OVARIAN RESPONSE TO GONADOTROPHIN RELEASING HORMONE IN NON-CYCLIC GOATS

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled "**OVARIAN RESPONSE TO GONADOTROPHIN RELEASING HORMONE IN NON-CYCLIC GOATS**" 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.

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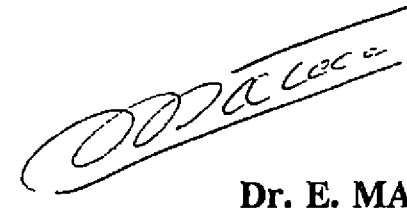
Certified that the thesis, entitled "**OVARIAN RESPONSE TO GONADOTROPHIN RELEASING HORMONE IN NON-CYCLIC GOATS**" is a record of research work done independently by **Dr. K.N. Aravinda Ghosh**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.



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(Dr. K.N. ARAVINDA GHOSH)

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Introduction

1. INTRODUCTION

Goats belong to the earliest group of animals domesticated for food and continue to contribute considerable share of animal protein for human consumption. The distribution of goat population throughout the world is unequal because of the geographical, historical and commercial diversity.

India has a very large and diverse genetic resource of goats. They contribute significantly to natural economy to a tune of 15,210 million rupees, through production of 3.2 per cent of the world milk (1.02 million metric tons), 30.3 per cent of meat (0.37 million metric ton), 8.04 per cent of skin (7600 metric ton) and about 50 metric ton of famous pashmina fibre which is the superior most cashmere in the world (Bhattacharya and Deoghare, 1989). In addition it contribute 8300 metric ton as manure which is of great value for low fertility soil.

In Kerala, goat husbandry is one of the most important livestock enterprise especially among the rural population. Backyard rearing of goats is very common. With the reduction of viable land and with the scarcity of labour, there is great scope to develop goat husbandry into an important rural industry in the state. Recently there has been considerable

revival of interest in goat farming and new research and development initiatives have now been taken up for improving the reproductive efficiency in goat breeding.

Reproductive behaviour of Indian goats varies considerably due to the large number of genetically distinct breeds inhabitating diverse geo-climatic zones. This is further influenced with a wide range of managemental systems associated with climate, tradition, social and economic constraints. The goats are spontaneous ovulating polyoestrous animals. Their cyclic sexual activity varies with geographical distribution. A number of Indian goats exhibit oestrus throughout the years, while others are seasonally polyoestrus.

Maximum reproductive efficiency, prevention of metabolic diseases and a cost efficient production are common goals for profitable goat husbandry in various systems. Nutritional and hormonal imbalances affect reproductive performance of female goats by delaying the onset of puberty and by depressing the regular cyclical ovarian activity.

There are varying reports on the role of minerals in controlling the ovarian activity in goats. Inspite of numerous research efforts, the intricate relationship between the nutrients and reproduction has not been fully understood. Reproductive problems caused by nutritional deficiency are

often complex and diagnosis from soil, plant or animal tissue samples have been inconclusive at times because of interactions, feed back dynamics and absorption interference with genetic adaptations. Infertility due to nutritional causes is usually characterized by a failure of oestrus or cessation of oestrous cycle. All essential minerals seem to be required for reproduction because of their cellular role in metabolism, maintenance and growth. Specific mineral and other nutrient requirements for optimal reproduction in goats have not been fully defined. Significant advancement in this area need to be based on the fundamental understanding of metabolism involving various nutrient elements and the specific role of each nutrient in reproductive organs. Hence it is proposed to investigate on the mineral status of goats during normal ovarian function and during anoestrum.

The oestrous cycle in goats is regulated by endocrine and neuroendocrine mechanisms involving the hypothalamic hormones, gonadotrophins and the steriods secreted by the ovary. Regulation of gonadotropin secretion during the oestrous cycle requires delicate balance between complex hormonal interactions. One of the component known to be having important influence is the Gonadotrophic releasing hormone (GnRH) elaborated from the hypothalamus. Changes in the rate of GnRH synthesis and release, as well as the rate of degradation of this compound, are additional factors that

modify its role in influencing the reproductive activity (O'Conner *et al*., 1984).

It is established that inadequate level or deficiency of GnRH leads to ovarian inactivity and consequent acyclicity in cattle, sheep and goats. It is also understood that the final phase of follicular growth and development are under the control of episodic LH secretion, and the ovarian dysfunction is mostly due to inadequate pattern of episodic LH secretion. Hence it can be inferred that administration of GnRH may be helpful to induce a frequency of LH episodes which may result in ovulation and corpus luteum formation. The success of GnRH therapy led to suggestion that it might be developed as a practical method of overcoming acyclicity in goats. Hence the present investigation is taken up with the object of studying the ovarian response to GnRH in noncyclic goats.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Pattern of oestrous cycle in does

Most of the Indian goats exhibit oestrus throughout the year (Kaura, 1952; Sahani, 1960; Rajkonwar and Borgohain, 1978; Singh and Sengar, 1978; Mazumdar and Mazumdar, 1983) while some are seasonally polyoestrus (Amble *et al.*, 1964; Mishra and Biswas, 1966; Singh and Singh, 1974; Singh *et al.*, 1978; Wani *et al.*, 1980, Singh *et al.*, 1985; Sharma, 1985 and Goel and Agrawal, 1988a). Many of the exotic breeds in the tropical climate, where length of day and night are almost equal, breed throughout the year (Nimo, 1972; Devendra and Burns, 1983; Wilson, 1989). Though goats are polyoestrus, seasonal peak in oestrous behaviour was appreciable. In desi goats of Bihar, the highest number of oestrus was observed during October and lowest during March (Mishra and Biswas, 1966; Singh *et al.*, 1978). Rajkonkar and Borgohain (1978) and Bhadula (1980) reported highest incidence of oestrus during the month of May in local goats of Assam.

Goel and Agrawal (1988b) in their study on Jamnapari, Barbari and Jakhrana does stationed at Mathura, observed that in Jamnapari and Barbari does, oestrous incidence was much higher from May to July (Average 22.57%) and September to October (Average 36.96%) in comparison to the rest of the year

when it was lowest. In Jakhrana does, though a higher incidence was observed in May to June (Average 37.74%) and October to December (Average 37.97%) compared to the rest of the year, the number of animals that exhibited oestrus during the latter period did not fall very low as in Jamnapari and Barbari. The authors concluded that there is a trend of seasonality in oestrous incidence in all the three breeds of goats maintained in North India.

In Malabari crossbred goats of Kerala, oestrus was exhibited throughout the year, a greater peak in July and lesser in November (Mukundan *et al* 1983 and Krishnakumar, 1992).

2.1.1 Length of oestrous cycle

The mean oestrous cycle length normally ranged from 17 to 22 days in Indian breeds (Ali *et al*., 1973; Mittal, 1981; Khan *et al*., 1981; Bhattacharya *et al*., 1981; Sharma, 1985; Mittal, 1988). However, abnormally short cycles were observed early in the breeding season usually associated with prematurely regressing corpus luteum or anovulation (Ott, 1981; Corteel *et al*., 1982; Riera, 1982 and Hafaz, 1987). Similarly, long interoestrous intervals are quite possible with aged animals (Camp *et al*., 1983). Suboestrus or extremely short oestrus are the prime factors for the occurrence of long inter oestrous interval (Van Rensburg, 1971).

Prasad and Bhattacharya (1979) reported an interoestrous interval of 19.18 ± 0.38 days in Barbari nannies. Singh and Sengar (1978) reported that Jamnapari, Beetal and Black Bengal goats exhibited 10 to 40 per cent short cycles, 50 per cent normal cycles and 30 to 40 per cent long cycles. But, Mittal (1981) after a similar study observed that they come into oestrus at an interval of 18.18 ± 0.31 days. The latter author also reported that in Jamnapari goats, the length of oestrous cycle was 17.29 ± 0.73 days. Similar observations were reported in Surti and Marwari goats (Sureshkumar *et al.*, 1988). But according to Setiadi *et al.* (1988) Jamnapari goats have an average interoestrous interval of 20.5 ± 3.6 days (ranged from seven to 28 days).

Mathai (1986) observed a mean oestrous cycle length of 21.174 days in Malabari crossbred goats. Similar oestrous cycle length was reported in native does (Ramachandriah *et al.*, 1986). Interoestrous interval reported was 24.2 ± 1.45 days in local Malvi goats (Qureshi *et al.*, 1991), 21.30 ± 0.28 days in Red Sokoto maradi goats (Pathiraja *et al.*, 1991), 20.01 ± 3.57 days in Guanzhong milch goats (LiJianwen, 1992) and 19.27 ± 1.94 days in Black Bengal goats (Jalaludeen, 1992). In Malabari crossbred goats of Kerala, the length of oestrous cycle was reported as 18 to 23 days (Krishnakumar, 1992).

2.1.2 Duration of oestrus

The duration of oestrus was usually 24 to 48 hours in most of the Indian breeds of goats (Mishra and Biswas, 1966; Sahani and Roy, 1967; Ali *et al.*, 1973; Khan *et al.*, 1978; Mittal, 1981 and 1988; and Goel and Agrawal, 1988b). Duration of oestrus in Pashmina goats was reported as 21 to 51 hours (Bhattacharya *et al.*, 1981), in Surti and Marwari goats as 34.8 to 54.6 and 33.1 to 49.7 hours respectively (Mehta *et al.*, 1991), in Maradi goats as 31.37 ± 0.24 hours (Pathiraja *et al.*, 1991) and in Guanzhong milch goats as 28.34 ± 8.47 hours (LiJianwen, 1992).

In Malabari crossbred goats of Kerala, the duration of oestrous was varying from 12 to 72 hours. Oestrus ceased by 36 hours in 84.8 per cent and by 48 hours in 93.26 per cent does (Krishnakumar, 1992).

Breed variations existed with regard to ovulation rate in goats (Bhattacharyya and Prasad, 1974; Rao and Bhattacharyya, 1980 and Goel and Agrawal, 1991). An ovulation rate of 1.6 each per cycle was reported in Norwagian goats (Lyngst, 1968) and 1.4 per cycle in nulliparous Barbari nannies (Prasad *et al.*, 1980), but a higher ovulation rate of 4.0 was recorded in Angora goats (Rao and Bhattacharyya, 1980). An ovulation rate of 1.68 ± 0.13 was reported in Sokota (Maradi) goats (Pathiraja *et al.*, 1991).

Achuthankutty and Raja (1971) observed that the right ovary was more active than the left ovary in Malabari goats. Similar findings were reported in Jakhrana goats (Goel and Agrawal, 1991). On contradictory, Li Jianwen (1992) reported higher incidence of ovulation in left ovary in Guanzhong milch goats.

2.2 Mineral status in cyclic and non-cyclic does

Adequate feed intake and nutrient requirements have for a long time recognized as important parameters determining the efficiency of goat reproduction. Among this, mineral elements are dietary essentials and they influence many of the metabolic activities of the living tissues, including those of endocrine glands which regulate reproductive function and fertility of animals.

There have been few research data concerning the macro and microelements in goats. The first concerted effort to assemble available information on mineral nutrient requirements was made under the aegis of the National Research Council (NRC, 1981). Updating of goat research concerning mineral elements is warranted to build up data banks that are specific for goats, since most of the earlier publications have mainly extrapolated data on cattle and sheep to apply to goat nutrient requirements in reproduction.

2.2.1 Calcium

Maintenance requirements of calcium in lactating Beetal goats in mid lactation were 0.54 g per kg metabolic weight and 1.16 g in diet per g calcium secreted in milk (Singh and Mudgal, 1987). For Swiss type goats the requirement was 1.25 g calcium per kg milk produced (Skjevdal, 1982) and for African and Asian breeds 0.88 to 2.04 g calcium per kg milk produced (Kessler, 1991). However, hypocalcemia was rarely reported and no cellular or molecular lesion for failure of calcium homeostatis in reproduction of goats have been identified (Reinhardt *et al.*, 1988).

Kessler (1991) reported the normal blood calcium level in Asian goats as 1.9 to 3.3 ± 0.2 nmol/L. Mehta *et al.* (1991) reported serum calcium level in short cycling and nomal cycling Surti goats as 12.81 ± 1.02 and 10.83 ± 1.76 mg per cent and in Marwari goats as 11.86 ± 0.72 and 9.80 ± 0.96 mg per cent, respectively.

Serum calcium level after feeding mineral supplements in lactating Sirohi ewes ranged from 10.02 to 10.5 mg per cent (Singh and Singh, 1992). However, Kumagai and White (1995) showed that mineral supplementation along with feeds had no significant effect on the reproductive performance of Marino ewes.

Nagam *et al.* (1990) reported that the calcium level was lower during the luteal phase, followed by a subsequent decline in early follicular phase in Surti and Marwari goats. However, Esso *et al.* (1990) observed that the stage of oestrous cycle had no significant effect on blood serum concentration of minerals. Similarly, Bhattacharyya *et al.* (1995) observed no significant variation in the calcium level during different stages of reproduction in goats, but the level showed an apparently increasing trend from day of oestrus towards late pregnancy. Blood calcium level recorded on the day of oestrus in goats was 9.40 ± 0.30 µg per cent (Bhattacharyya *et al.*, 1995).

2.2.2 Phosphorus

Anke *et al.* (1987) reported the daily phosphorus requirements of pregnant and lactating Swiss type goats as 3.0 g phosphorus per kg ration dry matter. They observed reduced growth, lowered conception rate and increased incidence of abortion and mortality when goats were fed on ration containing less than 2.0 g phosphorus per kg dry matter. Nagam *et al.* (1990) observed higher serum phosphorus level during luteal phase which decreased during follicular phase in both Surti and Marwari goats.

Schroeder *et al.* (1990) in a study on goats fed with phosphorus deficient diet, opined that the deletion of

phosphorus decreased the equilibrium dissociation constant but not maximum binding capacity of duodenal receptors in lactating Swiss type goats, while plasma calcium and alkaline phosphatase increased. However, Mehta *et al.* (1991) reported serum phosphorus level on the day of oestrus in short cycling and normally cycling Surti goats as 7.08 ± 0.51 and 7.20 ± 0.70 mg per cent, and in Marwari goats as 9.56 ± 1.25 and 9.37 ± 0.79 mg per cent, respectively.

Normal serum phosphorus level in exotic breeds of goats ranged from 1.4 to 2.8 ± 0.3 nmol/litre (Kessler, 1991). A peak serum phosphorus level on the day of oestrus (8.21 ± 0.31 mg per cent) compared to other days of oestrous cycle was reported in indigenous goats of Assam (Bhattacharyya *et al.*, 1995). Bis-wencel (1995) observed that supplementation of mineral mixture containing phosphates for over one year increased the blood inorganic phosphorus level in Saanen goats.

2.2.3 Copper

Underwood (1981) reported that plasma copper concentration in healthy ewes ranged from 9.4 to 23.6 micromol/litre; whereas the copper requirement was 6 ppm per kg dry matter when the molydenum concentration was below 1.5 ppm.

Requirement of copper was greatly influenced by other elements especially molybdenum (Lamand, 1984); and the normal range of 7 to 10 mg copper per kg dry matter was found adequate for goats. However, Humpharies *et al.* (1987) stated that 10 mg copper per kg dry matter was too high for Angora goats which produced toxicities. The mean serum copper level in Shal sheep of Iran was reported as 78.91 ± 9.49 microgram per cent (Zahari and Atminami, 1987). Reproductive problems like anoestrus in a goat herd was reported by Lofstedt *et al.* (1988); the mean serum copper concentration was 0.125 µg per ml in affected animals as compared to 0.45 µg per ml in unaffected ones. Serum copper level of goats of South Africa was reported to be varying at a range of 80 to 160 µg/dl (Niekerk *et al.*, 1990).

Sarker *et al.* (1991) observed that debilitated anaemic goats had significantly lower copper and cobalt levels than the healthy controls. Mehta *et al.* (1991) reported the serum copper level in short cycling and normally cycling Surti goats as 192.75 ± 37.09 mcg per cent and 121.56 ± 17.37 mcg per cent and in Marwari goats as 202.91 ± 21.2 and 214.87 ± 26.12 mcg per cent, respectively. Serum copper level after mineral supplementation was found ranging from 103 to 152 micrograms per cent in Sirohi does (Singh and Singh, 1992).

Haroun *et al.* (1992) observed mean serum copper level below normal (<9.4 micromol per litre) in seven Najdi sheep farm of Central Saudi Arabia affected with reproductive problems. The overall serum copper level in sheep of Semiarid tract of Rajasthan was reported as 82.33 ± 4.05 microgram per cent (Ghosal and Mathur, 1992) and in lactating ewes of Valle Medico, Spain was 79.8 ± 22.0 microgram per cent (Ramos and Fernandez, 1993).

Bhattacharyya *et al.* (1995) observed that the circulating levels of copper was significantly higher on the day of oestrus (2.84 ± 0.06 ppm) than the other stages of oestrous cycle in indigenous goats of Assam. Bis-Wencel (1995) observed that supplementation of copper sulphate along with other minerals significantly increased the serum copper level in Saanen goats.

2.2.4 Cobalt

National Research Council (1981) suggested that cobalt content of small ruminant rations should not exceed above 3 mg per kg dry matter. Studies by Clark *et al.* (1987) revealed that 0.04 mg cobalt per kg dry matter did not suppress daily weight gain in goats. Kessler (1991) suggested that 0.1 mg cobalt per kg dry matter was sufficient to meet the cobalt requirements of goats. However, the upper limit of cobalt tolerance have not been estimated in goats.

Kennedy *et al.* (1990) and Fisher and Mac Pherson (1990) described a specific radioimmunoassay method for estimating serum vitamin B_{12} and methylmalonic acid concentrations for monitoring cobalt level in sheep and goats and concluded that estimation of methylmalonic acid concentration was a more accurate method for diagnosis of cobalt deficiency in sheep and goats. Sarker and Mishra (1991) treated reproductive problems in a goat herd with oral supplementation of copper sulphate and cobalt sulphate for 10 days with remarkable success. Mbura *et al.* (1994) described that serum vitamin B_{12} concentration below 200 g/ml was indicative of cobalt deficiency in goats, exhibited as anoestrum. Supplementation of cobalt chloride along with other minerals significantly increased the blood cobalt level in Saanen goats (Biswecal, 1995).

2.2.5 Manganese

The normal serum manganese level in concentrate supplemented to Sriohi goats ranged from 36 to 48 microgram per cent (Singh and Singh, 1992). Groppel and Anke (1970 and 1975) showed that six mg manganese per kg dry matter induced reproductive problems in a goat herd and recommended a ration over 20 mg manganese per kg dry matter. A manganese level of 40 to 45 mg per kg dry matter was suggested fully adequate for goats (Akinsoyinu, 1985 and Wilkinson and Stark, 1987) for

healthy reproduction. Anke (1987) reported that 5.1 mg manganese per kg dry matter ration was adequate for goats during pregnancy to maintain normal birth weight to its offsprings. Bis-Wencel (1995) observed significant increase in the blood concentration of manganese in Saanen goats by supplementation of manganese chloride along with feed.

2.2.6 Zinc

The serum zinc concentration in sheep of semi-arid tract of Rajastan was found varying from 61.57 ± 3.2 to 77.65 ± 2.1 microgram per ml (Ghosel and Mathur, 1992). Serum zinc level with proper mineral supplementation in lactating Sirohi does varied from 194 to 231 microgram/ml (Singh and Singh, 1992) and concluded that the reproductive status of the lactating does improved with increasing level of concentrate supplementation.

Millet *et al.* (1964) reported that 40 mg zinc per kg dry matter did not induce any deficiency symptoms in goats. However, Chhabra and Arora (1985) showed that 15 mg zinc per kg dry matter resulted in some metabolic disorders in goats. It was seen that these disorders were corrected by 1800 IU vitamin A supplementation. Akinsoyuino (1985) recommended 45 to 50 mg zinc per kg dry matter fully adequate for goats.

Reproductive problems, mostly inactive ovaries, were reported due to zinc deficiency in Angora goats and when zinc sulphate was fed at a rate of 1.0 g per goat daily for seven days, the serum zinc level reached 140 microgram per ml (Rauter *et al.*, 1987). Niekerk *et al.* (1990) reported that serum zinc level less than 80 microgram per dl was indicative of marginal deficiency in sheep and goats. Vergnes *et al.* (1992) correlated the effect of dietary zinc deficiency on plasma inorganic phosphorus, calcium and alkaline phosphatase levels resulting in impaired growth and fertility in Romanov crossbred sheep.

2.3 Progesterone level

2.3.1 Progesterone level in cyclic animals

Serum progesterone level was reported to be varying during different phases of oestrous cycle in ewes and does (Pant *et al.*, 1972; Pathak *et al.*, 1990 and 1992). Stabenfeldt *et al.* (1969) reported that circulating serum progesterone concentration in Dorset-Rambowellet cross bred ewes was lowest on day two (0.1 ng per ml) with a small increase through day five (0.4 ng per ml). Progesterone level rose from 0.6 ng per ml on day six to 1.1 ng per ml on day nine; an abrupt rise was observed on day ten (2.4 ng per ml) and was maintained till day 16. Progesterone level declined

sharply on day 17 to 0.6 ng per ml; this was followed by the occurrence of oestrus within 24 hours. Progesterone level increased from 0.22 ± 0.01 ng/ml during oestrus to peak level of 2.94 to 6.75 ng per ml between day seven and 13 and from day 13 onwards, the level declined, however, progesterone level was basal before the onset of next oestrus in ewes (Pant *et al.*, 1972).

Sarda *et al.* (1973) found that in suffolk ewes, the progesterone level was low (0.1 ± 0.2 ng per ml) during the first three days of the cycle and began to increase on the day four, reaching a first peak on day ten (1.9 to 4.0 ng per ml). There was a decline to 1.0 to 1.8 ng per ml on day 12 or day 13 followed by a second peak on the day 14 or 15. The progesterone level began to decrease rapidly three to four days prior to onset of next oestrus, and attained a level of less than 0.4 ng per ml 24 to 48 hours before the onset of next oestrus. Just before, during and immediately after oestrus the concentration of progesterone was 0.1 to 0.2 ng per ml.

Hecker *et al.* (1974) reported that the progesterone concentration was one to two ng per ml on that day of oestrus and rose to four ng per ml on day four and five in cyclic ewes. They also opined that exogenous progesterone given early on the cycle caused earlier release of prostaglandin

F_2 alpha. Similarly low level of progesterone (0.2 to 0.5 ng per ml) was recorded on the day of oestrus and the level rose to three to four ng per ml on day five. This level was maintained until day 15 of the cycle after which the concentration fell rapidly to less than one ng per ml on day 16th of the oestrous cycle in ewes (Smith, 1975).

The circulating progesterone concentration in ewes was lowest during oestrus and for two days after oestrus (0.25 ± 0.01 ng per ml). A marked rise from day five (1.6 ± 0.14 ng per ml) to a peak of 3.70 ± 0.28 ng per ml between days seven and 13 followed by a decline over the preceeding day to the next oestrus (Pant *et al.*, 1977). Wheeler and Land (1977) studied the luteal function by assessing the progesterone concentration on day seven and 11 of the oestrous cycle. The mean progesterone concentration was 2.16 ± 0.206 ng per ml on day seven and 2.43 ± 0.167 ng per ml on day 11 of oestrous cycle in Merino ewes. Botha and Morgenthil (1980) recorded the plasma progesterone concentration in South African Merino ewes as 0.6 to 0.65 ng per ml during oestrous which increased gradually to 1.0 ng per ml during the first three days and the peak was 5.39 ng per ml on the day 12 of the cycle. From day 14 to 16, the progesterone levels decreased rapidly to reach a low point on the day of oestrus. Scaramezzi *et al.* (1980) stated that progesterone level was 3.1 ± 0.2 ng per ml on day 10 of the oestrous cycle in mountain ewes. Quirke *et al.*

(1981) noticed that the concentrations of progesterone in plasma was 10.5 ng per ml on day 10 of the oestrous cycle and less than 0.5 ng per ml on the day of oestrus in Galway adult ewes. However, serum progesterone concentrations in Yankasa ewes ranged from non-detectable levels on the day of oestrus to a peak of 1.86 ± 0.38 ng per ml at midcycle (Oyedipe *et al.*, 1986).

