages of the bovine estrous cycle by gross appearance of the corpus luteum. *Journal of Dairy Science*, 63(1), 155-160. [https://doi.org/10.3168/jds.S0022-0302\(80\)82901-8](https://doi.org/10.3168/jds.S0022-0302(80)82901-8)
20. Byers, S. L., Wiles, M. V., Dunn, S. L., & Taft, R. A. (2012). Mouse estrous cycle identification tool and images. *PloS one*, 7(4), e35538. <https://doi.org/10.1371/journal.pone.0035538>
21. Marcondes, F. K., Bianchi, F. J., & Tanno, A. P. (2002). Determination of the estrous cycle phases of rats: some helpful considerations. *Brazilian journal of biology*, 62, 609-614. <https://doi.org/10.1590/S1519-69842002000400008>
22. Rhodes, F. M., McDougall, S., Burke, C. R., Verkerk, G. A., & Macmillan, K. L. (2003). Invited review: Treatment of cows with an extended postpartum anestrous interval. *Journal of Dairy Science*, 86(6), 1876-1894. [https://doi.org/10.3168/jds.S0022-0302\(03\)73775-8](https://doi.org/10.3168/jds.S0022-0302(03)73775-8)
23. Baruselli, P. S., Reis, E. L., Marques, M. O., Nasser, L. F., & Bó, G. A. (2004). The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. *Animal reproduction science*, 82, 479-486. <https://doi.org/10.1016/j.anireprosci.2004.04.025>
24. Joshi, S., Bhawe, K., Potdar, V., Gaundare, Y., Punde, N., Shirsath, T., & Swaminathan, M. (2021). Performance of sex-sorted semen under Indian Small Holder Dairy Farming Systems. *Int. J. Curr. Microbiol. and Appl. Sci*, 10(02), 1335-1343. <https://doi.org/10.20546/ijemas.2021.1002.158>
25. Long, N. P., Park, S., Anh, N. H., Kim, S. J., Kim, H. M., Yoon, S. J., ... & Kwon, S. W. (2020). Advances in liquid chromatography–spectrometry-based lipidomics: a look ahead. *Journal of Analysis and Testing*, 4, 183-197. <https://doi.org/10.1007/s41664-020-00135-y>
26. Khurshid, Z., Warsi, I., Moin, S. F., Slowey, P. D., Latif, M., Zohaib, S., & Zafar, M. S. (2021). Biochemical analysis of oral fluids for disease detection. *Advances in clinical chemistry*, 100, 205-253. <https://doi.org/10.1016/bs.acc.2020.04.005>
27. Žuvela, P., Skoczylas, M., Jay Liu, J., Bączek, T., Kaliszan, R., Wong, M. W., & Buszewski, B. (2019). Column characterization and selection systems in reversed-phase high-performance liquid chromatography. *Chemical Reviews*, 119(6), 3674-3729. <https://doi.org/10.1021/acs.chemrev.8b00246>
28. Casali, B., Brenna, E., Parmeggiani, F., Tessaro, D., & Tentori, F. (2021). Enzymatic methods for the manipulation and valorization of soapstock from vegetable oil refining processes. *Sustainable Chemistry*, 2(1), 74-91.