FERTLITY TRIALS ON INDUCED OESTRUM IN REPEAT BREEDING CATTLE WITH PROLONGED OESTRUM

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

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2005

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DECLARATION

I hereby declare that this thesis, entitled **"FERTILITY TRIALS ON INDUCED OESTRUM IN REPEAT BREEDING CATTLE WITH PROLONGED OESTRUM"** is a bonafide record of research work done by me during the course of research and that the 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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CERTIFICATE

Certified that the thesis entitled **"FERTILITY TRIALS ON INDUCED OESTRUM IN REPEAT BREEDING CATTLE WITH PROLONGED OESTRUM"** is a record of research work done independently by **Dr. G. Jeba Sujana Dhas**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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ACKNOWLEDGEMENT

It gives me immense pleasure to record my sincere thanks and deep sense of gratitude towards my major advisor ***Dr. K.N. Aravinda Ghosh****, Associate Professor and Head, Veterinary College Hospital, College of Veterinary and Animal Sciences, Mannuthy and Chair person of the Advisory Committee for his pragmatic approach, meticulous guidance, incessant help, valuable suggestions and critical comments during the entire academic programme. It is a great opportunity to work under a affectionate, dynamic academician and clinician.*

I express my deep sense of gratitude to ***Dr. T. Sreekumaran****, Associate Professor and Head, Department of Animal Reproduction and member of the Advisory Committee for his constant supervision, valuable guidance and critical comments throughout the course of the academic programme and research work.*

I am deeply indebted to ***Dr. C. Ibraheem kutty****, Assistant Professor, Department of Animal Reproduction and member of the Advisory Committee for his fruitful discussion, enthusiastic spirit, valuable help and guidance rendered on me during the entire course of study.*

I express my heart full gratitude to ***Dr. K.D. John Martin****, Assistant Professor, Department of Veterinary Surgery and Radiology and member of the Advisory Committee for his lively classes, kind nature, valuable suggestions and encouragement throughout the course of study.*

I extend my heart felt thanks to ***Dr. Joseph Mathew****,* ***Dr. K.V. Athman****,* ***Dr. V. Vijayakumaran****,* ***Dr. Metilda Joseph****,* ***Dr. A.M. Vahida*** *and* ***Dr. G. Ajitkumar*** *for their lively suggestions, motivation and professional guidance.*

At one point or the other all the staff of the Department of Animal Reproduction were involved in this endeavor, I thank all of them.

The invaluable help rendered by the Department of statistics for statistical analysis is gratefully acknowledged.

I wish to express my thanks to ***Dr. E. Nanu****, Dean in charge, College of Veterinary and Animal Sciences, Mannuthy for providing facilities for the research work.*

My sincere thanks to ***Dr. R. Ganga Devi****, Associate Professor, University Livestock Farm, Mannuthy for providing me permission to conduct experiments in the farm.*

I sincerely acknowledge the invaluable help rendered by the staff and labourers of University Livestock Farm, Mannuthy.

My sincere thanks to Indian Council of Agricultural Research for providing me financial assistance in the form of Junior Research Fellowship without which my dream of M.V.Sc. would not have come true.

I owe my heart felt gratitude to ***Dr. Jebina Martha Phillip, Dr. R.S. Abilash, Dr. V. Uma, Dr. Leeba Chacko*** *and* ***Dr. Justin Davis*** *for their valuable help and support provided during the period of my research work.*

I sincerely acknowledge my colleagues ***Dr. Asha Merina Kuriakose, Dr. M. Ratheesh Babu*** *and* ***Dr. K. Magnus Paul*** *for their help and cordial company throughout the course of study.*

I wish to place on record the valuable help and fruitful company rendered to me by ***Dr. L. Deepthi, Dr. S. Deepa, Dr. Safna Issac, Dr. Sandhya Achuthan, Dr. N. Sathiaraj*** *and* ***Dr. S. Jagaveerapandian.***

Special thanks are due to ***Dr. T. Sujatha, Mr. K. Saravanan, Mr. A. Manickam, Dr. V. Jayanthi, Dr. J. Anton Roselin, Dr. P. Jenifer*** *and* ***Dr. R. Krishna Kumar*** *for their assistance, without which this research work would not have been successful.*

My unreserved gratitude goes to my friends ***Chitra akka, Kavitha akka, Rajathi, Deviakka, Reena, Smitha J.P. chechi, Sindhu chechi, Simi, Annie*** *and all fellow* ***P.G's of 2003 batch*** *for their frank friendship extended to me making every moment momentous.*

I extend my heart felt gratitude to all doctors who treated me and for the prayers of all people that gave me strength to complete this study in a successful manner.

I thank my parents, brother and relatives for their selfless love, care and constant encouragement extended in fulfilling this endeavour.

Above all, how could I thank for the mercies of the Lord .....

G.Jeba Sujana Dhas

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Introduction

1. INTRODUCTION

Repeat breeding is a major impediment factor for achieving the goal of one calf every year in dairy cows. According to Roberts (1986) a repeat breeding cow is a one that has normal or nearly normal oestrus cycles and oestrus periods and has been bred two or more times to a fertile bull yet failed to conceive. The incidence of repeat breeding is found to vary between 5.59 per cent (Selvaraju *et al.*, 2005 a) to 34.97 per cent (Velayudakumar, 2003). This situation is aggravated in case of small holders and marginal farmers owning two to five cows, mainly due to lack of managerial practices and proper technical know how. Apart from its clinical importance, its impact on the economy of dairy cattle is considerable.

The causes of repeat breeding include environment, management and animal factors. Alteration in endocrine environment has been suggested to be one of the intrinsic factors in repeat breeder cows. This alteration in hormonal balance results in various abnormalities like delayed or anovulatory conditions in farm animals (Srivatsva and Aahlawat, 1998) leading to fertilization failure (Bage *et al.*, 2002).

Episodic release of gonadotrophic hormones and preovulatory endocrine surge are the important oestrual events determining the pregnancy rate. Insufficient, asynchronous or absence of preovulatory surge of gonadotrophins might produce a variety of functional disorders leading to infertility. The concept of delayed ovulation implies asynchrony in the time relationship between a Luteinising Hormone (LH) surge and ovulation or insufficient LH surge to stimulate the series of events that culminate in

ovulation (Lee *et al.*, 1983). It is suspected that these deviations cause changes in the microenvironment of the preovulatory follicle and thereby affecting its final maturation leading to delayed ovulation and prolongation of oestrum (Bage *et al.*, 2002).

High conception rates in artificially inseminated cows can be achieved if ovulation occurs coincident with the presence of viable sperm in the oviduct. Ovulation in the cow occurs 10-12 hours after the end of behavioral oestrus and 18-26 hours after the ovulatory LH peak (Arthur *et al.*, 1996). Before and during oestrus several follicles develop but usually only one ovulates. Gonadotropin Releasing Hormone (GnRH) is capable of inducing LH release, which favours the sequence of events that ends up in ovulation. If ovulation is delayed in relation to time of insemination, either from errors of heat detection or physiological variation, pregnancy rates will be low.

Ovulatory disturbances constitute one of the major causes of repeat breeding among cross bred cattle of Kerala. An incidence of 8.89 per cent ovulatory disturbances among repeaters was reported by Iyer *et al.* (1992) in a detailed study conducted among animals presented in various infertility camps in Kerala. Various hormonal treatments were tried earlier to improve conception rate in these animals with varying results. Managerial efforts on heat detection and insemination at proper time can be made easier with induction of oestrus which may subsequently lead to improved fertility. Administration of GnRH and human Chorionic Gonadotropin (hCG) at the time of insemination has been investigated by various workers as a tool for obtaining synchrony between ovulation and insemination with the intention of enhancing pregnancy rates in repeat breeding cattle. It may be of relevance to

ascertain the possibility of improving the conception rate by administering GnRH or hCG during induced oestrum in repeat breeding animals.

The present study was aimed to evaluate the fertility in repeat breeding cattle with prolonged oestrum after oestrus induction using Prostaglandin F_2 alpha (PGF_2 alpha) and subsequent treatment with GnRH and hCG.

Review of Literature

2. REVIEW OF LITERATURE

Ovulatory disturbances contribute one of the major causes for repeat breeding among crossbred cows and heifers. Perusal of literature revealed that extensive work regarding ovulatory disturbances and various hormonal treatments for tackling the same have been undertaken in different parts of the world, however only a few trials have been conducted in Kerala.

2.1 OCCURRENCE OF REPEAT BREEDING

An incidence of 10 to 15 per cent repeat breeding among cows and buffaloes was reported by Dhanda and Gajbhiye (1987) in rural Gujarat and Haryana where as Pargoankar and Bakshi (1987) reported an incidence of eight and three per cent repeat breeding in Red Kandhari cows and its crossbreds respectively.

Shukla and Pandit (1989) observed an incidence of 18.01 percent repeat breeding in Gir breed and their crosses. The incidence was higher in heifers (23.5 per cent) than cows (6.23 per cent). Similarly Satheshkumar and Punniamurthy (2003) reported an incidence of 24.6 per cent repeat breeding among heifers in Thanjavur district of Tamil Nadu.

An incidence of 35 and 29.5 per cent repeat breeding among crossbred cows and heifers of Kerala with an overall incidence of 34.97 per cent was reported by Velayudakumar (2003). However, a lower incidence of 5.59 per cent was reported by Selvaraju *et al.* (2005 a) among cattle in Nammakkal district of Tamil Nadu.

2.2 OCCURRENCE OF PROLONGED OESTRUM

Gustaffson *et al.* (1986) noticed that the duration of oestrus was longer in repeat breeding heifers (31.50 ± 3.60 h) when compared to virgin heifers (23.84 ± 2.00 h). He observed that the interval from the onset of oestrus to preovulatory LH peak was longer in repeat breeding heifers (12.2 ± 2.8 h) than in virgin heifers (4.8 ± 1.5 h).

Stevenson *et al.* (1990) observed that the amount of LH released in preovulatory LH surge was less and its timings relative to the onset of oestrus were delayed in repeat breeding crossbred cows. Similarly Albinn (1991) reported that repeat breeding heifers had a longer duration of standing oestrus and more ovulatory disturbances than virgin heifers.

In a study conducted by Iyer *et al.* (1992) in 3427 cows presented at various infertility camps in Kerala, reported an incidence of 8.89 per cent ovulatory disturbances and stated that the higher incidence in cross breeds could be due to higher level of exotic inheritance. However, a higher incidence of 17.14 per cent anovulation and 11.43 per cent delayed ovulation in repeat breeder cows and heifers with an overall incidence of 28.57 per cent ovulatory defects was reported by Khanna and Sharma (1992).

Goley and Kadu (1995) observed that the duration required for ovulation in repeat breeder cows was 42.24 ± 3.54 h and the incidence of anovulation and delayed ovulation were 42.85 and 14.28 per cent respectively.

According to Hukeri (1995) delayed ovulation was one of the major causes of repeat breeding in crossbreds as only 66 per cent of the observed cows ovulated within 24 h of insemination.

High yielding Holstein cows having long intervals between oestrus and ovulation, and between LH surge and ovulation were associated with a markedly low preovulatory LH surge and lower than usual concentrations of oestradiol in the follicular phase before oestrus (Bloch *et al.*, 2001).