The blood progesterone level in Nellore deccani ewes averaged 0.31 ± 0.6 ng per ml at oestrus (Reddy *et al.*, 1989), the level rised to a maximum of 3.23 ± 0.19 ng per ml during dioestrus and then decreased to 0.51 ± 0.05 ng per ml after day 14. Similarly, Mugerwa *et al.* (1990) reported that the progesterone concentration was less than 1.0 ng per ml for two days prior and after the onset of oestrus and rose steadily to 5.0 to 5.6 ng per ml on days 10 to 14 of the cycle. A rapid decline was observed from day 15. In a study on crossbreed ewes the serum progesterone concentration was found less than 0.01 ng per ml during oestrus and it ranged from 1.5 to 2.5 ng per ml during day seven to 14 of the cycle (Rhodes and Nathanietz, 1990).

Baruah *et al.* (1987) estimated the average concentration of plasma progesterone in indigenous goats of Assam as 0.47 ± 0.09, 1.81 ± 0.07, 4.63 ± 0.1, 5.56 ± 0.12, 3.82 ± 0.13 and 0.50 ± 0.03 ng per ml on day 1, 4, 8, 12, 16 and 20 of

oestrous cycle, respectively. Plasma progesterone concentrations routinely were less than one ng per ml, beginning to increase from day four to five after the last day of standing oestrus, consistently remained high during the period of diestrum and decreased to less than one ng per ml 24 to 48 hours before the onset of next oestrus. Plasma progesterone concentration in West African Dwarf goats averaged 0.18 ± 0.04 ng/ml on the day of oestrus, 0.81 ± 0.22 and 1.13 ± 0.28 ng/ml on day four and five respectively, and 3.45 ± 0.12 ng/ml during early pregnancy (Akusa *et al.*, 1989). However, Wani (1989) reported that the plasma progesterone concentration in German dwarf breed goats increased from 0.25 ng per ml on day of oestrus to a peak of 10.3 ng/ml on day 12 of the oestrous cycle. Thereafter the values declined and were below 1.0 ng per ml on day 21 of the oestrous cycle.

The circulating levels of progesterone in cyclic Surti and Marwari goats were 0.5 ± 0.11 ng/ml and 0.76 ± 0.16 ng per ml respectively, on the day of oestrus; reached a peak on day nine to 13 of cycle as 2.93 ± 0.44 and 2.25 ± 0.58 ng per ml, respectively (Pathak *et al.*, 1992). El-Hommosy *et al.* (1991) observed lowest progesterone concentration on the day of oestrus as 0.7 ± 0.02 and 0.5 ± 0.4 ng per ml for Baladi and Anglo-Nubian goats, respectively and was highest on day 14 in the Baladis (5.4 ± 0.8 ng per ml) and on the day 10 in Anglo-Nubian goats (3.1 ± 1.5 ng per ml).

Hwang *et al.* (1994) observed a significantly higher serum progesterone level after day 10 of oestrus in Anglo-Nubian x Taiwan goats. The serum progesterone level in crossbred goats was <0.01 ng/ml on the day of oestrus increased to 7.80 ng/ml on day 10 and decreased rapidly during the last three days of the cycle (Sawada *et al.*, 1994). They also observed that plasma concentration of 20-hydroxy progesterone was also low (0.86 ng/ml) on the day of oestrus, increased gradually after oestrus and decreased in the last five days of the cycle. Progesterone concentration in Korean native goats averaged 0.40 ng/ml on the day of oestrus, increased gradually to 4.03 ng per ml by day 14 (Na *et al.*, 1994).

Leyva-Ocariz *et al.* (1995) observed similar progesterone levels in native and crossbred goats in a semi-arid zone of Venezuela, presenting higher values on day 15 and 12 (12 ng ml^{-1} and 10 ng ml^{-1} respectively). Progesterone concentration in both native and crossbred does on day 19 (2.10 ± 1.2 ng ml^{-1} and 1.8 ± 1.0 ng ml^{-1}) indicated that luteal regression had started. A significant difference between day four (2.049 ng/ml^{-1}) and day two in progesterone concentrations was found, indicating that luteal phase was established from day four until day 19.

Both the blood serum and whole milk progesterone levels during the oestrous cycle in thirty breeds of goats were

measured by solid phase radioimmunoassay technique (Oliveria *et al.*, 1992) and observed that the serum progesterone level was at undetectable levels (below 0.1 ng/ml) during oestrus and a peak level of 12.5 ng per ml during dioestrus; the milk progesterone levels were 0.0 and 4.81 ng per ml during early oestrus and dioestrus, respectively.

2.3.2 Progesterone level in non-cyclic animals

The serum progesterone was found significantly lower during non-breeding season, later increased from one month to 15 days prior to onset of breeding season in Rasa Argonesa ewes (Abecia *et al.*, 1996). Similarly, in Kargonuniko ewes, the mean serum progesterone level was very low during anoestrous period, later increased significantly to a higher mean progesterone level of 1.64 ± 0.1 ng per ml during breeding season (Menegotos *et al.*, 1995). On the contrary, Subra (1994) observed significantly higher serum progesterone level in Bakshi anoestrous ewes fed on poor ration compared to those fed with balanced ration (4.78 vs 0.933 ng/ml). A short term increase in progesterone concentrations before the onset of breeding season was reported in exotic breeds of goats (Ott *et al.*, 1980). The progesterone concentrations in plasma of German dwarf breed goats were under 1.0 ng per ml immediately before the onset of breeding season (Wani, 1989). The normal ovarian function was reported to be resumed in Serrana goats

during early breeding season when the progesterone concentration was above 0.5 ng ml^{-1} (Mascarenhas *et al.*, 1995). In anoestrous Angora goats, after exposure to males during early breeding period, Lorenzo *et al.*, (1996) observed a progressive increase in plasma progesterone concentration resulting in a short cycle of seven days in 79 per cent and two short cycles with a peak on day six and 12 in 16 per cent does.

The serum progesterone level in post partum anoestrous does (upto 90 days of kidding) were reported to be as low as 0.03 ng per ml (Traddi and Oliveria, 1992). Similarly, low serum progesterone level (less than one ng per ml) was reported during the first 32 days of kidding in adult Chilean creole goats (Parraquez *et al.*, 1995).

2.4 Induction of oestrus using Gonadotrophic Releasing Hormone

It is well understood that secretion of gonadotrophin is controlled by the neurohumoral substance, Gonadotrophin Releasing Hormone (GnRH). This decapeptide in doses of few nanogrammes is found to stimulate the release of Lutenizing Hormone (LH) and Follicle Stimulating Hormone (FSH) both *in vivo* and *in vitro* (Mauer *et al.*, 1972 and Webb *et al.*, 1977).

Clinical evaluations using synthetic GnRH indicated that it could induce FSH and LH secretion resulting in follicular growth, maturation, ovulation and corpus luteum formation in most of the domestic animals (McCann *et al.*, 1973). GnRH has been used successfully for induction of ovulation in women with secondary amonorrhea (Melvin *et al.*, 1973). Satisfactory results with GnRH or its analogues in inducing ovulation in anoestrous cattle was reported by Mauer *et al.* (1972), Zoldoy and Szenci (1977) and Madhavan and Raja (1983).

It has been suggested that the final phases of follicular growth and development are under the control of episodic LH secretion, and that ovarian inactivity in ewes and does are due to an inadequate pattern of episodic LH secretion (Yuthasastraksol *et al.*, 1977 and Baird, 1978). Similarly, Rahe *et al.* (1980) and Walters and Schallenberger (1984) stated that the increase in episodic secretion of LH primarily controls the final stages of development of preovulatory follicles and ovulation can be induced by administration of GnRH for a prolonged period to artificially induce a frequency of LH episodes (Webb *et al.*, 1977; Riley *et al.*, 1981 and McLeod *et al.*, 1985). This early success of GnRH therapy leads to suggest that it might be developed as practical method of overcoming cyclicity in sheep and goats.

McLeod *et al.* (1982a and b) demonstrated that GnRH induced FSH release could be increased in relation to LH by using low doses of GnRH and oestradiol 17 alpha pretreatment in ewes. It was shown that intermittant injection or continuous administration of low doses of GnRH in acyclic (anoestrous) ewes consistently induced normal ovulation (McLeod *et al.*, 1983) and that these induced ovulations were associated with normal pattern of gonadotrophin secretions and when the ewes were subjected to a period of progesterone treatment, the resultant corpora lutea were also functionally normal (McLeod and Haresign, 1984).

When ewes were subjected to continuous infusion of GnRH, mean LH concentrations were elevated for only 48 to 72 hours (Wright *et al.*, 1983) and that this short period of gonadotrophic stimulation was sufficient to fully develop preovulatory follicles in cyclic ewes. They also observed that both repeated injections and continuous infusions of GnRH resulted in high degree of synchrony in the timing of the pre-ovulatory LH peak and in the onset of oestrus in ewes. However, Rodway and Swift (1985) observed that a single dose of highly potent LHRH agonist was unable to produce normal luteal function or conception while pregnant mare serum gonadotrophin (PMSG) treated ewes exhibited oestrus, and few conceived.

It was observed that the mean FSH level was elevated for the first 2 hours of GnRH administration, but thereafter it declined progressively suggesting that the pattern of FSH secretion associated with GnRH induced ovulation was similar to that observed in naturally cycling ewes (Mc Leod and Haresign, 1984). Khalid *et al.* (1991a,b) studied the role of oestradiol in the regulation of pituitary FSH receptors by continuous infusion of GnRH in anoestrous ewes and established that an interaction between GnRH and oestradiol was necessary for an increase of pituitary GnRH receptor content in anoestrous ewes. Robin *et al.* (1994), however, observed that intramuscular administration of GnRH in lactating goats primed with progestagen was not as effective in regulating reproductive performance during anoestrus as were infusions of PMSG. PMSG treatment induced a preovulatory LH peak in a greater number of goats and a higher pregnancy rate than GnRH treated animals. Similarly, Ramos Mesa *et al.* (1995) treated crossbred anoestrous goats with progestagen impregnated sponge for nine days followed by GnRH, but observed no signs of increased serum progesterone in any of the ewes following GnRH treatment.

Beard and Hunter (1996) treated 15 anoestrous ewes with multiple injection of GnRH (250 μg i/v) followed by a bolus injection of GnRH (125 μg i/v) among which five were pre-treated with progesterone and 10 ewes received

progesterone 24 hours after the bolus injection. They found that in ewes pretreated with progesterone had normal luteal function. None of the ewes treated with progesterone after injection of GnRH had normal luteal phase. They inferred that a transient increase in progesterone prior to ovulation was associated with luteal phase of normal duration and that extended exposure to progesterone at about the time of ovulation prevents normal luteal phase and may result in short cycles.

However, Haresign *et al.* (1996) were successful in inducing oestrus in anoestrous ewes using a single intramuscular injection of progesterone followed by GnRH or by administration of intravaginal progesterone sponge for seven days followed by GnRH treatment, and observed high incidence of normal luteal function in intravaginal progesterone sponge primed GnRH treated ewes.

In anoestrous goats, oestrus can be induced by a treatment combining a vaginal sponge impregnated with a progestogen, followed by an intramuscular injection of PMSG approximately at sponge removal (Corteel, 1977; Patel *et al.*, 1984; Tamanini *et al.*, 1985; Goswamy *et al.*, 1989; Pathak *et al.*, 1992; Guven *et al.*, 1993; Robinson and Scaramuzzi, 1994 and Menegotos *et al.*, 1995). Progestagen-primed anoestrous goats treated with PMSG gave a satisfactory kidding

rate of 50 per cent (Corteel, 1975) and 65 per cent (Bretzlaff and Madrid, 1989). However, Corteel (1977) reported that progestagen sponges followed by PMSG treatment were generally less effective during the lactational peak. Dairy goats primed with a progestagen and treated with multiple intravenous injections of GnRH at the lactational peak presented kidding rates comparable to those obtained with PMSG after the lactational peak (Knight *et al.*, 1988). Cameron *et al.* (1988) showed that the administration of PMSG may lead to a biphasic response in goats such that upto several follicles may ovulate prematurely, even while the goats were receiving progestagen treatment through intravaginal sponges, with a large number of follicles ovulating approximately two days after the withdrawal of sponges.

GnRH has been used instead of PMSG to induce folicular maturation, ovulation and corpus luteum formation in anoestrous does (Bretzleff *et al.*, 1991). Moreover, GnRH seems to cause neither superovulation nor an immune response. However, multiple intravenous injections of GnRH were not practical for on farm use, and pharmacological doses of GnRH or its analogues injected intramuscularly would be more practical and effective.

In most of the earlier studies with GnRH, synthetic GnRH having identical structure to the natural molecule was used. In recent years, the potent GnRH agonist, Buserelin (D.Ser (But)6-LHRH-(1-9) nonapeptide ethylamide) has been used in several experiments (Von Rechenberg *et al.*, 1986; Lincoln, 1987 and McLeod *et al.*, 1991). The response to Buserelin treatment has persisted for a longer period than that associated with other synthetic GnRH (Von Rechenberg *et al.*, 1986).

Investigations in rats, rabbits, hamster and guinea pigs also proved that GnRH analogue, Buserelin possesses a considerable higher endocrine activity than natural GnRH. While GnRH consisted of 10 amino acids (decapeptide), the active principle of Buserelin contains only nine (nonapeptide). Pharmacologic studies utilizing both biological methods and radioactive labelling have confirmed that the action of buserelin was slightly more protracted than that of GnRH. This was explained to the delayed enzymatic breakdown of the nonapeptide (Product information, 1995, Hoechst India Pvt. Ltd., Pune).

2.5 Biometry of pituitary, ovary and tubular tract in GnRH treated does

The rate of follicular growth, maturation, ovulation and formation of corpus luteum depends on the level of pituitary

gonadotropins. Haresign *et al.* (1973) reported that in ewes given single intravenous injection of 150 μg or 300 μg GnRH during anoestrus and slaughtered four days after injection, there was a significant increase in the number of follicles, sized more than four mm mean diameter, but not in the number of follicles of smaller size. With 100 μg and 500 μg GnRH injected intramuscularly on the day of oestrus, greater follicular development during oestrous cycle has been observed by Bindon *et al.* (1971) and Bindon (1975). Foote and Hulet (1976) reported nonsignificant influence of progesterone on size or number of unovulated follicles.

McLeod and Haresign (1984) induced fertile oestrus in anoestrous ewes with low doses of GnRH and assessed the ovarian response by laproscopic studies. In anoestrous ewes prior to GnRH treatment, ovaries showed little or no evidence of follicular development. But after four days of GnRH treatment, all the ewes had ovulated and the resultant corpora lutea appeared macroscopically normal. the mean ovulation rate was 1.67 ± 0.13.

Administration of GnRH analogue, Buserelin resulted in an increase in body weight gain, early onset of puberty and a higher number of ovarian follicles in growing kids (Mathai, 1984). Growth of ovarian tissue and tubular genitalia were also higher in GnRH treated ones than the controls.

Singh and Madan (1986) treated 12, 20, 16 and 12 adult ewes with LHRH, PGF_2 alpha, progesterone and normal saline, respectively. There was increase in uterine weight in ewes treated with LHRH, PGF_2 alpha and progesterone, but the difference were non-significant. The thickness of body of uterus treated with PGF_2 alpha and progesterone was significantly higher than that of the controls. The ewes treated with PGF_2 alpha had significantly greater number of ovarian follicles than that in the other groups.

Khalid *et al.* (1991a) observed that in ewes slaughtered after 12 and 24 hours of GnRH infusion had a significantly higher pituitary GnRH binding content compared with those slaughtered after 94 hours of GnRH infusion and the controls. It was inferred that GnRH administration increased both LH concentration and GnRH binding and that the failure to maintain the pituitary responses thereafter was associated with a decrease in pituitary GnRH receptor content. However, no significant difference in the growth and number of follicles in goats treated with human chorionic gonadotrophin (HCG) six h after the onset of oestrus was observed (Dutta *et al.*, 1995).

2.6 Tissue enzymes

Reports on the correlation between endocrine and enzyme activities in pituitary, ovary and tubular genitalia were scanty in goats. However, it is known that significant change in enzymatic activity due to influence of sex steroids depends upon metabolic need for bringing about a specific biochemical change in the tissues (Singh and Madan, 1986).

2.6.1 Acid phosphatase (ACP)

Zamari (1980) and Roy and Saigal (1987) observed low ACP level in the luminal epithelium and endometrial glands in anoestrous ewes, but the level was found increasing from early pregnancy to mid pregnancy. The activity of ACP was very strong in maternal septae and cryptal epithelium and lowest in other tissue components of uterus. Bhattacharya and Saigal (1990) observed acid phosphatase activity highest on the lutein cell, followed by theca interna and interstitial cells in normally cycling goats.

Mathai and Nirmalan (1992a) observed no significant variation in the ACP level in serum, ovarian and uterine tissue during different phases of oestrous cycle in crossbred Malabari goats. Sureshkumar and Janakiraman (1993) observed that the level of ACP in ovarian tissue vary significantly during different phases of oestrous cycle, the activity was

higher on day two in the active ovary as compared with inactive ovaries.

2.6.2 Alkaline phosphatase (ALP)

Warnick and Walliace (1960), Goode *et al.* (1965) and Akino *et al.* (1969) reported higher ALP activity in the ovary and uterus during mid-luteal phase and lower activity at ovulation and late luteal phase in ewes. Dutta *et al.* (1968) reported a two fold increase in ALP activity in cervix during early dioestrum. Very low ALP level was reported in the vagina of anoestrous ewes (Ramachandriah *et al.*, 1978). A sudden increase in vaginal ALP activity from day three of oestrous cycle was reported in buffaloes (Rangaswamiah, 1981) and in ewes (Rao *et al.*, 1987).

Roy and Saigal (1987) observed intense ALP activity in the perinuclear area of endometrial surface and glandular epithelia and low ALP activity in the perimetrium, myometrium and endometrial stroma during early pregnancy in ewes. Patel *et al.* (1992) noted that the level of ALP was low upto day 20 of gestation period but later on tended to increase upto term in Surti and Marwari goats.

Mbassa and Paulson (1991) observed low serum ALP activity in adult cycling dwarf and Landrace goats (11.1 ± 2.4 microkat/L and 14.1 ± 9.4 microkat/L, respectively) compared

to young kids of the same breeds (18.3 ± 11.0 microkat/L and 37.6 ± 23.6 microkat/L respectively). Serum ALP during day one, five, nine, 13 and 17 of oestrous cycle in Surti goats was 16.18, 16.10, 21.88, 16.63 and 18.73 KAU per cent and in normally cycling Marwari goats was 20.48, 22.38, 20.95, 20.68 and 21.08 KAU per cent, respectively (Mehta *et al.*, 1991). Jaiswal and Mehta (1992) observed lowest ALP activity of 2.98 ± 0.84, 2.28 ± 0.45 and 1.76 ± 0.57 KAU per g of tissue in the uterus, cervix and vagina, respectively, on the day of ovulation in Marwari goats. ALP activity increased sharply from day three, reached peak (14.30 ± 1.66, 4.02 ± 0.59 and 4.99 ± 1.52 KAU per g of tissue, respectively) on day nine, and later declined from day 15 (5.02 ± 0.66, 3.78 ± 0.62 and 1.80 ± 1.03 KAU per g of tissue, respectively).

Mathai (1992 a&b) reported that the serum and ovarian ALP did not vary significantly during different phases of oestrous cycle in goats, however, the values were higher on days 14 and 18 of oestrous cycle. Further it was observed that the level of ALP in uterine tissues did not vary significantly. Sureshkumar and Janakiraman (1993) reported that the ALP in ovarian tissues vary significantly during different phases of oestrous cycle, the activity was significantly higher on day two in the active ovary as compared to inactive ovary. The normal serum ALP level in adult cycling Black bengal goats was recorded as 3.06 to 4.77 IU/dl (Behera *et al.*, 1993). Sharma

et al. (1995) observed no significant variation in the ALP activity in the ovarian tissues of Assam goats treated with HCG, HCG+PMSG, medoxyprogestrone + PMSG + HCG and control animals.

2.6.3 Lactic dehydrogenase (LDH)

Reports on the LDH level in the tissues of pituitary, ovary and uterus of goats were scanty. The LDH activity was reported to be the highest in chorionic epithelium in the placentome of cows (Friess and DeBarios, 1971). Roy and Saigal (1987) reported that the strongest LDH activity was observed in the perinuclear areas of the cryptal epithelium of uterus and was absent in perimetrium and endometrial stroma in pregnant ewes. The normal serum LDH level in adult cycling Black Bengal goats was 16.87 ± 0.83 to 25.62 ± 0.61 IU/dl, the level varied significantly between different age groups (Behera *et al.*, 1993).

Meghed *et al.* (1996) observed that the LDH activity in the oviductal washings of Egyptian buffaloes increased significantly during the follicular phase of oestrous cycle, the level being very low in animals with inactive ovaries.

Material and Methods

3. MATERIAL AND METHODS

The object of the study was to evaluate the effect of GnRH administration on ovarian activity and its usefulness in restoring normal cycles in adult non-cyclic goats.

Sixty healthy Malabari, Malabari x Saanen and Malabari x Alpine does aged one to four years, with a record of one or more kiddings and 45 days post-partum belonging to Goat Farm of Kerala Agricultural University, Mannuthy, Trichur were initially selected for the study. These animals were maintained in the farm under ideal conditions of feeding and management throughout the period of study. the study was conducted during the period 1993-96. These does were closely observed for a period of 60 days for the occurrence of oestrus. During this period, serum samples were collected from all the does for the estimation of minerals. From among these, eight cyclic does were included in Group I and sixteen non-cyclic does were included in Group II and III (eight each) at random.

3.1 Oestrous cycle

Heat detection among the does was performed at eight hour interval using vasectomized bucks. The animals in oestrus, detected by vasectomized bucks, were identified and subjected

to detailed gynaeco-clinical examinations. Observations on the duration of oestrus and intensity of oestrums were recorded as per the method followed by Mathai and Raja (1978). The period in between the onset and end of oestrous sign was considered as the duration of oestrus. The time interval between the initiation of two such successive oestruses detected was considered as the length of oestrous cycle.

3.2 Estimation of minerals

Approximately 20 ml of blood was collected at 7 AM from the jugular vein of all the 60 (25 cyclic and 35 non-cyclic) does at fortnightly intervals and the samples were allowed to clot for the separation of serum. The clotted blood was kept in a refrigerator for 24 hours. The clear serum was collected in aliquots and stored in a labelled air tight container at 0°C.

3.2.1 Estimation of calcium

Serum calcium was estimated by 0-cresolphthalein complexone method using calcium kit in photocolorimetor (Spectronic-20, Bosch and Lomb). 0.02 ml of serum (T), 0.02 ml of standard (S) and 0.02 ml of distilled water (Blank B) provided in the kit were taken separately and mixed individually with 2 ml each of buffer solution and colour reagent.

This was mixed well and allowed for absorbance of standard ($\frac{S}{B}$) and test (T) against Blank (B) on Spectronic-20 at 570 mm within 30 minutes.

Serum calcium level in mg per cent was calculated by applying the following formula:

$$\text{Serum calcium in mg per cent} = \frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times 10$$

3.2.2 Estimation of inorganic phosphorus

Inorganic phosphorus level in the serum was estimated using phosphorus kits (Stangen immunodiagnostics, Hyderabad) employing modified metol method (Morin and Prox, 1973).

Various reagents and serum were pipetted into clean, dry test tubes and labelled in three groups, Blank, standard and Test as per the following protocol.

Reagents	Blank (ml)	Standard (ml)	Test (ml)
Catalyst reagent	1.0	1.0	1.0
Molybdate reagent	1.0	1.0	1.0
Deionized water	0.1	-	-
Standard phosphorus solution	-	0.1	-
Serum	-	-	0.1
Metol reagent	1.0	1.0	1.0

The sample and reagents were mixed well and allowed to stand for 5 mts at room temperature. Deabsorbance values of test and standard were measured against Blank in Spectronic-20 at 680 nm within 30 mts.

