EFFECT OF BLOOD UREA NITROGEN, MINERAL STATUS AND UTERINE pH ON FERTILITY IN DAIRY COWS

SEENA. N. S

Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2008

Department of Animal Reproduction, Gynaecology and Obstetrics
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

DECLARATION

I hereby declare that the thesis entitled **"EFFECT OF BLOOD UREA NITROGEN, MINERAL STATUS AND UTERINE pH ON FERTILITY IN DAIRY COWS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy.
13/8/08

SEENA. N.S

CERTIFICATE

Certified that this thesis, entitled **"EFFCT OF BLOOD UREA NITROGEN, MINERAL STATUS AND UTERINE pH ON FERTILITY IN DAIRY COWS"** is a record of research work done independently by **Seena. N.S,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, associateship or fellowship to her.

13/8/08

Dr. K.V. Athman,
(Chairman, Advisory Committee)
Professor and Head,
University Veterinary Hospital,
Kokkalai, Thrissur,

Kokkalai.
13/8/08

CERTIFICATE

We, the undersigned members of Advisory Committee of **Seena. N.S**, a candidate for the degree of Master of Veterinary Science in Animal Reproduction, Gynaecology and Obstetrics, agree that the thesis entitled **"EFFCT OF BLOOD UREA NITROGEN, MINERAL STATUS AND UTERINE pH ON FERTILITY IN DAIRY COWS'** may be submitted by **Seena. N.S, in** partial fulfilment of the requirement for the degree.

13/8/08

Dr. K.V. Athman,
(Chairman, Advisory Committee)
Professor and Head
University Veterinary Hospital,
Kokkalai, Thrissur

13/8/08

Dr. T. Sreekumaran
Associate Professor and Head,
Department of Animal Reproduction,
Gynaecology and Obstetrics,
College of Veterinary and
Animal Sciences, Mannuthy
(Member)

Dr. G. Ajitkumar
Assistant Professor (SS),
Department of Animal Reproduction,
Gynaecology and Obstetrics
College of Veterinary and
Animal Sciences, Mannuthy
(Member)

13.8.08

Dr. A. D. Mercy,
Professor and Head,
Department of Animal Nutrition,
College of Veterinary and Animal
Sciences, Mannuthy
(Member)

External Examiner

Dr.R.C. RAJASUNDARAM, M.V.Sc., Ph.D.,
PROFESSOR
Veterinary University Peripheral Hospital
Tamilnadu Veterinary & Animal Sciences University
Madhavaram Milk Colony, Chennai - 600 051.

ACKNOWLEDGEMENT

It gives me immense pleasure to record my sincere thanks and deep sense of gratitude towards my guide and Chairman of the Advisory Committee, Dr. K. V. Athman, Professor and Head, Veterinary College Hospital, Kokkalai , for his affectionate guidance, valuable suggestions, generous support, personal attention and kindly help given in all possible ways during the entire period of my study. I recollect with great respect the interest he has bestowed upon my project and without his strong support and co-operation, the successful completion of this work would not have been possible.

With sincere gratitude and indebtedness, I would like to express my thankfulness to Dr. T. Sreekumaran, Associate Professor and Head, Department of Animal Reproduction, Gynaecology and Obstetrics and member of the advisory committee for his expert advice, meticulous guidance and whole-hearted support during the course of my study.

I was indeed fortunate for having Dr. G. Ajitkumar, Assistant Professor (ss), Department of Animal Reproduction, Gynaecology and Obstetrics as my minor advisor. No words can ever express my sincere gratitude for his constant support, diligent efforts, creative suggestions and prompt correction of thesis. I am deeply indebted to him for the generous and timely help given during the course of my research work starting from the preparation of synopsis till the submission of thesis.

I would like to express my heartfelt gratitude towards Dr. A. D. Mercy, Professor and Head, Department of Animal Nutrition for her pleasant co-operation, valuable guidance, prompt review of thesis and supporting attitude as a member of the Advisory Committee.

With great respect, I would like to express my deep sense of gratitude to Dr. K. N. Aravinda Ghosh,, Professor and Head, Veterinary College Hospital, Mannuthy, for his kind nature, valuable suggestions, readiness to help and constructive review of this manuscript.

I humbly place my respect and sincere thanks to Dr. Joseph Mathew, Dr. V. Vijayakumaran, and Dr. Metilda Joseph for their mental support and encouragement. I also remember with sincere gratitude to Dr. Shibu Simon , Dr. Hiron. M. Harshan and Dr .Abdul Azeez for the support and encouragement given during the later stages of preparation of this manuscript.

I acknowledge Dr. V. Prasad, Professor and Head, University Livestock Farm, Mannuthy for providing me permission to conduct my research in the farm.

I would like to express my sincere gratitude to the whole hearted help rendered by Mrs. K.S. Sujatha and Mrs. Mercy for statistical analysis.

I sincerely thank all the staff of Department of Animal Reproduction, Gynaecology and Obstetrics and University Livestock Farm, Mannuthy for their incessant help and support.

I am grateful to Dr. E. Nanu, Dean, College of Veterinary and Animal Sciences, Mannuthy, for providing the facilities to conduct the research.

I remember with gratitude, the help rendered by Dr.Jineshkumar.N.S for the conduct of my research in the Farm and for the technical assistance provided by him.

No words can ever express my sincere gratitude to my colleagues Dr. T. Rajeswary, Dr. Hari Narayanan and Dr. Julliet who helped me a lot in tiding over the difficulties encountered during the course of my study.

I recollect with sincere gratitude, the invaluable help, cordial company and support given by Dr. Deepthi. L, in every stage of my work.

I owe my heartfelt gratitude to my seniors Dr. N. Sathyaraj, Dr. G. Jeba Sujana Dhas and Dr. Safna Isaac. M for their warm support and affectionate encouragement.

Words are eloquent to express my deep sense of gratitude to my room mates and friends, Dr.Jessy.V and Dr. Bhagyalakshmi.P.S, who were always there to lend a helping hand whenever needed.

I owe a special word of thanks to all my junior PG students, Dr. Binoy.V.S and Dr.Sreejith.J.R in particular, for their fruitful company and willingness to help.

I would also like to thank my friends Drs. Sumi Cherian, Lekshmi.P and Asheene.U.S, who helped me a lot in coming out from many frustrating moments during the course of my study.

I am extremely grateful to Drs. Lekshmi.V, Archana. A, Ambily. V.R, Priya.P and Indu.K for the technical and timely help given for successful completion of this endeavor.

I fondly remember the help given by all of my PG class mates especially Drs. Elso John, Dhanya.V.Pai. Seena.K.M, Remya.V, Mariamma and Sheeje.V.M.

I thankfully remember Dr. K.V. Santhosh kumar and Dr. M. Vinod kumar for the mental support and encouragement provided whenever I was in need.

I sincerely acknowledge the non-teaching staff of our department for all the help given during the study.

I expresss my sincere gratitude to Mr.Ravi, M/S Peagles, Mannuthy for the efficient execution in preparation of this thesis.

My sincere gratitudes are also due to Ms.Sageetha and Ms. Radhika of Central lab,Ms.Dhanya of Dept. of Nutrition, Staff of Yescom and Mr.Suresh for the timely help given for successful completion of this endeavor.

I am thankful to Kerala Agriculture University for providing me the fellowship for the postgraduate study.

Words possess no enough power when I remember my beloved Mother, who left me during the course of my M.V.Sc.study, for joining the heavenly abode. I remember with a heavy heart, the love, care and steadfast support given even in her hard times of illness. I am dedicating this thesis to her loving memory.........

No words can implicitly express my deep sense of gratitude to my beloved Father, for his love, affection, encouragement, prayers and blessings. I owe very much to Him. I cannot confine my feelings to my dear Sister and Brother-in-law in a mere gratitude, who consoled me a lot during the hottest days, stayed with me, supported me and I am sure, without their strong support, the successful completion of this endeavor would not have been possible.

I am gratefully rememibering all those people who put their hand together for me within this endeavor. Whenever I needed help, I luckily found people to guide me, support me and help me and I am convinced of the fact that it is not merely a personal feat.

Above all, I give my highest gratitude to Almighty God for being my strength when I am weak and for surrounding me with great people who never falters to help me.

I kneel before Him for all He has given and...............

SEENA. N.S

Dedicated to
The Loving Memory of
My Mother

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

1. INTRODUCTION

Livestock rearing in India is almost exclusively a rural occupation with a complementary relationship to agricultural sector. Since time immemorial, cattle have been an integral part of Indian culture and way of life. As per National statistics, 2003 (GOI, 2006), India is bestowed with the largest number of cattle population in the world (185.18 million).

The trend of cattle population in India indicated a decline of 5.85 per cent during the past decade and one of the major reasons attributed to this is reduced reproductive efficiency. Success of dairy farming lies in ensuring proper and optimal reproductive rhythms of each individual animal in the herd within normal physiological limits. Any deviation in the breeding rhythm results in progressive economic loss due to increased days open, prolonged intercalving interval and reduced lifetime yield. During the last decade, assisted reproductive techniques and reproductive biotechnology played important roles in improving milk production. But, a drastic reduction in fertility was noticed paralleling the increase in production. The root cause for declining fertility is probably a combination of physiological, nutritional and managemental factors. Although the relative contribution of each factor leading to infertility can be debated, nutritional factors are found to be the most important (Ferguson and Chalupa, 1989). The marked increase in production during the last few decades was due to adjustments in nutritional and reproductive strategies.

It is a common practice among feed manufacturers who compute feed to increase the level of crude protein in the ration to 17 to 19 per cent on dry matter basis for better production. As there is shortage of feed and fodder resources in the country, farmers are also forced to feed the animals with compounded rations containing high protein and urea. Diets that are high in protein and urea support and stimulate milk production, yet they are found to be detrimental to reproductive performance. The exact mechanism by which a high protein diet affects fertility is unknown, but urea nitrogen level in blood (BUN), plasma, or milk greater than 19 mg/dl has been found to cause alterations in the uterine environment and reduction in fertility in lactating dairy cows and heifers. Successful embryo development depends on the nature of uterine environment. The uterine luminal milieu is dynamic and exhibits marked difference between stages of oestrous cycle as a consequence of ovarian steroidal regulation of endometrial secretion. Intake of high protein diet has been shown to alter the uterine pH and concentrations of other ions in uterine secretions which in turn affect fertility (Ferguson *et al.*, 1993 and Butler *et al.*, 1996).

The effect of dietary protein and urea on ovarian and uterine physiology can be easily monitored by analysing blood, plasma or milk urea nitrogen. Metabolism and utilization of dietary protein depends on the energy availability. Hence, detrimental effect of feeding high dietary protein on fertility will be augmented by negative energy balance. Long term effects of negative energy balance can impair the health of pre-ovulatory oocytes and follicles and reduce progesterone concentration after ovulation. A low blood level of total protein and glucose indicate poor protein-energy status of the animal.

There are also varying reports on the role of minerals in controlling ovarian cycle in cattle. Inspite of numerous research efforts, the intricate relationship between minerals and reproduction has not been fully understood. All essential minerals are required for reproduction because of their cellular roles in metabolism, maintenance and growth. Their deficiencies result in poor reproductive efficiency, which might be reflected on low blood levels of them.

Hence, the present investigation was undertaken with the following objectives.

1. To determine the effect of BUN and uterine pH on fertility under farm and field conditions

2. To correlate variation in mineral status with fertility

3. To determine the variation in BUN and uterine pH under farm and field conditions.

Review of Literature

2. REVIEW OF LITERATURE

2.1 PROTEIN NUTRITION OF LACTATING DAIRY CATTLE

The major cause of unsatisfactory breeding performance in dairy cattle was inadequate reproductive and nutritional management (Barton *et al.*, 1996). Strategies for meeting the nutritional requirements of high producing dairy cows had necessarily changed in conjunction with genetic gains. Milk production and dry matter intake of dairy cows were stimulated in response to increased intake of dietary protein; but, unfortunately, decreased fertility was often noticed with this nutritional strategy. Concerns about high dietary protein and reduced fertility were not new (Jordan and Swanson, 1979 and Sonderman and Larson, 1989), but more intensive investigations were carried out during the last decades (Gustafsson and Carlsson, 1993; Butler *et al.*, 1996; Darwash *et al.*, 1999; Rajala-Schultz *et al.*, 2001; Hammon *et al.*, 2005 and Rhoads *et al.*, 2006).

Butler (1998) observed that although the diets that were high in crude protein (17 to 19 %) supported and stimulated milk production in early lactation, it was found to be detrimental to reproductive performance in dairy cows.

Feeding diets containing 14 to16 per cent crude protein seemed to be most effective in maintaining or increasing pre partum dry matter intake (Vandehaar *et al.*, 1999 and Phillips *et al.*, 2003). Currently, the National Research Council recommended a diet with 13.8 to 20.8 per cent crude protein, 7.8 to 14.7 per cent rumen degradable protein and 2.8 to 8.9 per cent undegradable protein on dry matter basis for milk production ranging between 18.8 to 44 kg/day (Melendez *et al.*, 2003).

2.2 MONITORING PROTEIN METABOLISM IN RUMEN

Leng and Nolan (1984) reported that amino acid requirements of ruminants were met from ruminant microbes getting digested in the small intestine and from the dietary protein that was not degraded in the rumen, but was intestinally digestible (bypass protein).

According to Canfield *et al.* (1990), milk production and energy balance were based on the amount and ratio of amino acids and energy yielding nutrients absorbed from the gut. It was opined that when protein intake exceeded the requirement or when the protein consumed was highly degradable, large amounts of ammonia were produced and detoxified by the liver to urea. Melendez *et al.* (2003) stated that both these ammonia and urea interfered with reproduction at different time points in the reproductive process.

According to Butler (2005), conception and establishment of pregnancy were an ordered progression of interrelated events and processes within reproductive tract such as follicular development, ovulation, fertilization, embryo transport and development, maternal recognition and implantation. Ammonia, urea or some other toxic products of protein metabolism might intercede at one or more of these steps to impair reproductive efficiency.

2.2.1 Urea Nitrogen

According to Hammond (1992) and Hof *et al.* (1997), blood urea nitrogen (BUN) and milk urea nitrogen (MUN) concentrations could be used as a non-invasive tool for monitoring protein status in dairy cattle.

The BUN concentration was related to dietary crude protein intake, the percentage of rumen degradable and undegradable protein as well as protein - energy ratio in diet (De peters and Cant, 1992; Sniffen et al., 1992; Roseler *et al.*, 1993 and Baker *et al.*, 1995).

Measurement of BUN had provided a useful index for studying the association between metabolism of dietary protein and reproductive efficiency. Increased BUN concentrations were correlated with decreased fertility in dairy cows both in confinement and grazing herds (Butler, 1998; Westwood *et al.,* 1998 and Laven and Drew, 1999).

Melendez *et al.* (2003) opined that if more protein was consumed than that could effectively be utilised, primarily urea and secondarily ammonia were increased in blood and body fluids despite increase in hepatic activity for the enzymes of urea cycle. The BUN concentration in cattle ranged from 10 to 30 mg/dl (Rajala-Schultz and Saville, 2003 and Guo *et al.*, 2004).

2.2.1.1 Dietary and Nutritional Factors Affecting BUN Value

Nitrogen fertilization of pasture could affect BUN in grazing cattle due to increased forage nitrogen content (Carver *et al.,* 1978). According to Roseler *et al.* (1993), balanced diets for lactating dairy cows were associated with an average BUN concentration of 15 mg/dl and that an imbalance of degradable and undegradable protein increased BUN or MUN value beyond 19 mg/dl.

Carlsson *et al.* (1995) observed variations in the concentration of MUN from herd to herd and between cows in the same herd. It was opined that differences in feed composition and dairy cattle nutritional management practices were likely to be the major influence on MUN. The concentration was found to be lower when the cows were housed during winter than were let loose for grazing. Melendez *et al.* (2003) stated that the BUN concentration increased by 5.60 mg/dl for each kilogram of dietary crude protein intake.

2.2.1.2 Other Factors Affecting BUN

Peterson and Waldern (1981) observed that in dairy cows, BUN increased as the cows progressed from dry stage through pregnancy, early lactation and the lactating period. The BUN also increased with increasing age.

Fraser (1991) stated that any disease or body condition that reduced glomerular filtration, such as dehydration, heart disease and renal disease or any condition that increased protein catabolism resulted in an increased BUN level.

Elrod and Butler (1993) reported that sampling time was an important consideration for BUN and MUN. Peak BUN concentration occurred several hours after feeding and changes in MUN lagged behind changes in BUN by about 1 to 2 hours.

Gustaffsson and Palmquist (1993) concluded from the study conducted in Ohio dairy cows that a distinct diurnal pattern existed in BUN level, with the peak value related to and following the time of feeding by 3 to 5 hours.

Severe nutritional depletion as a result of prolonged under nutrition (Ward *et al., 1992*) or disease (Hayden *et al*, 1993) resulted in catabolism of tissue protein and resultant elevation of BUN. Carlsson *et al.* (1995) found that the BUN concentration was lower during the first month of lactation than in later lactation period.

Arunvipas *et al.* (2003) found that renal disease interfered with excretion of urea and resulted in high BUN concentrations.

2.2.1.3 BUN as a Predictor of Fertility

High concentrations of BUN in early lactation had been found to be negatively correlated with fertility (Gustaffsson and Carlsson, 1993; Larson *et al.*, 1997; Rajala-Schultz and Saville, 2003 and Guo *et al.*, 2004), but in other studies this correlation was less clear (Hojman *et al.*, 2004) or absent (Godden et *al.*, 2001 and Cotrill *et al.*, 2002). The mechanism by which BUN affect fertility was not always clear, because in most experiments only associations were established (Rajala-Schultz and Saville, 2003; Nousiainen *et al.*, 2004 and Rhoads *et al.*, 2006). Butler (2005) proposed estimation of BUN as a reliable means to monitor the protein status of dairy cows with some predictive value for fertility.

2.3 EFFECT OF PROTEIN NUTRITION ON FERTILITY

The effect of a high content of dietary crude protein on fertility was reviewed by Butler (1998) and concluded that increasing the crude protein in dairy diets from between 13 and 17 per cent to between 19 and 21 per cent lowered the conception rates from 53 to 65 per cent. It was also found that high levels of rumen degradable protein delayed the first ovulation or oestrus, reduced the conception rate after the first insemination, increased the number of days open and lowered the overall conception rate.

2.3.1 Associated Changes in Follicular Fluid

Sinclair *et al.* (2000) conducted a study in heifers that were fed with diets to generate high rumen ammonia and found elevated ammonia levels in follicular fluid and lowered oocyte cleavage rates.

Hammon *et al.* (2005) suggested elevated ammonia concentration in reproductive fluids as a factor affecting embryo development and fertility and found that ammonia and urea concentration in the follicular fluid was higher in those cows with high plasma urea nitrogen (PUN) value of greater than 20 mg/ dl.

2.3.2 Associated Changes in Oviductal Fluid

Elevated systemic concentrations of ammonia and/or urea might compromise early embryonic development in the oviduct (Mc Evoy *et al.,* 1997; O'Callaghan *et al.,* 1999; Gath *et al.,* 1999; Berasdinelli *et al.*, 2001 and Fahey *et al.*, 2001).

According to Kenny *et al.* (2002), elevations in systemic concentrations of ammonia or urea were likely to impair embryo survival in cattle as a consequence of disruptions to the oviductal fluid environment.

2.3.3 Progesterone Concentrations

Jordan and Swanson (1979) reported that cows fed with low crude protein during the breeding period had higher serum progesterone concentrations than those fed with high crude protein.

Plasma progesterone concentration during the mid-diestrus was approximately 30 per cent lower in cows with high PUN (Sonderman and Larson, 1989). It was found that non-pregnant cows with low progesterone had a mean MUN concentration of 23.3 mg/dl as against 21.5 mg/dl in pregnant cows.

Butler *et al.* (1996) found that plasma progesterone concentrations were greater in pregnant than non pregnant cows on day four and five. The PUN concentration on the day of AI was 18.7 ± 0.6 and 20.7 ± 0.6 mg/dl respectively for cows subsequently diagnosed as pregnant and non-pregnant.

Larson *et al.* (1997) observed that non-pregnant cows with low circulating progesterone concentration (<2ng/ml) had a higher MUN value (>21mg/dl) compared to non pregnant cows with high circulating progesterone concentration (>2ng/ml).

Butler (1998) opined that reduced concentrations of plasma progesterone during the early breeding period appeared to be a likely component of the reduction in fertility associated with feeding high dietary protein.

Butler (2000) opined that increased blood urea level from feeding high dietary protein might reduce fertility by interfering with normal inductive effects of progesterone on the uterine environment, there by providing suboptimal conditions for the support of embryo development.

2.3.4 Cleavage and Fertilization

On exposure of embryos produced *in vitro* to moderate concentrations of ammonia, Hammon *et al.* (2000) observed an increase in the proportion of degenerated ova and a decrease in the proportion of embryos that developed to blastocyst stage. De wit *et al.* (2001) found that exposure of oocytes to 6 milli molar of urea during *in vitro* maturation impaired meiosis and fertilization rate.

Ocon and Hansen (2003) showed that the proportion of embryos that developed to blastocysts was reduced by the addition of 21 mg/dl of urea into maturation medium.

2.3.5 Uterine Luminal Environment

Jordan *et al.* (1983) found that feeding large amounts of protein could alter the uterine environment by reducing concentrations of magnesium, potassium and phosphorus in uterine secretions.