Kutty and Ramachandran (2001) reported an incidence of 20 per cent ovulatory disturbances, in a study conducted among 132 repeat breeding cattle. Similarly Velayudakumar (2003) observed an incidence of 23.15 per cent ovulatory disturbances among repeat breeding cows and heifers.

2.3 SUBCLINICAL ENDOMETRITIS

Pateria and Rawal (1990) reported that uterine discharge from normal cows had low number of leucocytes to cause any change of colour where as the discharge from subclinical endometritis cases had moderate colour change and clinical cases showed intense colour reaction when subjected to white side test.

Presence of subclinical endometritis at the end of postpartum period has a significant effect on the number of days open. Average days open for endometritis positive cows were 154 days, compared to 115 days for cows without endometritis (Gilbert, 2002).

Krishnakumar *et al.* (2003) reported that the incidence and occurrence of subclinical endometritis was more common in natural service than artificial insemination. They opined that white side test could be used under field

conditions as a cost effective spot test in diagnosing the subclinical endometritis.

2.4 PGF_2 ALPHA TREATMENT

Reports vary with regard to onset of oestrum and ovulation following oestrus induction using different prostaglandin regimes. The time elapsed between PGF_2 alpha treatment and the onset of oestrus depends on the stage of the oestrus cycle at the time of PGF_2 alpha treatment. The mean interval to oestrus was 48 to 72 h when PGF_2 alpha was administered on oestrus cycle day five or day eight in dairy cows (Tanabe and Hann, 1984). Prostaglandin administration in mid cycle (day 8 to day 11) or later the luteal phase (day 12 to day 15) resulted in a mean time to estrus of 70 and 62 hours, respectively (King *et al.*, 1982, Stevenson *et al.*, 1984).

Moreno *et al.* (1986) reported that injection of 25 mg of PGF_2 alpha was sufficient to produce luteolysis in zebu cattle.

Goley and Kadu (1995) reported that the PGF_2 alpha corrected the uterine milieu and increased the conception rate in repeat breeder cows by preventing early embryonic mortality. Further they stated that it checked mild endometritis by increasing the phagocytic activity by uterine leucocytes and stimulatory actions on smooth muscles.

Mialot *et al.* (1999) recommended AI at the observed oestrum in PGF_2 alpha induced animals when the oestrus detection was conducted most satisfactorily. However, herds in which oestrus detection was poor the sequence of GnRH+PGF_2 alpha protocol and fixed time AI could be followed.

2.4.1 Oestrus response after PGF_2 alpha treatment

In a study conducted by Dailey *et al.* (1983) in postpartum dairy cows, administration of PGF_2 alpha at 112 days postpartum was capable of inducing oestrus within 6 days.

Odde (1990) observed all of the repeat breeder cross bred cows exhibiting oestrus following PGF_2 alpha treatment on the tenth day of cycle since functional corpus luteum was present in the ovaries of these animals.

The injection of PGF_2 alpha at day five of oestrus cycle caused immediate regression of the corpus luteum there by causing sharp decline in progesterone concentration to basal concentration within 24 h. This caused LH pulse frequency to increase causing a significant increase in oestradiol from the dominant follicle and the induction of oestrus and ovulation (Diskin *et al.*, 2002).

Administration of prostaglandin analogue, Tiaprost on day 10 of oestrus cycle resulted in 100 per cent oestrus induction in repeat breeding cattle (Selvaraju *et al.*, 2004).

2.4.2 Time taken for onset of oestrus after induction

The mean onset of oestrus in repeat breeder cows treated with PGF_2 alpha + GnRH and PGF_2 alpha + hCG were 63.71 ± 6.76 and 67.59 ± 5.00 h respectively (Goley and Kadu, 1995).

In a trial carried out to study the effect of administration of PGF_2 alpha for induction of oestrus in cows with clinical endometritis, Jacob *et al.* (1995)

noticed that the time taken for the expression of oestrus symptoms ranged from 36 to 120 h with an average of 58.95 h.

The interval from exogenous induced luteolysis or withdrawal of a progestagen treatment to oestrus and ovulation depends on the stage of follicular wave at time of luteolysis or at time of withdrawal of progestagen (Diskin *et al.*, 2002). They further observed that cattle with a dominant follicle showed oestrus with in 2-3 days, but those in which follicular growth was at the preselection stage, oestrus occurred after a period of 3-7 days.

Selvaraju *et al.* (2004) could not observe any significant difference on the onset of oestrus in animals induced using prostaglandin, followed by administration of hCG at 24 h prior to AI, during AI and four days after AI, the mean interval being 58.88 ± 0.75, 60.00 ± 0.77 and 59.63 ± 0.74 h respectively.

Administration of 0.98 mg of PGF_2 alpha analogue (Tiaprost) on day ten induced oestrum in 59.38 ± 0.81 h in repeat breeding animals (Selvaraju *et al.*, 2005 b).

2.4.3 Duration of oestrum

Nair and Madhavan (1984) reported that the duration of induced oestrus did not show marked variation from the normal oestrus in cross bred cows. However, a longer duration of 24-48 hours of oestrus response was observed in oestrus induced cows with endometritis than animals in natural oestrus (Jacob *et al.*, 1995).

Ajitkumar *et al.* (1996) studied the effect of single or double injection of PGF_2 alpha (25 mg Dinoprost) in heifers. The duration of oestrum ranged from 12 to 30 h and 18 to 36 h in single and double injection groups.

Selvaraju *et al.* (2004) observed that supplementation of hCG at the various stages following oestrus induction did not influence the duration of oestrus in repeat breeder cows. The duration of induced oestrus was 28.63 ± 0.65, 27.06 ± 0.95 and 27.5 ± 0.70 h in animals administered hCG at 24 h prior to AI, during AI and four days after AI respectively.

2.4.4 Intensity of oestrual signs

A high incidence of weak signs of oestrus was reported by Nair and Madhavan (1984) when oestrus was induced by using prostaglandin F_2 alpha where as Jacob *et al.* (1995) reported 66.66, 23.8 and 9.52 per cent of intense, medium and weak oestrus respectively in natural oestrus while the respective values in prostaglandin induced group were 66.66, 19.04 and 14.28 per cent.

Ajitkumar *et al.* (1996) reported that in PGF_2 alpha induced animals, there was marginal increase in vulval oedema, hyperemia of vaginal mucosa and oestrual discharge.

Administration of hCG at different stages following oestrus induction did not alter the intensity of oestrual signs in repeat breeder cows (Selvaraju *et al.*, 2004).

2.4.5 Fertility after PGF_2 alpha treatment

Oxender *et al.* (1974) obtained a conception rate of 78.94 per cent in cows, after administration of 30 mg PGF_2 alpha intramuscularly and inseminated at 70 and 88 h.

Duetscher *et al.* (1982) studied oestrus synchronization and pregnancy rates in beef cattle using single PGF_2 alpha. Out of 85 per cent lactating cows that exhibited oestrus a conception rate of 59 per cent was obtained.

Plunkett *et al.* (1984) induced oestrus using single injection of 25 mg PGF_2 alpha after detection of corpus luteum by ovarian palpation. The first service conception rate and overall conception rate were 43 and 86 per cent respectively.

Jacob *et al.* (1995) reported a conception rate of 52.27 per cent in crossbred cows, inseminated twice at an interval of 24 h in standing oestrus, induced by administration of 25 mg Dinoprost.

Ajitkumar *et al.* (1996) studied the effect of prostaglandin administration in heifers. The first insemination conception rate and overall conception rate were 33.33 and 66.67 per cent when inseminated 72 h post treatment while the respective values were 33.33 and 50 per cent when inseminated 96 h post treatment in heifers given single dose of PGF_2 alpha. A marginal increase in the overall conception rate was observed in heifers inseminated 72 h after the administration of the second dose of PGF_2 alpha, 12.5 per cent of animals conceived at first insemination while the overall conception rate was 62.5 per cent. The corresponding values at 92 h insemination were 25 and 50 per cent respectively.

A retrospective analysis was done by Strelow (1993) to determine the effect of PGF_2 alpha on conception rate in commercial dairy herds. The insemination rate within 6 days of treatment was 77.6 per cent. The conception rate for the treated animals (47.9 per cent) did not differ from that of non treated controls (47.2 per cent).

Leeba (2003) observed that induction of oestrus using 25 mg PGF_2 alpha analogue on tenth day of oestrum resulted in a first service conception rate of 42.86 and 50 per cent in cows and heifers respectively. The over all conception rate was 66.67 per cent after three consecutive oestrus periods.

Treatment of pleuriparous cows having subclinical endometritis with PGF_2 alpha resulted in a reduction in average days open from 160 to 119 and increase in first service conception rate from 22 to 45 per cent (Gilbert *et al.*, 2004).

Induction of oestrum using 0.98 mg of PGF_2 alpha analogue (Tiaprost) resulted in a first service conception rate of 43.75 per cent in repeat breeding animals (Selvaraju *et al.*, 2005 b).

2.5 EFFECT OF SEASON

Gwazdauskas *et al.* (1983) have stated that maximum environmental temperature on the day of oestrus had a significant relationship with oestrus activity at the first observation of heat. As maximum daily temperature increased to approximately 25°C, mounting activity also increased. However beyond 30°C a decline in mounting activity per hour occurred.

Heat stress has been shown to reduce reproductive efficiency, particularly in lactating dairy cows, both by reducing estrous

expression/detection and by decreasing conception rate (Stevenson *et al.*, 1984).

Ajitkumar *et al.* (1995) opined that season of the year did not influence oestrus response or conception rate in cows of Kerala. However, in heifers Ajitkumar *et al.* (1996) observed that the time taken for induction was least during rainy season and maximum during winter.

In both winter and summer GnRH increased the conception rate of cows with low body condition score at AI from 36.0 to 61.5 per cent, but did not affect the conception rate of cows with a high body condition score at AI (Kaim *et al.*, 2003).

2.6 EFFECT OF PARITY

Nakao *et al.* (1983) opined that GnRH treatment at the time of insemination significantly increased the first service pregnancy rate in dairy cows, especially in those on the first and third lactation.

Rosenberg *et al.* (1991) stated that administration of GnRH within 3 h of oestrus detection increased conception to 64.3 per cent in primiparous cows and 56.1 per cent in pluriparous cows. However, Ajitkumar *et al.* (1995) observed that parity of cows didnot influence oestrual response following oestrous synchronisation. Gilbert (2002) opined that parity of cows had no significant effect on the incidence of endometritis.

Ovulation response to GnRH teatment in postpartum dairy cows was influenced by parity and follicle size. Cows having follicle size less than 10-12 mm in diameter were much less likely to ovulate (Gumen and Seguin, 2003).

Conception rates were consistently greater during first lactation (27 per cent) than during later lactations (19 per cent) in cows treated with shortened ovsynch protocol (Stevenson *et al.*, 2003).

2.7 GnRH AND hCG TREATMENT

Lee *et al.* (1983) reported that administration of GnRH at the time of breeding induced an additional surge of LH that enhanced luteinisation of granulosa cells, which ensured adequate production of progesterone to maintain pregnancy.