Serum inorganic phosphorus level in mg per cent was calculated by applying the following formula.

$$\text{Serum inorganic phosphorus in mg\%} = \frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times 5$$

3.2.3 Estimation of microelements

Copper, cobalt, manganese and zinc level in the serum were estimated by Atomic Absorption Spectophotometry using a Perkin-Elmer 2380 model atomic absorption spectrophotometer (AAS).

3.2.3.1 Estimation of copper

As per the dilution rate recommended by the manufactures of the atomic absorption spectrophotometer (Weinstock and Uhlemann, 1981), the serum sample was diluted making up one ml of serum to two ml using deionized water.

Solution containing copper in 10 per cent aqueous glycerol was used as the standard for which 0.4 ml of copper stock standard solution containing 1000 ppm copper was made

upto volume with 10 per cent aqueous glycerol in a 100 ml volumetric flask.

The ten per cent aqueous solution of glycerol in deionized water was used as blank.

3.2.3.2 Estimation of cobalt

Undiluted serum as such was used for the estimation of cobalt

Working standard for cobalt estimation was prepared by making up 0.35 ml of stock standard solution containing 1000 ppm of cobalt in a 100 ml volumetric flask using 20 per cent aqueous glycerol, to yield a 3.5 ppm solution.

A 20 per cent aqueous solution of glycerol was used as the blank.

3.2.3.3 Estimation of zinc

As per the recommendations of the manufactures of AAS (Makino and Takahara, 1981) one millilitre of serum was diluted with four ml of deionized water.

Solution containing 1.0 ppm zinc in five per cent aqueous glycerol was prepared by making up 0.1 ml of stock standard

solution of zinc containing 1000 ppm zinc upto the mark using five per cent aqueous glycerol in a 100 ml volumetric flask.

The five per cent aqueous glycerol solution in deionized water used for the dilution of standard was used as the blank.

3.2.3.4 Estimation of manganese

Whole undiluted serum as such was used for manganese estimation.

The two ppm solution of manganese used as the working standard was obtained by making up 0.2 ml of stock standard solution of manganese containing 1000 ppm of manganese to 100 ml using 20 per cent aqueous glycerol in 100 ml volumetric flank.

The 20 per cent aqueous glycerol solution (the one used for preparation of manganese working standard) was used as the blank.

3.2.3.5 Conditions used for the operation of AAS

The AAS was set for operation as per the recommendations of the instrument manufactures (Perkin Elmer). The standard conditions for atomic absorption of various elements studied were furnished below.

Item	Copper	Cobalt	Zinc	Manganese
Wave length (nm)	324.8	279.5	213.9	279.5
Stat SEW (nm)	0.7	0.7	0.7	0.7
Flame gases*	A.AC	A.AC	A.AC	A.AC
Lamp hollow cathode	Copper	Cobalt	Zinc	Manganese
Lamp current	15 mA 25 mA	30 mA 40 mA	15 mA 20 mA	28 mA 30 mA
Time (seconds)**	0.2	0.2	0.2	0.2
Average	5	5	5	5

* Air - Acetylene

** Reading taken at every 0.2 seconds and 5 such readings averaged

3.2.3.6 General procedure for estimation of microelements

After setting the instrument to required specifications it was standardized using a reagent blank and the recommended working standard solution for each element. The instrument was them set to register a reading every 0.2 seconds and to display the average of five such readings. The working samples were then aspirated into the flame and the concentrations in ppm displayed directly were recorded. Between samples deionized water was aspirated to clean up the nebulizer system. Concentration of each element in the original sample was then computed using necessary dilution factors.

3.3 Progesterone estimation in cyclic and non-cyclic does

Approximately three ml of blood was collected from eight cyclic does (Group I) by jugular venipuncture using a dispossible 18 gauge hypodermic needle and syringe into clean, sterilized plain glass tubes on day one (first day of oestrus), four, six, ten, 14 and 18 of two consecutive oestrous cycles. Similarly, blood samples were collected from 16 noncyclic (Group II and III) does at day one and thereafter at weekly intervals on day eight, 15 and 22 for estimation of progesterone.

The serum was separated and stored in air-tight containers at -20°C until the progesterone level was estimated.

Progesterone concentration in the serum was estimated by Radio Immuno Assay (RIA), using a commercially available Coat-A-count progesterone kit (Diagnostic Products Corporation, Los Angles, California) at Radio Tracer Laboratory, Kerala Agricultural University. It is a solid phase RIA designed for the direct, quantitative measurement of progesterone in serum or plasma (Kubasik, 1984). A 100-tube kit for progesterone estimation contained 185 kBq (kilobecquerels) of radioactive ^{125}I labelled progesterone.

3.3.1 Principle

The Coat-A-count progesterone procedure is a solid-phase radioimmunoassay wherein ^{125}I-labelled progesterone competes with progesterone in the test sample for antibody sites for a fixed time. Because the antibody is immobilized to the wall of a polypropylene tube, simply decounting the supernatent suffices to terminate the competition and to isolate the antibody bound fraction of the radio labelled progesterone. Counting the tube in a gamma counter then yields a number, which converts by way of a calibration curve to a measure of progesterone present in the serum sample Progesterone determination by the Coat-A count method require neither extraction nor predilution.

3.3.2 Procedure

All the components in the progesterone assay kit were kept at room temperature.

Four plain (12x75 mm) polypropylene tubes were labelled for total counts (T) and non-specific binding (NSB) in duplicate. Fourteen progesterone Ab-coated tubes A (maximum binding) and B through G were labelled in duplicate. Additionally, antibody coated tubes were labelled in duplicate for controls and test samples.

Initially 100 microlitre of the zero calibrator A was pipetted into the NSB and A tubes and 100 microlitre of each of the calibraters B through G was added into corresponding labelled tubes. To every tube 0.1 ml of ^{125}I labelled progesterone was added. The samples were mixed well in a vortex mixer and incubated for three hours at room temperature. Later the tubes were decanted throughly using a foam decanting rack and allowed them to drain for two to three minutes.

Then the radioactivity was determined in a single channel gamma spectrometer (Electronic Corporation of India, Hydrabad).

3.3.3 Calculation

The average NSB-coated counts per minute (CPM) was first calculated for each pair of tubes.

Net counts = Average CPM minus Average NSB CPM

The binding of each pair of tubes as per cent of maximum binding (MB) was determined, with the NSB corrected counts of the A tubes taken as 100 per cent.

Per cent bound = (Net counts/Net MB counts) x 100

Using the logit-log graph paper provided in the kit, the per cent bound was plotted on the vertical axis against concentration on the horizontal axis for each of the calibrators B through G. Then a straight line was drawn approximating the path of these points. Progesterone concentration in the serum samples were estimated from this line by interpolation.

3.4 Gonadotrophic releasing hormone analogue administration in noncyclic does

A single dose of a potent GnRH analogue, RECEPTAL-VET (Hoechst Roussel Vet India Ltd., Pune) one millilitre was administered intramuscularly to all the eight noncyclic does in the Group II. Receptal (vet) is a synthetic gonadotrophic releasing hormone containing 0.0042 mg of buserelin acetate equivalent to 0.004 mg of buserelin per ml and 10 mg benzyl alcohol as antimicrobial additive. These animals were regularly followed up at eight hour intervals for observing the signs of oestrus using vasectomized teasor bucks. Those does which did not exhibit oestrus within 20 days of administration of Receptal were repeated with the same dose intramuscularly on the 21st day.

3.4.1 Observations on induced oestrous cycle

Time of onset of oestrus after GnRH administration, duration and intensity of induced oestrus and oestrous cycle length were recorded.

3.4.2 Progesterone and mineral estimation during induced oestrous cycle

Approximately 20 ml of blood was collected on day one, four, six, 10, 14 and 18 of induced oestrous cycle from four GnRH responded does. The progesterone and minerals, calcium, phosphorus, copper, cobalt, manganese and zinc were estimated by methods discussed in 3.3 and 3.4.

3.5 Slaughter studies on GnRH responded and noncyclic controls

Three does in Group II which responded to initial dose of GnRH were slaughtered on day 18 of induced cycle. From Group III, three non-cyclic does without treatment were selected at random and slaughtered. Immediately after slaughter pituitary, both ovaries, uterus and cervix of these animals were collected. Detailed biometry and weight of these organs were recorded. Ovaries were closely observed for presence of follicles and corpora lutea. The follicles were classified on the basis of their size as small (S) with less than 3 mm diameter, medium (M) with 3 to 6 mm and large (L) above 6 mm diameter. A score point of 1, 2 and 3 were marked for small, medium and large follicles, respectively.

3.6 Estimation of tissue enzymes

Approximately 500 mg of tissue from pituitary, left ovary, right ovary and uterus from ~~at~~ the ~~size~~ slaughtered animals were collected, weighed and its homogenate was prepared in a Potter Elvehjem type homogenizer using ice cold double distilled water. The final volume of the homogenate was made upto 50 ml. ACP, ALP and LDH level were estimated by spectrophotometry.

3.6.1 Estimation of acid phosphatase

A standard diagnostic reagent kit (Randox Laboratories Ltd., N. Ireland) was used for determination of acid phosphatase level using a modified king's method (King and Jagatheesan, 1959) by spectrophotometry.

Initially the spectrophotometer was adjusted to a wave length of Hg 405 nm with cuvette 1 cm light path at 37°C. The p-nitrophenyl phosphate (substrate) was reconstituted with citrate buffer. The absorbance of the sample against the kit reagent blank was recorded.

The total acid phosphatase activity in the tissues were calculated using the following formula.

$$\text{Total acid phosphatase in units/g of tissue} = \frac{101 \times \text{absorbance of sample} \times \text{dilution rate at } 37^{\circ}C}{\text{Weight of tissue in g}}$$

3.6.2 Estimation of alkaline phosphatase

The ALP enzyme activity in the tissues were estimated by a modified Kind and King's method (Verley, 1975) using a standard diagnostic kit (Randox Laboratories Ltd., N. Ireland).

Absorbance of the sample against blank was estimated at a wave length of Hg 405 nm, cuvette one cm light path of 37°C in a spectrophotometer. The ALP activity was calculated using the formula.

$$\text{Alkaline phosphatase (units/g of tissue} = \frac{3300 \times \text{absorbance nm/mt} \times \text{dilution rate}}{\text{Weight of tissue in g}}$$

3.6.3 Estimation of lactic dehydrogenase (LDH)

LDH level in the tissues of pituitary, ovaries and uterus of GnRH responded and non-cyclic controls were estimated using a standard diagnostic reagent kit supplied by Randox Laboratories Ltd., N. Ireland.

The absorbance in the test sample and blank were recorded in spectrophotometer adjusted at 365 nm wave length, one cm light path cuvette at 30°C. The LDH activity was calculated using the formula.

$$\text{LDH units/g of tissue} = \frac{9118 \times \text{absorbance 365 nm/mt} \times \text{dilution rate}}{\text{Weight of tissue in g}}$$

3.7 Analysis of data

For statistical analysis, arithmetic mean, standard deviation and standard error were calculated for each characteristics (Snedecor and Cochran, 1987). Normal deviation test (Z test) and chi-square tests were used to find the significant influence between parameters. Bivariate tables, statistical graphs and diagrams were also used to describe the nature of data.

Results

4. RESULTS

4.1 Pattern of oestrous cycle in Group I

The pattern of three consecutive oestrous cycle studied in eight cyclic does were tabulated in Table 1.

4.1.1 Length of oestrous cycle

The mean length of oestrous cycle I, II and III in cyclic does were 20.375 ± 1.495, 20.250 ± 1.854 and 20.000 ± 1.000 days, respectively. The overall mean oestrous cycle length was 20.313 ± 1.553 days.

4.1.2 Duration of oestrus

The mean duration of oestrus for the three consecutive cycles were 39.000 ± 9.950, 40.500 ± 9.836 and 33.000 ± 7.937 hours, respectively. The overall mean duration of oestrus for the three cycles was 37.500 ± 7.263 hours.

4.1.3 Oestrus signs

The oestrus were classified as mild, moderate and intense on the basis of the intensity of oestrus exhibited. The occurrence of mild, moderate and intense oestrus out of 24 oestruses observed in three consecutive cycles of eight

cyclic does were one (4.17%), nine (37.5%) and 14 (58.33%), respectively.

4.2 Mineral status in cyclic and non-cyclic does

The mean serum levels of macroelements, calcium and phosphorus and microelements copper, cobalt, manganese and zinc in cyclic and noncyclic does were shown in Table 2.

The mean serum calcium level in cyclic does was 9.410 ± 0.770 mg per cent and that of non-cyclic does was 9.22 ± 0.830 mg per cent. Eventhough the serum calcium level was higher in cyclic does, the difference was not sátistically significant.

The mean serum phosphorus level in cyclic does was 4.800 ± 0.260 mg per cent and in non cyclic does 4.770 ± 0.280 mg per cent. There was no significant difference in the levels of phosphorus between cyclic and non cyclic does.

The mean serum copper level in cyclic does was 1.160 ± 0.170 ppm and that of non cyclic does was 0.830 ± 0.110 ppm. On analysis there was highly significant ($P<0.01$) difference between serum copper levels in cyclic and non cyclic does.

The mean cobalt content was 0.066 ± 0.016 ppm in cyclic does as compared to 0.071 ± 0.010 ppm in non-cyclic does. The difference was observed to be statistically non-significant.

Cyclic does showed a mean manganese concentration of 0.023 ± 0.005 ppm. Non cyclic does showed a mean level of 0.030 ± 0.010 ppm. No significant difference in manganese content was observed between the two groups.

The serum zinc level was significantly lower ($P<0.05$) in cyclic does (1.180 ± 0.120 ppm) as compared to non cyclic does (1.510 ± 0.430 ppm).

The serum calcium, phosphorus, copper, cobalt and zinc in cyclic does between days showed no significant difference (Table 3). But serum manganese level showed significant difference ($P<0.05$) between days 15 and 30 and between 15 and 60.

In non cyclic does, there was no significant difference in the serum calcium, phosphorus, copper and zinc level between the days of sample collection (Table 4). But both serum cobalt and manganese levels showed significant difference between days 1 and 30 and 1 and 45.

4.3 Serum progesterone level

Serum progesterone levels were estimated in eight cyclic and sixteen non cyclic does using radioimmunoassay.

4.3.1 Serum progesterone level in cyclic does

The level of serum progesterone estimated in eight cyclic does on day one, four, six, ten, 14 and 18 of oestrous cycle I and II were tabulated in Table 5 and 6 and Fig.1.

The mean serum progesterone levels during the first oestrous cycle were 0.306 ± 0.111, 1.163 ± 0.406, 2.363 ± 0.745, 3.463 ± 1.062, 2.588 ± 0.670 and 0.978 ± 0.249 ng/ml on day one, four, six, ten, 14 and 18 respectively. The overall mean progesterone level for the oestrous cycle I was 1.810 ± 0.332 ng/ml (Table 5)

During oestrous cycle II, the mean serum progesterone level were 0.304 ± 0.078, 1.400 ± 0.415, 2.725 ± 0.857, 3.800 ± 0.808, 2.325 ± 0.721 and 0.763 ± 0.311 ng/ml on day one, four, six, ten, 14 and 18 respectively (Table 6). The overall mean progesterone level was 1.887 ± 0.339 ng/ml for the oestrous cycle II.

Comparison of mean serum progesterone level values between different days of first and second oestrous cycle showed significant variation except between day four and 18, six and 14 and 10 and 14 during oestrous cycle I and between day 6 and 14 during oestrous cycle II (Table 7).

The mean serum progesterone level between the same days of oestrous cycle I and II showed no significant difference (Table 8).

The mean serum progesterone concentration for the two consecutive oestrous cycle were 0.304 ± 0.087, 1.294 ± 0.382, 2.531 ± 0.758, 3.619 ± 0.794, 2.456 ± 0.430 and 0.871 ± 0.246 ng/ml on day one, four, six, ten, 14 and 18, respectively. The overall mean progesterone level for the two consecutive cycle was 1.848 ± 0.339 ng/ml (Table 9 and Fig.2).

4.3.2 Serum progesterone level in non-cyclic does

The mean serum progesterone level estimated on day one, eight, 15 and 22 in 16 non-cyclic does were 0.189 ± 0.111, 0.186 ± 0.107, 0.191 ± 0.109 and 0.189 ± 0.106 ng/ml, respectively (Fig.3). The overall mean serum progesterone in non-cyclic does was 0.190 ± 0.106 ng/ml (Table 10).

Comparison of mean values showed that there was no statistically significant variation between different days in the serum progesterone level in non-cyclic does (Table 11).

4.4 Response to GnRH administration

Out of eight non-cyclic does (Group II) treated with GnRH analogue (Buserelin), three (37.5%) responded to single dose,

one (12.5%) responded to second dose and four did not respond at all. The oestrous was exhibited at a mean of 87.000 ± 9.950 hours after the intramuscular administration of GnRH.

4.4.1 Pattern of oestrous cycle in GnRH responded does

The pattern of oestrous cycle in GnRH responded does were shown in Table 12.

4.4.1.1 Length of oestrous cycle

The mean length of oestrous cycle in GnRH responded does was 12.750 ± 0.830 days (Table 12) as compared to a higher value of 20.313 ± 1.553 days in cyclic does (Table 1). The mean length of oestrous cycle was significantly lower ($P<0.01$) in GnRH responded does as compared to cyclic does (Table 13).

4.4.1.2 Duration of oestrus

The mean duration of oestrous in GnRH responded does was 18.000 ± 4.240 hours (Table 12) as compared to 37.50 ± 7.263 hours in cyclic does (Table 1). The mean duration of oestrus in GnRH responded does was significantly lower ($P<0.01$) as compared to that in cyclic does (Table 13).

4.4.1.3 Oestrous signs

Out of four does which responded to GnRH analogue administration, three exhibited mild and one exhibited moderate oestrous signs (Table 12) whereas in cyclic does the intensity of oestrus was much higher.

4.4.2 Mineral status in GnRH responded does

There was no significant variation in the serum mineral status on different days of induced oestrous cycle, except for serum phosphorus (Table 14). The serum phosphorus level in GnRH responded does between day one and six and between one and 18 of induced oestrous cycle showed significant ($P<0.05$) difference.

The mean serum calcium level in GnRH responded does was 9.625 ± 0.390 mg per cent. There was no significant difference in the serum calcium level between GnRH responded and cyclic does (Table 15) and between GnRH responded and non-cyclic does (Table 16 and Fig.4).

The mean serum phosphorus level was found significantly higher ($P<0.01$) in GnRH responded does (5.375 ± 0.205 mg per cent) as compared to cyclic does (4.800 ± 0.260 mg per cent) and non-cyclic does (4.770 ± 0.280) (Table 15, 16 and Fig.5).

In GnRH responded does, a mean serum copper level of 1.123 ± 0.089 ppm was observed. Comparison of mean copper level showed no significant difference between GnRH responded and cyclic does (Table 15). But there was significantly higher ($P<0.01$) serum copper level in GnRH responded as compared to non-cyclic does (Table 16 and Fig.6).

The mean serum cobalt level in GnRH responded does was 0.063 ± 0.006 ppm (Table 15). Comparison of mean serum cobalt level between GnRH responded and cyclic does and with that of non-cyclic does showed no significant difference (Table 16 and Fig.7).

In GnRH responded does, mean serum manganese level of 0.025 ± 0.005 ppm was observed. The mean serum manganese level was found not significantly varying between cyclic and GnRH responded does (Table 15) and between non-cyclic and GnRH responded does (Table 16 and Fig.8).

The mean serum zinc level in GnRH responded does was found not significantly varying in comparison to cyclic (Table 15) and non-cyclic (Table 16 and Fig.9) does.

4.4.3 Serum progesterone level in GnRH responded does

The mean serum progesterone levels in four GnRH responded does on day one, four, six, ten, 14 and 18 of induced cycle

were 0.158 ± 0.026, 0.800 ± 0.177, 1.475 ± 0.334, 0.675 ± 0.238, 0.280 ± 0.030 and 0.120 ± 0.021 mg/ml, respectively (Fig.10). The overall mean serum progesterone level was 0.585 ± 0.139 mg/ml (Table 17).

Between different days of induced oestrous cycle, there was highly significant difference ($P<0.01$) in the serum progesterone except between day one and 18 and four and 10. There was significant difference ($P<0.05$) between day one and 18 but no significant difference between days four and 10 (Table 18).

Comparison of mean serum progesterone on different days of oestrous cycle between cyclic and GnRH responded does showed significant variation (Table 19). There was highly significant lower ($P<0.01$) serum progesterone levels on day one, ten, 14 and 18 and significantly lower ($P<0.05$) serum progesterone level on day 4 and 6 of induced cycle in GnRH responded does as compared to cyclic does.

The comparison of mean serum progesterone level in GnRH responded and cyclic does were represented in Fig.11 and Fig.12.

The overall mean serum progesterone level during induced oestrous cycle in GnRH responded does was 0.585 ± 0.139 ng/ml; which was significantly lower ($P<0.01$) as compared to the

overall mean progesterone level of normally cyclic does (1.848 ± 0.339 ng/ml) but significantly higher ($P<0.01$) as compared to non-cyclic does (0.190 ± 0.106 ng/ml) prior to treatment (Table 20).

4.5 Biometry of non-cyclic and GnRH responded does

For detailed study of ovarian response to GnRH administration, three non-cyclic does from Group II which responded to GnRH treatment and three non-cyclic does without treatment from Group III were slaughtered and immediately after slaughter pituitary, ovary and genitalia were collected for detailed biometry studies.

4.5.1 Biometry of pituitary and ovary

The detailed biometry of pituitary and ovary of non-cyclic and GnRH responded does were tabulated in Table 21 and 22.

The mean pituitary weight in GnRH responded does was 1.267 ± 0.055 g compared to a mean weight of 1.180 ± 0.108 g in non-cyclic does without GnRH treatments. The mean pituitary weight in GnRH responded does was higher compared to non-cyclic does but the difference was not statistically significant.

The mean weight of right and left ovary in GnRH responded does was 1.033 ± 0.065 and 1.168 ± 0.090 g and in non-cyclic does was 0.811 ± 0.116 and 0.918 ± 0.123 g, respectively. Though a slightly higher mean ovarian weight was noticed in GnRH responded does, no statistically significant difference was observed.

In three non-cyclic controls, the number of follicles in the right ovary was five (mean 1.667 ± 0.470), in left ovary was two (mean 0.667 ± 0.470) and the total number of follicles was seven (mean 2.333 ± 0.471).

In three GnRH responded does, the number of follicles in right and left ovary were 10 (mean 3.333 ± 0.200) and seven (mean 2.660 ± 0.745) respectively, the total number of follicles in both ovary together being 17 (mean 5.67 ± 0.943) Table 22.

All the seven follicles present in non-cyclic does were small (<3mm size) and obtained a score point of seven (mean 2.333 ± 0.471). But in GnRH responded does out of total 17 follicles, nine small, six medium (size between 3 to 6 mm) and two large (>6 mm size) were observed and a total score point of 27 (mean 8.667 ± 2.494) was obtained (Table 23). The mean number and size of follicles in the GnRH responded does was significantly higher ($P<0.01$) as compared to non-cyclic controls (Table 24).

A total of four small sized regressing corpora lutea were observed in three GnRH responded does but none in non-cyclic controls.

4.5.2 Biometry of uterus and cervix

The detailed biometry of uterus and cervix of three non-cyclic does and three GnRH responded does slaughtered were tabulated in Table 25 and 26.

The mean weight of uterus in non-cyclic does was 15.570 ± 1.089 and that in GnRH responded does was 21.933 ± 1.685 g. Though an increased weight of uterus was observed in GnRH responded does, no statistically significant difference was observed.

The mean length and thickness of body of uterus in non-cyclic controls was 40.000 ± 1.633 and 7.000 ± 0.477 mm and in GnRH responded does was 49.667 ± 6.233 and 8.667 ± 0.465 mm, respectively. Though the length and thickness of body of uterus was higher in GnRH responded does, no significant difference was however noticed.