Butler (1998) opined that successful embryo development depended on the nature of uterine environment. The uterine luminal mileu was dynamic and exhibited marked differences between the stages of oestrous cycle as a consequence of ovarian steroidal regulation of endometrial secretion. Intake of high dietary protein altered the concentration of ions in uterine environment which resulted in reduced fertility.

Hammon *et al.* (2005) found that in addition to urea, ammonia had also been found to be elevated in the uterine fluid in dairy cows fed with a high dietary protein. These animals had a high PUN value and the elevated ammonia directly impaired the embryo development.

Meza-Herrera *et al.* (2006) found that high levels of supplementation with undegradable protein at pre-breeding stages played a role in both uterine environment and survival of embryos at early stages of pregnancy in ewes.

2.3.6. Uterine pH

Elrod *et al.* (1993) stated that heifers fed with a diet containing 21.8 per cent crude protein had a lower uterine pH compared with heifers (pH 7.09) fed with a diet containing 15.5 per cent crude protein (6.79 *vs* 7.09). It was opined that the lower uterine pH might have resulted from an inhibition of endometrial carbonic anhydrase activity which was highly sensitive to alterations in ionic composition.

Elrod and Butler (1993) found that high dietary protein altered the uterine pH; it increased from 6.8 at oestrus to 7.1 on seventh day of oestrous cycle. But this increase failed to occur in both heifers and lactating cows fed with excess rumen degradable or undegradable protein. Concentrations of BUN above 19 mg/dl had been associated with altered uterine pH and reduced fertility in dairy cows (Butler, 1998). Eventhough the exact cause for the reduction in pH was unclear, Zhu *et al.* (2000) suggested that ureagenesis removed bicarbonate and reduced pH.

Melendez *et al.* (2003) reported that lowered uterine pH associated with high BUN or MUN value might have occurred as a result of altered magnesium, potassium and phosphorus in uterine secretions.

According to Ocon and Hansen (2003), culture of early bovine embryos at pH less than 7 reduced the cleavage rates suggesting the inhibitory effect of suboptimal uterine pH associated with high concentrations of PUN on *in vivo* early embryonic development. But, Hugentobler *et al.* (2004) reported a mean uterine pH of 6.96 during oestrus in normal healthy dairy cows.

Lowering of uterine pH on intra venous infusion of urea was explained as an effect of carbonic anhydrase by Rhoads *et al.* (2004). It was opined that uterine luminal pH was controlled by carbonic anhydrase enzyme and was involved in the selective transport of hydrogen and bicarbonate ions in exchange of sodium, potassium and chloride ions to modify the uterine luminal pH.

Meza-Herrera *et al.* (2006) found that uterine pH was affected by the level of protein in the feed of Rambouillet ewes; the high protein group exhibited a low uterine pH and high embryonic mortality.

2.3.7. Development of Embryo

Blanchard *et al.* (1990) found that early degeneration and poor development of embryos occurred in lactating dairy cows that were fed with excess rumen degradable protein.

Elrod and Butler (1993) reported that an extended luteal phase and inter-oestrus interval of 26 to 36 days was observed following breeding among the non-pregnant heifers fed with a high protein diet. The prolonged luteal phase observed might be due to early embryonic death that occurred after the critical period for maternal recognition of pregnancy.

Bishonga *et al.* (1996) reported that elevated circulating concentration of urea and ammonia resulting from feeding high rumen degradable protein exerted an adverse effect on early embryonic development. It was found that percentage of fertilized ova recovered from super ovulated cows was reduced after feeding with high rumen degradable protein.

Mc Evoy *et al.* (1997) reported that feeding excess quickly degradable nitrogen in sheep resulted in a significant reduction in the number of viable embryos collected. It was suggested that there was either a direct effect of ammonia on oocyte maturation and development and / or an indirect effect on the utero - oviductal environment.

Butler (1998) opined that dietary urea altered both the pH gradient across the cells of endometrium and increased the secretion of $PGF_{2\alpha}$ which might have interfered with embryo development and viability. It was also found that embryo quality and development was reduced in lactating cows fed excess rumen degradable protein.

2.4. INTERACTION BETWEEN EXCESS DIETARY PROTEIN AND NEGATIVE ENERGY BALANCE

Butler and Smith (1989) opined that excess rumen degradable protein might have exacerbated the negative energy balance and its negative effects on fertility. Loeffler *et al.* (1999) correlated the body condition score at the time of artificial insemination and fertility. It was found that cows with a body condition score of at least three at the time of insemination were most likely to become pregnant. With more extensive loss of body condition score, the reduction in conception rate became greater.

According to O'Callaghan and Boland (1999), metabolism and utilization of dietary protein depended on energy availability and the effect of feeding high dietary protein superimposed on the preceding effects of negative energy balance found to be reducing the fertility in dairy cows. Butler (2000) explained prolonged anoestrus in post partum dairy cows as a result of negative energy balance in early lactation, but understanding of the linkage between negative energy balance and suboptimum conception rates in normal ovulatory cyclic cows remained rather speculative.

Campanile *et al.* (2006) and Tamminga (2006) found that diets that were high in rumen degradable protein aggravated the negative energy balance, because of the energy involved in the detoxification and excretion of excess ammonia.

2.5. CHARACTERISTICS OF OESTROUS CYCLE

2.5.1. Duration and Intensity of Oestrus

Gustaffson *et al.* (1986) reported that the mean duration of oestrus was 31.50 ± 3.60 and 23.84 ± 2.00 hours in repeat breeder heifers and virgin heifers respectively. Harrison *et al.* (1990) and Verma *et al.* (1983) observed less intensity of oestrus in high producing cows compared to low producers.

According to Bearden and Fuquay (1997), the mean duration of oestrus was 21 hours in cows. Gordon (1996) and Hafez (2000) recorded that the duration of oestrus in cows ranged between 18 to 19 hours.

According to Orihuela (2000), the accuracy and efficiency of determining duration of oestrus by direct observation were affected by frequency, duration and timing of the observation periods. It was also opined that barn-housed cattle exhibited more duration of oestrus compared to pasture fed cattle since cattle maintained on pastures spent more time in feeding and hence had less time for exhibition of oestrus behaviours. Velayudhakumar (2003) reported ovulatory disturbances as a cause for prolongation of oestrual symptoms. Jeba (2005) noticed that the occurrence of prolongation of oestrual symptoms was lower under farm conditions than field conditions.

O'Connor *et al.* (2007) reported that nutrition, specifically energy balance has got an impact on exhibition of oestrual signs.

2.5.2 Characteristics of Cervical Mucus

2.5.2.1 Colour

Mehta (1986) observed that out of 21 mucus samples collected, only 54.17 per cent of the samples were clean and transparent in repeat breeder cows as against 71.43 per cent samples in fertile cows.

Sharma and Thripathi (1987) reported that the percentage of clear and cloudy mucus in normal and repeat breeder crossbred cows was 60.77 and 39.23 respectively and 41.66 and 58.34 per cent respectively in normal and repeat breeder cross bred cows.

According to Dev *et al.* (1997), clear cervical mucus was conducive for sperm penetration and conception whereas, turbidity arrested sperm motility in oestrual mucus.

2.5.2.2 Consistency

According to Sukhdeo and Roy (1971), the cervical mucus that flowed easily on a glass slide kept inclined at 45° angle was referred to as thin and that remained sticky as thick mucus. Merilan (1983) opined that viscosity of cervical mucus was directly related to degree of cross linkage between long chains of mucoproteins, which in turn depended on the hormonal status. At the beginning of follicular phase, the mucus was sparse in quantity and of high viscosity.

Sharma and Thripathi (1987) reported that the thin, medium and thick consistency of oestrual mucus was in 41.66, 58.33 and 20.00 per cent of healthy cows respectively as against 40.99, 50.00 and 9.01 per cent respectively of repeat breeder cows.

Rutlant *et al.* (2005) found that cervical mucus became more plentiful, watery, less viscous and easier to traverse by spermatozoa in the follicular phase of ovarian cycle. In the luteal phase of cycle, it became scanty, opalescent, viscous and consequently, not favourable to sperm passage.

2.5.2.3 Spinnbarkeit

Adhallikar (1986) opined that the spinnability values were higher in ovulated than in anovulated cows. Tsiligianni *et al.* (2001) reported that the mean spinnbarkeit value was 7.60 ± 0.40 cm for cervical mucus during oestrus in Friesian cows. Bennur *et al.* (2002) reported that the spinnbarkeit values were 11.38 ± 0.56 and 11.05 ± 1.33 cm for the oestrual mucus of pregnant and non-pregnant cows respectively with no significant difference.

2.5.2.4 Fern Pattern

Luktuke and Roy (1967) reported that the percentages of typical, atypical and nil types of fern pattern in cervical mucus of cows in oestrus were 63.10, 17.70 and 19.20 per cent respectively. Bugalia and Kohli (1982) observed the arborisation pattern of cervical mucus in oestrous and nymphomaniac Rathi cows. It was found that the typical and atypical fern patterns in oestrous cows were 80 and 20 per cent respectively, compared to 70 and 30 per cent in nymphomaniac cows.

Choudhary and Purbey (1983) reported a conception rate of 51.61 per cent in cows which evinced typical fern pattern of cervical mucus as against 10 per cent in cows with atypical fern pattern. However, none of the cows failed to evince fern pattern conceived.

According to Adhallikar (1986), the percentage of typical fern pattern in ovulatory and anovulatory oestrus were 75.50 and 24.50 respectively.

Mehta (1986) observed no significant difference in the occurance of typical fern pattern in cervical mucus of repeat breeding and fertile groups of crossbred cows, however, 4.76 per cent of the samples showed no fern pattern at all in repeat breeding animals.

Sharma and Tripathi (1987) observed that a large proportion (75 and 69.20%) of pregnant animals belonged to normal and repeat breeding groups had typical fern pattern at oestrus.

According to Salphale *et al.* (1993) reported that the typical, atypical and nil types of fern pattern were found to be 63.10, 17.70 and 19.20 per cent respectively in oestrual cervical mucus of cows. It was also found that typical fern pattern was only 27.28 per cent in repeat breeding cows. According to Dev *et al.* (1997), better crystallization pattern of cervical mucus was obtained in the period of maximum oestrogenic influence during oestrus.

2.6. BIOCHEMICAL COMPOSITION OF SERUM / PLASMA IN RELATION TO FERTILITY

2.6.1. Blood Urea Nitrogen

The BUN concentration was related to dietary crude protein intake, percentage of rumen degradable and undegradable protein and protein-energy ratio in the diet (Oltner and Wiktorson, 1983; Roselor *et al.*, 1993 and Baker *et al.*, 1995).

Parmar *et al.* (1986) found that the average blood urea level in regular breeding cows was 16.34 ± 3.04 mg per cent at luteal phase as against 22.39 ± 3.42 mg per cent at oestrus phase. The corresponding values in repeat breeding cows were 17.41 ± 2.61 mg per cent and 28.88 ± 1.87 mg per cent respectively. It was found that the difference between groups was significant at oestrus phase ($p<0.01$) and luteal phase ($p<0.05$). However, Howard *et al.* (1987) and Caroll *et al.* (1988) could not find a concomitant decline in fertility in cows with a high BUN (>24 mg/dl) level.

Many other studies clearly demonstrated that dairy cattle with elevated BUN level had reduction in fertility rate (Ferguson *et al.*, 1993; Blanchard *et al.*, 1990; Canfield *et al.*, 1990; Jonker, 1998). Elrod and Butler (1993) reported that BUN or MUN level of greater than 19 to 20 mg per cent had been associated with altered uterine environment and decreased fertility in lactating dairy cows and heifers. Butler (1998) reported that pregnancy rates were decreased by about 20 per cent when BUN was greater than 19 mg per cent.

Melendez *et al.* (2003) opined that the BUN concentration reflected protein metabolism in mammals and normal level in cattle ranged from 10 to 30 mg per cent.

Rajala-Schultz *et al.* (2001) found that pregnancy rate was reduced even at a BUN concentration of greater than 15.4 mg/dl.

2.6.2. Glucose

Sharma *et al.* (1984) observed statistically significant difference in the mean plasma glucose level between normally cycling (61.28 ± 5.85 mg %) and anoestrous cows (45.20 ± 6.30 mg %), while in repeat breeding cows, it was 57.15 ± 5.90 mg per cent. Prasad *et al.* (1984) opined low blood glucose level as an indicator of subnormal energy status.

Parmar *et al.* (1986) opined that blood glucose level was significantly higher in the early phase of oestrous cycle. It was found that the mean blood glucose level of normal cycling animal was 57 ± 4.21 mg per cent at luteal phase compared to 97.73 ± 9.36 mg per cent at oestrus phase. In repeat breeding cows it varied from 52.45 ± 5.16 mg per cent at luteal phase to 68.84 ± 10.11 mg per cent at oestrus phase.

Ramakrishna (1997) found significantly lower plasma glucose level in anoestrous cows indicating the possibility of under nutrition than disorders of digestion and assimilation. It was opined that relative hypoglycemia in cows might possibly affect expression of oestrus symptoms. It was also found that the mean blood glucose level in anoestrous cows was 47.09 ± 12.03 mg per cent as against 62.20 ± 3.23 mg per cent in normal cycling cows.

Yadav *et al.* (2004) could find a significant difference between glucose levels of normal cycling and anoestrous crossbred cows. It was observed that the mean blood glucose level of normally cycling crossbred cows was 62.3 ± 0.91 mg per cent against 49.44 ± 0.75 mg per cent in anoestrous cows.

2.6.3. Total Protein

Purohit and Bishnoi (1993) found that the serum total protein values were non-significantly higher in normal oestrous group compared to anoestrous group of cows. Pradhan *et al.* (1995) recorded a lower level of total protein in anoestrous crossbred cows.

Vhora *et al.* (1995) found that levels of total protein were significantly higher in normally cycling cows than in anoestrous cows. The average serum total protein level in normally cycling animals was 8.62 ± 0.13 g / dl compared to 6.82 ± 0.46 g/dl in anoestrus cows. It was also opined that serum proteins at a low level lead to deficiency of certain amino acids that were eventually needed for gonadotrophin synthesis and resultant impairment in reproduction.

Ramakrishna (1997) observed lowered total protein level in anoestrous cows. It was opined that inadequate protein intake; increased protein requirement during pregnancy and lactation could impair the synthesis of amino acids essential for gonadotrophins.

According to Bearden and Fuquay (1997), diets deficient in proteins had resulted in weak expression of oestrus, cessation of oestrus and repeat breeding. But feeding a ration containing more than 13 per cent crude protein resulted in lower reproductive efficiency by increasing the number of inseminations required per conception and days open.

Jayanthi *et al.* (2003) found that total serum protein was significantly lower (6.40 ± 0.05 g /dl) in repeat breeding cows.

2.7. MINERAL STATUS IN RELATION TO FERTILITY

2.7.1. Macro Mineral Composition

2.7.1.1 Calcium

Roberts (1971) reported that calcium deficiency did not cause reproductive failure in cattle. But Veldhis and Klase (1982) reported calcium as an integral part in steroid biosynthesis pathways of ovary and adrenal gland and was necessary for maintenance of normal fertility.

Sharma *et al.* (1984) observed mean serum calcium levels of 10.69 ± 2.05, 7.95 ± 1.08 and 9.85 ± 2.15 mg per cent in cyclic, anoestrous and repeat breeding crossbred cows respectively. Dabas *et al.* (1987) noticed a mean serum calcium level of 9.80 ± 0.50 and 11.50 ± 0.30 mg per cent in anoestrous and normally cycling cows respectively.

Ramakrishna (1997) reported that the mean serum calcium levels were 9.95 ± 0.25 and 9.85 ± 0.02 mg per cent in healthy and repeat breeder crossbred cows respectively.

According to Rajeev (1998), the mean serum calcium level in normally cycling heifers was 11.10 ± 0.30 mg per cent. The corresponding values for true anoestrous and repeat breeding crossbred heifers were 10.74 ± 0.13 and 10.80 ± 0.42 mg per cent respectively. There was no significant difference among the groups. Singh and Pant (1998) noticed a mean calcium concentration of 8.42 ± 0.22 and 8.24 ± 0.22 mg per cent in normal and repeat breeder cows. Arosh *et al.* (1998) reported that the mean serum calcium was 10.71 ± 0.36 mg per cent during oestrus in normally cycling crossbred cows.

Dutta *et al.* (2001) reported mean serum calcium levels of 10.72 ± 0.08, 9.54 ± 0.22 and 9.95 ± 0.18 mg per cent in normally cycling, anoestrous and repeat breeding cows respectively. It was opined that the low level of serum calcium in postpartum anoestrous and repeat breeding animals was due to failure of endocrine system to mobilize the body calcium and that in cyclic animals was due to fluctuating levels of oestrogen. On the contrary, Das *et al.* (2002) found no significant variation in serum calcium level between normal (10.50 ± 0.44 mg %) and repeat breeding (10.045 ± 0.327 mg %) animals.

Dhami *et al.* (2005) observed that the mean serum calcium concentration in the peripheral blood plasma of Holstein Friesian cattle varied significantly from 9.92 ± 0.59 to 12.16 ± 0. 73 mg per cent among different age groups with an overall mean of 11.53 ± 0.17 mg per cent. It dropped abruptly to 9.52 mg per cent soon after calving and then fluctuated around 10.50 mg per cent in lactating, anoestrous and repeat breeding cows.

2.7.1.2 Phosphorus

Sharma *et al.* (1984) observed serum phosphorus levels of 4.83 ± 0.33, 2.97 ± 0.23 and 4.76 ± 0.29 mg per cent in cyclic, anoestrous and repeat breeding crossbred cows respectively.

Dabas *et al.* (1987) noticed a serum inorganic phosphorus level of 6.50 ± 0.20 and 4.30 ± 0.31 mg per cent in cyclic and anoestrous cows respectively. According to Awasthi and Kharche (1987), repeat breeders had significantly lower (3.73 ± 0.29 mg %) inorganic phosphorus than normally cycling cows (5.06 ± 0.19 mg %).

Quayam *et al.* (1988) reported that marginal deficiency of phosphorus in blood serum was sufficient to cause disturbance in pituitary ovarian axis without manifestation of specific deficiency symptoms.

According to George (1995), the mean serum inorganic phosphorus level in normal fertile cows (7.53 ± 0.53 mg %) was significantly higher ($p < 0.05$) than the anoestrous group. Even though the repeat breeder group registered a lower value of 6.34 ± 0.44 mg per cent, the difference was not statistically significant.

Shrivastava and Kadu (1995) observed a lower level of inorganic phosphorus in serum of anoestrous cows than normally cycling cows.

The mean serum inorganic phosphorus level of normally cycling heifers was 4.87 ± 0.10 mg per cent while that of anoestrous and repeat breeders were 3.83 ± 0.09 and 3.52 ± 0.10 respectively. It was found that there was significant difference in serum phosphorus level of normally cycling group and anoestrous group (Rajeev, 1998).

Ramakrishna (1997) reported that the mean plasma inorganic phosphorus level was 4.51 ± 0.18 and 5.96 ± 0.18 mg per cent in repeat breeding and healthy crossbred cows respectively. However, Lopez *et al.* (2004) found that dietary phosphorus supplementation had no significant effect on intensity or duration of oestrus and ovarian activity.

Hurley and Doane (1989) opined that classical manifestations of phosphorus deficiency on reproduction involved alterations of oestrus, lowered conception rate, anoestrus and decreased ovarian activity. Radostits *et al.* (2000) opined that anoestrus, suboestrus, irregular oestrus and delayed sexual maturity were the consequences of phosphorus deficiency.

Dutta *et al.* (2001) reported that the inorganic phosphorus levels were 4.22 ± 0.07 and 3.62 ± 0.13 mg per cent in normal and repeat breeding cows respectively. Bearden and Fuquay (1997) opined that the deficiency of phosphorus could lead to anoestrus and delayed puberty.

Yadav *et al.* (2004) reported a serum inorganic phosphorus level of 5.49 ± 0.15 mg per cent in cyclic cattle and 3.27 ± 0.08 mg per cent in anoestrous animals.

2.8 SERUM MICRO MINERALS IN RELATION TO FERTILITY

2.8.1 Manganese

Parmar *et al.* (1986) noticed a mean manganese level of 0.19 ± 0.03 and 0.58 ± 0.08 µg/ml during oestrus phase in repeat breeding and normally cycling animals respectively.

According to George (1995), a marginal increase in the level of serum manganese level was noticed in normal fertile crossbred cows (0.06 ± 0.01ppm) compared to anoestrous (0.03 ± 0.05 ppm) and repeat breeding groups (0.04 ± 0.01 ppm).

Noakes *et al.* (2001) reported that reproductive disorders like anoestrus, poor follicular development, delayed ovulation, silent heat and reduced conception rate were noticed due to manganese deficiency.

According to Markandeya *et al.* (2002), manganese was required for activation of many enzyme systems in the body and was found to be involved specifically in the luteal tissue metabolism.

2.8.2 Zinc

George (1995) recorded a significantly higher serum zinc level in normally cycling crossbred cows (1.33 ± 0.15 ppm) compared to that of anoestrous cows (1.03 ± 0.98 ppm).

According to Rajeev (1998), the mean serum zinc level in normally cycling heifers was 1.61 ± 0.03 ppm. The corresponding values for true anoestrous and repeat breeding crossbred heifers were 1.17 ± 0.05 and 1.73 ± 0.06 ppm.