GnRH administration at insemination, 10 h after the onset of oestrus was capable of eliciting an endogenous surge of LH in most animals. Cows that responded with the intermediate surge of LH and subsequently became pregnant produced higher progesterone. Cows with extremely higher LH response or no LH response to GnRH and that did not conceive had lower progesterone production. High conception rates in cows injected with GnRH were probably the result of increased progestrone production to support pregnancy (Lee *et al.*, 1985).

Lucy and Stevenson (1986) found that when GnRH was administered concomitantly with the preovulatory LH surge, serum progesterone during 7 days after treatment was lower and slow to rise than when GnRH or saline treatment occurred after a spontaneous LH surge. They further opined that improved fertility after GnRH treatment might be associated with delayed or slowly rising concentrations of serum progesterone after ovulation.

Mee *et al.* (1990) observed that GnRH administration at first service postpartum failed to improve pregnancy rate, regardless of the timing of

GnRH administration or AI relative to the onset of oestrus. However, injection of GnRH early in oestrus and AI late in oestrus restored pregnancy rates to control values, which suggests an antifertility effect of GnRH, depending on when it is administered relative to the onset of oestrus and timing of the preovulatory effect of LH surge.

Rosenberg *et al.* (1991) reported that GnRH increased the fertility of cows when administered soon after the onset of oestrus. In cows that required more than one AI, GnRH improved fertility when AI was performed late in the oestrus period.

The administration of GnRH before PGF_2 alpha treatment reduced variability in the time interval to estrus after PGF_2 alpha injection (Twagiramungu *et al.*, 1992).

Administration of GnRH at oestrus in repeat breeding dairy cows increased concentration of progesterone soon after ovulation and maintained higher concentration of progesterone up to 40 d during pregnancy and was associated with higher embryonic survival until 42 to 56 d after AI. Increased progesterone after GnRH administration was due to an increased proportion of large luteal cells in the corpus luteum and possibly due to increased concentration and pulse frequency of FSH secretion (Mee *et al.*, 1993).

Wolfenson *et al.* (1994) suggested that usage of GnRH in cows 7 d prior to synchronisation of oestrus with PGF_2 alpha altered follicular development. Treatment with GnRH produced preovulatory follicle, which were more homogeneous, more oestrogen active and more dominant and there was a greater size difference between the preovulatory and subordinate follicles prior to oestrus.

Mann and Lamming (1995) reported that treatment with Buserelin on day 12 after mating, caused a reduction in oestradiol secretion, which reduced the stimulus for the development of the luteolytic mechanism. Faced with a weaker luteolytic signal, the chances of an embryo preventing luteolysis were increased and conception rate was improved.

Pursley *et al.* (1995) obtained synchronised ovulation in dairy cows within 8 h using a sequence of GnRH + PGF_2 alpha + GnRH (Ovsynch). This protocol allowed effective management in lactating dairy cows with difficulty of oestrus detection, and provided similar pregnancy rate when compared with those of classical reproductive management systems based on oestrus detection and hormonal therapy (Pursley *et al.*, 1997).

Administration of hCG or GnRH at the time of insemination in 30 animals with ovulatory disturbances resulted in cessation of behavioural oestrum within 24 and 30 h after treatment (Senthilkumar and Rajasekar, 1998).

Diskin *et al.* (2002) stated that the effect of GnRH on the existing follicle wave was dependent on the presence or absence of a dominant follicle.

Administration of GnRH at the onset of oestrus decreased the time interval from the onset of oestrus to the LH surge peak but it did not affect the interval from the peak of LH surge to ovulation (Kaim *et al.*, 2003).

Peters and Pursley (2003) stated that in order to control the time of LH surge and ovulation, the final GnRH administration of ovsynch must be given prior to a spontaneous LH surge to control time of AI.

2.7.1 Fertility after GnRH treatment

Nakao *et al.* (1984) noticed that treatment of 72 cows those failed to ovulate within 24-36 h after insemination, when reinseminated and treated intramuscularly with either GnRH or hCG resulted in ovulation rates and conception rates of 79.60 and 87.00 per cent and 46.90 and 26.10 per cent respectively.

Anderson and Malmo (1985) concluded that injection of 250 μg of synthetic GnRH at the time of service does not increase pregnancy rate to that service.

GnRH administration at the time of breeding resulted in a conception rate of 73 per cent (Lee *et al.*, 1983), 53 per cent (Roussel, 1988) in repeat breeding animals.

Administration of PGF_2 alpha in repeat breeding cattle produced a greater conception rate when oestrus was detected than after inseminations at 72 and 96 h in the absence of oestrus regardless of subsequent treatment with GnRH (Stevenson *et al.*, 1988).

Stevenson *et al.* (1990) reported that pregnancy rates of lactating dairy cows at third or fourth service were improved when 100μg of GnRH was administered intramuscularly following AI.

Administration of 2.5 ml of Receptal at the time of insemination in 40 crossbred cows having ovulatory disturbances due to delayed ovulation, resulted ovulation in 90 per cent of the cows giving a conception rate of 70 per cent (Iyer and Sreekumaran, 1992).

Archbald *et al.* (1993) observed that treatment with GnRH at or prior to insemination did not improve the pregnancy rate of repeat breeder dairy cows.

Ryan *et al.* (1994) stated that treatment of lactating dairy cows at pasture in a temperate climate with GnRH analogue, either at AI or on day 12 after AI, altered endocrine responses and ovarian follicle populations, but did not affect pregnancy rate.

Goley and Kadu (1995) obtained a conception of 57.74 per cent on treatment with 10 μg of Buserelin before AI after synchronization with PGF_2 alpha analogue in repeat breeder cows.

GnRH administration 12 h after onset of oestrus followed by AI induced 100 per cent ovulation and 75 per cent first service conception in repeat breeding cows (Selvaraj and Kumar, 2001).

High efficacy of oestrus synchronisation was observed in normal cows when GnRH was administered 48 h after PGF_2 alpha and the conception rate was 66 per cent (Doleiel *et al.*, 2002).

Synchronisation of oestrus using PGF_2 alpha preceded by progesterone, GnRH or both produced unexplainably differing pregnancy rate responses in heifers, with GnRH pretreated animals producing the lowest pregnancy rate (Richardson *et al.*, 2002).

Administration of 10μg Buserelin just before insemination resulted a conception rate of 20 per cent higher than control and GnRH was found to have no influence on subsequent serum progesterone level on fourteenth and twenty second day post insemination (Shelar *et al.*, 2002).

Srivatsava and Kharche (2002) noticed that administration of 20μg GnRH was more effective than 10μg and it improved the first service conception rate.

Coyan *et al.* (2003) made a study to compare the effectiveness of treatments combining GnRH and PGF_2 alpha, hCG and PGF_2 alpha combinations and double PGF_2 alpha administration for synchronisation of oestrus in cows. He opined that application of GnRH and hCG prior to synchronisation of oestrus with an injection of PGF_2 alpha may provide better results than two injection of PGF_2 alpha for oestrus synchronisation in cows.

Administration of GnRH in 24 h, 48 h and 72 h after PGF_2 alpha in cows bearing corpus luteum and atleast one largest follicle of 8-15 mm diameter produced a conception rate of 0, 50.00 and 12.50 per cent in the three groups respectively (Hartman *et al.*, 2003).

Administration of PGF_2 alpha and GnRH on the day of oestrum was found to increase the conception rate in repeat breeding cattle than the use of hCG (Velayudakumar, 2003).

2.7.2 Fertility after hCG treatment

Mehta *et al.* (1986) observed a conception rate of 83.33 per cent when repeat breeder cows and heifers with ovulatory disturbances were treated with 1500 IU of hCG during oestrus.

Swanson and Young (1990) observed that administration of 5000 IU hCG at the time of insemination did not result in increased fertility rate.

Goley and Kadu (1995) obtained a conception rate of 42.8 per cent on treatment with 1000 IU of hCG before AI after synchronisation with 25 mg of PGF_2 alpha in repeat breeder cows.

Replacing the second injection of GnRH agonist with an injection of hCG in the synchronisation protocol did not prevent a reduction in conception rate (Schmitt *et al.*, 1996).

Srivatsava and Aahlawat, (1998) stated that administration of 1500 IU of Chorulon intramuscularly within two minutes after insemination in repeat breeding cows produced a conception rate of 70 per cent compared to that of 37.5 per cent in saline treated control cows.

Fertility of postpartum cows was about 62 per cent when oestrus induction was done using PGF_2 alpha and hCG and fixed time insemination done after 16 to 18h of treatment (De Rensis *et al.*, 2002).

Sato *et al.* (2003) carried out a study to determine the efficacy of replacing the second GnRH with hCG in ovsynch protocol used for timed AI in cows. Conception rates were 52.4 per cent for cows treated with hCG and 51.2 per cent for cows treated with ovsynch.

A first service pregnancy rate of 66.66 per cent was obtained when hCG was administered on the day of oestrum in repeat breeder cows (Selvaraju *et al.*, 2003).

Administration of PGF_2 alpha, PGF_2 alpha and hCG at 48 and 72 h in repeat breeding cows produced first service conception rate of 43.75, 43.75 and 62.50 per cent respectively (Selvaraju *et al.*, 2004).

Materials and Methods

3. MATERIALS AND METHODS

The material for the present study consisted of crossbred cows and heifers belonging to University Livestock Farm, Mannuthy and those presented at Artificial Insemination Centre and Bull station, attached to the Department of Animal Reproduction, College of Veterinary and Animal Sciences, Mannuthy, Thrissur during the period from August 2004 to July 2005. Detailed data regarding the occurrence, onset, duration and intensity of prolonged oestrum among repeat breeders was collected using a suitable proforma. From among these, 40 animals (10 animals in each group) with the history of prolonged oestrus were selected randomly and grouped into four (Groups I to IV).

3.1 OCCURENCE OF PROLONGED OESTRUM

History of prolonged oestrum in the previous cycles of all repeat breeding cows and heifers were collected to determine the occurrence of prolonged oestrum. Animals having oestrus symptoms for more than 30 h were classified under prolonged oestrum.

3.2 DURATION AND INTENSITY OF PROLONGED OESTRUM

The period from begining to the end of the oestrus was recorded as the duration of oestrum. The intensity of oestrus was graded as high, medium and low based on behavioural symptoms and detailed clinico-gynaecological examination.

3.3 DETECTION OF SUBCLINICAL ENDOMETRITIS

Possibility of subclinical endometritis was ruled out by white side test in all the repeat breeders with history of prolonged oestrum as described by Pateria and Rawal, (1990). The uterine discharges were aseptically collected with a sterile pipette and syringe and mixed with equal volume of 5% NaOH. The mixture was heated up to the boiling point and the intensity of colour change was studied and graded as follows

1. Normal - Turbid or no colour.
2. Mild - Light yellow colour.
3. Moderate - Yellow colour.
4. Severe - Dark yellow colour.

Animals not exhibiting any signs of subclinical endometritis were selected for the study. On tenth day of cycle, detailed gynaecological examination was carried out. On ascertaining the presence of functional corpus luteum in one of the ovaries, the animals were randomly allotted to four groups of ten each and adopted the treatment.

Group I

Oestrum was induced using 25 mg dinoprost, a PGF_2 alpha analogue (Lutalyse) on the tenth day of cycle followed by artificial insemination twice at 24 h interval on detecting proper signs of oestrum, as confirmed by clinico-gynaecological examination.