The mean length and thickness of left and right uterine horns of non-cyclic does were 65.667 ± 3.298, 7.333 ± 0.477, 68.333 ± 11.027 and 7.333 ± 0.477 mm, respectively and that in GnRH responded does were 70.667 ± 4.779, 7.667 ± 0.466,

81.000 ± 6.481 and 8.000 ± 0.816 mm, respectively. The length and thickness of left and right uterine horns were higher in GnRH responded does, however no significant difference was observed.

The mean weight of cervix in non-cyclic and GnRH responded does were 5.983 ± 0.487 and 7.940 ± 0.553 g, respectively. There was no significant difference in weight of cervix between the two groups, eventhough an increased weight was observed in GnRH responded does.

The mean length and thickness of cervix of non-cyclic does was 32.667 ± 7.132 and 7.333 ± 0.477 and that in GnRH responded does was 46.333 ± 4.194 and 8.333 ± 0.477 mm, respectively. However, no significant difference in the length and thickness of cervix was observed between the two groups.

4.6 Tissue enzymes

Tissues of pituitary, ovary and uterus were collected from three non-cyclic controls and three GnRH responded does immediately after slaughter and tissue enzymes were estimated.

4.6.1 Acid phosphatase (ACP)

The mean ACP level in the tissues of pituitary, ovary and uterus of non-cyclic and GnRH responded does were presented in Table 27.

The mean ACP level in the pituitary of non-cyclic and GnRH responded does were 2.663 ± 0.203 and 3.852 ± 0.444 units/g of tissue, respectively. There was higher mean ACP level in GnRH responded does as compared to non-cyclic does, though statistically no significant difference was observed.

The mean ACP level in right and left ovary in non-cyclic controls were 2.240 ± 0.511 and 2.815 ± 0.576 and that in GnRH responded does were 3.490 ± 0.433, 2.014 ± 0.433 units/g, respectively (Table 30).

There was no significant differences in the ACP level between right and left ovary in non-cyclic does and between right and left ovaries in GnRH responded does (Table 31). Similarly, there was no significant difference in ACP level between right ovaries and between left ovaries of non-cyclic and GnRH responded does (Table 31). The mean ACP level in both ovaries together in non-cyclic and GnRH responded does were 5.055 ± 0.505 and 5.504 ± 0.479 units/g, respectively, but showed no statistically significant difference.

The mean ACP level in the uterus of non-cyclic and GnRH responded does were 1.903 ± 0.442 and 3.329 ± 0.208 units/g, respectively. No significant difference was observed between the two groups, though higher values were observed in GnRH responded does.

4.6.2 Alkaline phosphotase (ALP)

The mean ALP level in the tissues of pituitary, ovary and uterus in non-cyclic and GnRH responded does were tabulated in Table 28.

The mean ALP level in the pituitary of non-cyclic and GnRH responded does were 8.098 ± 2.958 and 8.509 ± 1.557 units/g, respectively. Though an increased ACP level was observed in GnRH responded does, no statistically significant difference was observed.

The mean ALP level in right and left ovary in non-cyclic were 28.730 ± 2.758 and 23.352 ± 7.809 units/g and that in GnRH responded does were 35.273 ± 8.068 and 25.167 ± 2.027 units/g, respectively (Table 30). There was no statistically significant difference in ALP level between right and left ovaries in non-cyclic and GnRH responded does and between right ovaries and between left ovaries of non-cyclic and GnRH responded does.

The mean ALP level in both ovaries together in non-cyclic and GnRH responded does were 52.082 ± 3.778 and 60.440 ± 4.268 units/g, respectively (Table 30). Though the total ALP of both ovaries was higher in GnRH responded as compared to non-cyclic does, no significant difference was observed (Table 31).

The mean ALP level in uterine tissue of non-cyclic and GnRH responded does were 26.497 ± 14.965 and 24.233 ± 6.310 units/g, respectively. No significant difference in uterine ALP was observed between the two groups.

4.6.3 Lactic dehydrogenase (LDH)

The mean tissue LDH level in pituitary, ovary and uterus of non-cyclic and GnRH responded does were tabulated in Table 29.

The mean LDH in the tissues of pituitary of non-cyclic and GnRH responded does were 86.253 ± 18.204 and 107.436 ± 20.538 units/g, respectively. There was higher mean LDH level in GnRH responded does as compared to non-cyclic does, but the difference was not significant.

The mean LDH in right and left ovary in non-cyclic controls were 25.185 ± 4.669 and 31.386 ± 10.005 and in GnRH responded does were 43.708 ± 3.984 and 41.964 ± 7.762 units/g,

respectively. There was no significant difference in the mean tissue LDH level between right and left ovary of non-cyclic and GnRH responded does. But, there was significant difference ($P<0.01$) in LDH level between the right ovary of non-cyclic and the right ovary of GnRH responded does (Table 31) but not between left ovaries.

The mean LDH level in both ovaries together in non-cyclic and GnRH responded does were 59.571 ± 7.385 and 85.672 ± 4.289 units/g, respectively (Table 30). This was significantly higher ($P<0.01$) in GnRH responded does as compared to non-cyclic controls (Table 31).

The mean LDH level in the uterus of non-cyclic and GnRH responded does were 41.252 ± 7.864 and 44.798 ± 17.884 units/g, respectively (Table 29). There was no significant difference in the LDH level in the uterus of non-cyclic and GnRH responded does.

Table 1. Pattern of oestrous cycle in cyclic does

Sl. No.	Oestrous cycle I			Oestrous cycle II			Oestrous cycle III		
	Duration of oestrus (h)	Intensity of oestrus	Oestrous cycle length (d)	Duration of oestrus (h)	Intensity of oestrus	Oestrous cycle length (d)	Duration of oestrus (h)	Intensity of oestrus	Oestrous cycle length (d)
1.	36	moderate	21	42	moderate	20	24	moderate	21
2.	48	intense	22	36	intense	21	36	intense	20
3.	48	intense	18	48	intense	18	48	moderate	19
4.	36	moderate	22	42	moderate	20	36	intense	21
5.	24	intense	21	24	intense	23	36	moderate	20
6.	48	intense	21	60	intense	22	36	mild	20
7.	48	moderate	18	36	intense	17	24	intense	18
8.	24	intense	20	36	intense	21	24	moderate	21
Total	312.000		163.000	324.000		162.000	264.000		160.000
Mean	39.000		20.375	40.500		20.250	33.000		20.000
S.D.	9.950		1.495	9.836		1.854	7.937		1.000

Oestrus signs: intense 14 Nos.
moderate 9 Nos.
mild 1 No.
Total 24 Nos.

Overall mean duration of oestrus 37.500
S.D. (h) 7.263

Overall mean length of oestrus cycle 20.313
S.D. (d) 1.553

Table 2. Comparison of serum mineral status between cyclic and non-cyclic does

Sl. No.	Mineral	Cyclic (25 Nos)	Non-cyclic (35 Nos)	Z value
1.	Calcium (mg%)	9.410± 0.770	9.220± 0.830	0.6026
2.	Phosphorus (mg%)	4.800± 0.260	4.770± 0.286	0.2584
3.	Copper (ppm)	1.160± 0.170	0.830± 0.110	6.4195**
4.	Cobalt (ppm)	0.066± 0.016	0.071± 0.010	0.4827
5.	Manganese (ppm)	0.023± 0.005	0.030± 0.010	1.3524
6.	Zinc (ppm)	1.180± 0.120	1.510± 0.430	2.3113*

* $P<0.05$
** $P<0.01$

Table 3a. Mean serum mineral status on different days in 25 cyclic does

Mineral	1st day	15th day	30th day	45th day	60th day
1. Calcium (mg%)	9.460± 0.720	9.570± 0.610	9.630± 0.650	8.730± 0.750	9.720± 0.450
2. Phosphorus (mg%)	4.660± 0.160	4.850± 0.250	4.800± 0.220	4.820± 0.290	4.910± 0.270
3. Copper (ppm)	1.080± 0.150	1.170± 0.160	1.150± 0.170	1.180± 0.160	1.190± 0.170
4. Cobalt (ppm)	0.063± 0.016	0.065± 0.019	0.067± 0.015	0.060± 0.019	0.066± 0.021
5. Manganese (ppm)	0.025± 0.005	0.020± 0.010	0.027± 0.005	0.024± 0.005	0.025± 0.010
6. Zinc (ppm)	1.260± 0.120	1.180± 0.110	1.150± 0.150	1.130± 0.050	1.180± 0.090

Table 3b. Z value comparison of serum mineral status between different days in 25 cyclic does

Sl. No.	Z value table comparison						
	Days	Calcium	Phos-phorus	Copper	Cobalt	Manga-nese	Zinc
1.	1&15	0.2288	0.5758	0.4082	0.0266	0.1055	0.3464
2.	1&30	0.9965	0.5635	0.8501	0.5412	0.4396	1.4149
3.	1&45	1.3703	0.7232	0.9946	0.8654	0.2180	1.6721
4.	1&60	1.4493	0.8822	1.5900	0.3927	0.0000	1.0943
5.	15&30	1.2292	0.1869	0.3657	0.2652	2.3320*	0.4881
6.	15&45	0.1146	0.8272	0.2271	0.6078	1.3074	0.8135
7.	15&60	0.2233	0.4769	0.4701	0.1284	2.2299*	0.0000
8.	30&45	0.3536	0.8916	0.1514	0.3912	0.8055	0.3536
9.	30&60	0.4585	0.3054	0.8619	0.1390	0.6476	0.6000
10.	45&60	0.1146	0.9827	0.7338	0.5058	0.3180	1.0000

* $P<0.05$

Table 4a. Mean serum mineral status on different days in 35 non-cyclic does

Mineral	1st day	15th day	30th day	45th day	60th day
1. Calcium (mg%)	9.160± 0.710	9.350± 0.670	9.130± 0.500	9.190± 0.520	9.240± 0.720
2. Phosphorus (mg%)	9.830± 0.180	4.850± 0.280	4.780± 0.150	4.680± 0.140	4.700± 0.210
3. Copper (ppm)	0.860± 0.110	0.800± 0.090	0.840± 0.100	0.830± 0.110	0.800± 0.150
4. Cobalt (ppm)	0.061± 0.010	0.064± 0.015	0.066± 0.010	0.066± 0.015	0.067± 0.020
5. Manganese (ppm)	0.020± 0.005	0.020± 0.010	0.020± 0.005	0.030± 0.005	0.026± 0.010
6. Zinc (ppm)	1.720± 0.110	1.540± 0.230	1.390± 0.250	1.470± 0.310	1.400± 0.180

Table 4b. Z value comparison of serum mineral status between different days in non-cyclic does

Sl. No.	Z value table comparison						
	Days	Calcium	Phos-phorus	Copper	Cobalt	Manga-nese	Zinc
1.	1&15	0.5058	0.0894	0.3717	0.0000	0.0000	0.5176
2.	1&30	0.1080	0.4823	0.5180	2.2404*	3.2404*	0.8278
3.	1&45	0.0748	1.4813	0.4404	2.2404*	2.2404*	0.6661
4.	1&60	0.2506	1.2778	1.1735	1.2404	1.2404	0.8206
5.	15&30	0.7777	0.6431	0.7478	1.2404	1.2404	1.0976
6.	15&45	0.6168	1.6665	0.6424	1.2404	1.2404	0.7267
7.	15&60	0.4050	1.4603	0.2794	1.2404	1.2404	1.2878
8.	30&45	0.1916	0.8038	0.0000	0.0266	1.3074	0.5481
9.	30&60	0.3661	0.6362	0.5588	0.0266	1.3074	0.0790
10.	45&60	0.1904	0.1825	0.4647	0.0266	1.3074	0.6171

* $P<0.05$

Table 5. Serum progesterone level (ng/ml) in cyclic does during oestrous cycle I

Sl. No.	Days of oestrus cycle						
	Day 1	Day 4	Day 6	Day 10	Day 14	Day 18	Average
1.	0.25	1.50	2.50	3.60	3.00	1.00	1.96
2.	0.21	1.00	2.70	3.80	2.10	0.42	1.70
3.	0.98	1.40	3.40	4.60	2.80	0.95	2.22
4.	0.50	1.20	1.90	2.80	2.20	0.85	1.58
5.	0.42	0.90	1.70	1.80	1.80	1.10	1.29
6.	0.38	0.50	1.00	2.20	1.90	1.20	1.20
7.	0.19	0.90	2.50	3.80	3.00	1.00	1.90
8.	0.32	1.90	3.20	5.10	3.90	1.30	2.95
Total	2.450	9.300	18.900	27.700	20.700	7.820	14.480
Mean	0.306	1.163	2.363	3.463	2.588	0.978	1.810
S.D.	0.111	0.406	0.745	1.062	0.670	0.249	0.332

Table 6. Serum progesterone level (ng/ml) in cyclic does during oestrous cycle II

Sl. No.	Days of oestrus cycle						
	Day 1	Day 4	Day 6	Day 10	Day 14	Day 18	Average
1.	0.20	1.20	2.70	3.80	3.10	0.80	1.97
2.	0.22	1.10	3.00	4.40	3.40	0.62	1.90
3.	0.23	1.60	3.90	4.70	1.10	0.52	2.01
4.	0.42	1.40	2.30	3.20	1.90	0.62	1.64
5.	0.36	1.00	1.90	2.50	2.00	0.90	1.56
6.	0.30	0.90	1.80	4.60	3.00	1.20	1.97
7.	0.30	1.80	2.00	2.80	2.00	0.24	1.52
8.	0.40	2.20	4.20	4.40	2.10	1.20	2.42
Total	2.430	11.200	21.800	30.400	18.600	6.100	15.096
Mean	0.304	1.400	2.725	3.800	2.325	0.763	1.887
S.D.	0.078	0.415	0.857	0.808	0.721	0.311	0.339

Table 7. Z value comparison of mean serum progesteron level between different days of oestrus cycle I and II

Sl. No.	Day comparison	Oestrus cycle I (Z value)	Oestrus cycle II (Z value)
1.	1&4	5.3870 **	6.8671 **
2.	1&6	7.2254 **	7.4434 **
3.	1&10	7.8224 **	11.3940 **
4.	1&14	8.8902 **	7.3732 **
5.	1&18	6.5217 **	3.7875 **
6.	4&6	3.7420 **	3.6816 **
7.	4&10	5.3522 **	6.9905 **
8.	4&14	4.8125 **	2.9418 **
9.	4&18	1.0277 NS	3.2498 **
10.	6&10	2.9435 **	2.9147 **
11.	6&14	0.5941 NS	0.9450 NS
12.	6&18	4.6650 **	5.6938 **
13.	10&14	1.8436 NS	3.6037 **
14.	10&18	6.0274 **	9.2808 **
15.	14&18	5.9595 **	5.2631 **

* $P<0.05$
** $P<0.01$
NS - Not significant

Table 8. Comparison of mean serum progesterone level between oestrous cycle I and II

Sl. No.	Day of oestrous cycle	Mean progesterone level (ng/ml)		Z value
		Oestrous cycle I	Oestrous cycle II	
1.	1	0.306± 0.111	0.304± 0.078	0.0393
2.	4	1.163± 0.406	1.400± 0.415	1.0800
3.	6	2.363± 0.745	2.725± 0.857	0.8434
4.	10	3.463± 1.062	3.800± 0.808	0.6682
5.	14	2.588± 0.670	2.325± 0.808	0.7070
6.	18	0.978± 0.249	0.763± 0.311	1.4278

Table 9. Mean serum progesterone level (ng/ml) of two consecutive cycles in cyclic does

Sl. No.	Days of oestrus cycle						
	Day 1	Day 4	Day 6	Day 10	Day 14	Day 18	Average
1.	0.225	1.450	2.600	3.700	3.050	0.900	1.97
2.	0.215	1.050	2.850	4.100	2.750	0.520	1.91
3.	0.205	1.500	3.550	4.650	1.950	0.740	2.12
4.	0.460	1.300	2.100	3.000	2.050	0.735	1.61
5.	0.380	0.950	1.800	2.150	1.900	1.000	1.37
6.	0.340	0.700	1.400	3.400	2.450	1.200	1.58
7.	0.245	1.350	2.250	3.300	2.500	0.620	1.71
8.	0.360	2.050	3.700	4.650	3.000	1.250	2.52
Total	2.430	10.350	20.250	28.950	19.650	6.925	17.790
Mean	0.304	1.294	2.531	3.619	2.456	0.871	1.848
S.D.	0.087	0.382	0.758	0.794	0.430	0.246	0.339

Table 10. Serum progesterone level (ng/ml) in non-cyclic does

Sl. No.	Days of oestrus cycle				
	1 day	8 day	15 day	22 day	Average
1.	0.10	0.11	0.14	0.12	0.12
2.	0.18	0.10	0.10	0.13	0.13
3.	0.10	0.10	0.14	0.11	0.11
4.	0.32	0.34	0.29	0.32	0.32
5.	0.22	0.20	0.25	0.22	0.22
6.	0.42	0.40	0.38	0.40	0.40
7.	0.10	0.10	0.10	0.10	0.10
8.	0.21	0.19	0.24	0.21	0.21
9.	0.10	0.10	0.11	0.11	0.11
10.	0.12	0.10	0.10	0.10	0.11
11.	0.21	0.29	0.30	0.27	0.27
12.	0.45	0.40	0.45	0.43	0.43
13.	0.16	0.10	0.12	0.13	0.13
14.	0.10	0.14	0.16	0.12	0.15
15.	0.14	0.19	0.11	0.15	0.15
16.	0.10	0.12	0.10	0.11	0.11
Total	3.030	2.980	3.060	3.030	3.040
Mean	0.189	0.186	0.191	0.189	0.190
S.D.	0.111	0.107	0.109	0.106	0.106

Table 11. Z value comparison of mean serum progesterone at weekly intervals in non-cyclic does

Sl. No.	Day comparison	Z value
1.	1 and 8	0.0337
2.	1 and 15	0.0223
3.	1 and 22	0.0000
4.	8 and 15	0.0567
5.	8 and 22	0.0345
6.	15 and 22	0.0228

Table 12. Pattern of oestrus and oestrous cycle in four GnRH responded does

Sl. No. *	Onset of oestrus after GnRH administration (hrs)	Duration of oestrus (hrs)	Oestrus signs	Length of induced oestrous cycle** (days)
1.	72	24	mild	13
2.	84	18	moderate	12
3.	96	18	mild	12
4.	96	12	mild	14
Total	348.000	72.000	-	51.000
Mean	87.000	18.000	-	12.750
S.D.	9.950	4.240	-	0.830

* Sl.No. 1-3 responded to single dose and Sl.No. 4 responded to second dose

** Length of induced oestrous cycle assessed by progesterone estimation

Table 13. Comparison of duration of oestrus and length of oestrous cycle in cyclic and GnRH responded does

Sl. No.	Oestrous cycle pattern	Cyclic (25 Nos)	GnRH responded (4 Nos)	Z value
1.	Mean duration of oestrus (hrs)	37.500± 7.263	18.000± 4.240	8.3160*
2.	Mean length of oestrous cycle (days)	20.313± 1.553	12.750± 0.830	4.3306**

* P<0.05
** P<0.01

Table 14. Z value comparison of mineral status between different days of induced oestrous cycle in GnRH responded does

Sl. No.	Z value table comparison						
	Days	Calcium	Phos-phorus	Copper	Cobalt	Manga-nese	Zinc
1.	1&6	0.1391	2.0948*	0.3090	0.3757	0.5410	0.4411
2.	1&14	0.1513	1.6951	0.1478	0.0000	1.0311	0.4667
3.	1&18	0.1427	2.0113*	0.1485	0.3464	1.2990	0.6243
4.	6&14	0.3248	0.3724	0.2137	0.3757	1.7321	0.0000
5.	6&18	0.3038	0.2832	0.5502	0.6518	1.7321	0.0000
6.	14&18	0.0000	0.1357	0.3601	0.3464	0.0000	0.0000

* $P<0.05$
** $P<0.01$

Table 15. Comparison of mineral status in cyclic and GnRH responded does

Sl. No.	Mineral	Cyclic (25Nos)	GnRH responded (4Nos)	Z value
1.	Calcium (mg%)	9.410± 0.770	9.625± 0.390	0.4932
2.	Phosphorus (mg%)	4.800± 0.260	5.375± 0.205	3.6148**
3.	Copper (ppm)	1.160± 0.170	1.123± 0.089	0.3693
4.	Cobalt (ppm)	0.066± 0.016	0.063± 0.006	0.3920
5.	Manganese (ppm)	0.023± 0.005	0.025± 0.005	0.6651
6.	Zinc (ppm)	1.180± 0.120	1.155± 0.091	0.3222

** $P<0.01$

Table 16. Comparison of mineral status in non-cyclic and GnRH responded does

Sl. No.	Mineral	Non-cyclic	GnRH responded	Z value
1.	Calcium (mg%)	9.220± 0.830	9.625± 0.390	0.9283
2.	Phosphorus (mg%)	4.770± 0.280	5.375± 0.205	4.0014**
3.	Copper (ppm)	0.830± 0.110	1.123± 0.089	4.9599**
4.	Cobalt (ppm)	0.071± 0.010	0.063± 0.006	0.5723
5.	Manganese (ppm)	0.030± 0.010	0.025± 0.005	0.3869
6.	Zinc (ppm)	1.510± 0.430	1.155± 0.091	1.5850

** P<0.01

↓

At what stage of the cycle ?