Das *et al.* (2002) reported that the mean serum zinc level in repeat breeder cows was significantly lower (1.8 ± 0.003 ppm) than that in normally cycling cows (2.09 ± 0.05 ppm). Saxena and Gupta (1995) found that the mean serum level of zinc was higher (1.72 ± 0.92 ppm) in cows that conceived within 120 days postpartum compared to those failed to conceive (1.05 ± 0.06 ppm) within this period. On the contrary, Khasatiya *et al.* (2005) reported a higher zinc level in infertile cows than in fertile cows.

2.8.3 Copper

Dabas *et al.* (1987) opined that the level of serum copper in anoestrous cows was very low compared to that of normally cycling animals. According to Hurley and Doane (1989) copper deficiency was associated with suboptimal ovarian activity, suboestrus and reduced conception rate in dairy cows.

Vadnere and Singh (1989) observed a significantly low mean serum copper level of 1.38 ± 0.11 ppm in post partum anoestrous crossbred cows.

According to Vhora *et al.* (1995), the serum copper level was 1.01 ± 0.37 and 0.73 ± 0.33 ppm in normally cycling and anoestrous cows respectively.

Saxena and Gupta (1995) found that the levels of serum copper were higher in crossbred cows which conceived during early postpartum period than those not conceived even after 120 days post-calving.

According to Das *et al.* (2002), the serum copper concentration in normally cycling cows was significantly higher (0. 97 ± 0.02 ppm) compared to repeat breeders (0.09 ± 0.01 ppm). Dutta *et al.* (2001) reported that the mean serum copper level was 1.11 ± 0.09 and 1.13 ± 0.02 ppm in normal and repeat breeder crossbred cows respectively.

Oestrus was observed in 90.3 per cent of anoestrous buffaloes following subcutaneous administration of 150 mg per cent of copper glycinate and conception occurred in 63.6 per cent of them (Randhawa *et al.*, 2004).

Khasatiya *et al.* (2005) studied the reproductive performance of postpartum fertile and infertile buffaloes and found a significant correlation of copper with service period and calving interval.

Materials and Methods

3. MATERIALS AND METHODS

Materials for the present study consisted of 40 apparently healthy and normally cycling crossbred dairy cows selected at random from those belonging to University Livestock Farm, Mannuthy and those brought for insemination at Artificial Insemination Centres at Mannuthy and Kokkalai ancillary to the Department of Animal Reproduction, Gynaecology and Obstetrics, Mannuthy. The study was conducted over a period of six months from October 2007 to April 2008. The selected cows were divided into two groups.

Group I

Group I consisted of 20 crossbred cows that were selected at random from University Livestock Farm, Mannuthy which were reared under ideal conditions of feeding and management.

Group II

Group II consisted of 20 crossbred cows selected at random from those brought for insemination at AI Centres, Mannuthy and Kokkalai, which were reared under field conditions.

Blood samples and uterine mucus were collected from all the animals in both groups at oestrus for estimation of biochemical parameters and uterine pH respectively.

All the animals in groups I and II were subjected to detailed clinico-gynaecological examination and observations were recorded. They were inseminated during the most appropriate period of oestrus and were subjected to pregnancy diagnosis at 60 days post insemination.

3.1 DETAILS OF FEEDING AND MANAGEMENT

The data regarding the type and quantity of feed and fodder fed to the farm animals selected were collected and recorded. The corresponding data of field animals selected were collected from the history narrated by the owner.

3.2 DURATION AND INTENSITY OF OESTRUS

3.2.1 Duration of Oestrus

The period from the beginning to the end of behavioural and clinical signs of oestrus *viz.* bellowing, oestrual discharge, mounting, vulval oedema and hyperemia of vestibular mucous membrane, was considered as the duration of oestrus.

3.2.2 Intensity of Oestrus

The intensity of oestrus was graded as high, medium or low based on the degree of clinical and behavioural signs.

3.3 PHYSICAL CHARACTERISTICS OF REPRODUCTIVE TRACT DURING OESTRUS

Physical characteristics of reproductive tract of all the animals during oestrus *viz.* oedema of vulval lips, hyperemia of vestibular mucous membrane, tonicity of uterus and nature of discharge (Stringy, watery and scanty) were recorded.

3.4 PHYSICAL PROPERTIES OF CERVICAL MUCUS

3.4.1 Colour and Consistency

The colour and consistency of mucus was studied by visual examination observing the clarity and consistency (Sharma and Thripathi, 1987).

3.4.2 Fern Pattern

The oestrual cervical mucus was uniformly spread on a clean, dry, grease free glass slide and the smear was allowed to dry at room temperature and examined under microscope for the fern pattern as suggested by Tsiligianni *et al.* (2001). Classification of fern pattern (Plate 1) was done as follows.

A true fern tree like appearance with clear venations and subvenations as primary, secondary and tertiary branching was considered as typical fern pattern.

Crystallisation of irregular pattern with clear venation with or without subvenations was considered as atypical fern pattern.

Crystallisation with no specific type of venations and subvenations was considered as nil fern pattern.

3.4.3 Spinnbarkeit Value

The elasticity of cervical mucus was measured by spinnbarkeit test. About 0.5 ml of cervical mucus was placed on a clean glass slide. Another slide was placed over the mucus on the slide and lifted vertically. Maximum length of the unbroken mucus thread so formed in between two slides was measured with a scale (Plate 2). This procedure was repeated twice for each sample and the mean value was recorded (Tsiligianni *et al.*, 2001).

3.5. COLLECTION OF UTERINE MUCUS FOR ESTIMATION OF pH

Uterine mucus was collected from the body of uterus by aspiration using a sterile intrauterine catheter connected to a 10 ml syringe. The mucus collected was then transferred into a sterile petri dish and pH was recorded immediately using a pH surface electrode[1] in a Bench pH meter[2] (Plate 3).

3.6 ESTIMATION OF BIOCHEMICAL PARAMETERS

From all the animals selected, 10 ml of blood was collected aseptically by jugular venepuncture and 3 ml was transferred into a dry sterile vial with sodium fluoride as anticoagulant (10mg/ml) and centrifuged at 3000 rpm for 10 min. The plasma thus obtained was separated and used for the estimation of blood glucose. The remaining portion of blood collected was then allowed to clot. The clotted blood was centrifuged at 3000 rpm for 10 min. and clear serum was aspirated without disturbing the clot fraction and stored in separate serum cryovials at -20°C till further estimation of total protein, blood urea nitrogen and minerals *viz.* calcium, phosphorus, manganese, zinc and copper.

3.6.1 Blood Urea Nitrogen

Serum urea nitrogen was estimated colorimetrically by modified Bertholot method as described in the reagent kit[1] in a UV visible spectrophotometer.

3.6.2 Glucose

The blood glucose level was estimated colorimetrically using glucose diagnostic reagent kit[1] in a UV visible spectrophotometer.

---

1. Eutech instruments Limited, Singapore
2. Cyberscan pH 2500, Singapore

3.6.3 Total Protein

Total protein in the serum was estimated colorimetrically by modified Biuret method as described in the reagent kit[1] in a UV visible spectrophotometer.

3.6.4 Mineral Status

Serum level of calcium, copper, manganese and zinc were estimated using Atomic Absorption Spectrophotometer[2].

Inorganic phosphorus level in serum was estimated by phosphomolybdate method using reagent kit[1] in a UV visible spectrophotometer.

3.7 CONCEPTION RATE

Pregnancy was ascertained at 60 days post-insemination by rectal examination. Conception rates in both the groups were compared in relation to each parameter.

3.8 STATISTICAL ANALYSIS

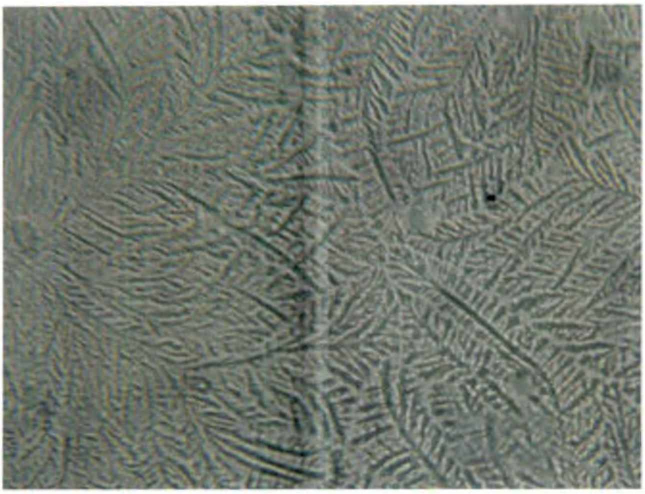
The data obtained were compiled and statistical analysis was carried out using paired *t*- test to find out the significant difference between the two groups (Snedecor and Cochran, 1994).

---

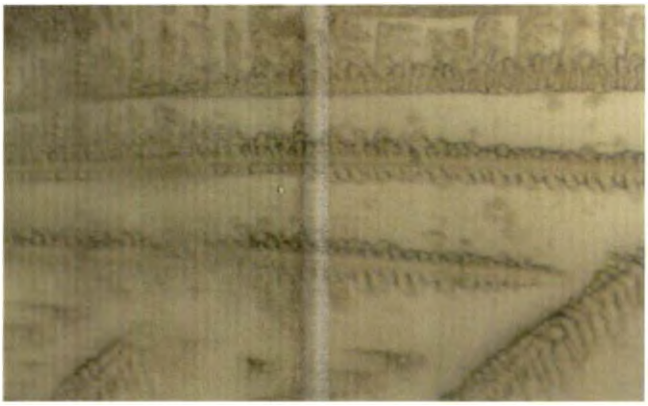
[1]Agappe Diagnostic Limited, Hyderabad

[2] Perkin- Elmer

A



B



C

Plate 1. Fern pattern of oestrual mucus
A. Typical type B. Atypical type C. Nil type

Plate 2. Measurement of spinnbarkeit value of cervical mucus

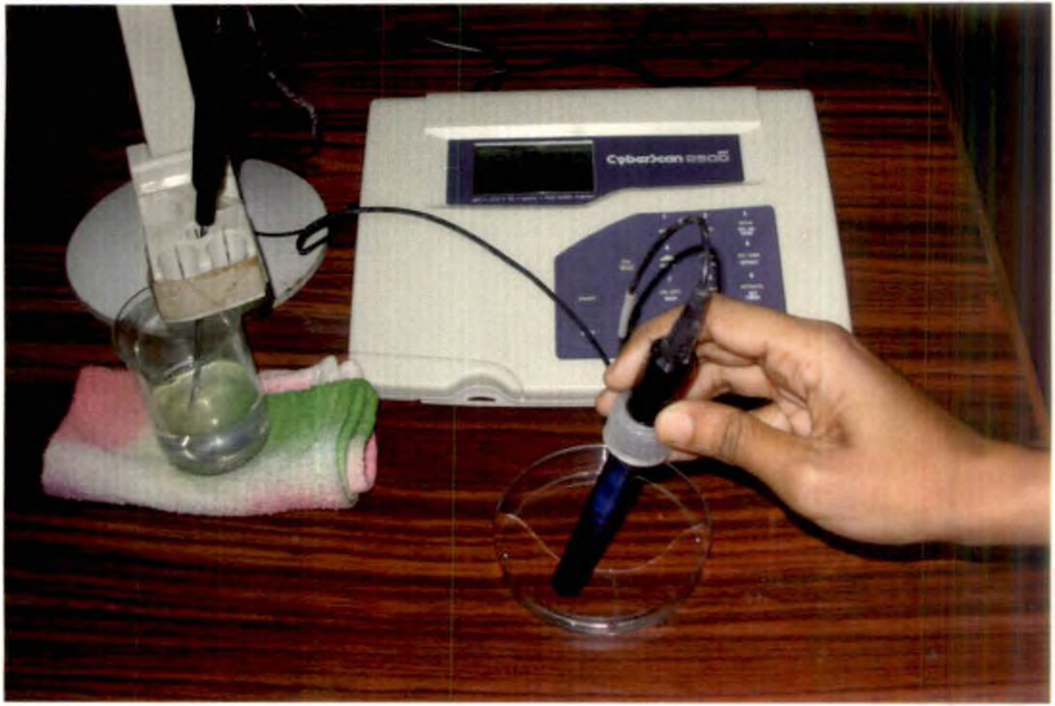


Plate 3. Measurement of pH of uterine mucus using surface electrode

Results

4. RESULTS

Results of the investigation carried out to find out the effect of uterine pH, blood urea nitrogen and mineral status on fertility in dairy cows are summarized in Tables 1 to13 and Fig.1 to 15.

4.1 DETAILS OF FEEDING AND MANAGEMENT

Analysis of details regarding feeding and management of all the cows under study revealed that those belonging to University Livestock Farm, Mannuthy (Group I) were maintained on a relatively high nutritious diet in order to boost up milk production. Their ration consisted of commercially available good quality compounded cattle feed, roughages (*viz.* green grass, straw and silage depending on availability) and mineral mixture.

Field animals selected for the present study (Group II) were found to be not identical in their dietary ration. Perusal of feeding history of field animals revealed that majority of the owners were offering locally available compounded cattle feed alone or as individual ingredients or a combination of both to their animals. The commonly fed concentrate materials included conventional feeds such as coconut cake, groundnut cake and cotton seed cake and unconventional feeds such as tamarind seed and tapioca waste. The roughages included green grass and straw based on availability. On close scrutiny of feeding details, it was found that most of the cows belonging to Group II were not fed with rations computed as per scientific requirements.

4.2 DURATION AND INTENSITY OF OESTRUS

4.2.1 Duration of Oestrus

Data presented in Table 1 and Fig.1 revealed that the duration of oestrus ranged from 18 to 32 hours with a mean of 26.10 ± 1.74 hours in Group I and 18 to 48 hours with a mean of 30.00 ± 2.10 hours in Group II.

4.2.2 Intensity of Oestrus

The percentage of animals exhibited high, medium and low intensity of oestrus was 40, 45 and 15 in Group I and 50, 40 and 10 in Group II respectively (Table 2 and Fig. 2).

4.3 PHYSICAL CHARACTERISTICS OF REPRODUCTIVE TRACT DURING OESTRUS

Physical characteristics of reproductive tract of all animals during oestrus *viz.* oedema of vulval lips, hyperemia of vestibular mucosa and tonicity of uterine horns are presented in Table 3 and Fig.3.

During oestrus, the percentage of animals with high, medium and low degree of vulval oedema was 50, 40 and 10 in Group I as against 55, 35 and 10 in Group II respectively.

The percentage of animals with high, medium and low degree of hyperemia of vestibular mucous membrane during oestrus in Group I was 35, 55 and 10 and in Group II was 50, 40 and 10 respectively.

The percentage of animals with high, medium and low degree of tonicity of uterus was 60, 30 and 10 and 50, 30 and 20 respectively in groups I and II.

4.4 CHARACTERISTICS OF CERVICAL MUCUS

The colour, consistency, fern pattern and spinnbarkeit value of cervical mucus during oestrus in groups I and II are presented in Table 4.

4.4.1 Colour

The percentage of animals with clear and cloudy discharge was 40 and 60 in Group I and 70 and 30 in Group II respectively. It was also found that the conception rate in animals with clear and cloudy mucus was 62.50 and 50 per cent respectively in Group I and 78.50 and 33.33 per cent respectively in Group II.

4.4.2 Consistency

The percentage of animals with stringy, watery and scanty mucus sample was found to be 40, 50 and 10 and 50, 35 and 15 in animals of groups I and II respectively. The conception rate in animals with stringy, watery and scanty mucus was 75, 40 and 50 per cent respectively in Group I and 60, 57.10 and 33.33 per cent respectively in Group II.

4.4.3 Fern Pattern

The fern pattern of cervical mucus samples were found to be typical, atypical and nil pattern (Plate 1) in 50, 30 and 20 per cent of animals in Group I as against 60, 30 and 10 per cent in Group II. The conception rate in animals with typical, atypical and nil fern pattern of cervical mucus was 70, 50 and 25 per cent respectively in Group I and 75, 50 and 50 per cent respectively in Group II.

4.4.4 Spinnbarkeit Value

The mean spinnbarkeit value was found to be 11.12 ± 0.54 and 12.44 ± 0.04 cm in animals of groups I and II respectively. It was also found that the mean spinnbarkeit value in conceived and non-conceived animals of Group I was 11.54 ± 0.93 and 10.55 ± 0.39 cm respectively as against 12.52 ± 1.49 and 11.95 ± 1.08 in Group II respectively.

4.5 UTERINE pH

Data regarding the uterine pH in animals of groups I and II are presented in Table 5 and Fig. 4. In the present study, the overall mean uterine pH was found to be 7.05 ± 0.14 and 7.07 ± 0.05 in animals of groups I and II respectively. It was also observed that the mean uterine pH value among conceived animals in groups I and II was 7.09 ± 0.04 and 7.10 ± 0.05 respectively and that of non-conceived animals in groups I and II was 7.01 ± 0.05 and 7.02 ± 0.11 respectively. An inverse relationship could also be obtained between BUN level in serum and uterine pH during oestrus. Statistical analysis revealed highly significant ($P<0.01$) correlation between uterine pH and BUN level is with a correlation co-efficient of $r = -0.896$ and $r = -0.753$ in groups I and II respectively (Table 7 and Fig.5 and 6).

4.6 BIOCHEMICAL COMPOSITION OF BLOOD

The biochemical composition of serum / plasma is presented in Tables 6 to 11 and Fig.5 to 9.

4.6.1 Blood Urea Nitrogen (BUN)

In the present study, the overall mean blood urea nitrogen level in animals of Group I was found to be 24.02 ± 0.85 mg per cent against 22.04 ± 0.96 mg per cent in Group II (Tables 6 and 11 and Fig.7). Statistical analysis showed a significant difference ($P<0.05$) between BUN level in animals of groups I and II. It was also found that the mean BUN level in animals of Group I which conceived (22.67 ± 1.12 mg %) was significantly lower ($P<0.05$) than that in animals failed to conceive (25.66 ± 1.14 mg %). Similarly, significant difference ($P < 0.05$) could be noticed between the mean blood urea nitrogen level in conceived (20.89 ± 1.19 mg %) and non-conceived animals (24.06 ± 1.39 mg %) of Group II. It was also noticed that conception rate of animals with a BUN level less than 20 mg/dl was 81.80 per cent and it reduced to 73.30 per cent in animals with a BUN level of 20 to 25 mg/dl and 35.71 per cent with a BUN level greater than 25 mg/dl (Table 8 and Fig.10).

4.6.2 Glucose

Data presented in Tables 9 and 11 and Fig. 8 revealed that the mean plasma glucose level was 54.69 ± 1.83 and 51.60 ± 0.93 mg per cent in Group I and II respectively. The mean plasma glucose level in conceived and non-conceived animals of Group I was 57.67 ± 2.62 and 51.06 ± 2.05 mg per cent respectively as against 51.62 ± 2.00 and 51.57 ± 1.02 mg per cent respectively in Group II.

4.6.3 Total Protein

The overall mean serum total protein in animals of Group I was found to be 6.64 ± 0.03 g per cent, which was significantly higher ($P<0.01$) when compared to 5.51 ± 0.21 g per cent in Group II (Tables 10 and 11 and Fig.9). Similarly, the mean serum total protein in conceived and non-conceived animals was 6.54 ± 0.18 and 6.82 ± 0.22 g per cent respectively in Group I as against 5.16 ± 0.29 and 5.94 ± 0.28 g per cent respectively in Group II.

4.7 MINERAL STATUS

The mean serum level of minerals *viz.* calcium, phosphorus, manganese, zinc and copper are presented in Table 12 and Fig.11-15.

4.7.1 Calcium

The overall mean serum calcium level in animals of groups I and II was 10.28 ± 0.19 and 9.83 ± 0.21 mg per cent respectively and the difference was found to be highly significant ($P<0.03$). It was also observed that the mean serum calcium level in animals of Group I which conceived was 10.64 ± 0.25 mg per cent as against 10.10 ± 0.27 mg per cent in animals not conceived. Similarly, the mean serum calcium level was 9.85 ± 0.23 and 9.33 ± 0.24 mg per cent respectively in animals of Group II.

4.7.2 Phosphorus

The overall mean serum phosphorus level in animals of groups I and II was found to be 4.84 ± 0.13 and 4.71 ± 0.15 mg per cent respectively. The mean value of serum phosphorus in conceived and non-conceived animals was 5.00 ± 0.16 and 4.64 ± 0.20 mg per cent respectively in Group I as against 4.84 ± 0.20 and 4.46 ± 0.19 mg per cent respectively in Group II.

4.7.3 Manganese

The overall mean serum manganese level in animals of groups I and II was 0.05 ± 0.004 and 0.04 ± 0.003 ppm respectively and the difference was found to be highly significant ($P<0.01$). The mean value of serum manganese in conceived and non-conceived animals was 0.05 ± 0.004 and 0.05 ± 0.005 ppm in Group I as against 0.04 ± 0.005 and 0.04 ± 0.004 mg per cent respectively in Group II.

4.7.4 Zinc

An overall mean serum zinc level of 1.39 ± 0.01 and 1.36 ± 0.01 ppm was observed in groups I and II respectively. The mean serum zinc level in conceived and non-conceived animals was 1.40 ± 0.01 and 1.38 ± 0.01 ppm in Group I as against 1.38 ± 0.02 and 1.34 ± 0.01 ppm respectively in Group II.