Group II

Oestrus induction was done using 25 mg dinoprost on tenth day of cycle and 10 μg buserelin, a GnRH analogue (Receptal) administered 48 h after PGF_2 alpha administration followed by artificial insemination twice at 24 h interval on detection of proper signs of oestrum.

Group III

Oestrus was induced using 25 mg dinoprost on tenth day of cycle and 1500 IU hCG (Chorulon) was administered 48 h after PGF_2 alpha administration followed by artificial insemination twice at 24 h interval on detection of proper signs of oestrum.

Group IV

Animals showing prolonged oestrum was subjected to insemination twice at 24 h interval after the onset of natural oestrum.

3.4 OESTRUS RESPONSE

In all the groups, oestrus response was studied in detail.

3.5 TIME TAKEN FOR INDUCTION OF OESTRUS

Group I, II and III repeat breeding animals were closely observed for the signs of oestrus after PGF_2 alpha injection and those found oestrus were confirmed by rectal palpation of internal genitalia. The interval from the administration of PGF_2 alpha to the onset of oestrum was recorded as the time taken for the induction of oestrus.

3.6 DURATION OF OESTRUS

Duration of oestrus was determined by close observation of clinical signs like cervical mucous discharge, vulval oedema, hyperemia of vaginal mucous membrane and bellowing. The period from the beginning to the end of exhibition of clinical signs was considered as the duration of oestrus.

3.7 INTENSITY OF OESTRUS

The intensity of oestrus was graded as high, medium and low from clinical and behavioural signs.

3.8 PHYSICAL CHANGES OF THE REPRODUCTIVE TRACT DURING OESTRUS

Physical changes in reproductive tract of animals in all groups, including oedema of vulval lips, hyperemia of vaginal mucosa, nature of discharge, cervical dilatation, tonicity of uterine horns and ovarian changes were recorded by detailed clinico-gynaecological examination.

3.9 CONCEPTION RATE

Pregnancy was confirmed by per rectal examination on 45-60 days after insemination and over all conception rate in each group was calculated. Conception rate in induced groups were compared with each other and with repeaters of group IV.

3.10 EFFECT OF PARITY

The observations in respect of animals classified according to parity were analysed.

3.11 EFFECT OF SEASON

The whole year was divided into three seasons, summer (Season I-March to June), rainy (Season II-July to September) and winter (Season III-October to February). The effect of season on conception rate was studied.

3.12 STATISTICAL ANALYSIS

The data obtained were compiled and subjected to statistical analysis as per Snedecor and Cochran (1985).

Results

4. RESULTS

Results of the investigations on fertility trials on induced oestrum in repeat breeding cattle with prolonged oestrum are presented in tables 1-13 and figures 1-16.

4.1 OCCURRENCE OF REPEAT BREEDING

Out of 817 crossbred cows and heifers presented for AI at Artificial Insemination center Mannuthy, 179 (21.91 per cent) animals were screened as repeat breeders, which includes 17.31 and 22.99 per cent heifers and cows, respectively (Table 1 and Fig. 1).

Among 193 animals investigated in University Livestock Farm, Mannuthy the incidence of repeat breeding was 19.17 per cent, which includes 15.68 and 20.42 per cent heifers and cows respectively (Table 2 and Fig. 2).

4.2 OCCURRENCE OF PROLONGED OESTRUM

Out of 179 repeat breeders, prolonged oestrum was exhibited by 47 (26.26 per cent) animals, which comprises of 38 cows (25 per cent) and 9 heifers (33.3 per cent) (Table 1 and Fig. 3).

Among 37 repeat breeding farm animals it was found that prolonged oestrum occurred in six cows (20.68 per cent) and two heifers (25 per cent) with an overall occurrence of 21.62 per cent (Table 2 and Fig. 4).

4.3 DURATION AND INTENSITY OF PROLONGED OESTRUM IN REPEAT BREEDING ANIMALS

Duration of prolonged oestrum ranged from 32 to 96 hrs with a mean of 65.65 ± 2.57 h. Out of the 40 repeat breeding animals selected for the study, 14 (35 per cent) animals exhibited oestrus length between 30-48 h, 18 (45 per cent) animals between 48-72 h, and 8 (20 per cent) animals between 72 and 96 h (Table 3 and Fig. 5). The intensity of oestrum was found to be higher in 31 (77.5 per cent), medium in eight (20 per cent) and low in one (2.5 per cent) animal (Table 4 and Fig. 6).

4.4 OESTRUS RESPONSE AFTER ADMINISTRATION OF PGF_2 ALPHA

Oestrus response after administration of lutalyse in groups I, II and III are presented in table 5. All the 30 animals (10 each) responded to the treatment by exhibiting oestrus signs and hence the efficacy was 100 per cent.

4.5 TIME TAKEN FOR INDUCTION OF OESTRUS

The time taken for induction of oestrus in group I, II and III animals were 52.7 ± 2.99 h, 51.7 ± 2.68 h and 52.0 ± 2.68 h respectively (Table 5 and Fig. 7). Analysis of data showed that there was no significant difference on time taken for induction of oestrus between the three groups.

4.6 DURATION OF OESTRUM AFTER PROSTAGLANDIN ADMINISTRATION

The duration of oestrus in groups I, II and III were 68.6 ± 3.75 h, 38.6 ± 3.75 h and 37.4 ± 3.75 h respectively (Table 5 and Fig. 7). Analysis

of data revealed that there was a significant difference in the duration of oestrus between group I and the other two groups (II and III).

4.7 PHYSICAL CHANGES OF THE REPRODUCTIVE TRACT DURING OESTRUS

Physical changes of reproductive tract of animals in all groups, including oedema of vulval lips, hyperemia of vaginal mucosa, tonicity of uterine horns and nature of discharge are presented in tables 6-9 and Fig. 8-11.

During natural oestrum the percentage of high, medium and low degrees of vulval oedema was 70, 20 and 10 in group I, 60, 30 and 10 in group II, 30, 50 and 20 in group III and 50, 30 and 20 in group IV animals respectively. The corresponding values during induced oestrum was 50, 20 and 30 in group I, 30, 50 and 20 in group II and 40, 40 and 20 in group III animals respectively.

The percentage of high, medium and low degrees of hyperemia of vaginal mucous membrane during natural oestrum was 60, 30 and 10 in group I, 60, 20 and 20 in group II, 50, 30 and 20 in group III and 40, 40 and 20 in group IV animals respectively. The corresponding data during induced oestrum was 40, 30 and 30 in group I, 20, 50 and 30 in group II and 40, 20 and 40 in group III animals respectively.

The stringy, watery and scanty nature of oestrual discharge observed in all the four groups were 60, 30 and 10 in group I, 60, 30 and 10 in group II, 70, 20 and 10 in group III and 50, 20 and 30 in group IV animals respectively during natural oestrum. During induced oestrum the nature of discharge in

the three groups was 60, 20 and 20 in group I, 60, 20 and 20 in group II and 30, 30 and 40 in group III animals respectively.

During natural oestrum the percentage of high, medium and low degrees of tonicity of uterus were 60, 30 and 10 in group I, 60, 30 and 10 in group II, 50, 30 and 20 in group III and 50, 20 and 30 in group IV animals respectively. The corresponding values during induced oestrum was 40, 50 and 10 in group I, 20, 50 and 30 in group II and 30, 50 and 20 in group III animals respectively.

4.8 INTENSITY OF OESTRUS AFTER PROSTAGLANDIN ADMINISTRATION

Intensity of oestrus in all the three groups are presented in table 4 and Fig. 13.

In group I, out of ten oestrus induced repeat breeding animals, 9 (90 per cent) showed high intensity of oestrus and one (ten per cent) showed medium intensity of oestrus.

In group II out of ten repeat breeding animals induced, two (20 per cent) showed high intensity of oestrus, five (50 per cent) showed medium intensity and three (30 per cent) showed low intensity of oestrus.

Out of the ten oestrus induced repeat breeding animals in group III, five (50 per cent) showed high intensity of oestrus, three (30 per cent) showed medium intensity and two (20 per cent) showed low intensity of oestrum.

4.9 COMPARISION OF DURATION OF OESTRUM IN OESTRUS INDUCED GROUPS

The mean duration of oestrum in group I, II and III repeat breeding animals were 65.8 ± 4.59, 66.8 ± 5.6 and 58.2 ± 5.71 h in natural oestrum and 68.6 ± 3.75, 38.6 ± 3.75 and 37.4 ± 3.75 h in induced oestrum (Table 10 and Fig.13). Analysis of data showed that there was no significant difference between repeat breeding animals in group I on duration of oestrum before and after induction (t = 0.83). There was a significant difference among group II (t = 7.33) and group III (t = 3.32) repeat breeding animals on duration of oestrum before and after induction.

4.10 CONCEPTION RATE DURING INDUCED OESTRUM IN REPEAT BREEDING ANIMALS

Conception rate during induced oestrum in repeat breeding animals with history of prolonged oestrum in group I, II, III and IV were presented in table 11 and Fig. 14.

In group I, ten repeat breeding animals were treated with PGF_2 alpha of which five (50 per cent) conceived.

In group II, of the ten repeat breeding animals treated with PGF_2 alpha and GnRH four (40 per cent) conceived.

In group III, ten repeat breeding animals were treated with PGF_2 alpha and hCG, of which four (40 per cent) animals conceived.

In group IV out of the ten repeat breeding animals which were inseminated at 24 h interval after the onset of oestrus, only three (30 per cent) animals concieved.

4.11 OVERALL CONCEPTION RATE

The overall conception rate for three consecutive oestrus in group I, II, III and control group were 60, 60, 50 and 40 percentage respectively (Table 11 and Fig.14).

The overall conception rate in heifers, primipara and pluripara after induction of oestrum were 42.85, 44.44 and 64.28 per cent respectively (Table 12 and Fig.15).

The overall conception rate during summer, rainy and winter seasons in repeat breeding animals showing prolonged oestrum were 46.15, 53.33 and 50.00 per cent respectively (Table 13 and Fig.16).

Table 1. Occurrence of repeat breeding and prolonged oestrum among cows and heifers presented in AI centre, Mannuthy.

Animals	No. of animals screened	Repeat breeding animals		Repeat breeding animals showing prolonged oestrum	
		Number	Per cent	Number	Per cent
Cows	661	152	22.99	38	25
Heifers	156	27	17.31	9	33.3
Total	817	179	21.91	47	26.26

Table 2. Occurrence of repeat breeding and prolonged oestrum among cows and heifers belonging to University Livestock Farm, Mannuthy.

Animals	No. of animals screened	Repeat breeding animals		Repeat breeding animals showing prolonged oestrum	
		Number	Per cent	Number	Per cent
Cows	142	29	20.42	6	20.68
Heifers	51	8	15.68	2	25
Total	193	37	19.17	8	21.62

Table 3. Duration of prolonged oestrum in repeat breeding animals.