Table 17. Serum progesterone level (ng/ml) during induced oëstrous cycle in GnRH responded does

Sl. No.	Days of oestrus cycle						
	Day 1	Day 4	Day 6	Day 10	Day 14	Day 18	Average
1.	0.150	0.950	1.300	0.500	0.120	0.130	0.525
2.	0.200	0.850	2.000	0.800	0.100	0.100	0.675
3.	0.160	0.900	1.500	1.000	0.800	0.150	0.752
4.	0.120	0.500	1.100	0.400	0.100	0.100	0.387
Total	0.630	3.200	5.900	2.700	1.120	0.480	2.339
Mean	0.158	0.800	1.4875	0.675	0.280	0.120	0.585
S.D.	0.026	0.177	0.334	0.238	0.030	0.021	0.139

Table 18. Comparison of serum progesterone level between different days of oestrous cycle in GnRH responded does

Sl. No.	Day	Mean progesterone level	Day	Mean progesterone level	Z value
1.	1	0.158±0.260	4	0.800±0.177	6.2157**
2.			6	1.475±0.334	6.8091**
3			10	0.675±0.238	3.7402**
4.			14	0.280±0.030	5.3228**
5.			18	0.120±0.021	1.9693*
6.	4	0.800±0.177	6	1.475±0.334	3.0929**
7.			10	0.675±0.238	0.7300NS
8.			14	0.280±0.030	5.0170**
9.			18	0.120±0.021	6.6079**
10.	6	1.475±0.334	10	0.675±0.238	3.3786**
11.			14	0.280±0.030	6.1722**
12.			18	0.120±0.021	7.0129**
13.	10	0.675±0.238	14	0.280±0.030	2.8521**
14.			18	0.120±0.021	4.0234**
15.	14	0.280±0.030	18	0.120±0.021	7.5677**

* P<0.05
** P<0.01
NS - Not significant

Table 19. Comparison of mean serum progesterone on different days of oestrous cycle between cyclic and GnRH responded does

Sl. No.	Day	Mean serum progesterone level		Z value
		Cyclic	GnRH responded	
1.	1	0.304 ± 0.087	0.158 ± 0.026	2.994**
2.	4	1.294 ± 0.382	0.800 ± 0.177	2.444*
3.	6	2.531 ± 0.758	1.475 ± 0.334	2.427*
4.	10	3.619 ± 0.794	0.675 ± 0.238	6.624**
5.	14	2.456 ± 0.430	0.280 ± 0.030	8.281**
6.	18	0.870 ± 0.246	0.120 ± 0.021	5.533**

* $P<0.05$
** $P<0.01$

Table 20. Comparison of overall mean progesterone level between non-cyclic, cyclic and GnRH responded does

Sl. No.	Mean serum progesterone level (ng/ml)			Z value
	Non-cyclic	Cyclic	GnRH responded	
1.	0.190±0.106	1.848±0.339	-	17.131**
2.	0.190±0.106	-	0.585±0.139	5.892**
3.	-	1.848±0.339	0.585±0.139	6.537**

** P<0.01

Table 21. Biometry of pituitary and ovaries of non-cyclic does

Sl. No.	Pituitary weight (gms)	Right ovary			Left ovary		
		Weight (gms)	No.of follicles	No.of corpus luteum	Weight (gms)	No.of follicles	No.of corpus luteum
1.	1.265	0.930	2	nil	0.755	1	nil
2.	1.145	0.852	2	nil	1.050	0	nil
3.	1.200	0.650	1	nil	0.950	1	nil
Total	3.55	2.432	5.000	0.00	2.755	2.000	0.00
Mean	1.18	0.811	1.667	0.00	0.918	0.667	0.00
S.D.	0.108	0.166	0.470	0.00	0.123	0.470	0.00

Table 22. Biometry of pituitary and ovaries of GnRH responded does

Sl. No.	Pituitary weight (gms)	Right ovary			Left ovary		
		Weight (gms)	No.of follicles	No.of corpus luteum	Weight (gms)	No.of follicles	No.of corpus luteum
1.	1.350	0.950	5	1	1.250	2	1
2.	1.250	1.050	3	0	1.205	2	1
3.	1.200	1.100	2	0	1.050	3	1
Total	3.750	3.100	10.0	1.00	3.505	7.000	3.00
Mean	1.267	1.033	3.33	0.333	1.168	2.660	1.00
S.D.	0.055	0.065	0.20	0.472	0.090	0.745	0.00

Table 23. Correlation between the number and size of follicles in non-cyclic and GnRH responded does

Sl. No.	Non-cyclic					GnRH responded				
	Total number and size of follicles of right and left ovary					Total number and size of follicles of right and left ovary				
	S	M	L	Total	Score point	S	M	L	Total	Score point
1.	3	-	-	3	3	3	2	2	7	13
2.	2	-	-	2	2	3	2	-	5	7
3.	2	-	-	2	2	3	2	-	5	7
Total	7	-	-	7	7	9	6	2	17	27
Mean	2.333	-	-	2.333	2.333	3.000	2.000	0.667	5.667	8.667
S.D.	0.471	-	-	0.471	0.471	0.000	0.000	0.000	0.943	2.494

S = Small follicles below 3 mm (1 score point)
M = Medium follicles between 3-6 mm (2 score points)
L = Large follicles above 6 mm (3 score points)

Table 24. Z value comparison of mean number and size of follicles in the ovaries of non-cyclic and GnRH responded does

Sl. No.	Follicle parameter	Z value
1.	Number of follicles	3.528**
2.	Size of follicles	4.720**

** P<0.01

Table 25. Biometry of uterus and cervix of non-cyclic does

Sl. No.	Uterus							Cervix		
	Weight (gms)	Body of uterus		Left horn of uterus		Right horn of uterus		Weight (gms)	Length (mm)	Thickness (mm)
		Length (mm)	Thickness (mm)	Length (mm)	Thickness (mm)	Length (mm)	Thickness (mm)			
1.	15.75	40.0	7.0	70.0	7.0	82.0	7.0	6.650	35.0	8.0
2.	16.805	38.0	7.0	65.0	8.0	68.0	8.0	5.780	40.0	7.0
3.	14.155	42.0	8.0	62.0	7.0	55.0	7.0	5.520	23.0	7.0
Total	46.710	120.000	22.000	197.000	22.000	205.000	22.000	17.950	98.000	22.000
Mean	15.570	40.000	7.000	65.667	7.333	68.333	7.333	5.983	32.667	7.333
S.D.	1.089	1.633	0.477	3.298	0.477	11.027	0.477	0.487	7.132	0.477

Table 26. Biometry of uterus and cervix of GnRH responded does

Sl. No.	Uterus						Cervix		
	Weight (gms)	Body of uterus		Left horn of uterus		Right horn of uterus		Weight (gms)	Length (mm)	Thickness (mm)
		Length (mm)	Thickness (mm)	Length (mm)	Thickness (mm)	Length (mm)	Thickness (mm)			
1.	20.140	43.0	9.0	73.0	8.0	75.0	8.0	7.250	42.0	8.0
2.	21.480	48.0	8.0	64.0	7.0	78.0	7.0	8.605	45.0	8.0
3.	24.180	58.0	9.0	75.0	8.0	90.0	9.0	7.965	52.0	9.0
Total	65.800	149.000	26.000	212.000	23.000	243.000	24.000	23.820	139.000	25.000
Mean	21.933	49.667	8.667	70.667	7.667	81.000	8.000	7.940	46.333	8.333
S.D.	1.685	6.233	0.465	4.779	0.466	6.481	0.816	0.553	4.194	0.477

Table 27. Acid phosphatase level (units/g of tissue) in non-cyclic and GnRH responded does

Sl. No.	Non-cyclic controls				GnRH responded does			
	Pituitary	Right ovary	Left ovary	Uterus	Pituitary	Right ovary	Left ovary	Uterus
1.	2.715	2.920	2.453	2.193	4.039	3.931	2.000	3.607
2.	2.393	1.839	2.932	1.276	4.274	2.981	1.983	3.105
3.	2.881	1.961	2.061	2.239	3.244	3.559	2.060	3.275
Total	7.989	6.720	7.446	5.708	11.557	10.471	6.043	9.987
Mean	2.663	2.240	2.815	1.903	3.852	3.490	2.014	3.329
S.D.	0.203	0.511	0.576	0.442	0.444	0.433	0.433	0.208

Table 28. Alkaline phosphatase level (units/g of tissue) in non-cyclic and GnRH responded does

Sl. No.	Non-cyclic controls				GnRH responded does			
	Pituitary	Right ovary	Left ovary	Uterus	Pituitary	Right ovary	Left ovary	Uterus
1.	6.089	25.256	30.106	9.616	6.327	24.532	24.530	20.734
2.	10.086	32.003	12.406	45.991	9.840	37.302	27.911	33.039
3.	8.118	28.931	27.543	23.884	9.361	43.984	23.059	18.871
Total	24.293	86.190	70.055	79.491	25.528	105.818	75.500	72.698
Mean	8.098	28.730	23.352	26.497	8.509	35.273	25.167	24.233
S.D.	2.958	2.758	7.809	14.965	1.557	8.068	2.027	6.310

Table 29. Lactic dehydrogenase level (units/g of tissue) in non-cyclic and GnRH responded does

Sl. No.	Non-cyclic controls				GnRH responded does			
	Pituitary	Right ovary	Left ovary	Uterus	Pituitary	Right ovary	Left ovary	Uterus
1.	102.200	28.351	44.928	50.303	104.825	40.100	42.080	20.724
2.	95.780	22.386	28.168	31.134	116.401	49.266	51.414	50.092
3.	60.780	33.818	21.062	41.321	101.082	41.757	32.397	63.569
Total	258.760	84.555	94.158	122.758	322.308	131.123	125.891	134.395
Mean	86.253	25.185	31.386	41.252	107.436	43.708	41.964	44.798
S.D.	18.204	4.669	10.005	7.864	20.538	3.984	7.762	17.884

Table 30. Comparison of mean tissue enzyme level in the right and left ovary of non-cyclic and GnRH responded does

Tissue enzyme	Non-cyclic controls			GnRH responded does		
	Right ovary	Left ovary	Total	Right ovary	Left ovary	Total
ACP	2.240± 0.511	2.815± 0.576	5.055± 0.505	3.490± 0.433	2.014± 0.433	5.504± 0.479
ALP	28.730± 2.758	23.352± 7.809	52.082± 3.778	35.273± 8.068	25.167± 2.027	60.440± 4.268
LDH	28.185± 4.669	31.386± 10.005	59.571± 7.385	43.708± 3.984	41.964± 7.762	85.672± 4.289

Table 31. Z value comparison of ACP, ALP and LDH in the right and left ovaries of non-cyclic and GnRH responded does

Sl. No.	Comparison of tissue enzymes	ACP (Z value)	ALP (Z value)	LDH (Z value)
1.	Non-cyclic right ovary and left ovary	1.8048	0.9184	0.4100
2.	GnRH right ovary and left ovary	1.6081	1.7181	0.2827
3.	Non-cyclic right ovary and GnRH right ovary	0.5279	1.0852	3.5767**
4.	Non-cyclic left ovary and GnRH left ovary	0.1024	0.3182	1.1814
5.	Total enzyme level in both ovaries in non-cyclic and GnRH	0.4492	1.6175	3.9277**

** P<0.01

Fig.1 **Serum Progesterone level (ng/ml) of eight cyclic does (Group 1)**

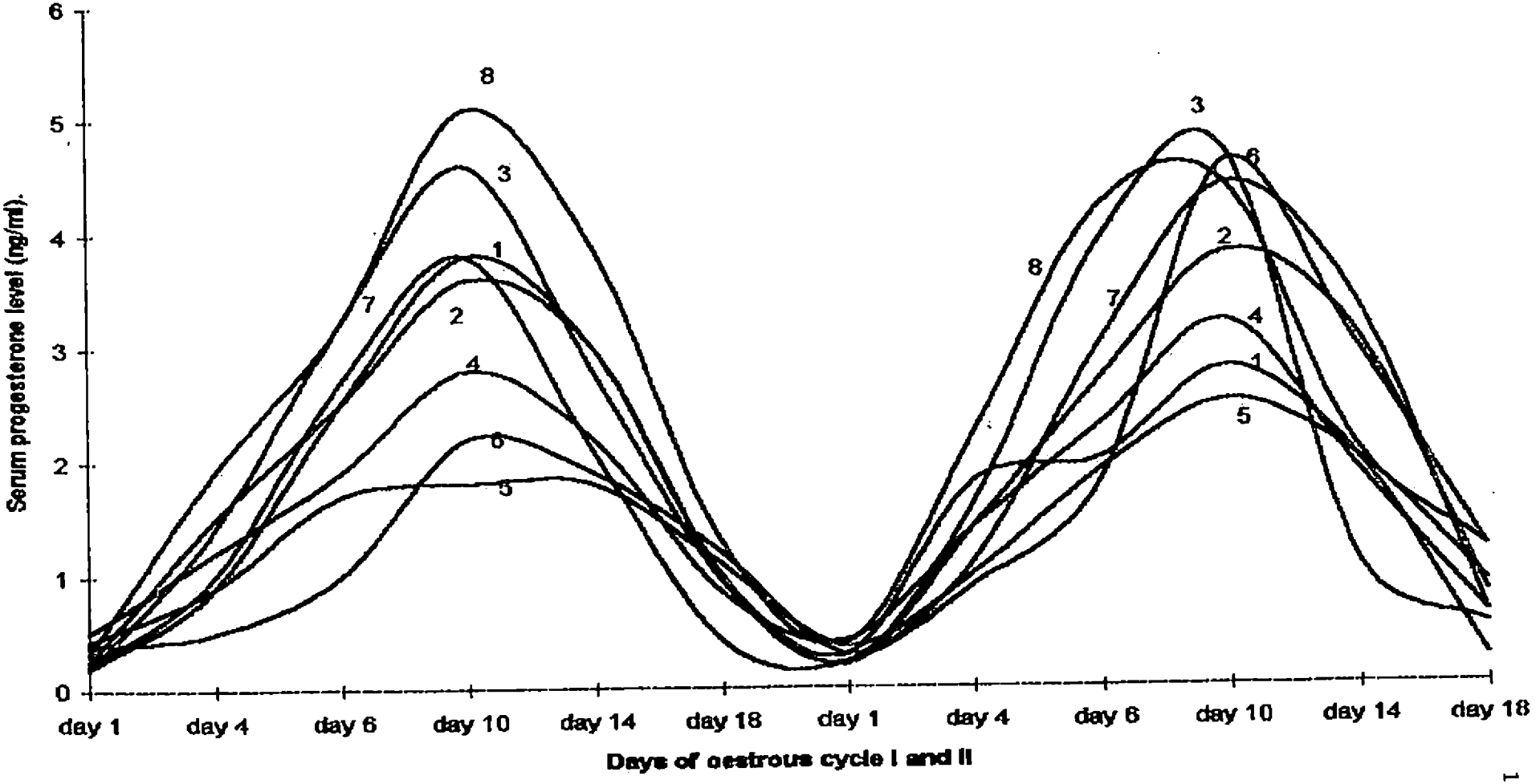


Fig.2 **Mean serum progesterone level (ng/ml) of two consecutive oestrous cyclesin cyclic does**

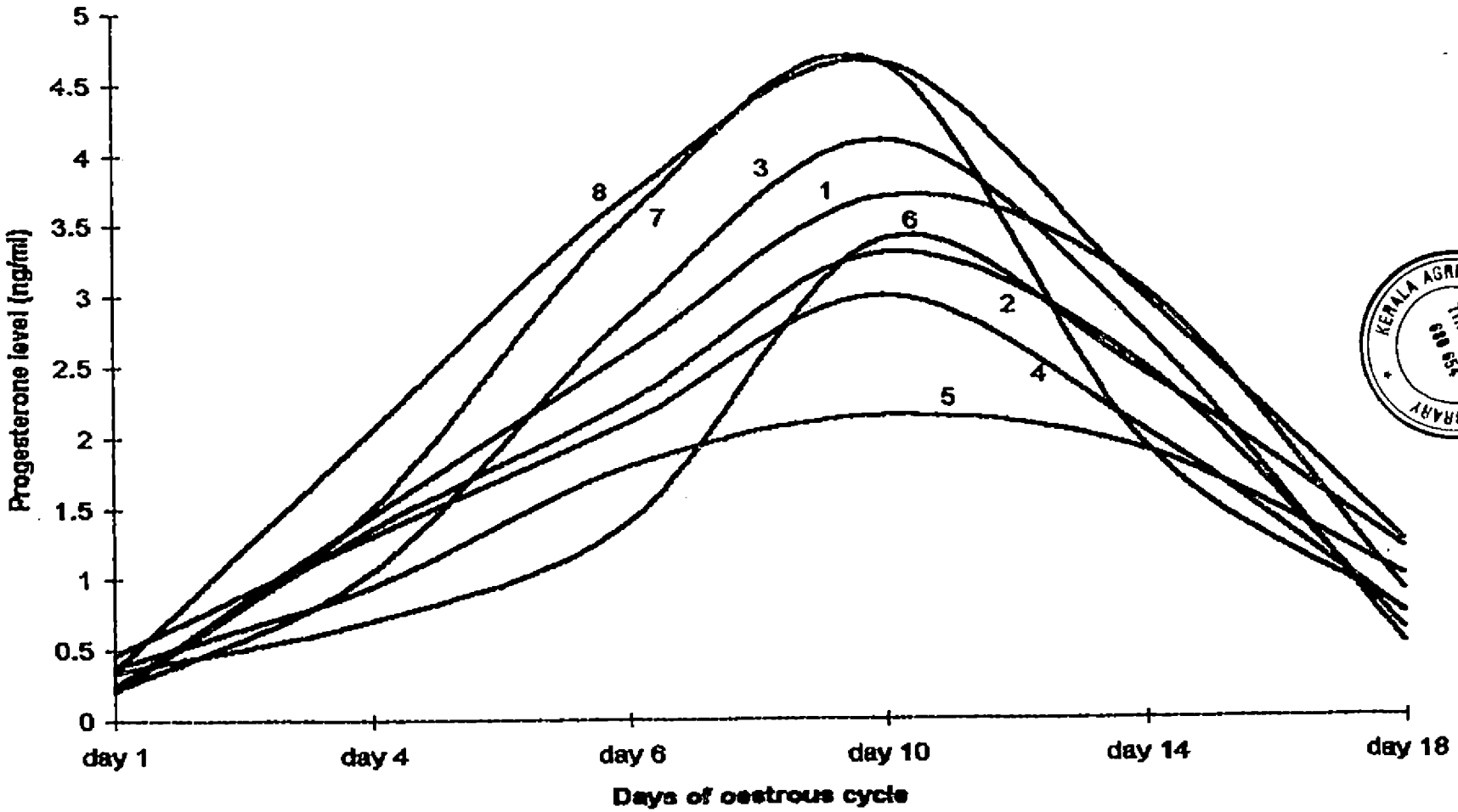


Fig.3 **Mean serum progesterone level (ng/ml) in non-cyclic does**

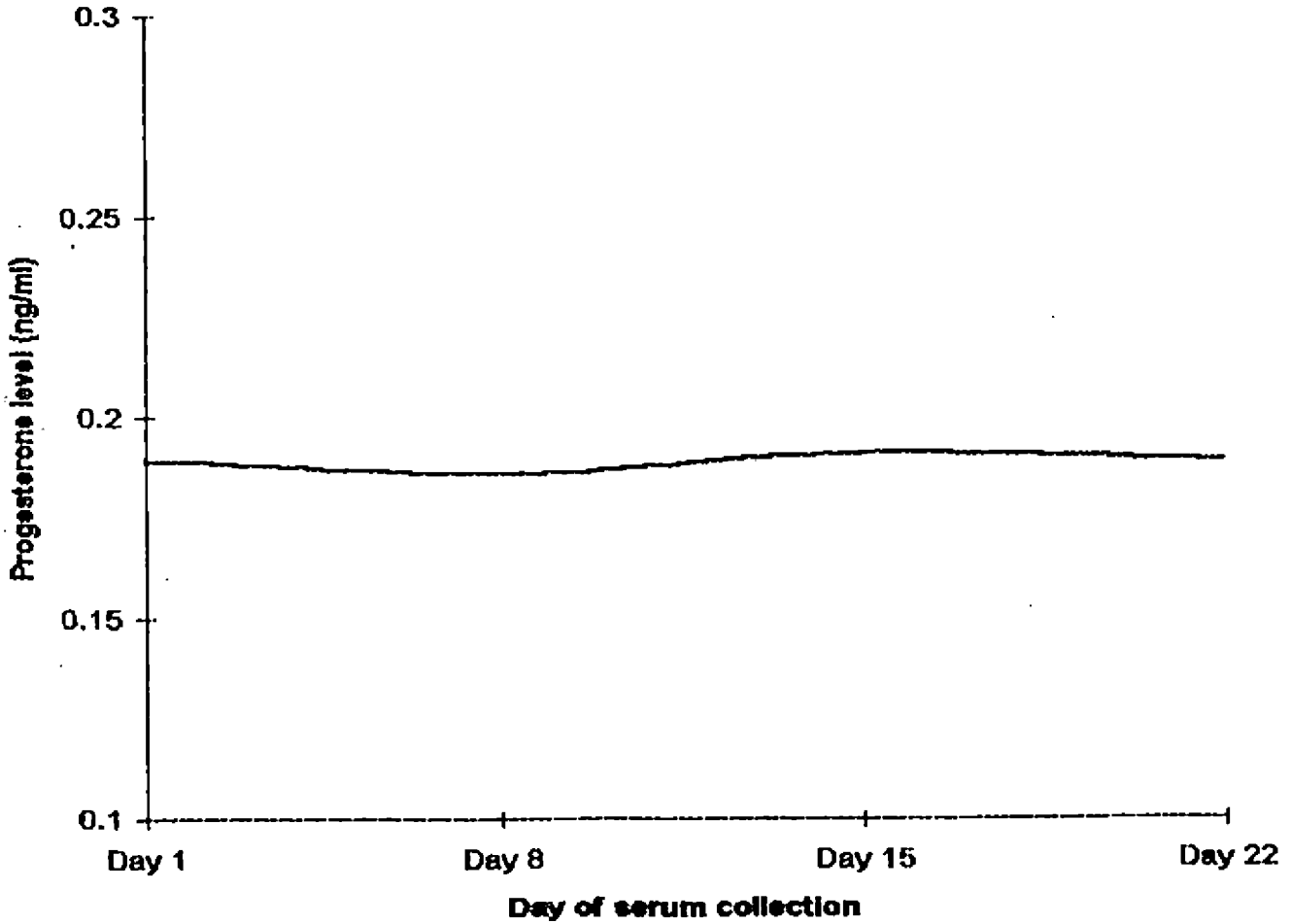


Fig.4 **Mean serum Calcium level (mg %) in cyclic, non-cyclic and GnRH responded does**

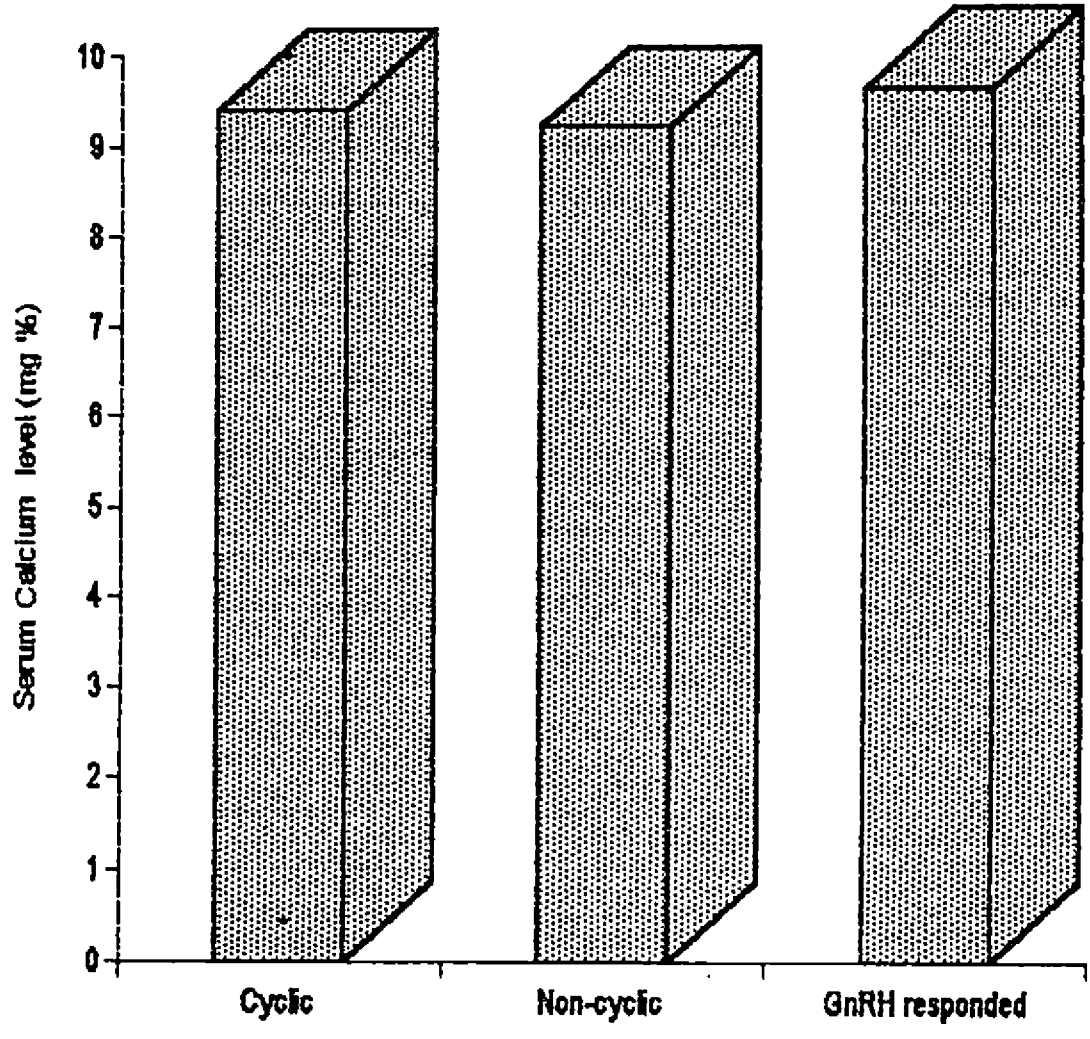


Fig.5 Mean serum phosphorus level (mg %) in cyclic, non-cyclic and GnRH responded does