4.7.5 Copper

An overall mean serum copper level of 1.19 ± 0.01 and 1.17 ± 0.01 ppm was observed in Group I and II animals respectively. The mean serum copper level in conceived and non-conceived animals was 1.18 ± 0.02 and 1.16 ± 0.01 ppm in Group I as against 1.19 ± 0.01 and 1.19 ± 0.02 ppm respectively in Group II.

4.8. CONCEPTION RATE

The conception rate among animals of groups I and II are presented in Table 13. The overall conception rate was found to be 55 per cent in Group I as against 65 per cent in Group II.

Table 1. Duration of oestrus among animals of groups I and II

Groups	Duration of oestrus (hours)	
	Range	Mean ± SE
Group I	18 - 32	26.10 ± 1.74
Group II	18 - 48	30.00 ± 2.10

Table 2. Intensity of oestrus among animals of groups I and II

Groups	Intensity of oestrus					
	High		Medium		Low	
	No. of animals	Percentage	No. of animals	Percentage	No. of animals	Percentage
Group I	8	40	9	45	3	15
Group II	10	50	8	40	2	10

Table 3. Physical characteristics of reproductive tract during oestrus in animals of Groups I and II

Characteristics		Group I		Group II	
		No. of animals	Percentage	No. of animals	Percentage
Vulval oedema	High	10	50	11	55
	Medium	8	40	7	35
	Low	2	10	2	10
Hyperemia of vestibular mucosa	High	7	35	10	50
	Medium	11	55	8	40
	Low	2	10	2	10
Tonicity of uterus	High	12	60	10	50
	Medium	6	30	6	30
	Low	2	10	4	20

Table 4. Characteristics of cervical mucus during oestrus in animals of groups I and II

Oestrual characteristics		Groups					
		Group I No. of animals (%)			Group II No. of animals (%)		
		Conceived	Not conceived	Overall	Conceived	Not conceived	Overall
Colour	Clear	5 (62.50)	3 (37.50)	8 (40)	11 (78.50)	3 (21.40)	14 (70)
	Cloudy	6 (50)	6 (50)	12 (60)	2(33.33)	4(66.66)	6(30)
Consistency	Stringy	6 (75)	2 (25)	1(40)	7 (60)	3 (40)	10(50)
	Watery	4(40)	6 (60)	1 (50)	4(57.10)	3(42.8)	7 (35)
	Scanty	1(50)	10 (50)	2 (10)	1(33.33)	2 (66.66)	3 (15)
Fern pattern	Typical	7 (70)	3 (30)	10(50)	9 (75)	3 (25)	12(60)
	Atypical	3 (50)	3 (50)	6(30)	3 (50)	3 (50)	6 (30)
	Nil	1 (25)	3 (75)	4 (20)	1 (50)	1 (50)	2 (10)
Spinnbarkeit value	Mean ± SE (cm)	11.54 ± 0.93	10.55 ± 0.39	11.12 ± 0.54	12.52 ± 1.49	11.95 ± 1.08	12.44 ± 0.04

Table 5. Uterine pH in animals of groups I and II

Uterine pH					
Group I			Group II		
Animal no.	Conceived	Not Conceived	Animal no.	Conceived	Not conceived
1.	-	6.86	1.	7.16	-
2.	7.13	-	2.	6.83	-
3.	-	7.16	3.	7.6	-
4.	7.13	-	4.	7.03	-
5.	-	7.06	5.	-	7.14
6.	7.02	-	6.	7.08	-
7.	7.23	-	7.	7.13	-
8.	6.76	-	8.	-	6.78
9.	6.81	-	9.	7.04	-
10.	-	6.88	10.	7.06	-
11.	7.14	-	11.	-	7.08
12.	-	6.84	12.	7.23	-
13.	7.23	-	13.	-	6.93
14.	-	7.04	14.	-	7.16
15.	7.07	-	15.	-	7.53
16.	7.18	-	16.	6.93	-
17.	-	6.91	17.	-	6.53
18.	-	7.23	18.	7.24	-
19.	-	7.18	19.	6.98	-
20.	7.30	-	20.	7.08	-
Mean ± SE	7.09 ± 0.04	7.01 ± 0.05	Mean ± SE	7.10 ± 0.05	7.02 ± 0.11
Overall mean ± SE	7.05 ± 0.14		Overall mean ± SE	7.07 ± 0.05	

Table 6. Blood urea nitrogen level (mg/dl) in animals of groups I and II

Blood urea nitrogen (mg/dl)					
Group I			Group II		
Animal no.	Conceived	Not Conceived	Animal no.	Conceived	Not conceived
1.	-	29.32	1.	17.20	-
2.	22.44	-	2.	28.63	-
3.	-	24.12	3.	16.20	-
4.	20.08	-	4.	23.02	-
5.	-	26.65	5.	-	23.20
6.	24.60	-	6.	18.22	-
7.	22.48	-	7.	18.03	-
8.	29.03	-	8.	-	27.12
9.	28.73	-	9.	21.03	-
10.	-	28.13	10.	20.03	-
11.	20.64	-	11.	-	24.02
12.	-	28.46	12.	18.20	-
13.	19.29	-	13.	-	27.24
14.	-	26.32	14.	-	18.20
15.	24.72	-	15.	-	20.63
16.	19.16	-	16.	28.30	-
17.	-	26.03	17.	-	28.03
18.	-	17.84	18.	28.03	-
19.	-	24.08	19.	16.66	-
20.	18.24	-	20.	26.03	-
*Mean ± SE	22.67 ± 1.12	25.66 ±1.14	*Mean ± SE	20.89 ± 1.19	24.06 ± 1.39
*Overall mean ± SE	24.02 ± 0.85		*Overall mean ± SE	22.04 ± 0.96	

* Significant at $P<0.05$

Table 7. Correlation between Uterine pH and blood urea nitrogen in animals of groups I and II

Group I				Group II	
Animal no.	Uterine pH	Blood urea nitrogen(mg/dl)	Animal no.	Uterine pH	Blood urea nitrogen(mg/dl)
1.	6.86	29.32	1.	7.16	17.20
2.	7.13	22.44	2.	6.83	28.63
3.	7.16	24.12	3.	7.60	16.20
4.	7.13	20.08	4.	7.03	23.02
5.	7.06	26.65	5.	7.14	23.20
6.	7.02	24.60	6.	7.08	18.22
7.	7.23	22.48	7.	7.13	18.03
8.	6.76	29.03	8.	6.78	27.12
9.	6.81	28.73	9.	7.04	21.03
10.	6.88	28.13	10.	7.06	20.03
11.	7.14	20.64	11.	7.08	24.02
12.	6.84	28.46	12.	7.23	18.20
13.	7.23	19.29	13.	6.93	27.24
14.	7.04	26.32	14.	7.16	18.20
15.	7.07	24.72	15.	7.53	20.63
16.	7.18	19.16	16.	6.93	28.30
17.	6.91	26.03	17.	6.53	28.03
18.	7.23	17.84	18.	7.24	16.66
19.	7.18	24.08	19.	6.98	26.03
20.	7.30	18.24	20.	7.08	20.10
*Mean ± SE	7.05 ± 0.14	24.02 ± 0.85	*Mean ± SE	7.07 ± 0.05	22.04 ± 0.96

Correlation between uterine pH and BUN is significant at $P < 0.01$

Table 8. Conception rate among animals of groups I and II in relation to BUN level (mg/dl) level on the day of AI

BUN level(mg/dl)		No. of animals		Conception rate (%)
		Conceived	Not conceived	
<20 mg/dl	Group I (n=4)	3	1	75
	Group II (n=7)	6	1	85.7
	Overall (n= 11)	9	2	81.8
20-25 mg/dl	Group I (n=8)	6	2	75
	Group II (n=7)	5	2	71.4
	Overall (n= 15)	11	4	73.3
>25 mg/dl	Group I (n=8)	2	6	33.3
	Group II (n=6)	3	3	50
	Overall (n= 14)	5	9	35.71

Table 9. Plasma glucose level (mg/dl) in animals of groups I and II

Animal no.	Group I		Animal no.	Group II	
	Conceived	Not Conceived		Conceived	Not conceived
1.	-	61.70	1.	54.30	-
2.	64.61	-	2.	52.13	-
3.	-	54.70	3.	41.5	-
4.	49.30	-	4.	51.20	-
5.	-	56.66	5.	-	58.12
6.	64.80	-	6.	52.28	-
7.	56.50	-	7.	54.26	-
8.	56.50	-	8.	-	49.78
9.	68.50	-	9.	54.93	-
10.	-	48.53	10.	51.03	-
11.	45.15	-	11.	-	49.30
12.	-	52.75	12.	56.20	-
13.	52.35	-	13.	-	43.15
14.	-	44.55	14.	-	58.24
15.	62.50	-	15.	-	50.13
16.	68.55	-	16.	51.42	-
17.	-	46.50	17.	-	52.31
18.	-	42.50	18.	53.10	-
19.	-	51.61	19.	48.50	-
20.	45.68	-	20.	50.30	-
Mean ± SE	57.67 ± 2.62	51.06 ± 2.05	Mean ± SE	51.62 ± 2.00	51.57 ± 1.02
Overall mean ± SE	54.69 ± 1.83		Overall mean ± SE	51.60 ± 0.93	

Table 10. Serum total protein level (g/dl) in animals of groups I and II

Animal no.	Group I		Animal no.	Group II	
	Conceived	Not Conceived		Conceived	Not conceived
1.	-	6.86	1.	6.02	-
2.	7.20	-	2.	5.20	-
3.	-	5.80	3.	4.81	-
4.	5.90	-	4.	6.80	-
5.	-	7.50	5.	-	4.31
6.	7.60	-	6.	4.34	-
7.	5.91	-	7.	5.96	-
8.	6.20	-	8.	-	7.20
9.	5.80	-	9.	6.74	-
10.	-	6.71	10.	5.02	-
11.	6.90	-	11.	-	5.84
12.	-	6.90	12.	4.21	-
13.	6.10	-	13.	-	6.82
14.	-	7.50	14.	-	5.80
15.	7.2	-	15.	-	6.30
16.	6.91	-	16.	5.4	-
17.	-	6.71	17.	-	5.28
18.	-	6.22	18.	4.04	-
19.	-	7.23	19.	4.34	-
20.	6.20	-	20.	4.26	-
Mean ± SE	6.54 ± 0.18	6.82 ± 0.22	Mean ± SE	5.16 ± 0.29	5.94 ± 0.28
*Overall mean ± SE	6.64 ± 0.03			5.51 ± 0.21	

* Significant at P<0.05

Table 11. Blood urea nitrogen (mg/dl), glucose (mg/dl) and total protein (g/dl) in animals of groups I and II (Mean ± SE)

Conception status	Blood urea nitrogen (mg/dl)		Glucose (mg/dl)		Total protein (g/dl)	
	Group I	Group II	Group I	Group II	Group I	Group II
Conceived	22.67 ± 1.12	20.89 ± 1.19	57.67 ± 2.62	51.62 ± 2.00	6.54 ± 0.18	5.16 ± 0.29
Not conceived	25.66 ± 1.14	24.06 ± 1.39	51.06 ± 2.05	51.57 ± 1.02	6.82 ± 0.22	5.94 ± 0.28
Overall	24.01 ± 0.85	22.04 ± 0.96	54.69 ± 1.83	51.60 ± 0.93	6.64 ± 0.14	5.51 ± 0.21

Table 12. Serum mineral status in animals of groups I and II (Mean ± SE)

Conception status	Calcium (mg/dl)		Phosphorus (mg/dl)		Manganese (ppm)		Zinc (ppm)		Copper (ppm)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Conceived	10.64 ± 0.25	10.10 ± 0.27	5.00 ± 0.16	4.84 ± 0.20	0.05 ± 0.004	0.04 ± 0.005	1.40 ± 0.01	1.38 ± 0.02	1.18 ± 0.02	1.19 ± 0.01
Not conceived	9.85 ± 0.23	9.33 ± 0.24	4.64 ± 0.20	4.46 ± 0.19	0.05 ± 0.005	0.04 ± 0.004	1.38 ± 0.01	1.34 ± 0.01	1.16 ± 0.01	1.19 ± 0.02
Overall	10.28 ± 0.19	9.83 ± 0.21	4.84 ± 0.13	4.71 ± 0.15	0.05 ± 0.004	0.04 ± 0.003	1.39 ± 0.01	1.36 ± 0.01	1.19 ± 0.01	1.17 ± 0.01

Table 13. Conception rate among animals of groups I and II

Groups	No: of animals			Conception rate (%)
	Conceived	Not conceived	Total	
I	11	9	20	55
II	13	7	20	65

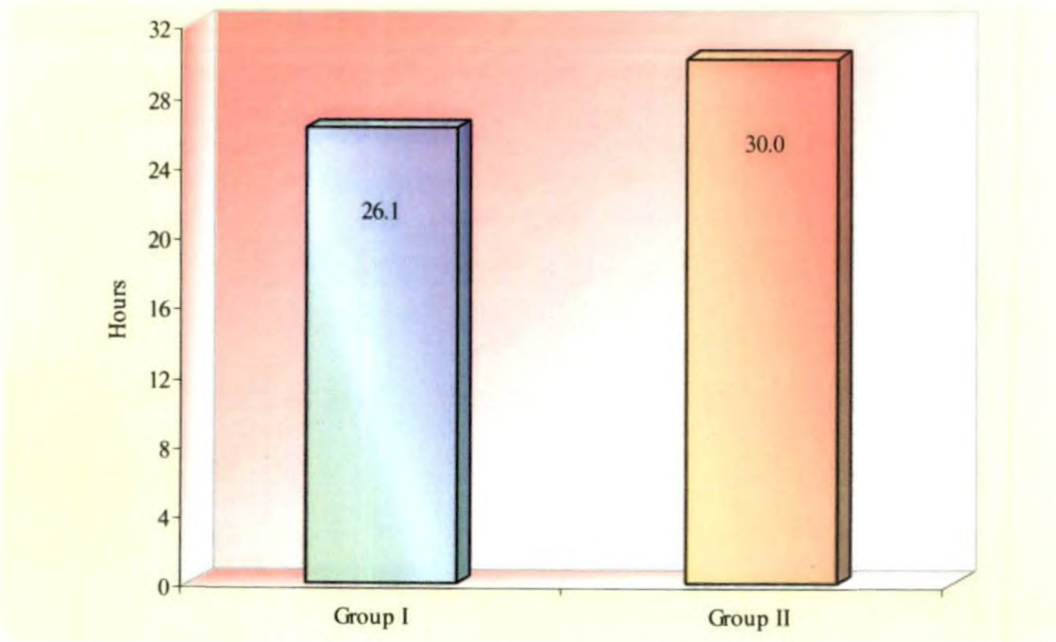


Fig.1. Duration of oestrus among animals of groups I and II

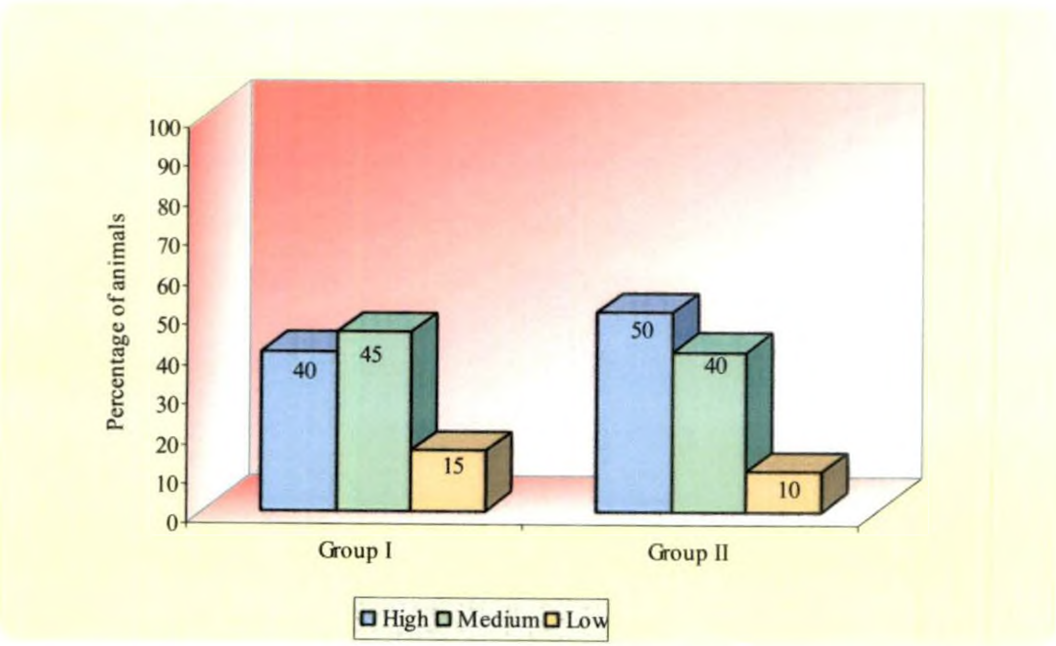


Fig. 2. Intensity of oestrus among animals of groups I and II

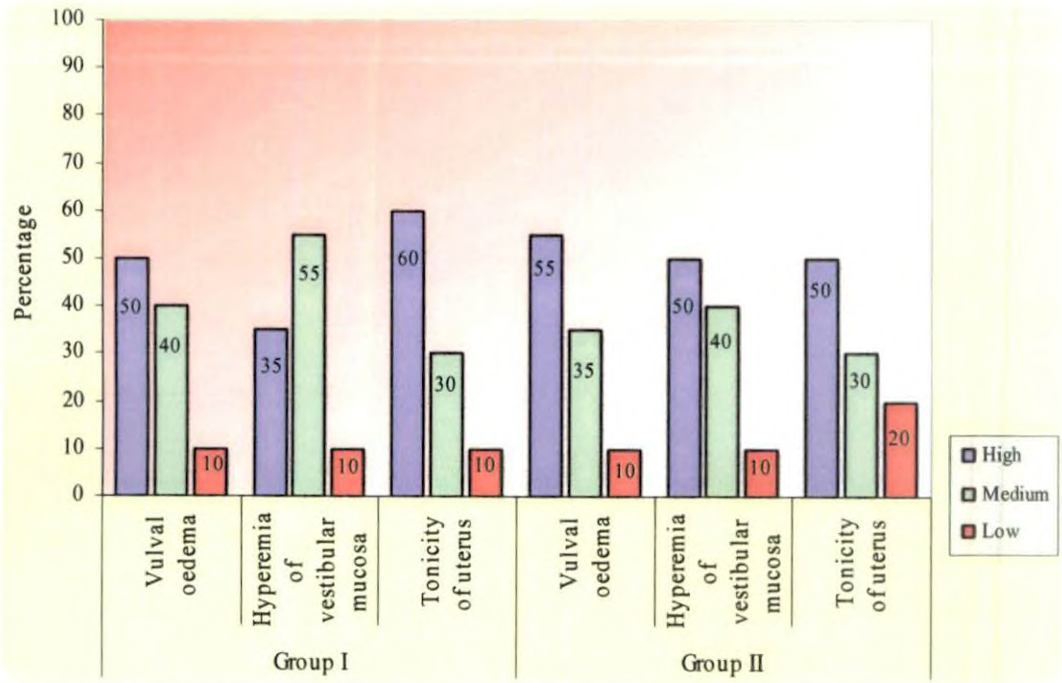


Fig. 3. Physical characteristics of reproductive tract during oestrus among animals of groups I and II