Total number of animals	Duration of prolonged oestrum (hours)							
	Range	Mean±SE(hours)	>30-48 h		48-72 h		>72 h	
			Number	Per cent	Number	Per cent	Number	Per cent
40	32-96 h	65.65±2.57	14	35	18	45	8	20

Table 4. Intensity of oestrus prior to induction and after induction using Dinoprost tromethamine and treatment with GnRH and hCG

Degree of Intensity	Intensity of oestrum prior to induction		Intensity of oestrum					
			Group I		Group II		Group III	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
High	31	77.5	9	90	2	20	5	50
Medium	8	20	1	10	5	50	3	30
Low	1	2.5	-	-	3	30	2	20

Table 5. Oestrus induction using Dinoprost tromethamine (Lutalyse) in repeat breeding animals.

	Group I	Group II	Group III
Number of animals treated	10	10	10
Number of animals responded to $PGF_2\alpha$ treatment	10(100%)	10(100%)	10(100%)
Time taken for induction of oestrus (Mean ± SE hours)	52.7 ±2.99	51.7±2.68	52.0± 2.68
Duration of oestrum (Mean ± SE hours)	68.6 ± 3.75	38.6 ± 3.75	37.4 ± 3.75

Table 6.Vulval oedema in repeat breeders with prolonged oestrum during natural and induced oestrum

Groups	Vulval oedema											
	During natural oestrum						During induced oestrum					
	High		Medium		Low		High		Medium		Low	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Group I	7	70	2	20	1	10	5	50	2	20	3	30
Group II	6	60	3	30	1	10	3	30	5	50	2	20
Group III	3	30	5	50	2	20	4	40	4	40	2	20
Group IV	5	50	3	30	2	20	-	-	-	-	-	-

Table 7.Hyperemia of vaginal mucous membrane in repeat breeders with prolonged oestrum during natural and induced oestrum

Groups	Hyperemia of vaginal mucous membrane											
	During natural oestrum						During induced oestrum					
	High		Medium		Low		High		Medium		Low	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Group I	6	60	3	30	1	10	4	40	3	30	3	30
Group II	6	60	2	20	2	20	2	20	5	50	3	30
Group III	5	50	3	30	2	20	4	40	2	20	4	40
Group IV	4	40	4	40	2	20	-	-	-	-	-	-

Table 8.Nature of oestrual discharge in repeat breeders with prolonged oestrum during natural and induced oestrum

Groups	Nature of oestrual discharge											
	During natural oestrum						During induced oestrum					
	Stringy		Watery		Scanty		Stringy		Watery		Scanty	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Group I	6	60	3	30	1	10	6	60	2	20	2	20
Group II	6	60	3	30	1	10	6	60	2	20	2	20
Group III	7	70	2	20	1	10	3	30	3	30	4	40
Group IV	5	50	2	20	3	30	-	-	-	-	-	-

Table 9.Tonicity of uterus in repeat breeders with prolonged oestrum during natural and induced oestrum

Groups	Tonicity of uterus											
	During natural oestrum						During induced oestrum					
	High		Medium		Low		High		Medium		Low	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Group I	6	60	3	30	1	10	4	40	5	50	1	10
Group II	6	60	3	30	1	10	2	20	5	50	3	30
Group III	5	50	3	30	2	20	3	30	5	50	2	20
Group IV	5	50	2	20	3	30	-	-	-	-	-	-

Table 10. Comparision of duration of oestrus in natural oestrus and during induction in repeat breeders with prolonged oestrum

Groups	Duration of oestrum in natural oestrus (Mean±SE hours)	Duration of oestrum after induction (Mean±SE hours)
Group I	65.8 ± 4.59	68.6 ± 3.75
Group II	66.8 ± 5.6	38.6 ± 3.75
Group III	58.2 ± 5.71	37.4 ± 3.75
Group IV	66.5 ± 5.5	-

Table 11. Fertility during induced oestrum and subsequent oestrum in repeat breeders and in control animals

Groups	Number of animals	Conception rate after first insemination		Overall conception rate after three oestrus	
		Number	Per cent	Number	Per cent
Group I (PGF_2 alpha alone)	10	5	50	6	60
Group II (PGF_2 alpha + GnRH)	10	4	40	6	60
Group III (PGF_2 alpha + hCG)	10	4	40	5	50
Group IV (Control)	10	3	30	4	40

Table 12. Effect of parity on conception rate in oestrum induced repeat breeders showing prolonged oestrum

Parity	No. of animals	Conception rate after first insemination		Overall conception rate after three oestrus	
		Number	Per cent	Number	Per cent
Heifers	7	2	28.57	3	42.85
Primiparous	9	3	33.33	4	44.44
Pluriparous	14	7	50	9	64.28

Table 13. Effect of season on conception rate in repeat breeding cattle showing prolonged oestrum

Season	No. of animals	Conception rate following first insemination		Overall conception rate after three oestrus	
		Number	Per cent	Number	Per cent
Season I (March – June) Summer season	13	5	38.46	6	46.15
Season II (July - September) Rainy season	15	6	40	8	53.33
Season III (October – February) Winter season	12	4	33.33	6	50

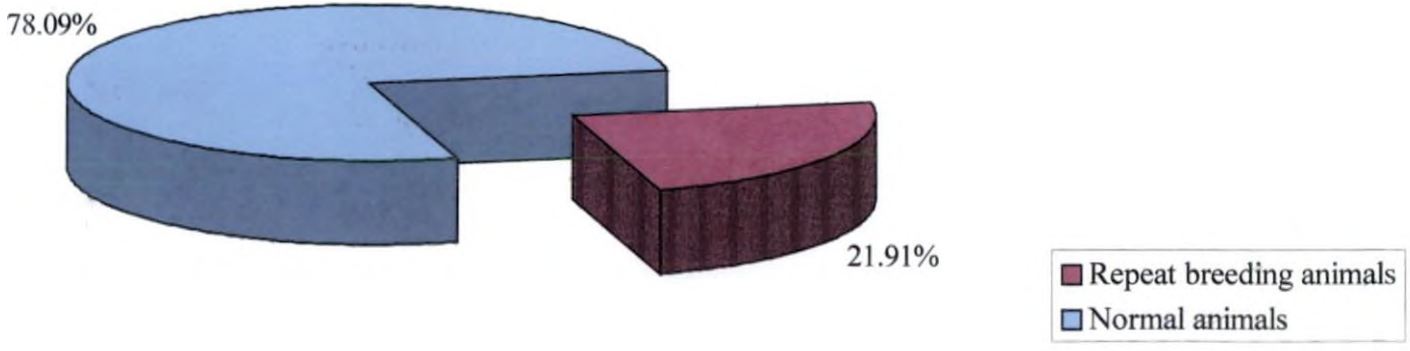

Fig.1 Occurence of repeat breeding among cows and heifers presented for artificial insemination in AI Centre of Veterinary College, Mannuthy

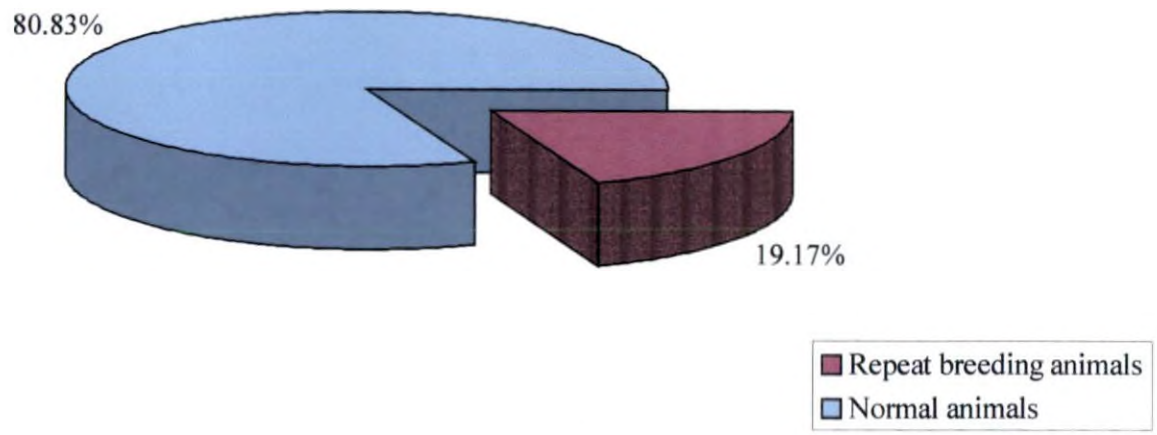

Fig.2 Occurernce of repeat breeding among cows and heifers presented for AI in University Livestock Farm, Mannuthy

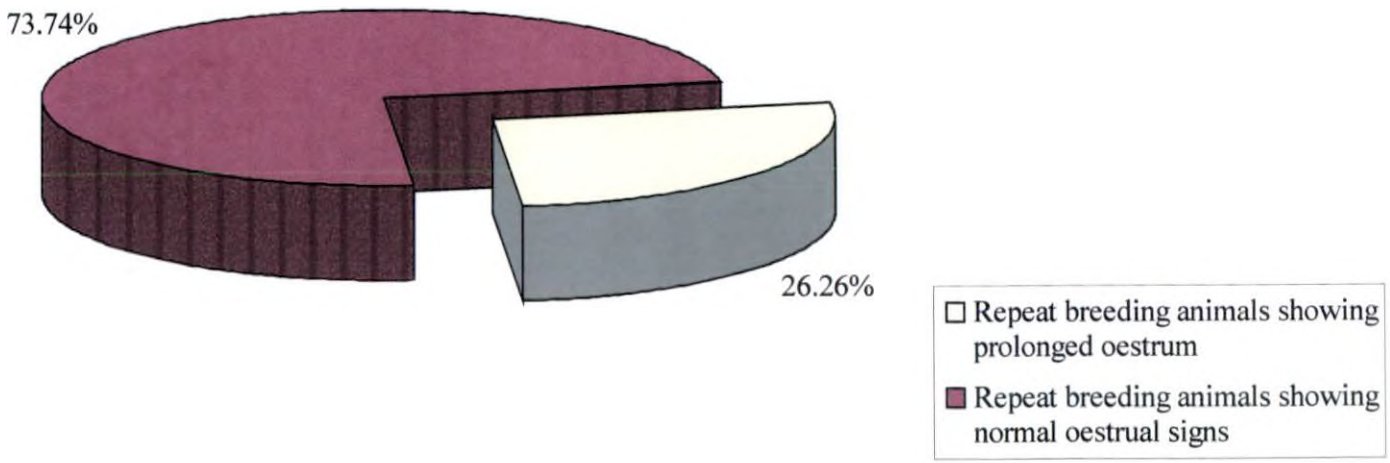

Fig.3 Occurence of prolonged oestrum among repeat breeding cows and heifers presented in AI centre, Mannuthy