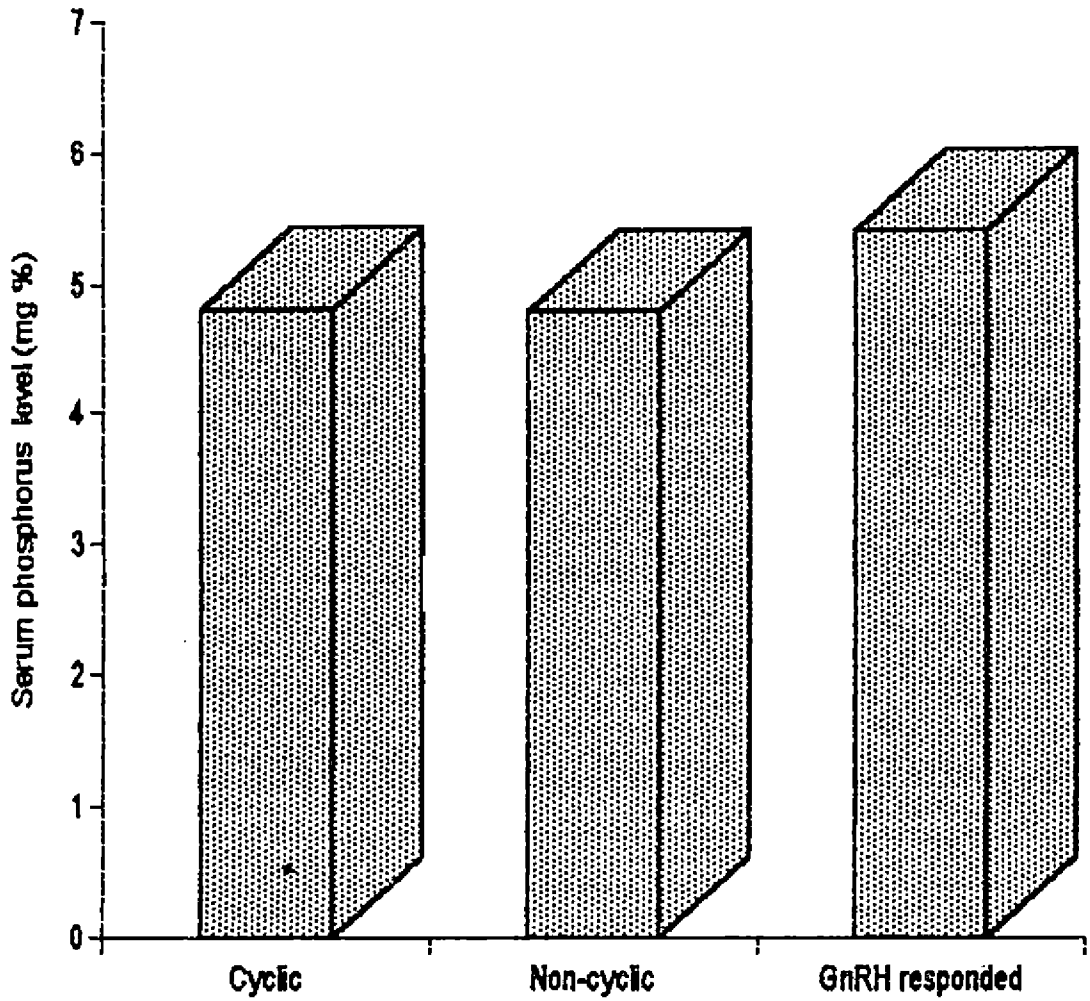


Fig.6 Mean serum copper level (ppm) in cyclic, non-cyclic and GnRH responded does

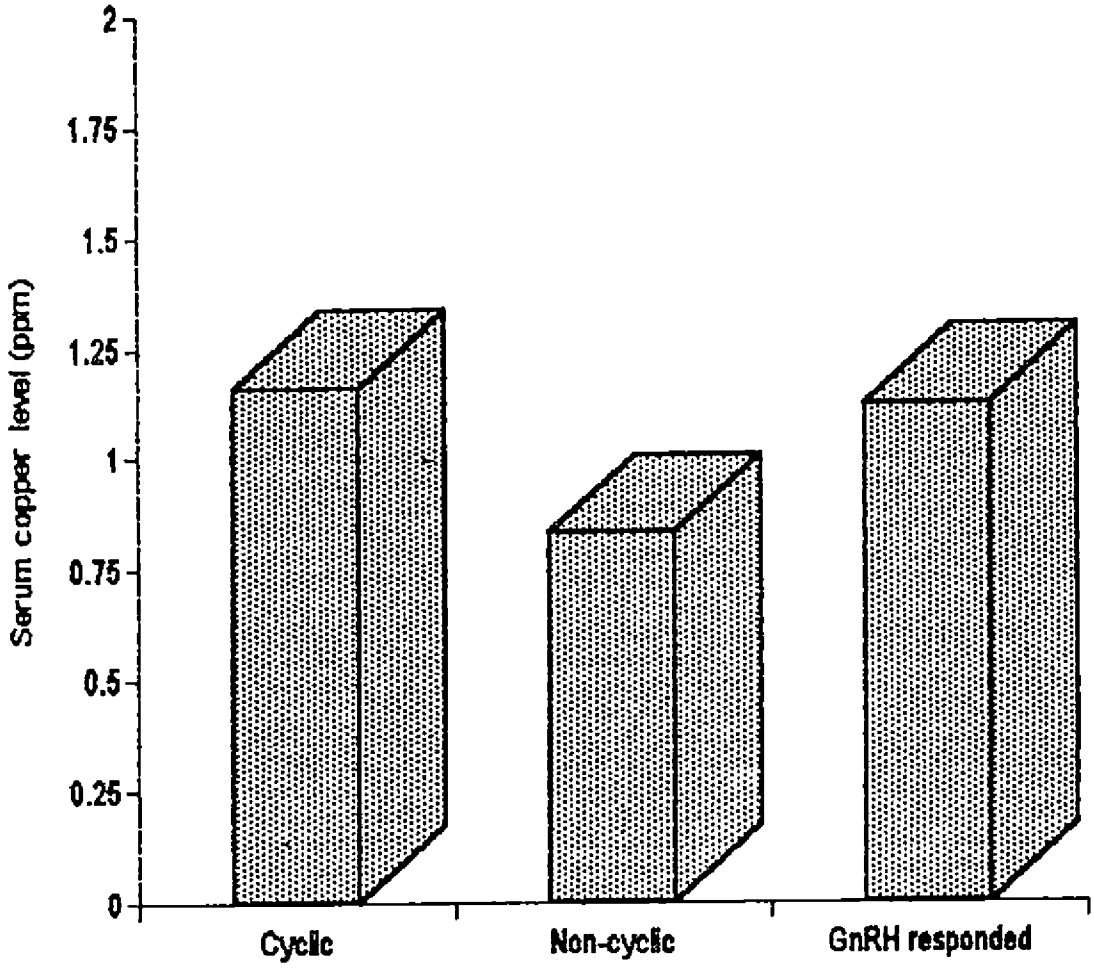


Fig.7 **Mean serum cobalt level (ppm) in cyclic, non-cyclic and GnRH responded does**

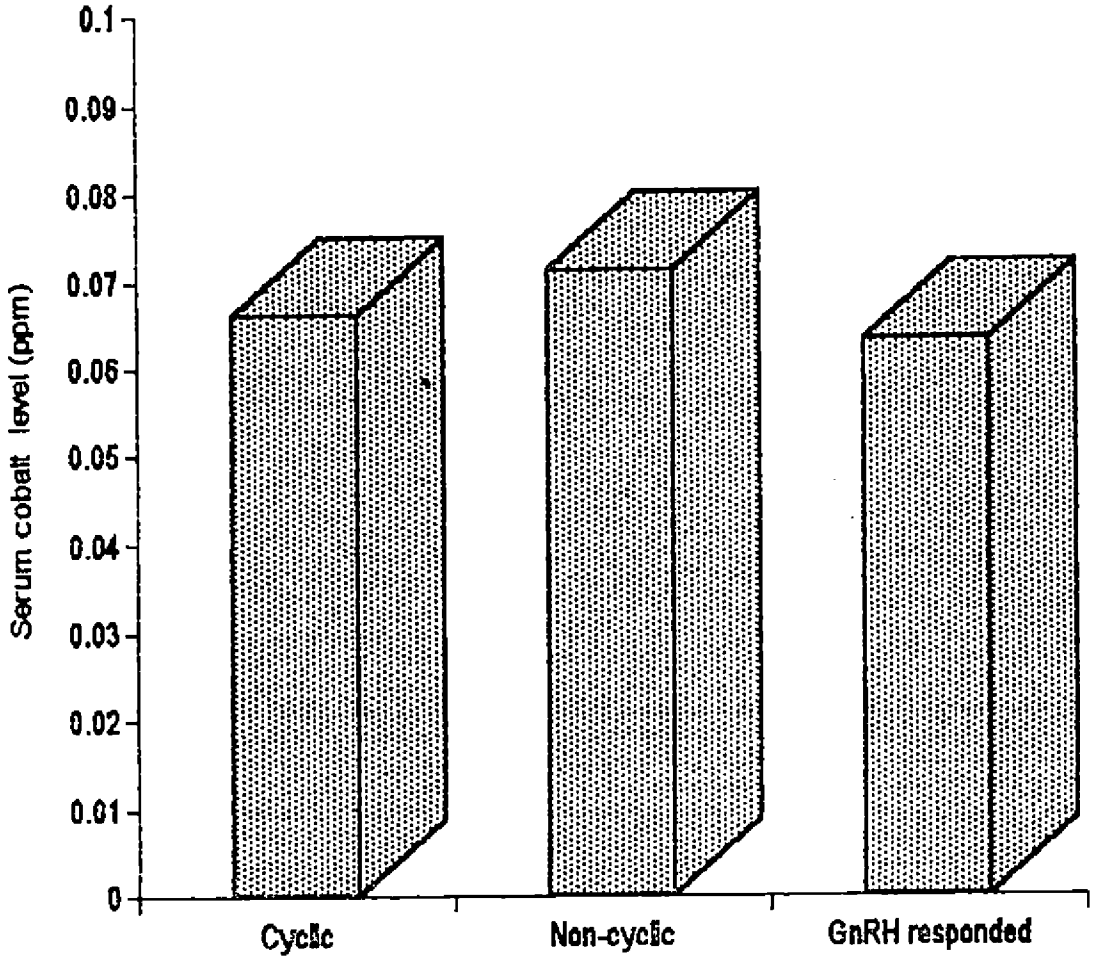


Fig.8 Mean serum manganese level (ppm) in cyclic, non-cyclic and GnRH responded does

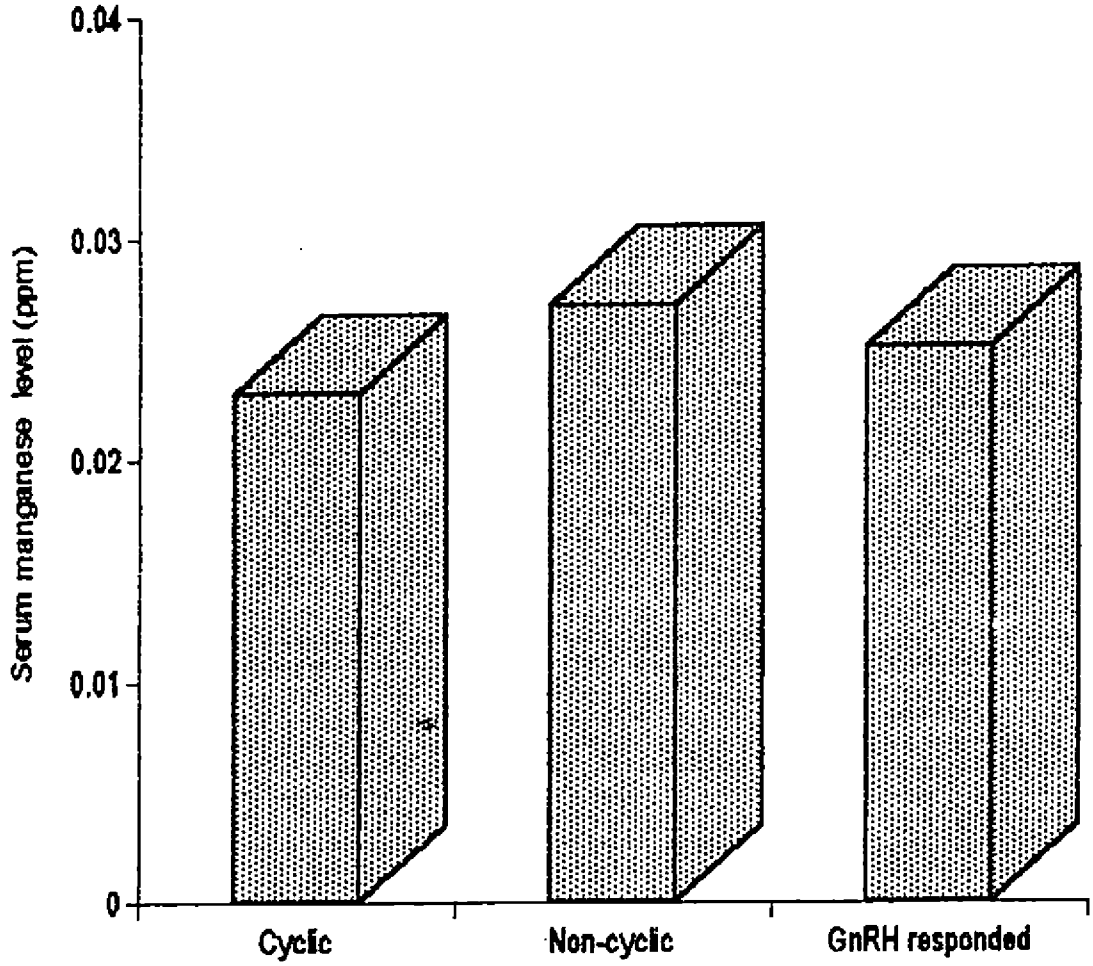


Fig. 9 Mean serum zinc level (ppm) in cyclic, non-cyclic and GnRH responded does

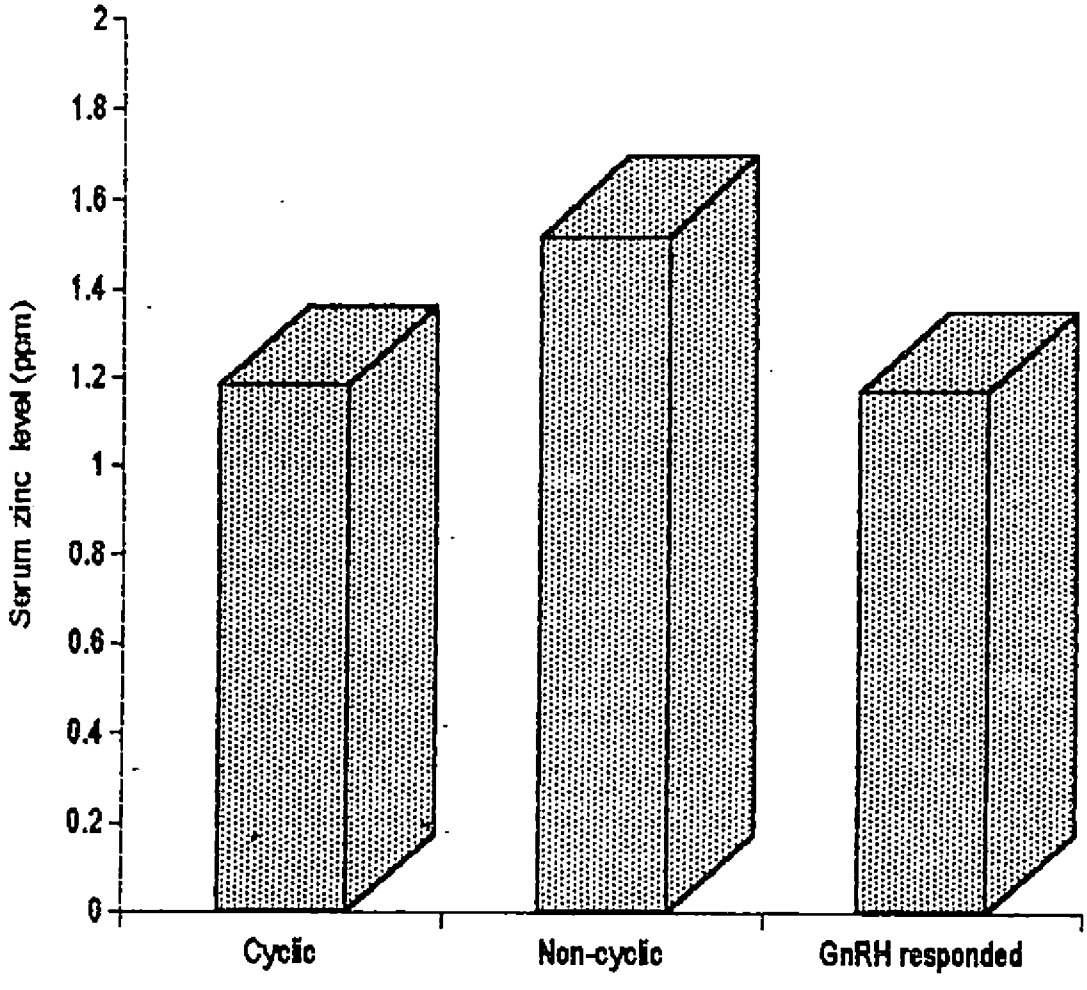


Fig.10 **Serum progesterone (ng/ml) during induced oestrous cycle in GnRH responded does**

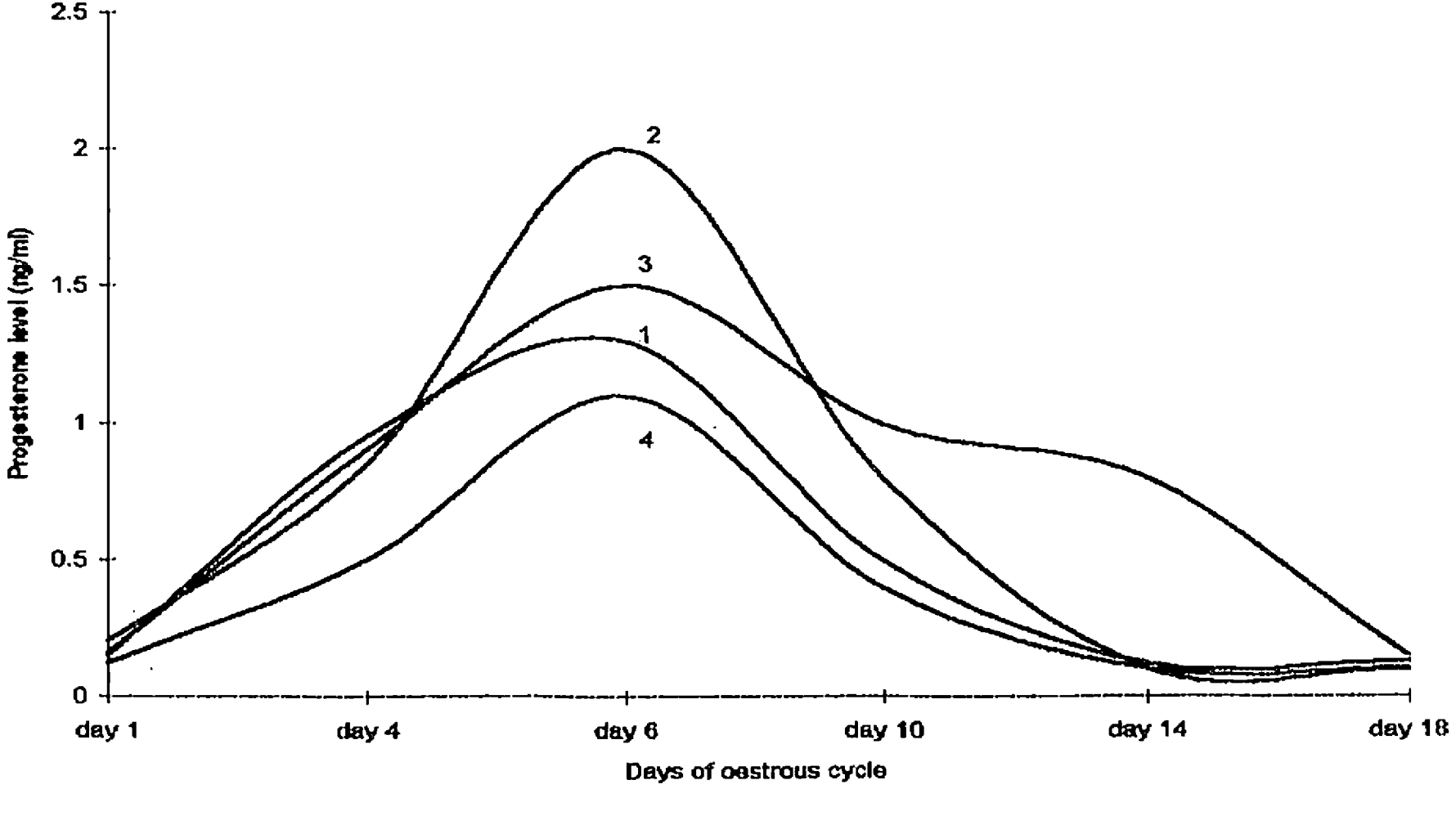


Fig.11 A comparison of serum progesterone level (ng/ml) of cyclic and GnRH responded does