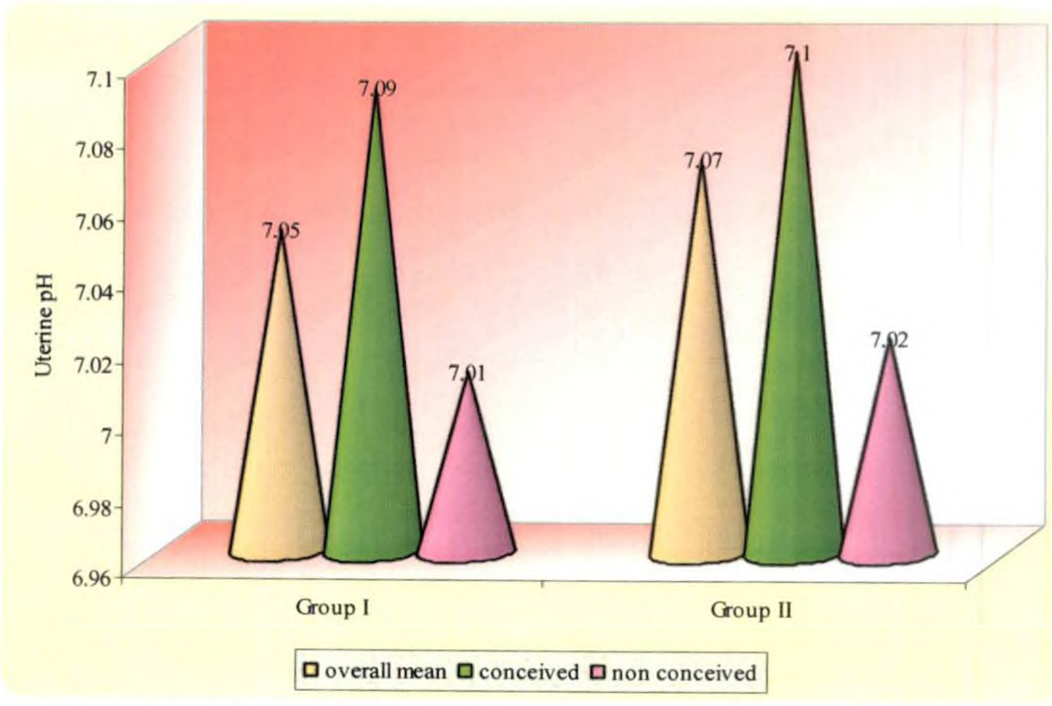


Fig. 4. Uterine pH in animals of groups I and II

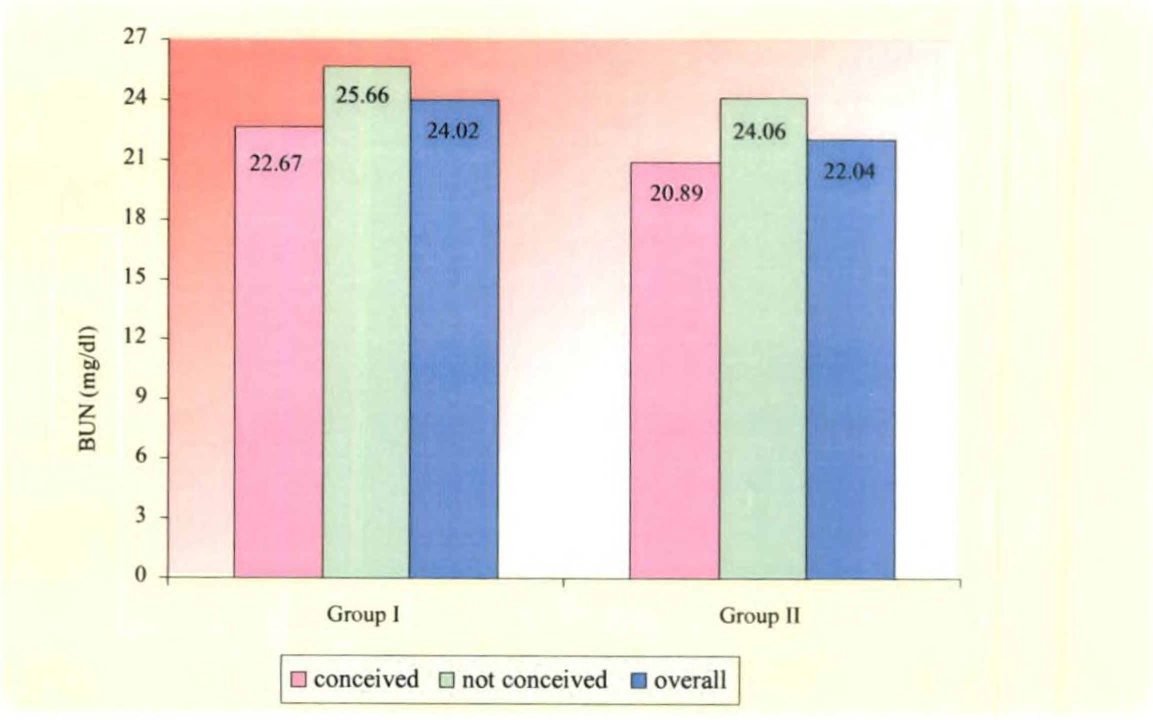


Fig. 5. Blood urea nitrogen level (mg/dl) in animals of groups I and II

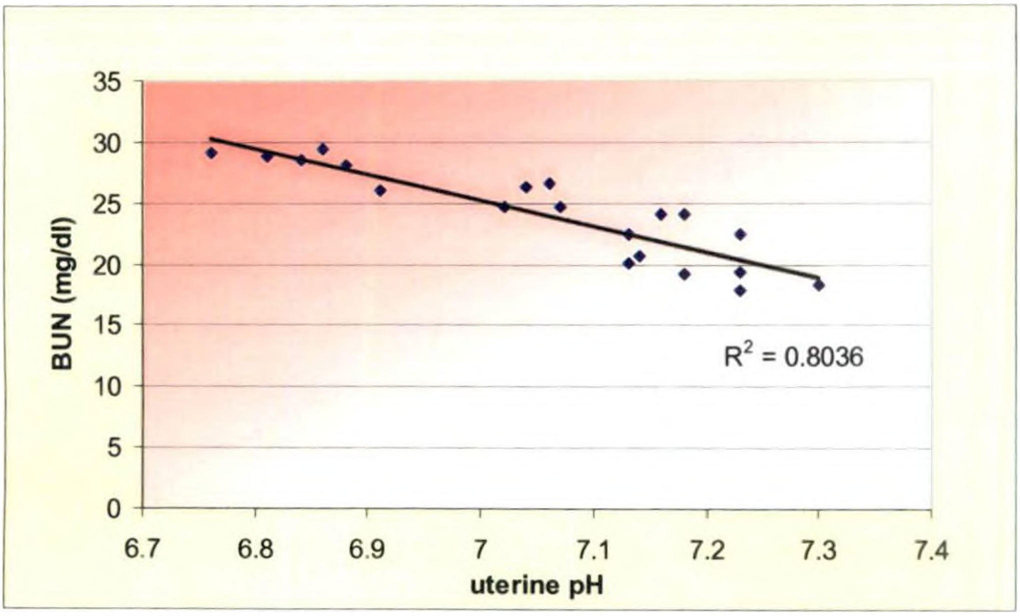


Fig.6. Blood urea nitrogen level and uterine pH in animals of Group I

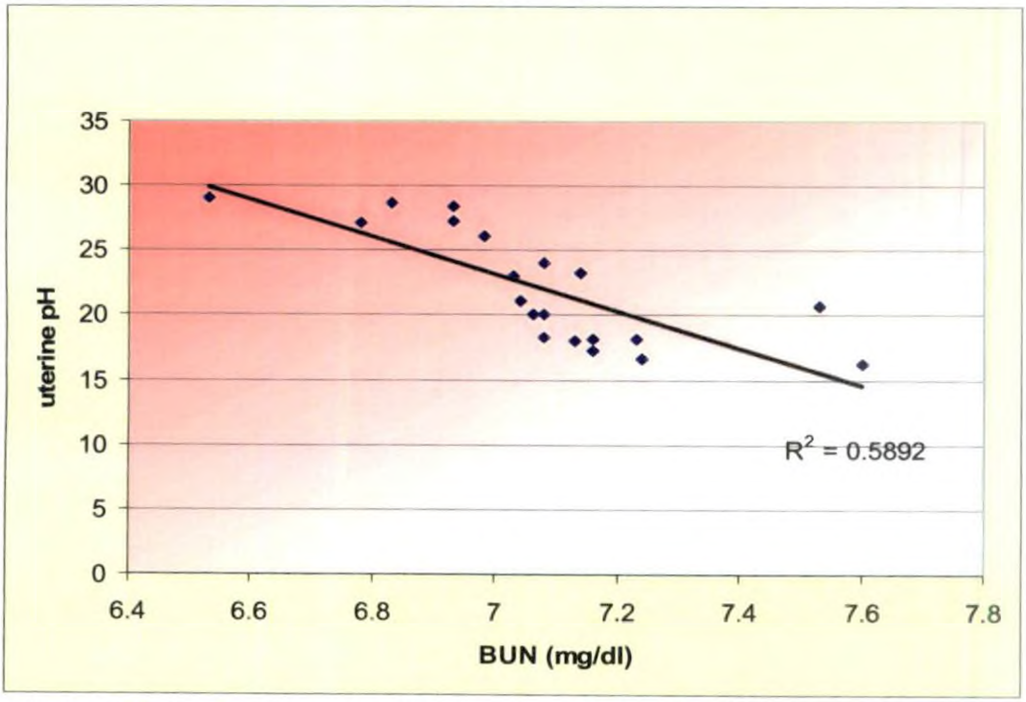


Fig.7. Blood urea nitrogen level and uterine pH in animals of Group II

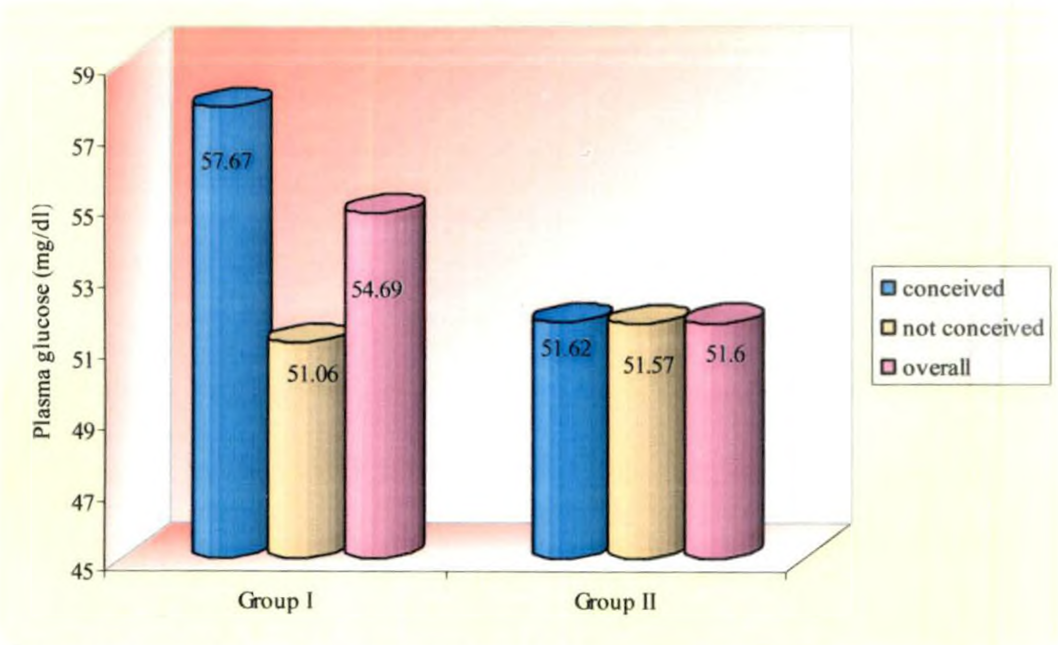


Fig.8. Plasma glucose level (mg/dl) in animals of groups I and II

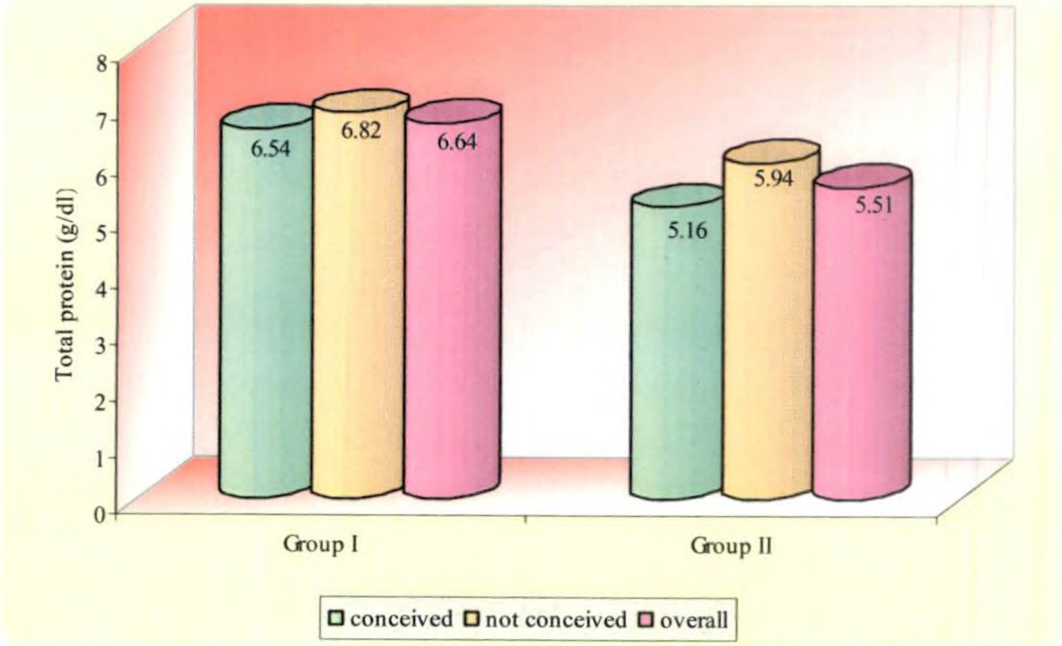


Fig. 9. Serum total protein level (mg/dl) in animals of groups I and II

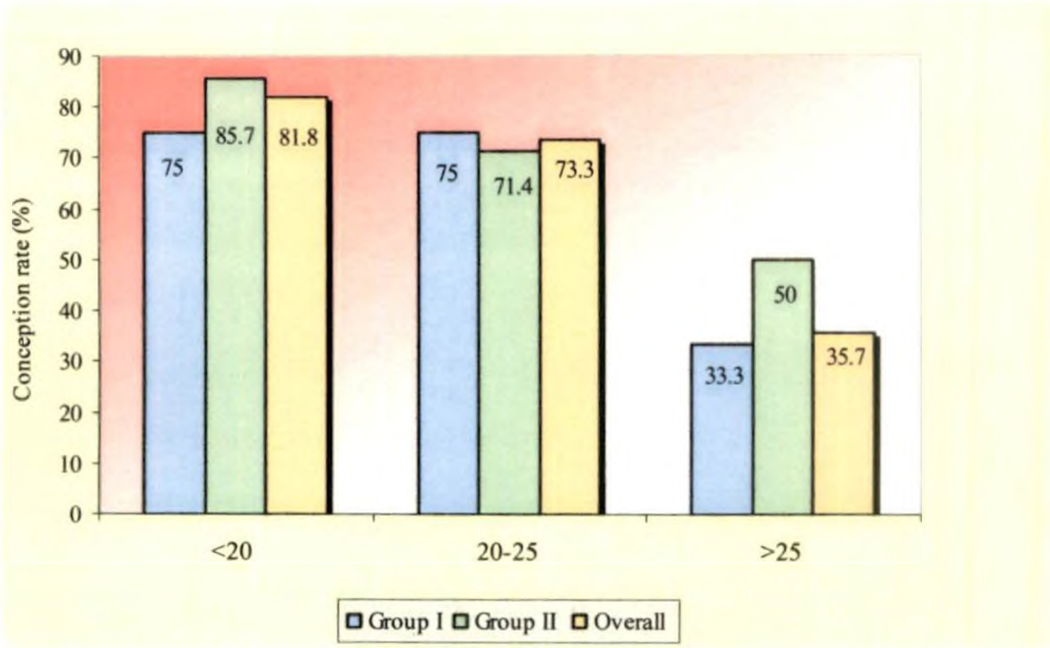


Fig. 10. Conception rate among animals of groups I and II in relation to BUN level (mg/dl) level on the day of AI