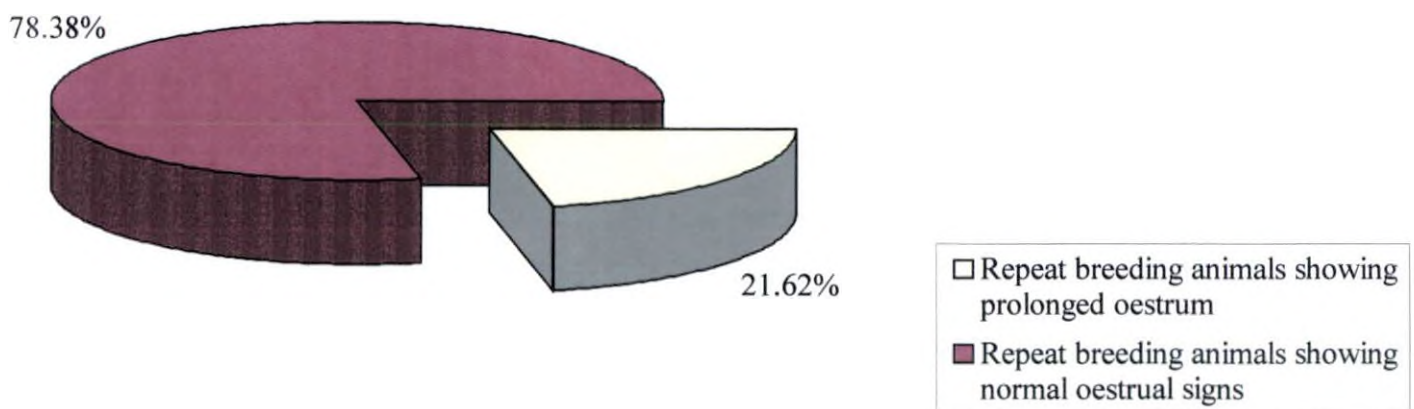

Fig.4 Occurence of prolonged oestrum among repeat breeding cows and heifers belonging to University Livestock Farm, Mannuthy

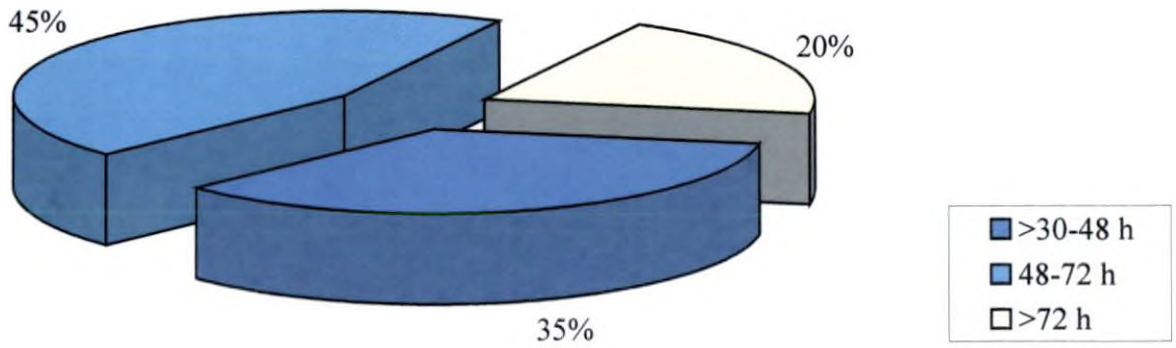

Fig.5 Duration of prolonged oestrum in repeat breeding cows and heifers

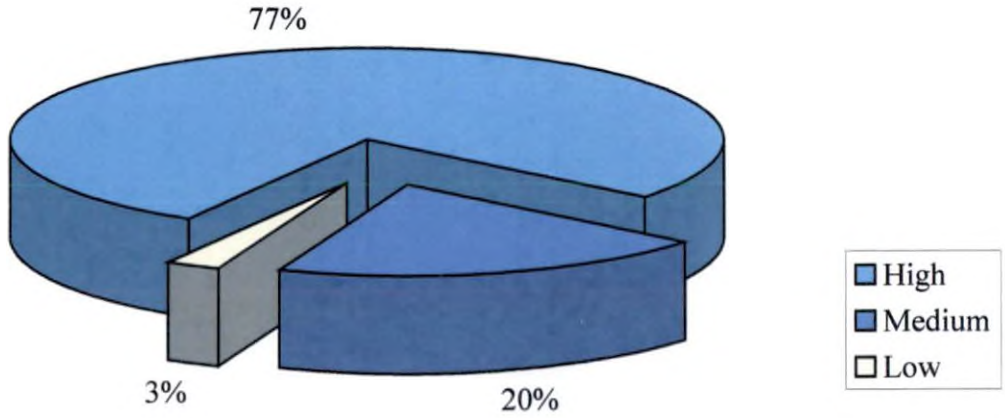

Fig.6 Intensity of oestrus in repeat breeding animals showing prolonged oestrum

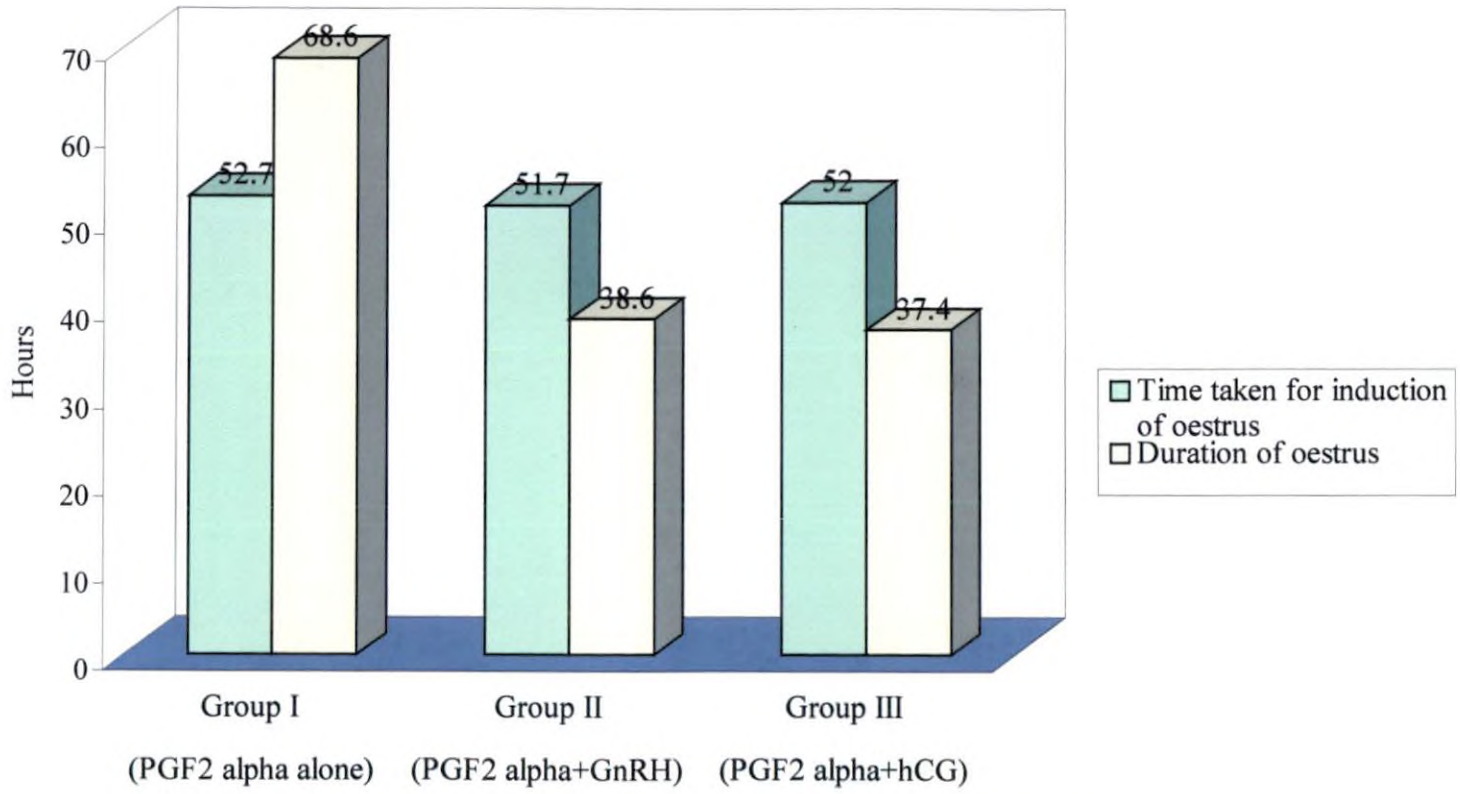

Fig. 7 Time taken for induction and duration of oestrus in Group I, II and III repeat breeding animals showing prolonged oestrum

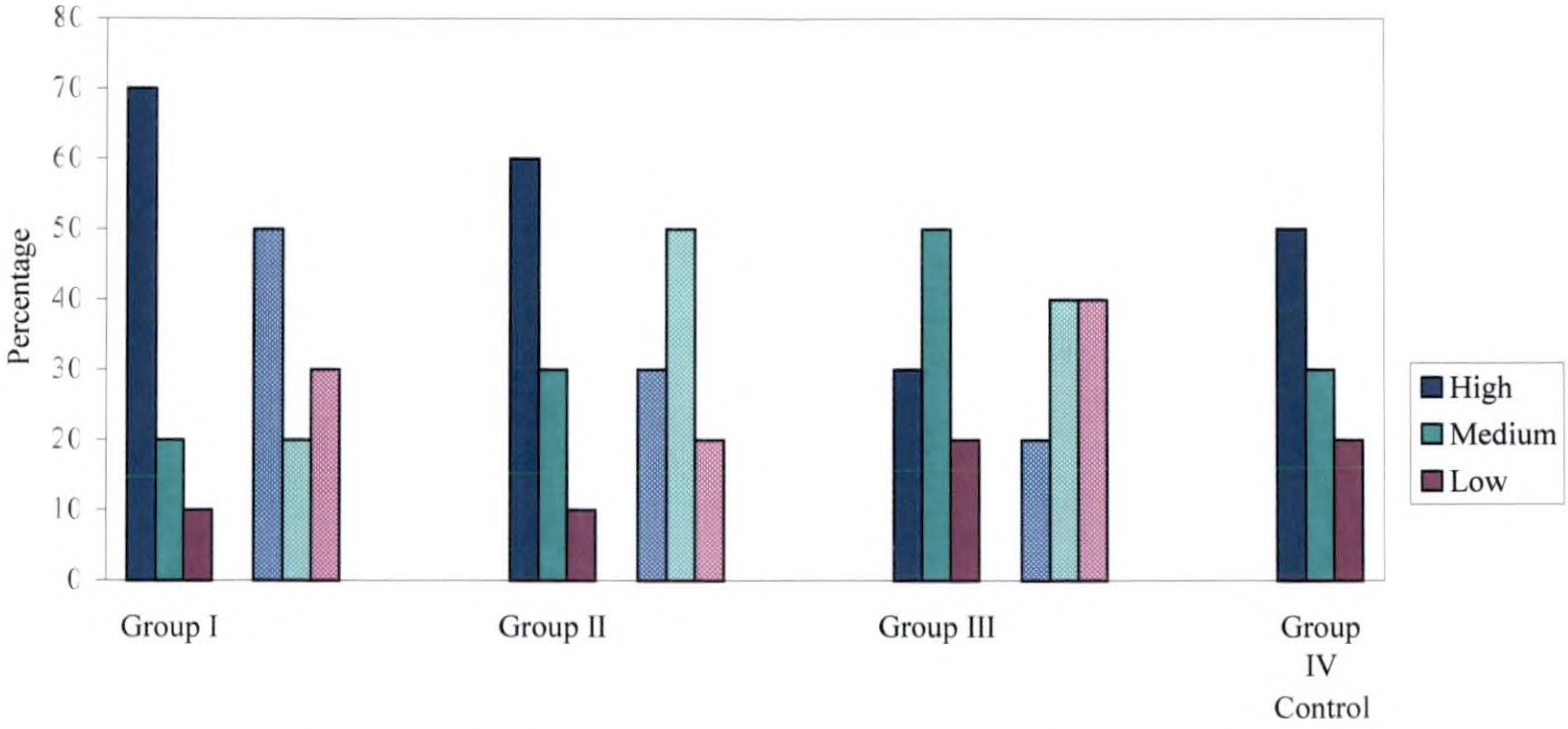

Fig.8 Vulval Oedema in repeat breeders with prolonged oestrum during induced and natural oestrum