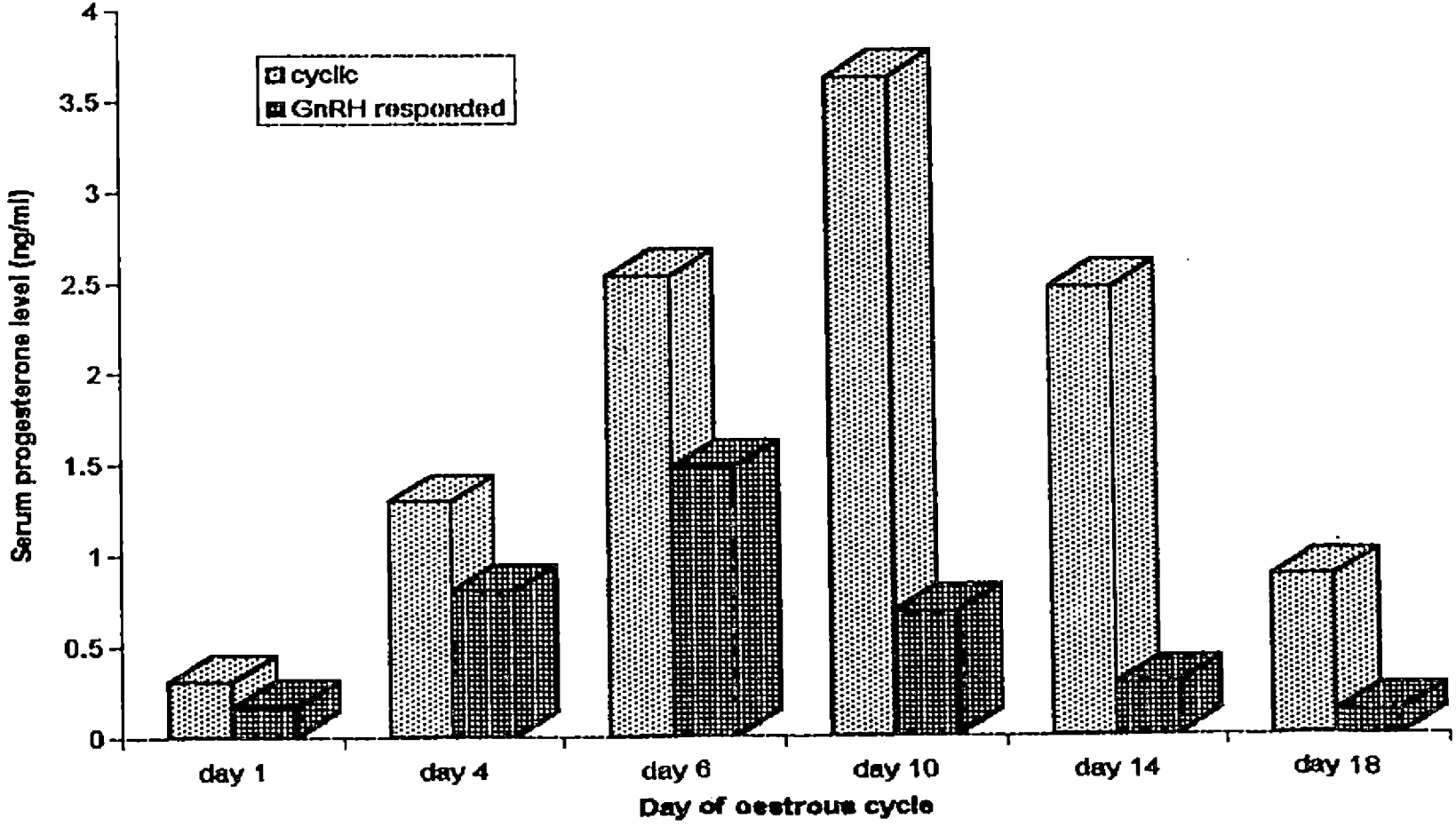
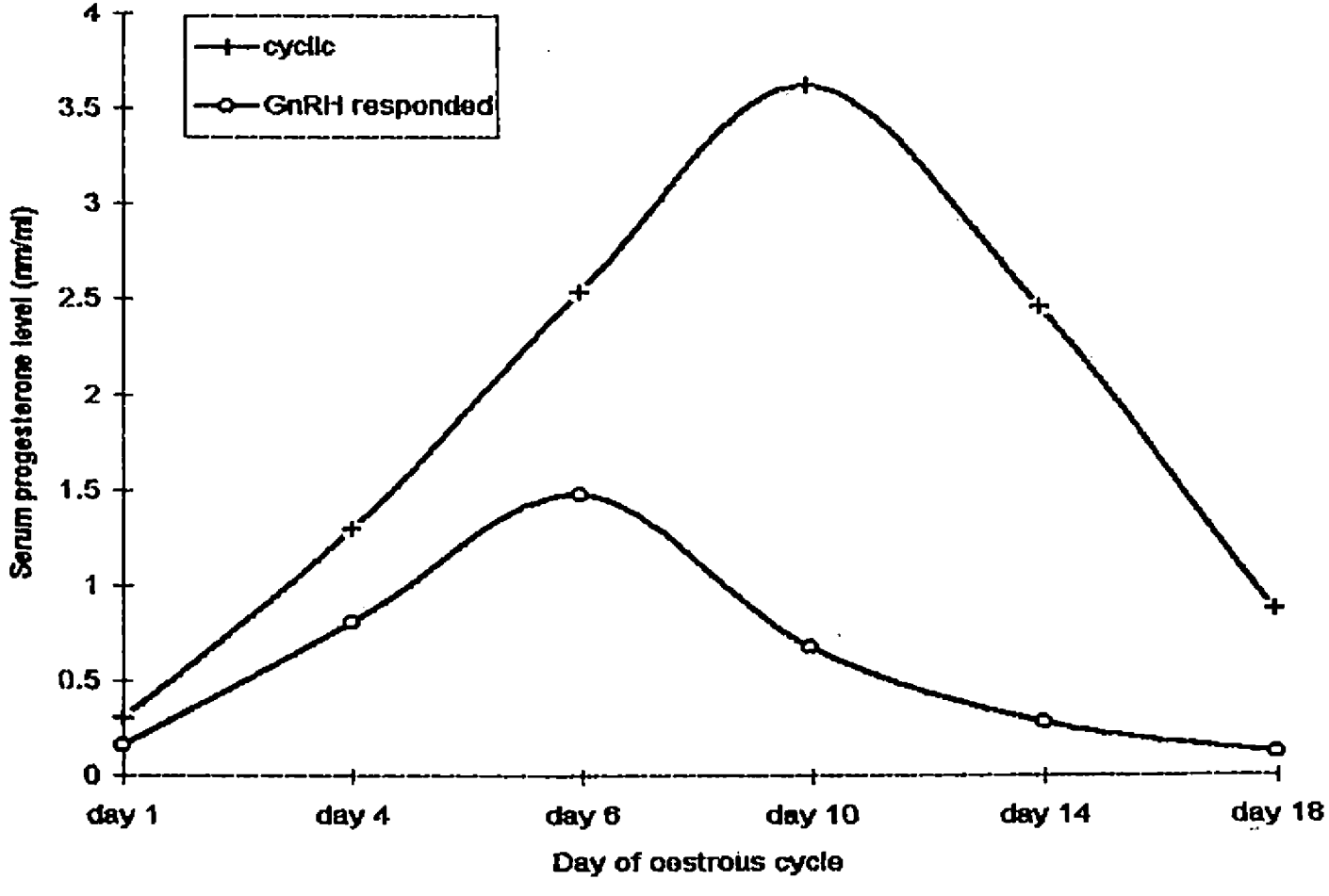


Fig.12 **A comparison of serum progesterone level (ng/ml) of cyclic and GnRH responded does**



Discussion

5. DISCUSSION

It is well understood that GnRH controls the secretion of gonadotropins which in turn regulates the production and activity of gonadal hormones. Clinical use of GnRH for inducing oestrus in acyclic goats in less understood. Hence this investigation was carried out to study the effect of administration of synthetic analogue of GnRH, Buserelin on the pattern of oestrous cycle, mineral status, serum progesterone and tissue enzyme profiles.

5.1 Oestrous cycle

Observations on the pattern of oestrous cycle in does were subjected to detailed discussion.

5.1.1 Length of oestrous cycle

The mean length of oestrous cycle in cyclic does in the present study was 20.313 ± 1.553 days (Table 1). This was well within the normal range of 17 to 22 days as reported in most of the Indian breeds. A mean oestrous cycle length of 21.174 days was reported in malabari goats (Mathai, 1986), 21.400 days in native does (Ramachandriah *et al.*, 1986), 21.30 ± 0.28 days in Red Marwadi goats (Pathiraja *et al.*, 1991), 24.2 ± 1.5 in local Malvi goats (Quereshi *et al.*, 1991) and 21.01 ± 3.57 days in Gurnzhong milch goats (LiJianwen, 1992)

and 19.27 ± 1.94 days in Black Bengal goats (Jalaludeen, 1992).

Occurrence of 10 to 20 per cent of short and long cycles in Barbari goats (Prasad and Bhattacharya, 1979), in Beetal and Black Bengal goats (Singh and Sengar, 1979) and in Surti and Marwari goats (Sureshkumar *et al.*, 1988) was reported. Occurrence of short cycles was reported in nullipara and animals in immediate post partum and this was due to various extrinsic factors. It is also observed in animals at the beginning of the breeding season (Ott, 1981, Corteel *et al.*, 1982 and Riera, 1982). In post partum anoestrous does, short cycles were observed on resumption of sexual activity (Riera, 1982). Prolonged oestrous cycle length was reported in aged animals (Camp *et al.*, 1983) and in suboestrus and underfed animals (Van Rensburg, 1971). However, in the present study the incidence of short cycle and long cycle are not observed because only apparently healthy kidded does during their prime age of reproductive status, under ideal feeding and managemental practices were selected for the present study.

5.1.2 Duration of oestrus

The mean duration of oestrus observed in cyclic does under present study was 37.50 ± 7.263 hours. The duration of oestrus was reported in pashmine goats as 21.51 hours (Bhattacharrya, 1981), in Surti and Marwari goats as 34.8 to

54.6 and 31.1 to 49.7 hours respectively (Mehta *et al.*, 1991), in Maradi goats as 21.37 ±0.44 hours (Patheraja *et al.*, 1991) and in Guanzhong milch goats as 24.34 ± 8.47 hours (LiJaiwen, 1992). The duration of oestrus in Malabari crossbred was varying from 12 to 72 hours and in 84.8 per cent of them, oestrus ceased by 36 hours (Mathai, 1986 and Krishnakumar, 1992). The duration of oestrus observed in the present study is well in agreement with the reports of earlier workers.

Breed, age, season and presence of male are the factors which can influence the duration of oestrus (Mishra and Biswar, 1966; Van Rensberg, 1977 and Pineda, 1989). In the present study, healthy Malabari crossbreds of aged one to four years maintained under identical feeding and management conditions, with presence of buck throughout the period of experiment might be the reasons for less variation in duration of oestrus. Seasonal effect on the occurrence of oestrus was not observed since Malabari goats are reported to breed throughout the year with greater peak during July and lesser one during November (Krishnakumar, 1992).

5.1.3 Oestrous signs

Malabari crossbred does in the present study showed mild (4.16%), moderate (33.3%) and intense (62.5%) oestrous signs. Similar observation of mild, moderate and intense oestrous

signs were reported in Barbari goats (Prasad and Bhattacharya, 1979) and Malabari crossbreds (Mathai, 1986).

5.2 Mineral status in cyclic and non-cyclic does

For successful reproduction, a proper synergism between anabolic and catabolic reaction is essential and trace elements have a key role in maintenance of reproductive efficiency. Hence this study was conducted in cyclic and non-cyclic does to correlate the influence of various minerals in ovarian activity.

5.2.1 Calcium

Serum calcium level in cyclic does was 9.410 ± 0.770 mg per cent and that in non-cyclic does was 9.220 ± 0.830 mg per cent in the present study. No significant difference was observed between the two groups. The values of blood calcium level observed were similar to one reported by Kessler (1991) and Battacharyya *et al.* (1995).

Present study showed no significant difference in the serum calcium level on different days both in cyclic and noncyclic does (Table 3). Similarly, no significant variations in the calcium level during different phases of reproduction in goats (Bhattacharyya, 1995) and in ewes (Esso *et al.*, 1990). In contrast, Nagam *et al.*, (1990) observed variation

in the serum calcium level during different phases of reproduction in goats.

Mehta *et al.* (1991) reported higher serum calcium level in short cycling and normally cycling Surti goats as 12.81 ± 1.02 and 10.83 ± 1.75 mg per cent and in Marwari goats as 11.86 ± 0.72 and 9.80 ± 0.96 mg per cent, respectively.

The present study agree with the findings of Bhattacharrya *et al.* (1995) and Esso *et al.* (1990) and shows that blood calcium levels are not usually affected in goats with inactive ovaries. Hence it is inferred that supplementation of calcium along with feed may not have significant effect on the reproductive performance of goats, as reported by Kumagai and White (1995) in Marino ewes.

5.2.2 Phosphorus

No significant difference in serum phosphorus level between cyclic (4.80 ± 0.26 mg per cent) and non-cyclic does (4.77 ± 0.28 mg per cent) was observed. The values of serum phosphorus obtained in the present study was lower than that reported by Mehta *et al.* (1991) in Surti goats and Bhattacharrya *et al.* (1995) in indigenous goats of Assam. This may be due to breed difference.

Nagam *et al.* (1990) and Mehta *et al.* (1991) observed significant difference in the serum phosphorus level in different phases of oestrus cycle in short cycling and normally cycling surti goats. However, in the present study no variation in the serum phosphorus level was obtained between different days.

There are few reports showing the important role of phosphorus in the reproductive function of ruminants (Cates and Christenson, 1983 and Brooks *et al.*, 1984). Anke *et al.* (1987) reported reduced growth, lowered conception rate and increased incidence of abortion and mortality in lactating Swiss-type goats when fed on a phosphorus deficient diet. Reinhardt *et al.* (1988) reported that usually no cellular or molecular lesions on failure of phosphorus homeostasis have been identified in goats. Similarly no primary hormone deficiency has been identified interfering with phosphorus absorption and retention. Hence from the present study it is concluded that variation in phosphorus level rarely occur in goats as a serious factor for acyclicity in goats.

5.2.3 Copper

Malabari crossbred non-cyclic does in the present study showed a significantly lower serum copper level (0.83 ppm) as compared to cyclic does (1.16 ± 0.17 ppm). Serum copper level observed in the cyclic does in the present study agrees with

the findings of Singh and Singh (1992) who reported normal serum copper level in Sirohi goats ranging from (1.03 to 1.52 ppm). However, Mehta *et al.* (1991) reported a higher serum copper level of 192.75 ± 37.09 mcg per cent and 121.56 ± 17.37 mcgm per cent in short cycling and normally cycling Surti goats. In sheep, a lower copper level of 78.91 ± 9.49 microgram per cent was reported by Zahari and Atminani (1987) and 80-160 microgram per cent by Niekerk *et al.* (1990). Bhattacharyya *et al.* (1995) observed a significantly higher copper level on the day of oestrus compared to the other phases of oestrous cycle in goats. However, no significant difference was noticed in the serum copper level between days in the present study.

Loftedt *et al.* (1988) and Sarker *et al.* (1991) observed low serum copper levels in goats affected with reproductive problems. Similar findings were reported in ewes by Horoun *et al.* (1992). They also established that oral supplementation of copper sulphate along with feed may increase the serum copper level in sheep and goats.

In the present study the lower copper level observed in non-cyclic does agrees with observation of Loftedr *et al.* (1988) and Sarker *et al.* (1991).

The relationship between plasma copper level and fertility has been reported to be inconsistent (Kappel *et al.*,

1984). Copper related reproductive disorders are mostly due to actual deficiency or interference to copper utilization (Allcroft and Parker, 1949). Requirement of copper is greatly influenced by interaction with other elements such as sulphur, iron, calcium, zinc and especially molybdenum (lamand, 1984). Molebdenum is thought to reduce the capacity of liver to store copper (Blackermore and Venn, 1950). High levels of calcium, phosphorus and zinc have been reported to be accompanied by low levels of copper (Saba *et al.*, 1987).

Copper is an integral component of metalloenzymes which play a significant role in metabolic functions including those of endocrine organs. Copper is also found to modulate the prostaglandin E2 receptor binding thus regulating the release of luteinizing hormone (Barnea *et al.*, 1985).

In the present study, low serum copper level in non-cyclic does as compared to cyclic does indicate hypocuprosis and resultant interference in reproductive function.

5.2.4 Cobalt

In the present study, no significant difference in the serum cobalt level was observed between cyclic (0.662 ± 0.158 ppm) and non cyclic does (0.071 ± 0.010 ppm). The serum cobalt level was found significantly varying between different days. However, reports on serum cobalt level in goats are

often lacking for comparison. Prasad *et al.* (1989) and Chauhan *et al.* (1991) reported low serum cobalt in cows affected with reproductive problems. Joy George (1995), Vhora *et al.*, (1995) and Rajeev (1998) observed significant difference in cobalt level in anoestrous as against fertile cows.

Sarker and Mishra (1991) claimed reasonable success with oral supplementation of cobalt sulphate for treating reproductive problems in a goat herd, indicating the role of cobalt in goat reproduction.

Cobalt is an integral part of Vit. B_{12} and plays a major role in reproductive function. It is necessary for microbial synthesis of cobalamin and deficiency will lead to impaired synthesis of Vit. B_{12}. It is reported that supplementation of cobalt may help to alleviate higher incidence of anoestrum and poor conception rate (Hidiroglou, 1979). Selective uptake of free cobalt versus cobalamin by cellular organelles suggests that the metabolic effect of cobalt may not be limited to its requirement for cobalamin synthesis (Georgievskii, 1981). Mburu *et al.* (1994) described that serum B_{12} concentration below 200 pg was indicative of cobalt deficiency in goats.

However, in the present study, no significant difference in the serum cobalt level was observed in non cyclic as compared to fertile cyclic does. Hence it is concluded that

though cobalt is essentially required for reproduction in goats, its deficiency is a rare cause of ovarian dysfunction in Malabari crossbreds.

5.2.5 Manganese

No significant difference in serum manganese level was observed between cyclic (0.023 ± 0.005 ppm) and non-cyclic does (0.030 ± 0.010 ppm) in the present study. Singh and Singh (1992) reported similar values of serum manganese level as 0.036 to 0.048 ppm in Sriohi goats. It is clear that the manganese level is within the normal range in both cyclic and non-cyclic does.

Though manganese deficiency is rare in ruminants dietary deficiency and reduced tissue concentration can lead to impaired reproductive function characterised by anoestrum, an novulation and reduced fertility (Wilson, 1966).

Reproductive disorders such as acyclicity, more services per conception and disturbed foetal development in goats have been attributed to manganese deficiency (Groppel and Anke, 1971). Many of the gross effects of manganese deficiency can be explained in terms of the effect of manganese on mucopolysaccharide synthesis (Underwood, 1977). Deficiency also effects several manganese metalloenzymes including hydroxylase, kinase, decarboxylase and transferases.

Manganese has been implicated explicitly in synthesis of steroid hormones. Gonadotropins like HCG may influence manganese transport and modify its availability to different tissues or organs (Underwood, 1977). Hence it is concluded that though manganese is essential for reproduction, its role in causing acyclicity in does is not clear.

5.2.6 Zinc

Present study revealed that the serum zinc level was lower in cyclic (1.180 ± 0.120 ppm) as compared to non-cyclic does (1.510 ± 0.430 ppm). Niekerk *et al.* (1990) showed that serum zinc level less than 80 microgram/dl indicated marginal deficiency in sheep and goats. Serum zinc level in goats of West Bengal was 0.83 and 0.96 ppm during dry and wet seasons (Bhattacharrya *et al.*, 1995). However, Singh and Singh (1992) observed a higher serum zinc level with proper concentrate supplementation in lactating Sirohi does varying from 1.940 ± 2.310 ppm.

Present study revealed that in both cyclic and non-cyclic does serum zinc level was within the normal range as reported by Niekerk *et al.* (1990) and Bhattacharrya *et al.* (1995), though a significant difference existed between the two groups.

It is reported that zinc deficiency may lead to defects in prostaglandin metabolism in ruminants (Hidiroglou, 1979). Reproductive problems, mostly inactive ovaries was reported due to zinc deficiency in Angora goats (Rauter *et al.*, 1987) Zinc, being an integral part of many proteins and enzymes, has been attributed to several important biological processes. Some of these enzymes may be important in their function on reproductive tissue, but little is known about how zinc deficiency influences manifestations of zinc dependent functions. A role in reproduction may involve zinc as an essential component or activator of enzymes involved in steriodoenesis. Zinc may act indirectly through the pituitary to influence gonadotrophic hormone or directly through complexing with specific ligands on the gonads (Apagar, 1985).

In the present study, though serum zinc level varied significantly between cyclic and non-cyclic does, both are within the normal limits. It is inferred that though zinc is essential for reproduction variation in zinc level may not effect the ovarian function.

5.3 Serum progesterone level

A study of serum progesterone profiles was carried out by RIA using serum collected from eight cyclic and sixteen

non-cyclic does and the results derived are subjected to detailed discussion.

5.3.1 Serum progesterone level in cyclic does

In the present study, there was significant difference in the levels of serum progesterone on different days of oestros cycle I and II. The mean serum progesterone of the two consecutive oestros cycles on day four, six, ten and 14 of cycle 1.294 ± 0.382, 2.531 ± 0.758, 3.619 ± 0.794 and 2.456 ± 0.430 ng/ml respectively. This was higher than the levels of progesterone on days one and 18 of the oestrous cycles 0.304 ± 0.087 and 0.871 ± 0.246 ng/ml respectively.

The trend in serum progesterone level was noted rising from day four of the cycle reaching a peak on day 10 and declining from day 14 of cycle. Similar observations were made by Baruah *et al.* (1987) and Pathak *et al.* (1990) in cyclic Surti and Marwari goats, respectively.

During day one and 18 of oestrous cycle, serum progesterone concentration (0.304 ± 0.087 and 0.871 ± 0.246 ng/ml respectively) were less than one ng per ml. This is in agreement with observations made by Wani (1989) in German Dwarf breed of goats, Ding *et al.* (1990) in Haimen goats, El. Hommosy *et al.* (1991) in Anglo Nubian goats and Leyva-Ocariz *et al.* (1995) in native and crossbred goats in the tropical

semiarid zone of Venezuela. This indicates that corpus luteum activity is very less on day one and day 18 of the cycle.

Pant *et al.* (1977) indicated that at the end of luteal phase, the serum progesterone level declined acting as a primary signal for preovulatory gonadotropin surge for the onset of next cycle.

A significant increase in the level of serum progesterone on day four of cycle (1.294 ± 0.382 ng/ml) indicated that luteal tissue was formed by day four of cycle and its activity started. Further increased level of serum progesterone on day six, ten and 14 of oestrous cycle (2.531 ± 0.758, 3.619 ± 0.794 and 2.456 ± 0.430 ng/ml, respectively) indicated the presence of more active corpora lutea in the ovaries on these days. The serum progesterone level reached peak on day 10 of cycle indicating further growth of CL on day 10 of cycle. The serum progesterone level started declining from day 14 showing that corpus luteum regression has started by day 14 of cycle. Similar observations were reported in cyclic does (Baruah *et al.*, 1987; Wani, 1989, Ding *et al.*, 1990; Pathak *et al.*, 1990; El Hommosy *et al.*, 1991 and Leyva-Ocariz *et al.*, 1995) and in ewes (Pant *et al.*, 1977; Wheeler and Land, 1977; Reddy *et al.*, 1989).

5.3.2 Serum progesterone in non-cyclic does

The mean progesterone level in non cyclic does was 0.189 ± 0.107 ng/ml which was significantly lower as compared to mean progesterone level of cyclic does (1.848 ± 0.339 ng/ml). The mean progesterone level in non cyclic does was much less than the lowest progesterone level of day one oestrous cycle of cyclic does. This indicates less luteal tissue and low ovarian activity in non cyclic does. Similarly progesterone levels less than 0.5 ng/ml were reported immediately before the onset of breeding season in exotic breeds of goats (Ott *et al.*, 1980; Wani, 1989; and Mascarenchas *et al.*, 1995). Much lower progesterone level (less than 0.1 ng/ml) was reported during the non breeding season in exotic breeds (Abecia *et al.*, 1991; Sawada *et al.*, 1994 and Leyra Ocariz, 1995).

Malabari crossbred goats exhibit oestrus throughout the year with a greater peak in July and lesser one in November (Mukundan, 1983; Mathai, 1986; Krishnakumar, 1992). Hence the mean serum progesterone level 0.189 ± 0.107 ng/ml in non-cyclic does indicated low ovarian inactivity which can be corrected by suitable treatments. Ovarian inactivity may occur due to severe hormonal deficiency, especially gonadotropins (Hafaz, 1987).

5.4 Response to GnRH administration in non cyclic does

Single dose of one ml GnRH analogue, Baserelin (4 microgram) administered intramuscularly induced oestrus in three out of eight and a second dose induced oestrus in one of non-cyclic does in the present study (Table 12). The oestrus was exhibited at a mean of 87.0 ± 9.95 hours after the administration of GnRH.

Success rates on induction of oestrous in acyclic does using GnRH, progestagen pessaries, intravaginal progestagen sponges, FSH and pregnant mare serum gonadotropin (PMSG) vary greatly. In 15 anoestrous ewes treated with continuous administration of GnRH, all showed oestrus and ovulated (McLeod *et al.*, 1983). Similarly, in another trial, McLeod and Haresign (1984) induced oestrus in 22 out of 24 ewes with GnRH continuous infusion. Knight *et al.* (1988) and Bretztaff and Madrid (1989) who obtained good response to multiple intravenous injection of GnRH in progestagen primed lactating dairy goats. Rubin *et al.* (1994) induced oestrus in anoestrous progestagen primed ewes with either single or double intramuscular injection of GnRH and obtained 75 per cent success. In another trail, Robin *et al.* (1994) obtained a better oestrous induction rate in 85 per cent of ewes using PMSG than GnRH. Rodway and Swift (1985) got less response to LHRH than PMSG.

In the present study, four out of eight noncyclic does exhibited oestrus after intramuscular injection of GnRH analogue, Buserelin. Number of does responding to GnRH in the present study was comparatively less, than the result obtained with progesterone priming. Progesterone status prior to the treatment may have mild effect on the magnitude of the initial LH concentration duration of increased plasma LH concentration or to the frequency of LH episodes (McLeod *et al.*, 1991).

Further, in the present study response observed was comparatively less probably due to single injection of GnRH analogue, whereas trials with continuous infusion or multiple intermittent administration of GnRH showed better result (Knight *et al.*, 1988).

A single dose of GnRH analogue administration in the present study, might have resulted in less LH peak to induce oestrus and ovulation in the non-responded doe. It can also be inferred that in non cyclic does, the hypothalamus is not properly primed with endogenous GnRH, and hence intramuscular injection of Buserelin at the dose choosen in the present study might not have been sufficient to activate the hypothalano pituitary gonadal axis.