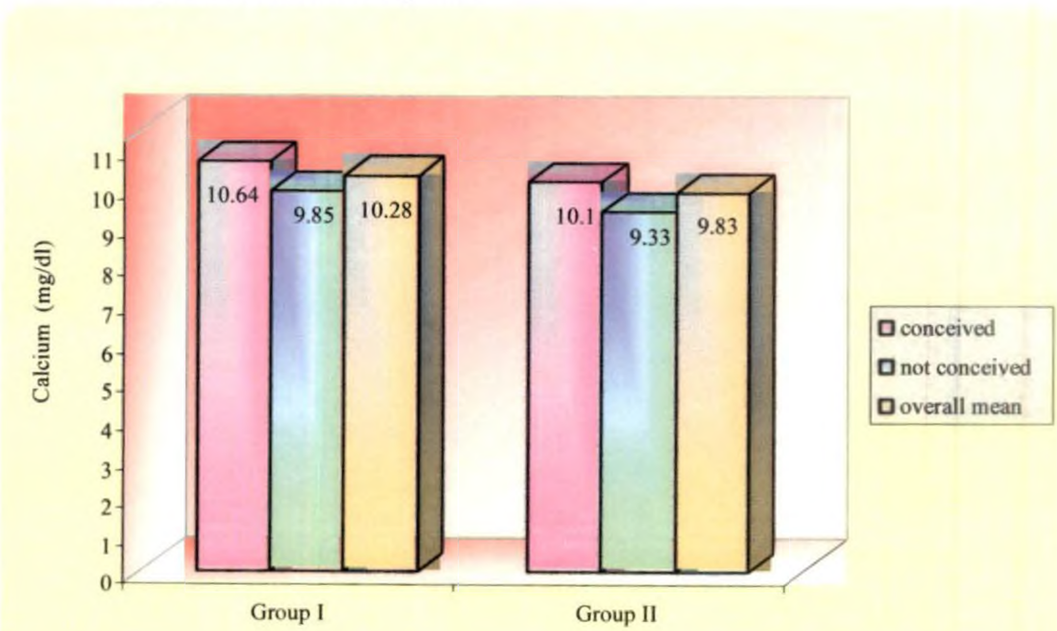


Fig .11. Serum calcium level (mg/dl) in animals of groups I and II

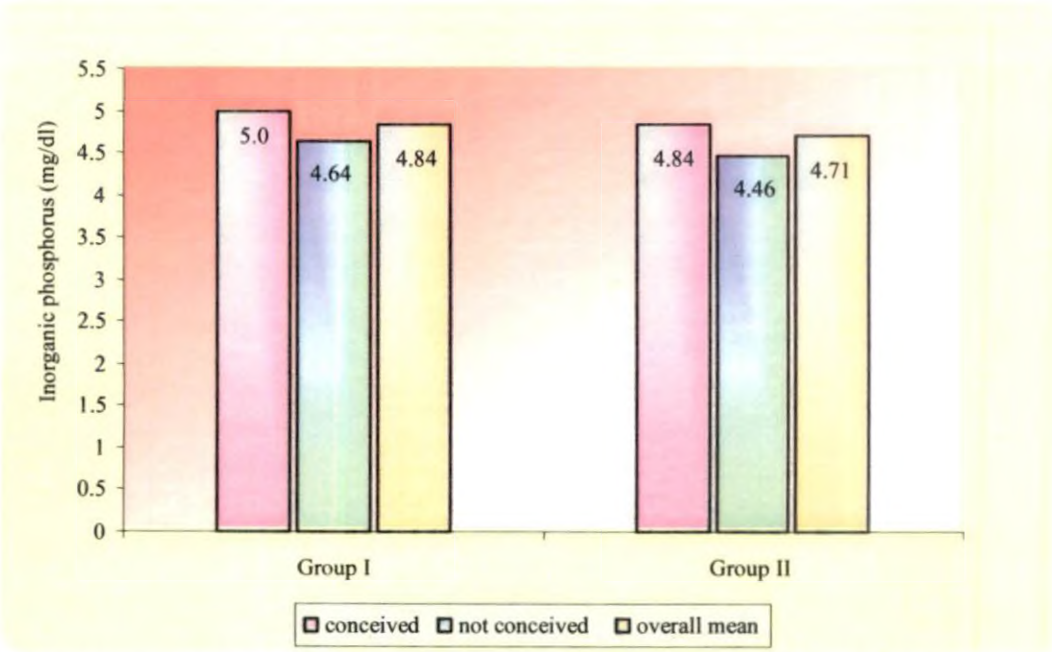


Fig.12. Serum inorganic phosphorus (mg/dl) in animals of groups I and II

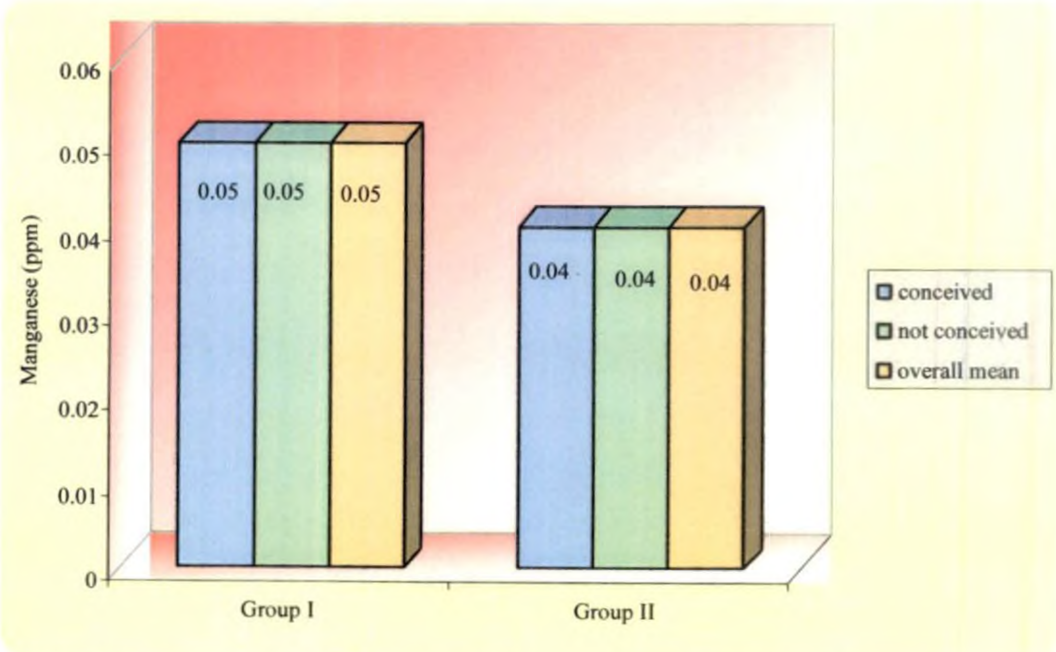


Fig. 13. Serum manganese level (ppm) in animals of groups I and II

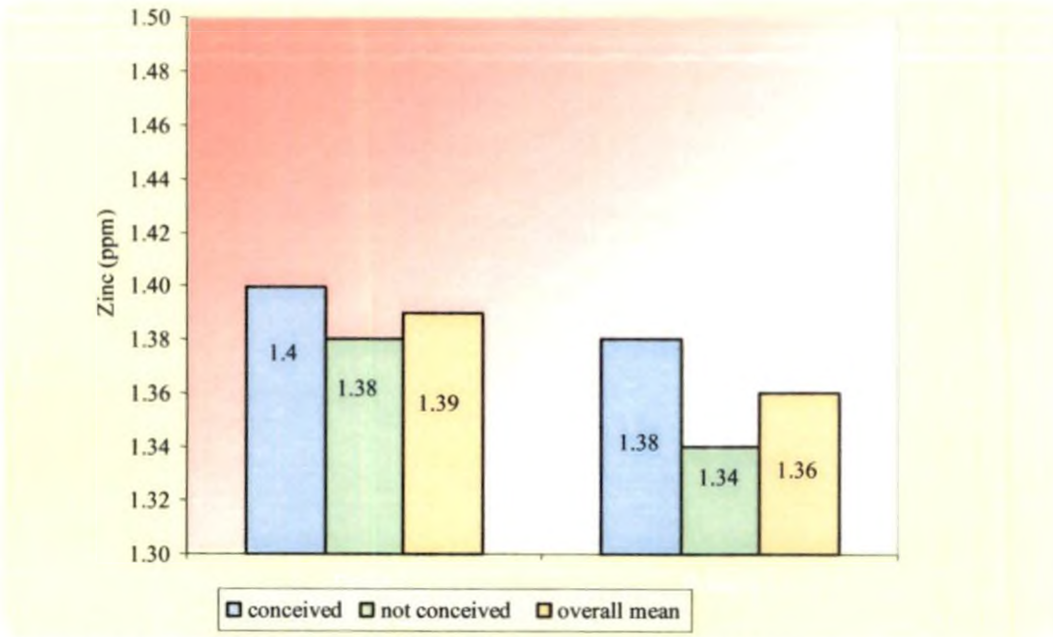


Fig. 14. Serum zinc level (ppm) in animals of groups I and II

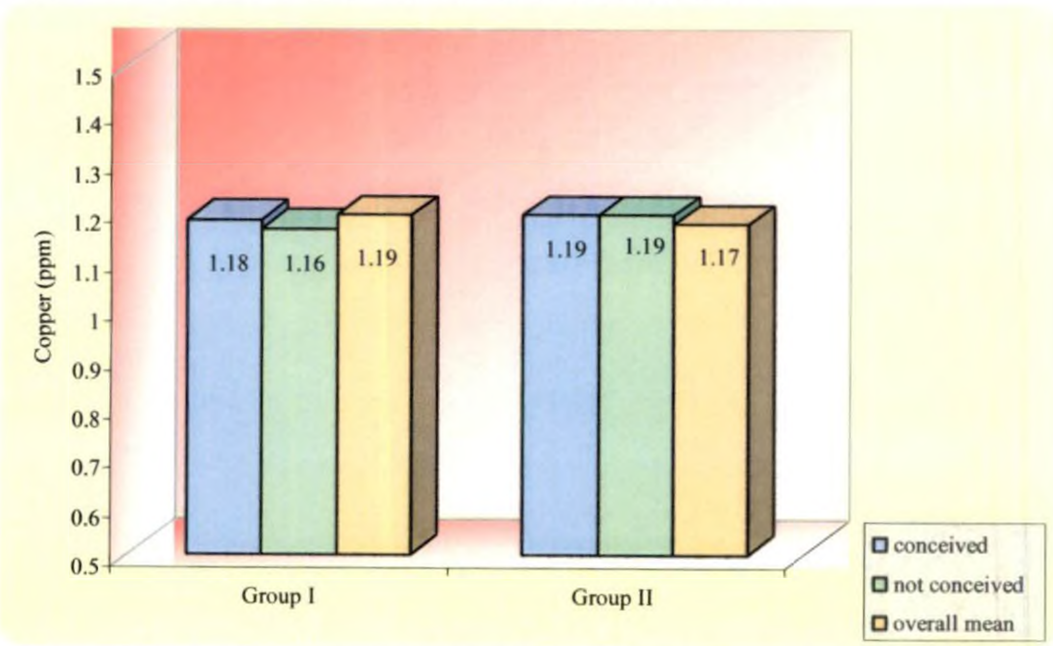


Fig. 15. Serum copper level (ppm) in animals of groups I and II

Discussion

5. DISCUSSION

Reproductive failure or infertility in dairy cattle is multifactorial in nature. The risk factors that collectively interact and exert influences on fertility include managemental, environmental, metabolic and nutritional problems. Among these, nutritional factors play a very important role and a good nutritional management can improve dairy herd fertility (Ferguson and Chalupa, 1989). In an effort to sustain optimum milk production, dairy producers often increase the nutrient density of dairy cow diets to compensate for suboptimum intakes. This situation may lead to protein intakes in excess of recommendations or requirements and can be associated with decline in fertility (Canfield *et al.*, 1990 and Elrod and Butler, 1993). The intake of high dietary protein can result in elevated blood levels of ammonia, urea or both. Many evidences have accumulated suggesting that increased BUN concentrations are highly related with fertility in dairy cows (Butler, 1998; Westwood *et al.*, 1998 and Gath *et al.*, 1999).

The present investigation was taken up with the objective of studying the effect of BUN and uterine pH on fertility in dairy cows under farm and field conditions and also for correlating the mineral status with fertility.

The material for the present study consisted of apparently healthy and normally cycling crossbred dairy cows, 20 each from those belonging to University Livestock Farm, Mannuthy and those brought for insemination at Artificial Insemination Centres at Mannuthy and Kokkalai ancillary to the Department of Animal Reproduction, Gynaecology and Obstetrics.

5.1. DETAILS OF FEEDING AND MANAGEMENT

Perusal of data regarding feeding and management of cows selected for the present investigation revealed that those belonging to University Livestock Farm, Mannuthy (Group I) were maintained in a relatively high nutritious diet in order to sustain high milk production. It was observed that a scientific feeding practice was followed in the Farm. But compared to farm animals, the feeding practice followed by owners of cows selected from the field was found to be unscientific, even though majority of them were feeding their animals with locally available cattle feed or individual ingredients alone or in combination. On detailed stratification of the types and quantity of ration offered by each owner, it was observed that most of the cows belonging to Group II were not fed with a ration computed as per scientific requirements of energy, dry matter and crude protein. This finding agrees with Chattergee (2007) who found that feeding practice and nutritional status in dairy cattle reared by rural house hold farmers are unscientific. On the contrary, Singh and Pant (1998) noticed that the different feeding practices followed in farm and field conditions did not significantly affect the production and reproduction parameters. According to Spain *et al.* (2007), nutritional status and nutritional management were the most essential components for better production and reproduction in dairy cows.

5.2. DURATION AND INTENSITY OF OESTRUS

5. 2. 2. Duration of Oestrus

Perusal of data presented in Table 1 revealed that the duration of oestrus ranged from 18 to 32 hours with a mean of 26.10 ± 1.74 hours in Group I and 18 to 48 hours with a mean of 30.00 ± 2.10 hours in Group II. The duration of oestrus did not vary significantly between groups I and II, though a slightly higher value was recorded in Group II. The present finding agrees with Jeba (2005), who noticed that the occurrence of prolongation of oestrual symptoms was lower under farm conditions than field conditions. According to Orihuela (2000), the accuracy and efficiency of determining duration of oestrus by direct observation were affected by frequency, duration and timing of the observation periods. It was also opined that barn-housed cattle exhibited more duration of oestrus compared to pasture fed cattle since cattle maintained on pastures spent more time in feeding and hence had less time for exhibition of oestrus behaviours. Velayudhakumar (2003) reported ovulatory disturbances as a cause for prolongation of oestrual symptoms.

The comparatively lower value of duration of oestrus observed in Group I animals may either be due to the better managemental and nutritional conditions provided in the farm which might have reduced the occurance of delayed ovulation among Group I animals or due to the variation in housing and management factors between farm and field animals.

5. 2. 3. Intensity of oestrus

In the present study, cows exhibited high, medium and low intensity of oestrus were 40, 45 and 15 per cent respectively in Group I and 50,40 and 10 per cent in Group II. Ajitkumar (1994) also obtained almost similar data on intensity of oestrus in crossbred cows during natural oestrus. O'Connor *et al.* (2007) reported that nutrition, specifically energy balance had an impact on exhibition of oestrual signs. But in the present study, more number of animals showed higher intensity of oestrum in Group II than Group I even though Group I animals were maintained under good nutrition. However, Harrison *et al.* (1990) reported that intensity of oestrus expression was less in high producing cows when compared to low producers which agrees with the present finding since most of the animals in Group I were high producers.

5.3. PHYSICAL CHARACTERISTICS OF REPRODUCTIVE TRACT DURING OESTRUS

Perusal of data in Table 3 revealed that the percentage of cows with high, medium and low degrees of vulval oedema was 50, 40 and 10 in Group I against 55, 35 and 10 in Group II. The percentage of cows with high, medium and low degrees of hyperemia of vestibular mucous membrane during oestrus in Group I was 35, 55 and 10 and 50, 40 and 10 in Group II animals respectively. The percentage of high, medium and low degrees of tonicity of uterus were 60, 30 and 10 and 50, 30 and 20 respectively in animals of groups I and II. The percentage of animals with high degree of vulval oedema and hyperemia of vestibular mucous membrane was more in Group II compared to Group I. But percentage of animals with high degree of uterine tonicity was higher in Group I. The difference noticed with respect to physical characteristics of reproductive tract between groups in the present study may be due to variation in parity, breed, nutritional status and agro- climatic conditions as reported by Rao and Kodagali (1983).

5.4. CHARACTERISTICS OF CERVICAL MUCUS

5. 4. 1 Colour

In the present study, the percentage of animals with clear and cloudy cervical mucus was 40 and 60 in Group I and 70 and 30 in Group II respectively. Safna (2007) also observed almost similar percentage of animals with clear and cloudy mucus during oestrus. It was also observed in the present study that the conception rate in animals with clear mucus was high in both groups compared to those with cloudy mucus. The present finding is thus in agreement with Umashankar *et al.* (1984), who observed a higher percentage of cloudy mucus in repeat breeders compared to normal healthy cows. According to Dev *et al.* (1997), clear cervical mucus was conducive for sperm penetration and conception whereas, turbidity arrested sperm motility in oestrual mucus.

5. 4. 2 Consistency

The percentage of animals with stringy, watery and scanty mucus sample was found to be 40, 50 and 10 and 50, 45 and 5 in animals of groups I and II respectively. Also, the conception rate in animals with stringy, watery and scanty mucus was 75, 40 and 50 per cent respectively in Group I and 60, 57.10 and 33.33 per cent respectively in Group II. Sukhdeo and Roy (1971) reported that a greater amount of stringy mucus occurred during the period of maximum oestrogenic influence. This endorses the fact that cows should be inseminated during periods with stringy mucus for better conception.

5. 4. 3 Fern Pattern

The fern pattern of cervical mucus samples were found to be typical, atypical and nil pattern in 50, 30 and 20 per cent of animals of Group I as against 60, 30 and 10 per cent of Group II. Thus it could be inferred that majority of the animals exhibited typical type of fern pattern during oestrus. This finding agrees with Luktuke and Roy (1967) who also recorded higher percentage of animals with typical fern pattern (63.10%) in cervical mucus of cows during oestrus.

In the present study, better conception rate was obtained in animals with typical fern pattern in both groups compared to atypical and nil fern pattern. Hence the present finding is in agreement with Choudhary and Purbey (1983) who reported 51.61 and 10.00 per cent conception respectively in cows with typical and atypical fern pattern in cervical mucus. On the contrary, Mehta (1986) observed no significant difference in the occurrence of typical fern pattern in cervical mucus of repeat breeding and fertile group of crossbred cows. According to Dev *et al.* (1997), better crystallization pattern of cervical mucus was obtained in the period of maximum oestrogenic influence during oestrus.

5. 4. 4 Spinnbarkeit Value

The mean spinnbarkeit value was found to be 11.12 ± 0.54 and 12.44 ± 0.04 cm in animals of groups I and II respectively, without variation between groups. It was also found that the mean spinnbarkeit value in conceived and non-conceived animals of Group I was 11.54 ± 0.93 and 10.55 ± 0.39 cm respectively as against 12.52 ± 1.49 and 11.95 ± 1.08 in Group II respectively. The value obtained in the present study is comparable with the findings of Bennur *et al.* (2002) who recorded the spinnbarkeit values as 11.38 ± 0.56 and 11.05 ± 1.33 cm for the oestrual mucus of conceived and non-conceived cows respectively. On the contrary, lower spinnbarkeit value (7.60 ± 0.40 cm) was recorded by Tsiligianni *et al.* (2001) during oestrus in Friesian cows.

5.5 UTERINE pH

The mean uterine pH of 7.05 ± 0.14 and 7.07 ± 0.05 obtained in the present study in animals of groups I and II were comparable with the findings of Kane *et al.* (2002) who observed a range of 7.00 to 7.05 for uterine pH during oestrus in dairy cows. On the contrary, Hugentobler *et al.* (2004) reported a mean uterine pH of 6.96 during oestrus in normal healthy dairy cows. Elrod and Butler (1993) and Butler (1998) reported that protein content in the diet of dairy cows could reduce the uterine pH and decline fertility. But in the present study, the mean value of uterine pH did not vary much between two groups even though they were maintained on different protein and nutritional status. However, comparison between conceived and non-conceived animals of groups I and II revealed that the mean uterine pH in non-conceived animals (7.01 ± 0.05 and 7.02 ± 0.11 in groups I and II respectively) was slightly lower than that in conceived animals (7.09 ± 0.04 and 7.10 ± 0.05 in groups I and II respectively). This agrees with the reports of Elrod *et al.* (1993) who noticed that reduction in uterine pH was associated with decline in fertility. According to Jordan *et al.* (1983), the lower uterine pH resulted from an inhibition of endometrial carbonic anhydrase which was sensitive to the alterations in ionic composition of uterine fluid. The slightly lower value of uterine pH obtained in non-conceived animals in the present study suggests that the mechanisms regulating the composition of uterine fluid might have affected by some dietary protein metabolite.

In the present study, an inverse relationship could also be obtained between BUN level in serum and uterine pH during oestrus. Statistical analysis revealed that correlation between uterine pH and BUN level is highly significant ($P<0.01$) with a correlation co-efficient of r = -0.896 and r = - 0.753 in groups I and II respectively. Perusal of data presented in Table 7 and Fig. 6, 7 revealed that as the BUN level in cows increased, uterine pH reduced slightly. This agrees with the findings of Melendez *et al.* (2003) who also noticed an inverse relationship between BUN and uterine pH. Zhu *et al.* (2000) reported that ureagenesis removed bicarbonate ions in uterine lumen and reduced the uterine pH.

5.6 BIOCHEMICAL COMPOSITION OF SERUM / PLASMA

5.6.1 Blood Urea Nitrogen (BUN)

In the present study, the mean blood urea nitrogen level in animals of Group I (24.01 ± 0.96 mg %), was found to be significantly higher ($P<0.05$) than that in Group II (22.04 ± 0.96 mg %). Feeding practices followed in the farm and field conditions were found to be different and the farm animals selected for the present study were found to be maintained under a nutritious, protein rich diet compared to selected field animals. Thus the present findings agree with that of Oltner and Wiktorson (1983) and Roselor *et al.* (1993), who opined that BUN concentration was related to dietary protein intake, percentage of rumen degradable and undegradable protein and protein-energy ratio in the diet. The mean value of BUN level recorded in the present study is comparable to that (22.39 ± 3.42 mg %) reported by Parmar *et al.* (1986), in normally cycling crossbred dairy cows.

In the present study, a significant interaction could also be observed between BUN level in serum and conception rate. It was found that conception rate of animals with a BUN level less than 20 mg/dl was 81.80 per cent and it reduced to 73.30 per cent in animals with a BUN level of 20 to 25 mg/dl and 35.71 per cent with a BUN level of greater than 25 mg/dl. The mean BUN level varied significantly ($P<0.05$) between conceived and non-conceived animals of both groups, suggesting a relationship between the BUN level and low conception rate. Even though the present finding agrees well with many of the previous reports (Ferguson *et al.,* 1993; Baker *et al.*, 1995; Butler *et al.*,1996 and Hammon *et al.,* 2005), Howard *et al.* (1987) and Carroll *et al.* (1988) could not find a concomitant decline in fertility in cows with a high BUN value.

The reduced conception rate observed with elevated BUN level in animals of groups I and II may be due to fertilization failure/early embryonic death occurred due to the toxic effect of ammonia or urea or both.

5.6.2 Glucose

In the present study, the mean plasma glucose level observed in Group I during oestrus (54.69 ± 1.83 mg%) was slightly higher than that observed in Group II (51.60 ± 0.93 mg%). Prasad *et al.* (1984) opined low blood glucose level as an indication of subnormal energy status. The mean value of plasma glucose recorded during oestrus in the present study is in agreement with Sharma *et al.* (1984) who also obtained a similar value in normally cycling cows during oestrus. However, higher values were recorded by Yadav *et al.* (2004) and Ramakrishna (1997) during oestrus (63.035 ± 0.80 and 62.2 ± 3.23 mg% respectively) in dairy cows. On the contrary, Rao *et al.* (1981) reported a lower value of blood glucose level (42.23 ± 1.35 mg %) in cows during oestrus.

On comparing the plasma glucose level between conceived and non-conceived animals of groups I and II, a marginal increase in plasma glucose level could be noticed in conceived animals compared to non-conceived animals of Group I. But, almost similar values were recorded in conceived and non-conceived animals of Group II. Shrivastava and Kharche (1986) found significant difference between the mean plasma glucose level of healthy and repeat breeding cows during oestrus. But contrary to this, Singh and Pant (1998) observed almost similar plasma glucose level in conceived and non-conceived cows during oestrus.

The higher mean glucose level observed in the present study in cows of Group I may be due to the better feeding practices followed in the Farm. The lower blood glucose level obtained in non-conceived animals suggests the effect of subnormal energy status on pituitary function and biochemical activity of hormones involved in normal reproduction.

5.6.3 Total Protein

The mean serum total protein in animals of Group I was found to be 6.64 ± 0.03 g per cent, which was significantly higher (P<0.01) when compared to 5.51 ± 0.21 g per cent in Group II. However, almost similar mean value of serum total protein was recorded in conceived and non-conceived animals of both groups I and II. Vohra *et al.* (1995) opined that proteins at a low level in circulation lead to deficiency of certain amino acids that are eventually needed for gonadotrophin synthesis and resultant impairment in reproduction. In the present study comparatively higher mean serum total protein observed in Group I might be due to increased biosynthesis of protein for milk production as most of them were high yielders or due to relatively high protein diets fed to this group of animals.

5.7 MINERAL STATUS

5.7.1 Calcium

The mean serum calcium level in cows of Group I was significantly higher (P<0.03) than that in cows of Group II. However, the level in both groups was within the normal range of 8 to 12 mg/dl (Radostits *et al.*, 2000). A slight increase in the value of mean serum calcium was observed in conceived animals compared to non-conceived animals. The present finding is in agreement with many previous researchers (Sharma *et al.*, 1984; Rajeev, 1998; Dutta *et al.*, 2001 and Das *et al.*, 2002) and all of them could observe significant difference in the value of mean serum calcium level between fertile and infertile animals. On the contrary, Roberts (1971) reported that calcium deficiency normally did not cause reproductive failure in cattle. But Veldhis and Klase (1982) reported calcium as an integral part in steroid biosynthesis pathways of ovary and adrenal gland and was necessary for maintenance of normal fertility. The significantly higher value of serum calcium level in animals of Group I may be due to the better feeding, management and mineral supplementation provided in the farm.

5.7.2 Phosphorus

The mean serum phosphorus level in cows of groups I and II did not show a marked variation, but a slightly higher value could be obtained in conceived animals compared to non-conceived animals. Different authors conclusively reported variation in serum phosphorus level between healthy and infertile animals (Sharma *et al.*, 1984; Dabas *et al.*, 1987; George, 1995; Rajeev, 1998 and Dutta *et al.*, 2001), and was attributed as a major reason for reproductive problems in dairy cattle. Even though there was no significant difference in the mean phosphorus level between conceived and non-conceived animals of groups I and II, the slightly higher value obtained in conceived animals indicates that marginal deficiency of phosphorus in blood is sufficient to cause disturbances in the pituitary-ovarian axis without manifestation of specific deficiency symptoms (Quayam *et al.*, 1988).

5.7.3 Manganese

The mean serum manganese level in animals of group I was significantly higher ($P<0.01$) than that in animals of Group II. However, the serum level of manganese did not vary much between conceived and non-conceived animals of groups I and II. According to Markandeya *et al.* (2002), manganese was required for activation of many enzyme systems in the body and was found to be involved specifically in the luteal tissue metabolism. Parmar *et al.* (1986) and Das *et al.* (2002) reported that the manganese level in blood was higher in healthy animals as compared to infertile animals. The significantly higher value obtained in Group I may be due to the better nutritional and managemental conditions provided in the Farm.