Plain- Natural oestrum
Dotted- Induced oestrum

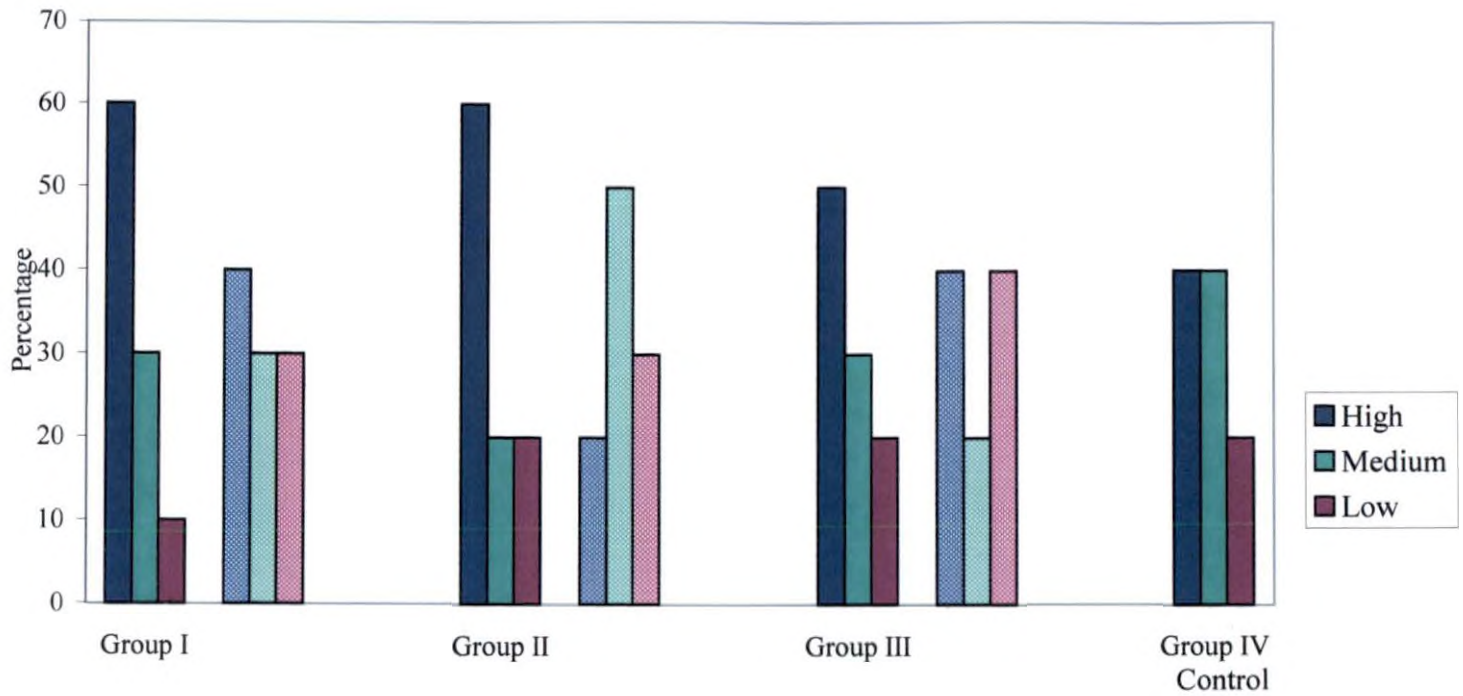

Fig.9 Hyperemia of vaginal mucous membrane in repeat breeders with prolonged oestrum during natural and induced oestrum

Plain- Natural oestrum
Dotted- Induced oestrum

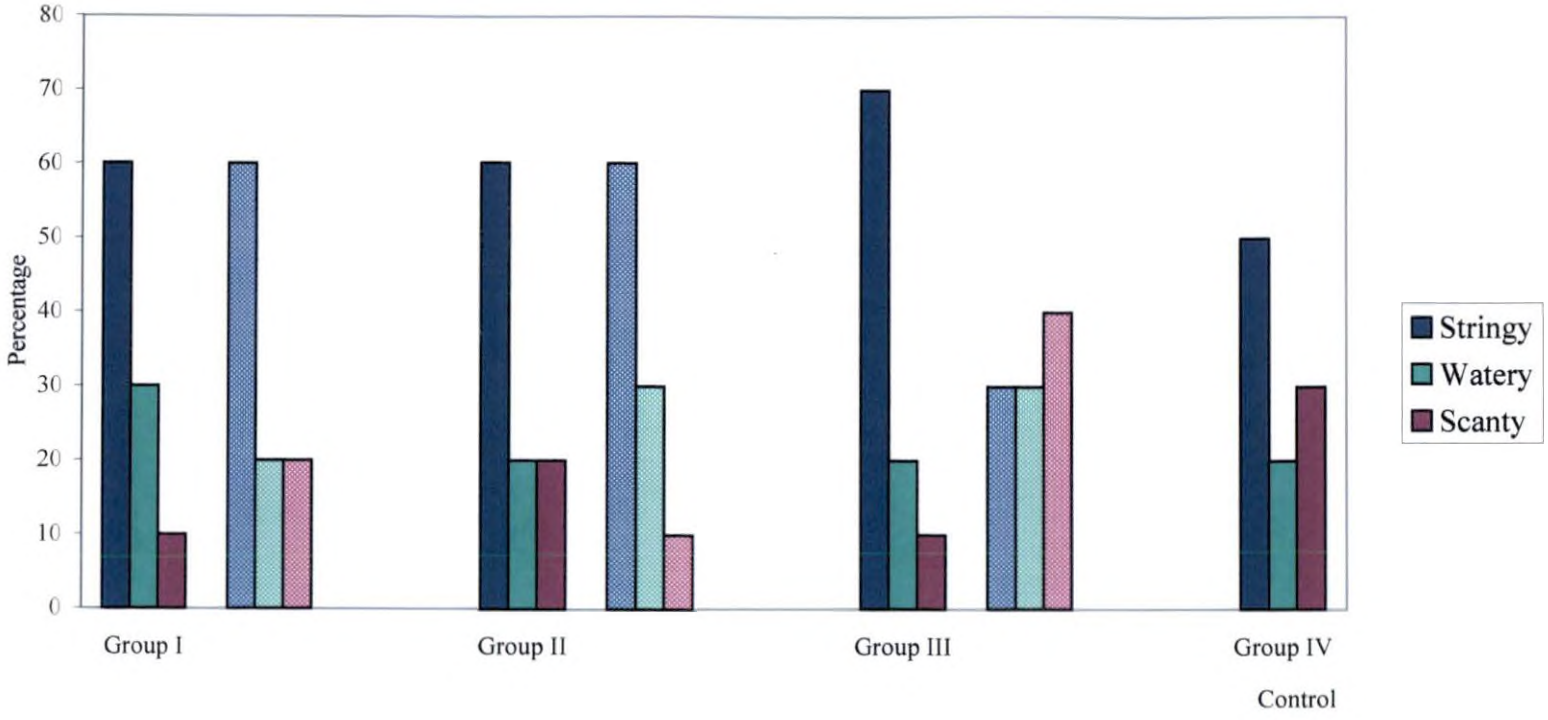

Fig.10 Nature of oestrual discharge in repeat breeders with prolonged oestrum during natural and induced oestrum

Plain- Natural oestrum
Dotted- Induced oestrum

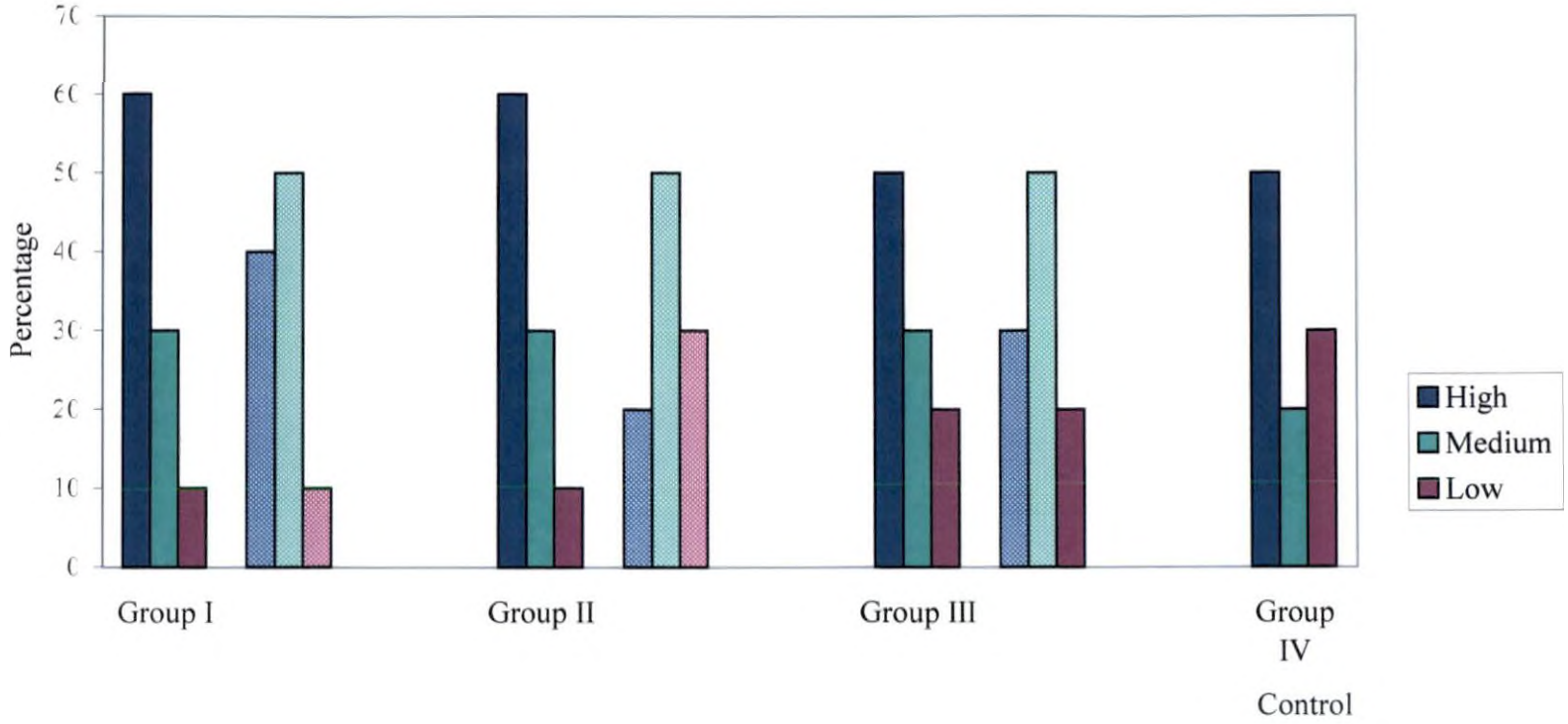

Fig.11 Tonicity of uterus in repeat breeders with prolonged oestrum during natural and induced oestrum