The moderate success observed in Buserelin administration for inducing oestrus in the present study shows that the GnRH analogue may be having a prolonged half life than pure form of

FSH or PMSG which may explain a better sychrony of LH peaks due to ovarian stimulation occurring for a longer period of time thus initiating ovulation and corpus luteum formation (McLeod *et al.*, 1991).

5.4.1 Oestrous cycle in GnRH responded does

5.4.1.1 Oestrous cycle length

The mean oestrous cycle length in GnRH responded does was significantly shorter (12.75 ± 0.829 days) as compared to the cycle length in ~~non~~ cyclic does without treatment. The occurrence of oestrous cycle of short duration was reported after GnRH treatment in anoestrus ewes (Wright *et al.*, 1983 and 1989 and Rodway and Swift, 1985) and in does (Knight *et al.*, 1988 and Bretztaff *et al.*, 1991). In goats at the onset of breeding an LH peak of low amptitude was observed at oestrus, that was followed by a short oestrous cycle (Camp *et al.*, 1982). Similarly, an abnormally short luteal phase resulting in short oestrous cycle length has been documented after GnRH induced ovulation in anoestrous cows (Webb *et al.*, 1977; Carter *et al.*, 1980; and Troxel *et al.*, 1983). However, Mc Leod (1984) observed normal oestrous cycle in ewes that were subjected to progesterone treatment prior to continuous administration of GnRH.

In the present study, short oestrous cycle observed after GnRH analogue treatment was in agreement with findings of Knight *et al.* (1988) and Bretztaff *et al.* (1991). Thus it is inferred that single dose of GnRH administration in non cyclic does might have induced oestrus and ovulation, but the luteotrophic support during early stages of corpus luteum formation was inadequate which might have resulted in its premature regression leading to shorter oestrous cycle length.

5.4.1.2 Duration of oestrus

In the present study, the duration of oestrus in GnRH responded does was 18.0 ± 4.243 hours and in non cyclic controls was 37.50 ± 7.263 hours. The duration of oestrus in GnRH responded does was significantly shorter as compared to non cyclic controls. Similar findings of short duration of oestrus was reported in GnRH induced oestrus in does (Knight *et al.*, 1991 and Bretztaff *et al.*, 1991) and in ewes (Wright *et al.*, 19 and Rodway and Swift, 1985).

It could be inferred that the single dose of GnRH analogue administration in the present study might have resulted in less follicular growth and maturation leading to a short duration of oestrus.

5.4.1.3 Oestrus signs

Intensity of oestrus signs observed in GnRH responded was less as compared to cyclic does in the present study. A mild to moderate oestrual signs were observed in GnRH responded as against moderate to intense oestrual signs in normally cyclic does. Similar observations were reported in GnRH responded does (Knight *et al.*, 1988 and Bretztaff *et al.*, 1991) and in prostaglandin oestrus induced ewes (Ali *et al.*, 1995 and Beck *et al.*, 1996).

In the present study it could be inferred that the GnRH administration in non cyclic does might have resulted inadequate pattern of FSH and LH release leading to lesser follicular growth. This might have contributed to weak oestrous signs due to lower threshold of oestrogens in these follicles.

5.4.2 Mineral status in GnRH responded does

It was observed that serum phosphorus and copper level was significantly higher in GnRH responded does as compared to cyclic does and non cyclic controls.

There are many reports favouring the role of phosphorus in reproduction in ruminants (Cates and Christensen, 1983 and Brook *et al.*, 1984). The phosphorus is an integral component

of nucleic acids, nucleotides, phospholipids and some proteins. It is required for transfer and utilization of energy and normal phospholipid metabolism. The role of phosphorus and phospholipid dependent proteinkinase in the phospholipid and cyclic AMP synthesis may be crucial in mediating hormonal action (Hurdley and Decane, 1989). In non cyclic does GnRH administration might have indirectly resulted in changes in serum phosphorus level or might have directly influenced the phosphorus homeostasis, resulting in increased level.

Significantly higher copper level was seen in GnRH responded does compared to untreated non cyclic does. Copper is reported to modulate the PGF_2 alpha receptor binding and this regulates the release of LHRH (Barnea *et al.*, 1985) GnRH administration might have indirectly influenced the metabolic function including those of endocrine glands.

It could be inferred that GnRH administration influenced the level of phosphorus and copper in non cyclic does, probably due to increase of gonadotropins and steriod hormones. Hence it can be concluded that mineral status exert a powerful effect on reproductive system in goats, the responses are partly to the changes in gonadotropin and gonadal hormones.

5.4.3 Serum progesterone level in GnRH responded does

Serum progesterone level was found lower in all days of induced oestrous cycle in GnRH responded does as compared to that in normally cyclic does. Similar observation were reported in GnRH induced oestrous cycle in does (Knight *et al.*, 1988 and Bretztaff *et al.*, 1991) and in ewes (McNelly *et al.*, 1981; Mc Leod *et al.*, 1983). The reason for occurrence of lower progesterone in GnRH responded does can be attributed to reduced number and size of corpora lutea/lutein cells formed and its premature regression.

Serum progesterone level was found rising from day 4, reaching a peak on day six and then declined during GnRH induced oestrous cycle. Thus highest values of progesterone was found on day six (1.475 ± 0.334 ng/ml) in GnRH induced oestrous cycle whereas on day 10 (3.619 ± 0.794) in normally cyclic does. This shows that luteal activity is highest on day six of induced oestrous cycle whereas on day 10 of normally cycling does. It can be inferred that in GnRH responded does, corpora lutea with reduced number and size, or defective or less functional are formed, and later its premature regression has occurred resulting in short cycles. Occurrence of similar short cycles were reported in GnRH treated anoestrous does (Knight *et al.*, 1988 and Bretztaff *et al.*, 1991), in anoestrous ewes (Mc Nelly, 1981 and Mc Leod

et al., 1983) and in dairy cows (Webb *et al.*, 1977; Carter *et al.*, 1980 and Troxel *et al.*, 1983).

One possible cause of luteal failure or defective corpus luteum formation is due to insufficient luteotropic support at the time of or after the preovulatory luteinising hormone (LH) surge. In anoestrous ewes, single GnRH administration resulted in an LH surge that was lower than that in natural oestrus and ovulation was followed by formation of less functional corpus luteum (Croghton *et al.*, 1975). Thus it can be concluded that GnRH analogue administration can induce oestrous in acyclic does and these induced oestrous cycle are associated with formation of less functional corpora lutea and occurrence of short cycles.

5.5 Biometry of pituitary and reproductive organs in non cyclic and GnRH responded does

The present study revealed increase in weight and size of pituitary, ovary, uterus and cervix in GnRH responded does as compared to non cyclic controls. However, the difference were not significant. Similar observations of increase in weight and size of ovary, uterus and cervix were observed after GnRH treatment in ewes (Singh and Madan, 1986). Haresign (1975) observed that the uterus and cervix of GnRH treated ewes were typical of anoestrous ewes, eventhough there was significant

increase in weight and size of ovaries due to follicular development.

The increase in weight and size of ovary, uterus and cervix observed in GnRH responded does in the present study are in agreement with the findings of Singh and Madan (1986). This increase in weight and size can be attributed to the follicular growth due to FSH and LH activity following GnRH administration. Increase in weight and size of uterus and cervix can be attributed to the direct action of oestrogens. One of the most important effect of oestrogen is marked by the structural growth of endometrium and endometrial glands resulting in increase in weight and size of uterus.

There was significant ($P<0.01$) increase in the number and size of follicles in the ovary of GnRH responded does as compared to noncyclic controls. Similar increase in number and size of follicles in GnRH treated ewes were reported (Bindon *et al.*, 1991; Bindon, 1975 and Singh and Madan, 1986).

In the ovaries of all the three GnRH responded does slaughtered, regressing corpora lutea of the previous induced oestrous cycle were observed but none in untreated non cyclic does. This shows that the initial dose of GnRH has induced ovulation and the resultant corpora lutea were present as remanant during the next oestrous cycle. These findings confirm that GnRH analogue, Baserelin was effective for

inducing ovulation and for further corpus luteum formation in non cyclic goats.

5.6 Tissue enzymes

ACP, ALP and LDH levels in the tissues of pituitary, ovaries and uterus of non cyclic controls and GnRH responded does were compared for detailed discussion.

5.6.1 Acid phosphatase (ACP)

Present study revealed no significant difference in the level of ACP in the pituitary, ovary and uterus of GnRH responded does to that in non cyclic does. Similarly, no significant difference in the ACP level within and between the right and left ovaries of GnRH responded does and non cyclic controls (Table 31).

Level of ACP was reported to be highest in the epithelial cells of the endometrium and endometrial glands (Mathai, 1982) and in the luteal cells of cyclic ovaries (Bhattacharyya and Saigal, 1990) in goats. However, Mathai and Nirmalan (1992a) and Bahura *et al.* (1993) observed no significant variation in the tissue ACP level during different phases of oestrous cycle. Sureshkumar and Janakiraman (1993) reported significantly higher ACP level in the tissues of ovary and uterus on the day of ovulation as compared to anoestrous

ovaries in Marwari goats. In the present study also, a non significant increase in the level of tissue ACP was observed in the tissue of ovary and uterus of GnRH responded does as compared to non cyclic controls. It can be inferred that under the influence of increased steriods the enzyme levels might have increased showing increased metabolic activity after GnRH administration. Further, remnants of corpora lutea of the previously induced cycle might have contributed for this ACP increase.

Since reports of pituitary ACP level in goats are lacking, the values could not be compared.

5.6.2 Alkaline phosphatase (ALP)

No significant difference in the tissue ALP level in the pituitary, ovary and uterus of GnRH responded does as compared to that in non cyclic controls was observed.

Intensity of ALP activity in the goat ovaries was reported to be higher during luteal phase (Ramachandirah, 1980 and Singh and Rajya, 1982). Similarly, higher ACP activity was reported in the uterus during luteal phase (Singh and Rajya, 1982). Mathai and Nirmalan (1992a) and Sharma *et al.* (1995) observed no significant variation in the level of ACP during different phases of oestrous cycle in goats. However, Sureshkumar and Janakiraman (1993) observed significantly

higher ovarian ALP level on the day of ovulation as compared to inactive ovaries in Marwari goats. Present study also shows higher levels of ALP in the ovaries and uterus of GnRH responded does as compared to non cyclic controls.

It can be confirmed that under the influence of increased steriods, mostly oestrogen, the ALP levels might have increased showing an increase in metabolism following GnRH treatment.

5.6.3 Lactic dehydrogenase (LDH)

There was no significant variation in the level of LDH in the tissues of pituitary and uterus in GnRH responded does as compared to non cyclic controls. Ovarian LDH level between right and left ovaries in GnRH responded and non cyclic does also showed no significant difference. But there was significant difference in ALP between the right ovaries of GnRH responded and non cyclic does. LDH level in both ovaries together also showed significant difference between GnRH responded and non cyclic controls.

There are very few reports showing the tissue LDH level in goats available for comparison. Meghed *et al.* (1996) reported very low LDH activity in oviductal washings of Egyptian buffaloes with inactive ovaries as compared to active ovaries. Higher levels of ovarian tissue LDH in GnRH

responded does could be attributed to the stimulation of ovaries under the influence of GnRH administration. It is stated that even a mild change in the tissue enzyme level of LDH is dependent upon the metabolic need for bringing about a specific biochemical change in the tissues of ovary and uterus, mostly under the influence of steriod hormones (Meghed *et al.*, 1996). Hence it is inferred that an increase in LDH in the ovary and uterus of GnRH responded does could be attributed to action of steriods, mostly oestrogen.

Summary

6. SUMMARY

With the object of the studying the effect of GnRH administration on ovarian activity in non-cyclic adult goats and evaluating its usefulness in restoring normal cycles, an investigation was carried out. The serum progesterone and mineral profile in cyclic and non-cyclic does before and after the administration of GnRH was also estimated with a view to correlate its influence on the reproductive status.

A total of sixty healthy Malabari, Malabari x Saanen and Malabari x Alpine does aged one to four years, with a record of one or more kiddings and 45 days postpartum belonging to Goat Farm of Kerala Agricultural University, Mannuthy were closely observed for a period of 60 days for the occurrence of oestrus. The does in oestrus, detected by vasectomized bucks were identified and subjected to detailed clinico-gynaecological examination. Observations on the duration and intensity of oestrus and length of oestrous cycle were recorded. From among these, eight cyclic does (Group I) and sixteen non-cyclic does (eight each in Group II and III) were selected at random.

Serum samples were collected from all the cyclic and non cyclic does at fortnightly interval for the estimation of macro and microminerals. The serum calcium was estimated by

employing modified 0-cresolphthalein complexone method using standard calcium kit and the inorganic phosphorus was estimated by modified metol method using standard phosphorus kits. Serum microelements copper, cobalt, manganese and zinc were estimated by Atomic Absorption Spectrophotometry.

Serum progesterone concentration in cyclic and non cyclic does was estimated by Radio Immuno Assay (RIA), using commercially available Coat-A Count progesterone kit (Diagnostic Products Corporation, Los Angles, California). The progesterone activity was determined using a single channel gama spectrometer.

A single dose of potent GnRH analogue, Burerelin (RECEPTAL-VET Hoechst Roussel Vet India Ltd., Pune) one millilitre was administered intramuscularly to eight non cyclic does. A second dose was administered to those does which failed to exhibit oestrus within 20 days of administration of Receptal. Out of eight non cyclic does treated, three responded to single dose, one responded to a second dose and four did not respond at all. The oestrus was exhibited at a mean of 87.000 ± 9.950 hrs after the administration of GnRH analogue.

Three does in Group II which responded to GnRH treatment and three noncyclic does in Group III without treatment were slaughtered and their pituitary ovaries, uterus and cervix

were used for the detailed biometry and for the estimation of tissue enzymes. The tissue ACP, ALP and LDH were estimated using standard diagnostic kits.

The mean length of oestrous cycle and duration of oestrus in cyclic does were 20.313 ± 1.553 days and 37.500 ± 7.263 hours, respectively while in GnRH responded does were 12.750 ± 0.830 days and 18.000 ± 4.240 hrs, respectively. The mean length of oestrous cycle and duration of oestrus were significantly lower ($P<0.01$) in GnRH responded does as compared to cyclic does.

The mean serum calcium level in cyclic, non cyclic and GnRH responded does were 9.410 ± 0.770, 9.220 ± 0.830 and 9.625 ± 0.390 mg per cent, respectively. There was no significant difference in the serum calcium level between the three groups.

The mean serum phosphorus level was found significantly higher ($P<0.01$) in GnRH responded does (5.375 ± 0.205 mg per cent) as compared to cyclic does (4.800 ± 0.260 mg per cent) and non-cyclic does (4.770 ± 0.280 mg per cent).

Comparison of mean serum copper level showed no significant difference between cyclic (1.160 ± 0.170 ppm) and GnRH responded does (1.123 ± 0.089 ppm). But there was significantly higher serum copper level in the GnRH responded

and cyclic does as compared to non-cyclic does (0.830 ± 0.110 ppm).

The mean serum cobalt level in cyclic, non-cyclic and GnRH responded does were 0.066 ± 0.016, 0.071 ± 0.010 and 0.063 ± 0.006 ppm, respectively. There was no significant difference in the serum cobalt level between the three groups. Similarly, no significant difference in the serum manganese level was observed between cyclic (0.023 ± 0.005 ppm), non-cyclic (0.030 ± 0.010 ppm) and GnRH responded does (0.025 ± 0.005 ppm).

The serum zinc level was significantly higher in non-cyclic does (1.510 ± 0.430 ppm) as compared to cyclic (1.180 ± 0.120 ppm), and GnRH responded does (1.155 ± 0.091 ppm).

The mean serum progesterone levels in eight cyclic does for the two consecutive oestrous cycles were 0.304 ± 0.087, 1.294 ± 0.382, 2.531 ± 0.758, 3.619 ± 0.794, 2.456 ± 0.430 and 0.871 ± 0.246 ng/ml on day one, four, six, 10, 14 and 18 respectively. The serum progesterone level was found elevated from day one to four and six, reached a peak on day 10, then declined on day 14 and reached lower values on day 18 of oestrous cycle. The overall mean serum progesterone in cyclic does was 1.848 ± 0.339 ng/ml.

The mean serum progesterone level estimated at weekly intervals (Day one, eight, 15 and 22) in sixteen noncyclic does (Group II and III) were 0.189 ± 0.111, 0.186 ± 0.107, 0.191 ± 0.109 and 0.189 ± 0.106 ng/ml, respectively. The overall mean serum progesterone was 0.190 ± 0.106 ng/ml.

The mean serum progesterone concentration in GnRH responded does on day one, four, six, 10, 14 and 18 of induced cycle were 0.158 ± 0.026, 0.800 ± 0.177, 1.475 ± 0.334, 0.675 ± 0.236, 0.280 ± 0.030 and 0.120 ± 0.021 ng/ml, respectively. The serum progesterone level was found elevated from day one to four, reached a peak on day six and declined on day 10 of cycle. There was significantly lower progesterone level in GnRH responded does on all days of oestrous cycle as compared to that in cyclic does.

The overall mean serum progesterone level during induced oestrous cycle in GnRH responded does was 0.585 ± 0.139 ng/ml which was significantly lower as compared to the overall mean progesterone level of normally cyclic does (1.848 ± 0.339 ng/ml) but significantly higher as compared to non-cyclic does prior to treatment (0.190 ± 0.106 ng/ml).

Detailed biometry of pituitary, ovary uterus and cervix of three untreated noncyclic does and three GnRH responded does were carried out. There was no significant increase in the weight of pituitary and weight and size of ovary, uterus

and cervix in GnRH responded does as compared to cyclic does. The mean number and size of follicles were found significantly higher in GnRH responded as compared to untreated non-cyclic does. This confirms that GnRH administration has resulted in increased follicular growth and maturation in GnRH responded does.

The mean ACP level in the tissues of pituitary, right ovary, left ovary and uterus in GnRH responded does were 3.382 ± 0.444, 3.490 ± 0.433, 2.014 ± 0.433 and 3.329 ± 0.208 units/g and that in non cyclic does were 2.663 ± 0.203, 2.240 ± 0.511, 2.815 ± 0.576 and 1.903 ± 0.442 units/g, respectively. There was higher mean ACP level in GnRH responded does as compared to noncyclic does, though statistically significant difference was not observed.

The mean ALP level in the tissues of pituitary, right ovary, left ovary and uterus in GnRH responded does were 8.509 ± 1.557, 35.273 ± 8.068, 25.167 ± 2.027 and 24.233 ± 6.310 units/g and in untreated noncyclic does were 8.098 ± 2.958, 28.730 ± 2.758, 23.352 ± 7.809 and 26.497 ± 14.965 units/g, respectively. Though an increased ALP level was observed in GnRH responded does, no statistically significant difference was noticed.

The mean LDH level in the pituitary, right ovary, left ovary and uterus in GnRH responded were 107.436 ± 20.538,

43.708 ± 3.984, 41.964 ± 7.762 and 44.798 ± 17.884 units/g, and in untreated non-cyclic does were 86.253 ± 18.204, 25.185 ± 4.669, 31.386 ± 10.005 and 41.252 ± 7.864 units/g, respectively. No significant difference in the tissue LDH was observed between the two groups, though higher levels were observed in GnRH responded does. The mean LDH level in both ovaries together in non-cyclic and GnRH responded does were 59.571 ± 7.315 and 85.672 ± 4.289 units/gm, respectively. This was significantly higher ($P<0.01$) in GnRH responded does as compared to non-cyclic controls.

It can be inferred that the GnRH against, Buserelin was effective for inducing oestrus in 50 per cent non-cyclic does. However, the length of induced oestrous cycle, duration of oestrus and intensity of oestrus were significantly lower than that of cyclic does. The serum progesterone levels in GnRH responded does on all days of induced cycle were significantly lower as compared to that in cyclic does. Biometry studies showed no significant increase in weight and size of ovaries due to increased follicular growth and maturation. Presence of corpus luteum in the ovaries of GnRH responded does confirm ovulation following GnRH administration. The non-significant increase in the tissue enzymes ACP, ALP and LDH in the ovary and uterus, reflect increased metabolic activity within these organs subsequent to GnRH administration. The present study confirms that GnRH administration in non-cyclic does may

reactivate the ovary for increased follicular growth, follicular maturation, ovulation and corpus luteum formation. But the CL formed, probably, was inadequate to present a progesterone level similar to normally cyclic does and hence short oestrous cycles occurred.

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OVARIAN RESPONSE TO GONADOTROPHIN RELEASING HORMONE IN NON-CYCLIC GOATS

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ABSTRACT OF A THESIS

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ABSTRACT

The study was conducted to evaluate the effect of Gonadotropic Releasing Hormone (GnRH) on ovarian activity and its usefulness in restoring normal oestrous cycle in adult non-cyclic goats.

A total of 60 healthy Malabari, Malabari x Saanen and Malabari x Alpine does aged one to four years, with a record of one or more kiddings and 45 days post partum belonging to Goat Farm of Kerala Agricultural University, Mannuthy were closely observed for a period of 60 days for the occurrence of oestrus and serum samples were collected at fortnightly intervals for the estimation of macro and micro minerals. Serum progesterone profile in eight cyclic and sixteen non-cyclic does, selected at random, were estimated by Radioimmunoassay using a coat-A-count progesterone kit.

Out of eight non-cyclic does treated with potent GnRH anologue (Buserelin) three responded to single dose and one responded to a second dose. The oestrus was exhibited at a mean of 87.000 ± 9.950 h after the administration of GnRH analogue. The mean length of oestrous cycle and duration of oestrus in cyclic does were 20.313 ± 1.553 days and 37.500 ± 7.263 hrs while in GnRH responded does were 12.750 ± 0.830 days and 18.000 ± 4.240 hrs, respectively.

The mean serum phosphorus level was found significantly higher in GnRH responded does (5.375 ± 0.205 mg per cent) as compared to cyclic does (4.800 ± 0.260 mg per cent) and non-cyclic does (4.770 ± 0.280 mg per cent). There was significantly higher serum copper level in GnRH responded (1.123 ± 0.089 ppm) and in cyclic does (1.160 ± 0.170 ppm) as compared to non-cyclic controls (0.830 ± 0.110 ppm). The serum zinc level was significantly higher in non-cyclic (1.510 ± 0.430 ppm) as compared to cyclic (1.180 ± 0.120 ppm) and GnRH responded does (1.155 ± 0.091 ppm). There was no significant difference in the serum calcium, cobalt and manganese level between the three groups.

The mean serum progesterone in cyclic does for the two consecutive cycles was 0.304 ± 0.087, 1.294 ± 0.382, 2.531 ± 0.758, 3.619 ± 0.794, 2.456 ± 0.430 and 0.871 ± 0.246 ng/ml and in GnRH responded does was 0.158 ± 0.026, 0.800 ± 0.177, 1.475 ± 0.334, 0.675 ± 0.236, 0.280 ± 0.030 and 0.120 ± 0.021 ng/ml on day one, four, six, ten, 14 and 18 of cycle, respectively.

The overall mean serum progesterone during induced cycle in GnRH responded does was 0.535 ± 0.139 ng/ml which was significantly lower as compared to cyclic does (1.848 ± 0.339 ng/ml) but significantly higher as compared to untreated non-cyclic does (0.190 ± 0.106 ng/ml).

Detailed biometry studies of pituitary ovary, uterus and cervix of GnRH responded does showed non-significant increase in the size and weight as compared to untreated non-cyclic does. The mean number and size of follicles were found significantly higher in GnRH responded as compared to non-cyclic does.

The mean tissue ACP, ALP and LDH in the pituitary, ovary and uterus of GnRH responded does showed non significant increase as compared to untreated non-cyclic does. The mean LDH level in both ovaries together was significantly higher in GnRH responded does as compared to non-cyclic does.

The present study confirms that GnRH administration in non-cyclic does has reactivated the ovary by increased follicular growth, maturation and corpus luteum formation. However, the length of induced oestrous cycle, duration and intensity of oestrus was significantly less in GnRH responded does.