5.7.4 Zinc

The mean serum zinc level obtained in animals of Group I was slightly higher than that in cows of Group II. Comparison between conceived and non-conceived animals also recorded a slightly higher value in conceived. Almost similar values were recorded by George (1995) and Rajeev (1998). The results are also comparable with the findings of Saxena and Gupta (1995) who reported a higher value of mean serum zinc in cows which conceived than those not conceived. Dutta *et al.* (2001) and Das *et al.* (2002) also reported that the mean serum zinc level was significantly higher in normal fertile cows than infertile cows. However, statistically insignificant variation was reported by George (1995) and Singh *et al* (2004). The lower serum zinc level in non-conceived animals could better be explained from the reports of Reece (2004) as zinc deficiency could alter the synthesis of prostaglandin which might have affected reproduction.

5.7.5 Copper

The mean serum copper level did not vary significantly between groups, even though a slightly higher value was obtained in animals of Group I compared to Group II.

Comparison between conceived and non-conceived animals also revealed a slight increase in the level of serum copper in conceived animals of Group I, but almost similar values were obtained in conceived and non-conceived animals of Group II. Dutta *et al.* (2001) reported almost similar value of serum copper in crossbred dairy cows during oestrus. According to Khasatiya *et al.* (2005), copper level in circulation appeared to be influenced by hormones in circulation.

The findings in the present study suggest the need for a good nutritional management for improved fertility in dairy cows. The significantly high BUN level obtained in non-conceived animals indicates the negative association between BUN and fertility. As the metabolism of excess rumen degradable or undegradable protein results in high urea production, monitoring of BUN concentration has proved to be useful in associating the impact of excess dietary protein and conception rate. The inverse relationship between BUN and uterine pH obtained in the present study indicates the possible alterations in uterine luminal environment, if BUN level has become elevated. However, more studies are required for better understanding of the mechanism by which urea or ammonia affect the uterine pH and ultimately viability of embryos.

Summary

6. SUMMARY

With the objective of studying the effect of BUN and uterine pH on fertility in dairy cows under farm and field conditions and also for correlating the mineral status with fertility, an investigation was carried out using apparently healthy and normally cycling crossbred dairy cows, 20 each from those belonging to University Livestock Farm, Mannuthy (Group I) and those brought for insemination at Artificial Insemination Centres at Mannuthy and Kokkalai (Group II). After collecting the details of feeding and management, all the selected cows were subjected to clinico-gynaecological examination and observations were made. Blood samples and uterine mucus were collected from all the animals at oestrus for estimation of biochemical parameters and uterine pH respectively. They were inseminated during the most appropriate period of oestrus and were subjected to pregnancy diagnosis at 60 days post insemination.

All the cows in Group I were found to be maintained in a relatively high nutritious diet in order to sustain high milk production. On the other hand, Group II animals were fed with rations not computed as per scientific requirements of energy, dry matter and crude protein.

The duration of oestrus ranged from 18 to 32 hours with a mean of 26.10 ± 1.74 in Group I and 18 to 48 hours with a mean of 30.00 ± 2.11 in Group II. Intensity of oestrus was high, medium, and low in 40, 45, and 15 and 50, 40 and 10 per cent in animals of groups I and II respectively.

The percentage of cows with high, medium, and low degree of vulval oedema was 50, 40 and 10 in Group I against 55, 35 and 10 in Group II. The percentage of cows with high, medium and low degree of hyperemia of vestibular mucous membrane during oestrus in Group I was 35, 55 and 10 and 50, 40 and 10 in Group II animals respectively. The percentage of high, medium and low

degrees of tonicity of uterus was 60, 30 and 10 and 50, 30 and 20 respectively in animals of groups I and II.

The percentage of animals with clear and cloudy cervical mucus was 40 and 60 in Group I and 70 and 30 in Group II respectively. It was also observed that the conception rate in animals with clear mucus was high in both groups compared to those with cloudy mucus. The percentage of animals with stringy, watery and scanty mucus sample was found to be 40, 50 and 10 and 50, 45 and 5 in animals of groups I and II respectively. The conception rate in animals with stringy, watery and scanty mucus was 75, 40 and 50 per cent respectively in Group I and 60, 57.10 and 33.33 per cent respectively in Group II. The fern pattern of cervical mucus samples were found to be typical, atypical and nil pattern in 50, 30 and 20 per cent of animals in Group I as against 60, 30 and 10 per cent in Group II. The mean spinnbarkeit value of cervical mucus was found to be 11.12 ± 0.54 and 12.44 ± 0.04 cm in animals of groups I and II respectively. The value was found to be 11.54 ± 0.93 and 10.55 ± 0.39 cm and 12.52 ± 1.49 and 11.95 ± 1.08 cm in conceived and non-conceived animals of groups I and II respectively.

The uterine pH was found to be 7.05 ± 0.14 and 7.07 ± 0.05 in animals of groups I and II respectively. Comparison between conceived and non-conceived animals of groups I and II revealed that the mean uterine pH in non-conceived animals (7.01 ± 0.05 and 7.02 ± 0.11 in groups I and II respectively) was slightly lower than that in conceived animals (7.09 ± 0.04 and 7.10 ± 0.05 in groups I and II respectively). Also, an inverse relationship could be obtained between BUN level in serum and uterine pH during oestrus. Correlation between uterine pH and BUN level is highly significant ($P<0.01$) with a correlation co-efficient of $r = -0.896$ and $r = -0.753$ in groups I and II respectively.

The mean blood urea nitrogen level in animals of Group I (24.02 ± 0.85 mg %), was found to be significantly higher ($P<0.05$) than that in Group II (22.04

± 0.96 mg%). It was found that conception rate in animals with a BUN level less than 20 mg/dl was 81.80 per cent and it reduced to 73.30 per cent in animals with a BUN level of 20 to 25 mg/dl and 35.71 per cent with a BUN level of greater than 25 mg/dl. The mean BUN level varied significantly (P<0.05) between conceived and non-conceived animals of both groups.

The mean plasma glucose level observed in Group I during oestrus (54.69 ± 1.83 mg%) was slightly higher than that observed in Group II (51.60 ± 0.93 mg%). A marginal increase in plasma glucose level could be noticed in conceived animals compared to non-conceived animals of Group I. The mean serum total protein in animals of Group I was found to be 6.64 ± 0.03 g per cent, which was significantly higher (P<0.01) when compared to 5.51 ± 0.21 g per cent in Group II.

The mean serum calcium level in Group I (10.28 ± 0.19 mg%) was significantly higher (P<0.03) than that in cows of Group II (9.83 ± 0.21 mg%). Also, a slight increase in the value of mean serum calcium was observed in conceived animals compared to non-conceived animals. The mean serum phosphorus level in cows of groups I and II (4.84 ± 0.13 and 4.71 ± 0.15 mg% respectively) did not show a marked variation, but a slightly higher value could be obtained in conceived animals compared to non-conceived animals.

The mean serum manganese level in animals of groups I and II was 0.05 ± 0.004 and 0.04 ± 0.003 ppm respectively and the difference was found to be highly significant (P<0.01). The mean value of serum manganese in conceived and non-conceived animals was 0.05 ± 0.004 and 0.05 ± 0.005 ppm in Group I as against 0.04 ± 0.005 and 0.04 ± 0.004 mg per cent respectively in Group II. A mean serum zinc level of 1.39 ± 0.01 and 1.36 ± 0.01 ppm was observed in groups I and II respectively. Comparison between conceived and non-conceived animals also recorded a slightly higher value in conceived.

A mean serum copper level of 1.19 ± 0.01 and 1.17 ± 0.01 ppm was observed in Group I and II animals respectively. The mean serum copper level in conceived and non-conceived animals was 1.18 ± 0.02 and 1.16 ± 0.01 ppm in Group I as against 1.19 ± 0.01 and 1.19 ± 0.02 ppm respectively in Group II. The overall conception rate in Group I was found to be 55 per cent as against 65 per cent in Group II.

It was concluded from the present study that,

1. The mean duration of oestrus was slightly higher in field animals compared to farm animals. Behavioural and physical signs associated with oestrus were more pronounced in field animals than farm animals. A better conception rate was observed in those with clear and stringy mucus with typical type of fern pattern during oestrus.

2. The mean uterine pH was slightly lower in non-conceived animals compared to conceived animals in both groups and an inverse relation could be noticed between uterine pH and BUN level.

3. A significantly higher mean BUN level was noticed in farm animals compared to animals selected from field conditions and a reduced conception rate was observed with elevated BUN level.

4. The mean plasma glucose and serum total protein level was found to be higher in farm animals compared to field animals. The mineral profile of selected animals revealed a significantly higher value of serum calcium and manganese in farm animals compared to field animals.

Hence the present study suggests the need for a good nutritional management to improve fertility in dairy cows. The benefits of feeding excess dietary protein and urea to maintain peak milk production should be compared with potential negative effects on fertility.

References

REFERENCES

Adhallikar, V.P. 1986. Studies on cervico-vaginal mucus and physico-biochemical properties of cross bred cows at mid oestrus for prediction of ovulation and fertility. M.V.Sc. Thesis Abstract. *Indian J. Anim. Reprod.* 7: 152.

Ajitkumar, G. 1994. Management of oestrous cycle in crossbred cattle using prostaglandin. M.V.Sc. thesis, Kerala Agricultural University, Trichur, 135 p.

Arosh, J.A., Kathiresan, D., Devanathan, T.G., Rajasundaram, R.C and Rajasekaran, A. 1998. Blood biochemical profile in normal cyclical and anoestrus cows. *Indian J. Anim. Sci.* 68: 1154-1156.

Arunvipas, P., Dohoo, I.R., Van Leeuwen, J.A and Keefe, G.P. 2003. The effect of non-nutritional factors on milk urea nitrogen levels in dairy cows in Prince Edward Island, Canada. *Preven. Vet. Med.* 59 : 83–93.

Awasthi, M.K and Kharche , K.G. 1987. Studies on some blood constituents in normal cycling, fertile and infertile repeat breeder cross bred cows. *Indian J. Anim. Reprod.* 8:95-97.

Baker, L.D., J.D. Ferguson and Chalupa, W.1995. Responses in urea and true protein of milk to different protein feeding schemes for dairy cows. *J. Dairy Sci.* 78:2424-2434.

Barton, B.A., Rosario, H.A., Anderson, G.W., Grindle, B.P and Caroll, D.J.1996. Effects of dietary crude protein, breed, parity and health status on the fertility of dairy cows. *J. Dairy Sci.* 79:2225-2236.

Bearden, H.J. and Fuquay, J.W. 1997. *A Text Book of Applied Animal Reproduction- Hormonal control of oestrus cycle.* Fourth edition. 90-92p.

Bennur, P.C, Honnappagol, S.S and Tandle, M.K. 2002. Effect of physico-chemical properties of cervicovaginal mucus on fertility in cows. *Indian Vet. J.* 81:1069.

Berasdinelli, J.G., Weng, J., Burfening , P.J and Adair,R. 2001. Effect of excess degradable intake protein on early embryonic development, ovarian steroids and blood urea nitrogen on days 2,3,4 and 5 of the oestrus cycle in mature ewes. *J. Anim. Sci.* 79:193-199.

Bishonga, C., Robinson, J.J., Mc.Evoy, T.G., Findlay, D.A., Aitken, R.P and Robertson, E. 1996. Excess dietary urea intake in ewes and its effect on ovulation rate and embryo development. *Jpn. J. Vet. Res.* 44:139 - 151.

Blanchard, T., Love, L., Takeda, T., Henderson, B., Hasler, J and W. Chalupa 1990. Effect of dietary crude protein type on fertilization and embryo quality in dairy cattle. *Am. J. Vet. Res.* 51:905-908.

Broderick, G.A and Clayton, M.K. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of Milk Urea Nitrogen. *J. Dairy Sci.* 80:2964–2971

Bugalia, N.S and Kohli, I.S. 1982. Blood picture and arborisation pattern of cervical mucus in nymphomaniac Rathi cows. *Indian J. Anim. Reprod.* 2:23-25.

Butler, W.R., and Smith, R.D. 1989. Interrelationships between energy balance on postpartum reproductive function in dairy cattle. *J. Dairy Sci.* 72:767–783.

Butler, W.R., Calaman, J.J and Beam, S.W. 1996. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. *J. Anim. Sci.* 74:858.

Butler, W.R. 2000. Nutritional interactions with reproductive performance in dairy cattle. *Anim. Reprod. Sci.* 60:449-457.

Butler, W.R. 1998. Effect of protein nutrition on ovarian and uterine physiology in dairy cattle: review. *J. Dairy Sci.* 81: 2533–2539.

Butler, W.R. 2005. Relationship of dietary protein and fertility. *Adv. Dairy Technol.*17:159 -168.

Campanile, G., Negulia, G., Di palo, R., Gasparrini, B., Pacelli, C., D'Occhio, M.J and Zicarelli, L. 2006. Relationship of body condition score and blood urea and ammonia to pregnancy in Italian Mediterranean buffaloes. *Theriogenology* 46:57-62.

Chattergee, A., Singh, R.B., Saha, R.C and Roy, P.K. 2007. Feeding practice and nutritional status in dairy cattle in rural households of old alluvial zone in West Bengal. *J. Dairy Sci.* 60: 408-412.

Canfield, R.W., Sniffen, C.J., Butler, W.R., 1990. Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. *J. Dairy Sci.* 73, 2342–2349.

Carlsson, J., Bergstrom, J and Pehrson, B. 1995. Variations with breed age seasonyield stage of lactation and herd in the concentration of urea in bulk milk and individual cow's milk. *Acta Vet. Scand.* 36: 245-254.

Caroll, D.J., Barton, B.A., Anderson, G.W and Smith, R.D. 1988. Influence of protein intake and feeding strategy on reproductive performance in dairy cows. *J. Dairy Sci.*71: 3470-3481.

Carver, L.A., Barth, K. M., McLaren, J. B., Fribourg, H. A., Connell, J. T and J. M. Bryan. 1978. Plasma urea nitrogen levels in beef steers grazing nitrogen-fertilized bermudagrass and orchardgrass-ladino pastures. *J. Anim. Sci.* 47:927-934.

Choudhary, S.K and Purbey, L.N. 1983. Oestrus and its relation to conception in rural cattle. *Indian J. Anim.Reprod.*4:21-23.

Cottrill, B., Biggadike, H.J., Collins, C and Laven, R.A. 2002. Relationship between milk urea nitrogen concentration and the fertility of dairy herds. *Vet. Rec.* 151: 413-416.

Dabas, Y. P. S., Singh, S.P and Saxena, O.P. 1987. Serum concentration of minerals in anoestrus cows and buffaloes. *Indian J. Anim. Reprod.* 8: 98-101.

Darwash, A.O., Lamming, G.E and Wooliams, J.A. 1999. The potential for identifying heritable endocrine parameter associated with fertility in post partum dairy cows. *Anim. Sci.* 68:333-347.

Das, S., Bandopadhya, S.K., Basu, S., Ghosh, B.B and Dattagupta, R. 2002. Blood mineral profile of normal cyclic and repeat breeder cross bred cows under rural condition. *Indian J. Anim. Reprod.* 23: 167-169.

De peters, E. J and Cant, J. P.1992. Nutritional factors influencing the nitrogen concentration of bovine milk: a review. *J. Dairy Sci.* 75:2043-2070.

DeWit, A.A., Cesar, M.L nad Kruip, T.A. 2001. Effect of urea during in vitro maturation on nuclear maturation and embryo development of bovine cumulus-oocyte complexes. *J. Dairy Sci.* 84:1800-1804.

Dev, S., Pangawkar, G.R., Sharma, R.K and Verma, H.K. 1997. Sperm penetration in relation to physical characteristics of buffalo oestrual mucus. *Inter. J. Anim. Sci.* 12:89-91.

Dhami, A.J., Lakum, P.D., Patel, P.M., Panchal, M.T and Kavani, F.S. 2005. Blood biochemical profile in relation to age and reproductive status of Holstein Friesian cattle reared under tropical climate. *Indian J. Anim. Reprod.* 26:34 -38.

Dutta, A., Baruah, B.C., Sarmah, B.C., Baruah, K.K and Goswami, R.N. 2001. Macro mineral levels in cyclic, post partum anestrous and repeat breeding local cows in lower Brahmaputra valley of Assam. *Indian J. Anim. Reprod.*22: 41- 44.

Elrod, C. C., and W. R. Butler. 1993. Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. *J. Anim. Sci.* 71:694–701.

Elrod, C.C., Van Amburgh, M. and Butler, W.R. 1993. Alterations of pH in response to increased dietary protein in cattle are unique to the uterus. *J. Anim. Sci.* 71:702–706.

Fahey, J., Boland, M.P. and O'Callalghan, D. 2001. The effects of dietary urea on embryo development in superovulated donor ewes and on early embryo survival and development in recipient ewes. *Anim. Sci.* 72: 395-400.

Ferguson, J.D., and W. Chalupa. 1989. Impact of protein nutrition on reproduction in dairy cows. *J. Dairy Sci.* 72:746–766.

Ferguson, J.D., Galligan, D.T., Blanchard, T. and Reevesm M.1993. Serum urea nitrogen and conception rate: the usefulness of test information. *J. Dairy Sci.* 76:3742–3746.

Fraser, C.M. 1991. *The Merck Veterinary Manual.* Seventh edition. Merck and Company. Rahway. NJ.

Gath, V., Lonergan, P., Boland, M.P., O'Callaghan, D., 1999. Effects of diet type on establishment of pregnancy and embryo development in beef heifers. *Theriogenology* 51: 224 - 228.

George, J.1995. Phosphorus and trace element status of anoestrus and repeat breeder crossbred cows. M.V.Sc. thesis. Kerala Agricultural University, Mannuthy, 79p.

Godden, S.M., Kelton, D.F., Lissemore, K.D., Walton, J.S., Leslie, K.E and Lumsden, J.H. 2001. Milk urea testing as a tool to monitor reproductive performance in Ontario dairy herds. *J. Dairy Sci.* 84: 1878-1885.

Gordon, I. Controlled reproduction in cattle and buffaloes. Cab international walling Ford. U.K. p 450.

GOI (Government of India). 2006. Basic Animal Husbandry Statistics. NDDB home page (On-line). Available :www.nddb.org/statistics.html.

Guo, K., Russek-Cohen, E., Varner, M.A. and Cohn, R.A. 2004. Effects of milk urea nitrogen and other factors on probability of conception of dairy cows. *J. Dairy Sci.* 87:1878-1885.

Gustaffson, A.H and Carlsson, J. 1993. Effects of silage quality, protein evaluation systems and milk urea content on milk yield and reproduction in dairy cows. *Livest. Prod. Sci.* 37: 91-105.

Gustaffson, A.H and Palmquist, D.L. 1993. Diurnal variation in rumen ammonia, serum urea and milk urea in dairy cows at high and low yields. *J. Dairy Sci.* 76:475-484.

Gustaffson, H., Larsson, K., Kindahl, H and Madez, A. 1986. Sequential endocrine changes and behaviour during estrus and metoestrus in repeat breeder and virgin heifers. *Anim. Reprod. Sci.* 10:261-273.

Hafez, E.S.E. and Hafez, B.2000. *Reproduction in Farm Animals*. Seventh edition. Lippincott Williams and Wilkins, Philadelphia. 509 p.

Hammon, D.S., Holyok, G.R and Dhiman,T.R. 2005. Association between blood plasma urea levels and reproductive fluid urea nitrogen and ammonia concentrations in early lactating dairy cows. *Anim. Repro. Sci.* 86:195-204.

Hammon, D.S., Wang, S and Holyok, G. K. 2000. Effects of ammonia during different stages of culture on development of *in vitro* produced bovine embryos. *Anim. Reprod. Sci.* 59: 23-30.

Hammond, A. C. 1983a. Effect of dietary protein level, ruminal protein solubility and time after feeding on plasma urea nitrogen and the relationship of plasma urea nitrogen to other ruminal and plasma parameters. *J. Anim. Sci.* 57(Suppl. 1):435.

Hammond, A. C. 1992. Update on BUN and MUN as a guide for protein supplementation in cattle. In: 3rd Annual Florida Ruminant Nutrition Symposium, University of Florida, Gainesville, FL. pp. 9-18.

Harrison, R.O, Ford, S.P., Young, J.W. 1990. Increased milk production versus reproductive and energy status of high producing dairy cows. *J. Dairy Sci.* 73: 2749.

Hayden, J.M., Williams, J.E and Collier, R.J. 1993. Plasma growth hormone, insulin-like growth factor, insulin, and thyroid hormone association with body protein and fat accretion in steers undergoing compensatory gain after dietary energy restriction. *J. Anim. Sci.* 71: 3327-3338.

Hof, G., Vervoorn, M.D and Lenaers, P.J. 1997. Milk urea nitrogen as a tool to monitor the protein nutrition of dairy cows. *J. Dairy Sci.* 80: 3368-3376.

Hojman, D., Kroll, O., Adin, G., Hanochi, Bone and Ezra, E. 2004. Relationships between milk urea and production, nutrition and fertility in Israeli dairy herds. *J. Dairy Sci.* 87: 1001-1011.

Howard, H.J., Aalseth, E.P., Adams, G.D., Bush, L.J., McNew, R.W and Dawson, L.J. 1987. Influence of dietary protein on reproductive performance on reproductive performance of dairy cows. *J. Dairy. Sci.* 70:1563-1571.

Hurley, W.C and Doane, R.M.1989. Recent developments in the role of vitamins and minerals in reproduction. *J. Dairy Sci.* 72: 784-804.

Hugentobler, S., Morris, D.G., Kane, M.T. and Sreenan, J.M. 2004. In situ oviduct and uterine pH in cattle. *Theriogenology* 61:1419-1427.

Jayanthi, N., Chelalpandian, M and Balachandran, S. 2003. Blood biochemical profile of repeat breeding cows in Thirunelveli region of Tamil Nadu. *Indian Vet. J.* 80:939 - 940.

Jeba, G.S.D. 2005. Fertility trials on induced oestrus in repeat breeding cattle with prolonged oestrum. M.V.Sc. thesis, Kerala Agricultural University, Trichur, 135 p.

Jonker, J.S., Kohn, R.A and Erdman, R.A. 1998.Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. *J. Dairy Sci.*81:2681-2692.

Jordan, E.R., and Swanson, L.V. 1979. Effect of crude protein on reproductive efficiency, serum total protein and albumin in the high-producing dairy cow. *J. Dairy Sci.* 62:58–63.

Jordan, E.R., and Swanson, L.V. 1979. Serum progesterone and luteinizing hormone in dairy cattle fed varying levels of crude protein. *J. Anim. Sci.* 48:1154–1158.

Jordan, E.R., Chapman, T.E. Holtan, D.W. and Swanson, L.V. 1983. Relationship of dietary crude protein to composition of uterine secretions and blood in high-producing dairy cows. *J. Dairy Sci.* 66: 1854–186.

Kane, K.K., Creighton, K.W., Peterson, M.K., Hallford, D.M., Remmenga, M.D and Hawkins, D.E. 2002. Effects of varying levels of undegradable intake of protein on endocrine and metabolic functions of young postpartum beef cows. *Theriogenology* 57: 2179 –2191.

Kenny, D.A., Humpherson, P.G., Leese, Moris, T.G., Tomos, A.D., Diskin, M.G. and Sreenan, J.M. 2002. Effect of elevated concentrations of ammonia and urea on the metabolic and ionic composition of oviductal fluid in cattle. *Biol. Reprod.* 66:1797-1804.

Khasatiya, C.T., Dhami, A.J., Ramani, V.P., Savalia, F.P and Kavani, F.S. 2005. Reproductive performance and mineral profile of postpartum fertile and infertile Surti buffaloes. *Indian J. Anim. Reprod.* 26: 145- 148.

Larson, S.F., Butler, W.R and Currie, W.B. 1997. Reduced fertility associated with low progesterone post breeding and increased milk urea nitrogen in lactating cows. *J. Dairy Sci.* 80: 1288-1295.

Laven, R.A and Drew, S.B. 1999. Dietary protein and the reproduction performance of cows. *Vet. Rec.* 145: 687–695.

Leng, R. A and Nolan, J.V. 1984. Nitrogen metabolism in rumen. *J. Dairy Sci.* 67 :1072-1089.

Loeffler, S.H., De Vries, M.J., Schukken, Y.H., De Zeeuw, A.C., Dijkhuizen, A.A., De Graaf, F.M., Brand, A., 1999. Use of AI technician scores for body condition, uterine tone and uterine discharge in a model with disease and milk production parameters to predict pregnancy risk at first AI in Holstein dairy cows. *Theriogenology* 51: 1267–1284.

Lopez, H., Wu, Z., Satter, L.D and Wiltbank, M.C. 2004. Effect of dietary phosphorus concentration on estrus behaviour of lactating dairy cows. *Theriogenology* 61: 437–445.

Luktuke, S.N and Roy, D.J. 1967. Studies on cervical mucus pattern in relation to fertility in bovines. *Indian J. Vet. Sci.* 37:26-31.

Markandeya, N.M., Bhikane, A.V and Bharkad, G.P. 2002. Clinical response to microminerals with Vitamin Et al. supplementation in anoestrus Deoni cows. *Indian J. Anim. Reprod.* 23:77-78.

Mc Evoy, T.G., Robinson,J.J., Aikten,R.P., Findley,P.A and Robertson, I.S. 1997. Dietary excess of urea influences the viability and metabolism of preimplantation sheep embryos and may affect fetal growth among survivors. *Anim. Reprod. Sci.* 47:71-90.

Mehta, G.B. 1986. A study on repeat breeding conditions in crossbred (K x J & K x HF) cattle with special reference to cervical mucus. M.V.Sc.Thesis Abstract. *Indian J. Anim. Reprod.* 2: 102-103.

Melendez, P., Hernandez, J., Bartolome, J., Risco, C.A., Staples, C and Thatcher, W.W. 2003. Milk, Plasma and Blood urea nitrogen concentrations, dietary protein and fertility in dairy cattle. *J. Am. Vet. Med. Assoc.* 223: 628-634.

Melendez, P., Donovan, A and Hernandez, J. 2000. MUN and infertility in Florida Holstein cows. *J. Dairy Sci.* 83:459-463.

Merilan, C.P. 1983. Evaluation of bovine cervical mucus during estrous cycle by nuclear magnetic resonance. *J. Dairy Sci.* 66:1184-1188.

Meza-Herrera, C.A., Ross, T., Hawkins, D and Hallford, D. 2006. Interactions between metabolic status, pre-breeding protein supplementation, uterine pH and embryonic mortality in ewes: preliminary observations. *Trop. Anim. Hlth. Prod.* 38:407-413.

Noakes, D.E., Parkinson, T.J., England, G.C.W and Arthur, G.H. 2001. *Arthur's Veterinary Reproduction and Obstetrics.* Eighth edition. W.B. Saunders Company, Philadelphia, 868p.

Nousiainen, J., Shingfield, K.J and Hernandez, J. 2004. Evaluation of milk urea nitrogen as a diagnostic of protein feeding. *Anim. Reprod. Sci.* 87:386-398.

O'Callaghan, D.O and Boland, M.P. 1999. Nutritional effects on ovulation, embryo development and the establishment of pregnancy in ruminants. *Anim. Sci.* 68: 299–314.

O'Connor, L.M. 2007. Estrus detection. In: Youngquist, R.S and Threlfall,W.R. *Current Therapy in Large Animal Theriogenology.* Second edition. Elsvier Saunders, Missouri, pp.273-274

Ocon, O.M and Hansen, P.J. 2003. Disruption of bovine oocytes and preimplantation embryos by urea and acidic pH. *J. Dairy Sci.* 86: 1194-1200.

Oltner, R. and Wiktorson, H. 1983. Urea concentrations in milk and blood as influenced by feeding varying amounts of protein and energy to dairy cows. *Livest. Prod. Sci.* 10: 457-467.

Orihuela, A. 2000. Some factors affecting the behavioural manifestation of oestrus in cattle: a review. *Appl. Anim. Behav. Sci.* 70:1-16.

Parmar, K.S., Mehta, U.M and Patel, J.M.1986. Biochemical profile of repeat breeding cross bred cattle in relation to different phases of oestrus cycle. *Indian J. Anim. Reprod.* 7:31- 35.

Peterson, R.G. and Waldern, D. E. 1981. Repeatabilities of serum constituents in Holstein-Friesians affected by feeding, age, lactation, and pregnancy. *J. Dairy Sci.* 64:822-831.

Phillips, G.J., Citron, T.L., Sage, J.S., Cummins, K.A., Cecava, M.J and McNmara, J.P. 2003. Adaptations in body muscle and fat in transition dairy cattle fed differing amounts of protein and methionine hydroxy analog. *J. Dairy Sci.* 86:3634-3647.

Pradhan, J., Mohanty, B.N., Ray, S.K.H and Mohanty, D.N. 1995. A comparative study of haemoglobin, Copper and Zinc concentration of post partum anoestrus cows. *Indian J. Anim.Reprod*.16:28-31.

Prasad, J.K., Singh, B., Gupta, H.P and Kumar, M. 2007. Plasma mineral profile in relation to pregnancy rate in repeat breeding cows. *Indian J. Anim. Reprod.* 28: 69-71.

Prasad, R.S., Kharche, K.G. and Shrivastava, O.P. 1984. Studies on Blood Glucose, Cholesterol and Total leukocyte count in anestrus cross bred cows. *Indian J. Anim. Reprod.* 4: 10-14.

Purohit, G.N. and Bishnoi, B.L. 1993.Some blood biochemical studies in Rathi's cows and heifers before and after induction of oestrus with fertivet. *Indian J. Anim.Reprod.*14: 74-76.

Quayam, S.A., Devanathan, T.G and Pattabiraman, S.R.1988. Studies on the influence of mineral, biochemical and heamatological concentrations at five days post partum oestrus in buffaloes. *Indian Vet. J* .65:236 - 238.

Radostits, O,M., Gay, C,C., Blood, D.C. and Hinchcliff, K.W. 2000.*Veterinary Medicine*. A text book of the diseases of cattle, sheep, pigs, goats and horses. Ninth edition, W.B. Saunders, Philadelphia, 1502p.

Rajala-Schultz, P.J. and Saville, W.J.A. 2003. Sources of Variation in Milk Urea Nitrogen in Ohio Dairy Herds. *J. Dairy Sci.* 86:1653–1661.

Rajala-Schultz, P.J., Saville, W.J.A.., Frazer, G.S. and Wittum, T.E. 2001. Association between milk urea nitrogen and fertility in Ohio dairy cows. *J. Dairy Sci.* 84:482-489.

Rajeev, R. 1998. Growth and reproductive performance of crossbred heifers in selected areas. M.V.Sc. thesis, Kerala Agricultural University, Mannuthy, 140p.

Ramakrishna, K.V.1997. Comparative studies on certain biochemical constituents of anoestrus crossbred Jersey rural cows. . *Indian J. Anim. Reprod.*18: 332-335.

Randhawa, C.S., Randhawa, S.S., Singh, N. and Sidhu, S.S. 2004. Copper supplementation and fertility response in anoestrus buffaloes – A clinical trial. *Indian J. Anim. Reprod.* 25: 41- 42.

Rao, D.G., Prasad, A.B.A., Krishna, V.J and Rao, K.S. 1981. Studies on some biochemical constituents of blood in Ongole cows. *Indian Vet. J.* 58: 870-873.

Rao, N.M and Kodagali, S.B. 1983. Onset of oestrus, oestrus signs and optimum time for AI in Surti Buffaloes. *Indian J. Anim. Sci.* 53: 553-555.

Rathour, K.K., Pandit, R.K., Agarwal, O.G., Ouadri, M.A and Shrivastava, O.P. 2005. Some blood biochemical indices and fertility following treatment of anoestrus in Murrah buffaloes. *Indian J. Anim. Reprod.* 26: 129-132.

Reece, W.O. 2004. *Duke's Physiology of Domestic Animals*. Twelfth edition. Panima Publishing Corporation, New Delhi, 999p.

Rhoads, M.L., Gilbert, R.O., Lucy, M.C and Butler, W.R. 2004. Effects of urea infusion on uterine luminal environment of dairy cows. *J. Dairy Sci.* 87:2896-2901.

Rhoads, M.L., Rhoads, R.P., Gilbert, R.O., Toole, R.E and Butler.W.R. 2006. Detrimental effect of high plasma urea nitrogen levels on viability of embryos from lactating dairy cows. *Anim. Repro. Sci.* 91:1-10.

Roberts, S.J. 1971. *Veterinary Obstetrics and Genital Diseases.* Second edition. W.B. Saunders Company, Philadephia, 776p.

Roseler, D. K., Ferguson, J.D., Sniffen, C.J. and Herrema, J. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. *J. Dairy Sci.* 76:525-534.

Rutlant, J., Lopez-Bejar, M and Lopez-Gatiuz, F. 2005. Ultrastructural and rheological properties of bovine vaginal fluid and its relation to sperm motility and fertilization: a review. *Reprod. Dom. Anim.* 40: 79-86.

Safna, I. M. 2007. Non-antibiotic therapy for subclinical endometritis in repeat breeder cattle. M.V.Sc. thesis, Kerala Agricultural University, Trichur, 68 p.

Salphale, G.V., Kadu, M.M., Fasihuddin, M and Kadu, M.S. 1993. Study of some physical properties of estrual cervical mucus in synchronized, normal and repeat breeder cows with reference to fertility. *Indian J. Anim. Reprod.* 14: 77-78.

Saxena, M.S and Gupta, S.K. 1995. Variations in the plasma levels of copper and Zinc in cross bred cows with early and delayed post partum conception. *Indian J. Anim. Reprod.* 16: 25-27.

Selvaraj, P., Harendrakumar and Mahmood, P. 2002. Physico-enzymatic characteristics of cervical mucus with reference to fertility in repeat breeder cows. *Indian J. Anim. Reprod.* 23: 54 -56.

Sharma, M.C., Shanker, U., Gupta, O.P., Verma, R.P. and Mishra, R.R. 1984. Biochemical studies in cyclic, anoestrus and repeat breeding cross bred cows. *Indian J. Anim.Reprod.* 4: 51-53.

Sharma, V.K and Thripathi, S.S. 1987. Physico chemical properties of cervical mucus in relation to conception in normal and repeat breeder cross bred cows. *Indian J. Anim.Reprod.*8:39-42.

Shrivastava, H.K. and Kharche, K.G.1986.Studies on some blood constituents in normal and abnormal cycling buffaloes. *Indian J. Anim.Reprod.*7:62-65.

Shrivastava, O.P. and Kadu, M.S. 1995. Blood biochemical profiles in normal cyclic and delayed pubertal crossbred heifers. *Indian J. Anim. Reprod.*13:147-149.

Sinclair, K.D., Kuran, M., Gebbie, F.E., Webb, R. and McEvoy, T.G. 2000. Nitrogen metabolism and fertility in cattle: II. Development of oocytes recovered from heifers offered diets differing in their rate of nitrogen release in the rumen. *J. Anim. Sci.* 78:2670-2680.

Singh, M and Pant, H. C.1998. Blood biochemical profile of normal and repeat breeder cows in Himachal Pradesh. *Indian J. Anim.Reprod.*19: 156-157.

Singh, A., Saxena, M.S and Prasad, J.K. 2004. Levels of trace minerals, glucose and haemoglobin in relation to occurrence of post-parturient oestrum in buffaloes. *Indian J. Anim. Reprod.* 25: 58-60.

Sniffen, C.J., O'Connor, J.D and Van Soest, P.J. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *J. Anim. Sci.* 70:3562-3577.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods.* Eight edition. Oxford and IBH Publishing Company, Calcutta, 564 p.

Sonderman, J.P. and Larson, L.L.1989. Effect of dietary protein and exogenous gonadotropin releasing hormone on circulating progesterone concentration and performance of Holstein dairy cows. *J. Dairy Sci.* 72: 2179-2183.

Spain, J.N., Lucy, M.C and Hardin, D.K. 2007. Effects of nutrition on reproduction in dairy cattle. In: Youngquist, R.S and Threlfall, W.R. *Current therapy in large animal theriogenology.* Saunders elsvier, St. Louis, Missouri. 442-449 p.

Sukhdeo and Roy. 1971. Investigations on repeat breeding cows and buffaloes - studies on physical properties of cervical mucus. *Indian Vet. J.* 48: 479-484.

Tamminga, S. 2006. The effect of the supply of rumen degradable protein and metabolisable protein on negative energy balance and fertility in dairy cows. 96: 227-239.

Tsiligianni, Th., Karagiannidis, A., Brikas, P and Saratsis, P.H. 2001. Physical properties of bovine cervical mucus during normal estrus and induced (progesterone and/ or $PGF_{2\alpha}$) estrus. *Theriogenology* 55:629-640.

Umashankar, Sharma, M.C., Verma, R.P. and Gupta, O.P.1984. Studies of cervical mucus in cyclic and repeat breeding crossbred cows. *Indian J. Anim. Reprod.* 4: 41-42.

Vadnere, S.V and Singh, S. 1989. Blood plasma levels of Iodine, Calcium, Inorganic phosphorus, Copper and Iron in post partum anoestrus cross bred cows. *Indian J. Anim. Reprod.* 10: 145-146.

Vandehaar, M.J., Yousif, G., Sharma, B.K., Herdt, T.H., R.S. Emery, M.S. Allen, and Liesman, J.S. 1999. Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cows in the periparturient period. *J. Dairy Sci.* 82:1282–1295.

Velayudhakumar, R. 2003. Effect of human chorionic gonadotropin, gonadotropin releasing hormone and prostaglandin F 2α in augmenting conception rate of repeat breeder cattle. M.V.Sc. thesis, Kerala Agricultural University, Trichur, 85 p.

Veldhis, J.D. and Klase, A.S. 1982. Mechanisms by which calcium ion regulate steroidogenic action of leutinising hormone in isolated ovarian cells *in vitro*. *Endocrynology* 111:1-4.

Verma, R.P., Saxena, S.K and Mishra, R.R. 1983. Oestrus pattern of cross bred cattle in tropics. *Indian Vet. J.* 60: 235-236.

Vhora, S.C., Dindorkar, C.V and Kaikini, A.S. 1995. Studies on blood serum levels of certain biochemical constituents in normal cycling and anoestrus crossbred cows. *Indian J. Anim. Reprod.* 16: 85 -87.

*Ward, J.R., Henricks, D.M., Jenkins, T.C. and Bridges, W.C. 1992. Serum hormone and metabolite concentrations in fasted young bulls and steers. *Domestic Anim. Endo.* 9:97-103.

*Westwood, C.T., Lean, I. J and Kellaway, R. C. 1998. Indications and implications for testing of milk urea in dairy cows: A quantitative review. Part 1. Dietary protein sources and metabolism. *N. Z. Vet. J.* 46:87–96.

Yadav, V.P., Singh, A.P., Kunju, V., Akhtar, M.H., Roy, G.P. and Singh, C. 2004. Study on incidence of anoestrus and blood biochemical constituents in non cyclic and cyclic cross bred cows. *Indian J. Anim. Reprod.* 25 :116-119.

Zhu, L.H., Armentano, L.E., Bremmer, D.R., Grummer, R.R. and Bertics, S.J. 2000. Plasma concentrations of urea, ammonia, glutamine around calving and the relation to hepatic triglyceride to ammonia removal and blood acid-base balance. *J. Dairy Sci.*83: 734-740.

* Originals not consulted

EFFECT OF BLOOD UREA NITROGEN, MINERAL STATUS AND UTERINE pH ON FERTILITY IN DAIRY COWS

SEENA. N. S

Abstract of the thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2008

Department of Animal Reproduction, Gynaecology and Obstetrics
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

ABSTRACT

An investigation was carried out with the objective of studying the effect of BUN and uterine pH on fertility in dairy cows under farm and field conditions and also for correlating the mineral status with fertility using 40 crossbred dairy cows selected at random during oestrus, 20 each from those belonging to University Livestock Farm, Mannuthy (Group I) and those brought for insemination at Artificial Insemination Centres at Mannuthy and Kokkalai (Group II). All the cows in Group I were found to be maintained in a relatively high nutritious diet computed as per the scientific feeding standards compared to Group II animals.

Detailed clinico-gynaecological examination was carried out and blood samples and uterine mucus were collected from all the selected cows during oestrus for estimation of biochemical parameters and uterine pH respectively. They were inseminated during the most appropriate period of oestrus and were subjected to pregnancy diagnosis at 60 days post insemination. Conception rates in both the groups were compared in relation to each parameter.

The mean duration of oestrus was slightly higher in Group II (30.00 ± 2.11 hours) compared to Group I (26.10 ± 1.74 hours). Intensity of oestrus was high, medium and low in 40, 45 and 15 per cent of animals respectively in Group I and 50, 40 and 10 per cent in Group II. Physical changes of reproductive tract *viz.* degree of vulval oedema and hyperemia of vestibular mucous membrane were more pronounced in animals of Group II compared to Group I, where as degree of tonicity was high in Group I compared to Group II. Characteristics of cervical mucus were also found to be affecting fertility. Better conception rate was obtained in animals with clear and stringy cervical mucus exhibiting typical type of fern pattern. Spinnbarkeit value did not vary much between conceived and non-conceived animals.

Uterine pH did not show a marked variation between groups, even though a slightly higher value was recorded in Group I. But, an inverse relationship could be obtained between BUN level in serum and uterine pH during oestrus. Correlation between uterine pH and BUN level was highly significant ($P<0.01$) with a correlation co-efficient of $r = -0.896$ and $r = -0.753$ in groups I and II respectively. The mean blood urea nitrogen level in animals of Group I was significantly higher ($P<0.05$) than that in Group II. The BUN level also varied significantly ($P<0.05$) between conceived and non-conceived animals of both groups. *A marginal increase in plasma glucose level could be noticed in* conceived animals compared to non-conceived animals of Group I. The mean serum total protein was significantly higher ($P<0.01$) in Group I compared to Group II. The serum level of minerals *viz.* calcium, phosphorus, manganese, zinc and copper were also correlated with fertility. The mean level of serum calcium and manganese varied significantly between groups I and II, but there was no significant difference in serum phosphorus, zinc and copper between two groups. Also, a slightly higher mean value was observed for serum calcium, phosphorus, manganese and zinc in conceived animals compared to non-conceived. But serum copper level did not vary between conceived and non-conceived animals.

In light of these findings, it can be concluded that the elevation in systemic concentration of urea is likely to impair fertility in dairy cows as a consequence of alterations in uterine environment. The benefits of feeding excess dietary protein and urea to maintain peak milk production should be compared with potential negative effects on fertility. Hence, a good nutritional management is essential for improved fertility in dairy cows.