Plain- Natural oestrum
Dotted- Induced oestrum

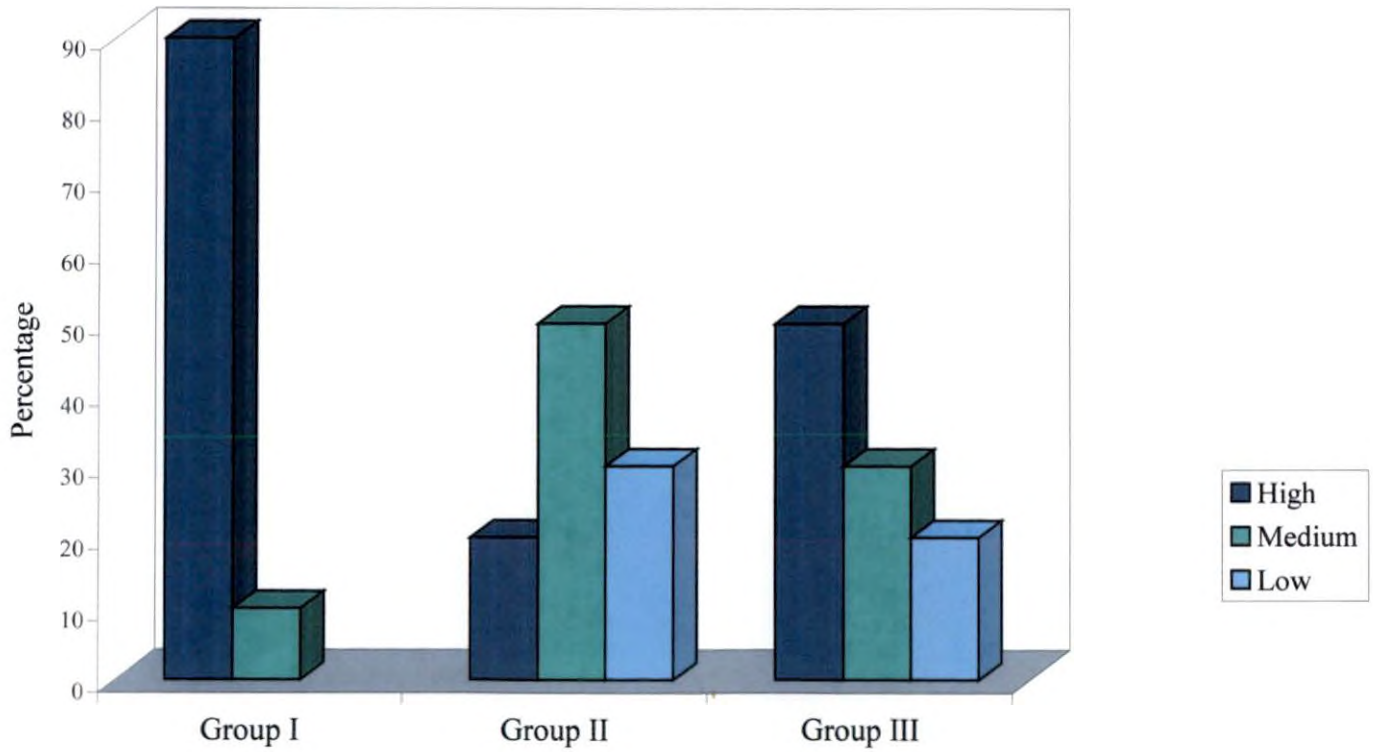

Fig.12 Intensity of oestrus after induction using Dinoprost tromethamine and treatment with GnRH and hCG

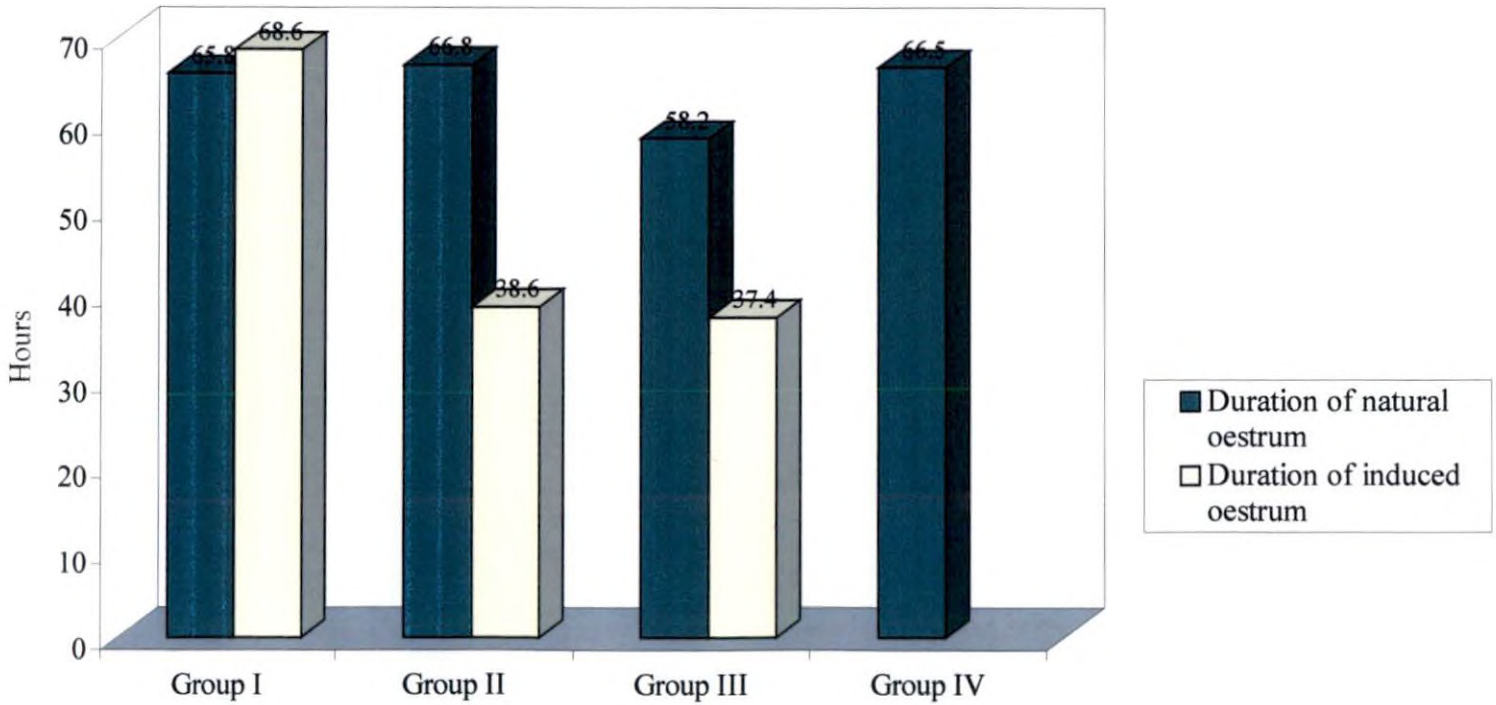

Fig.13 Duration of oestrus in natural and induced oestrum in repeat breeding animals showing prolonged oestrum

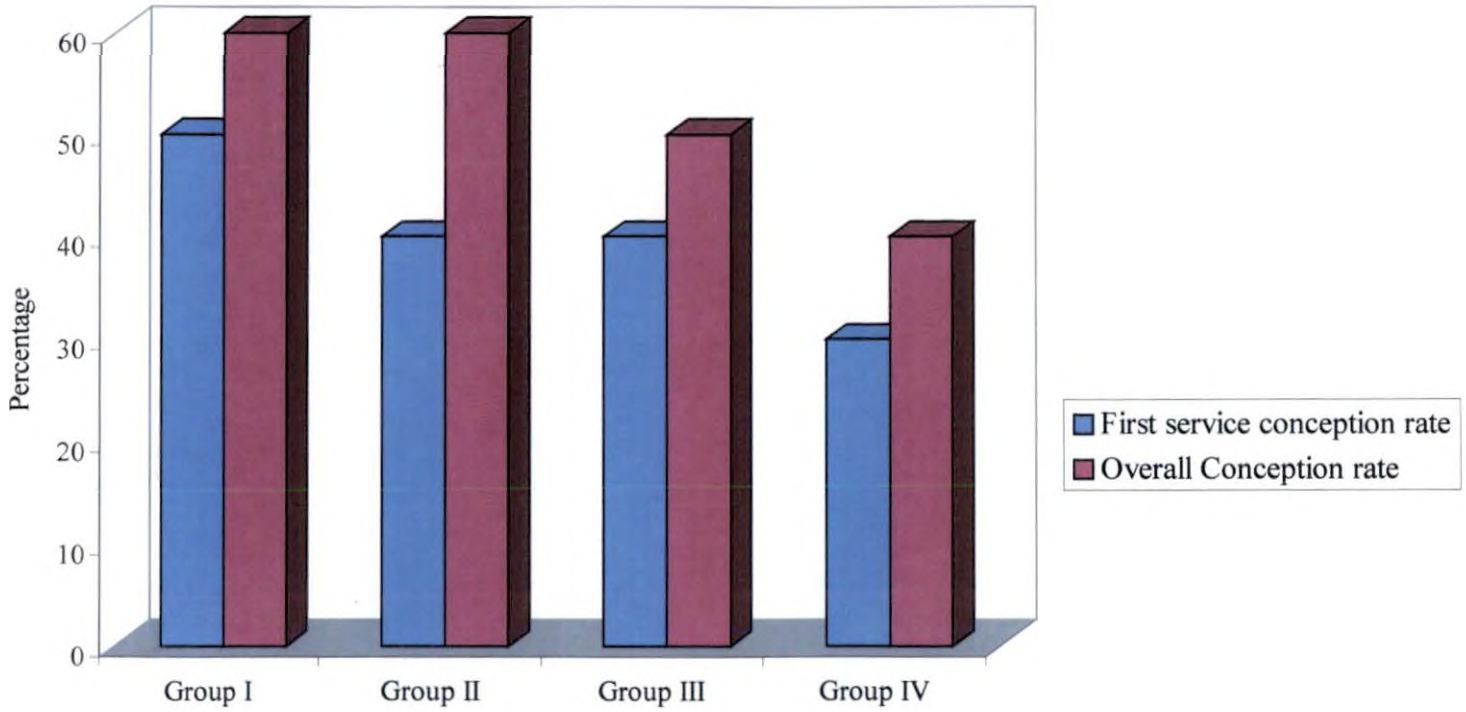

Fig.14 Conception rate during induced oestrum and subsequent oestrum in treatment and control groups

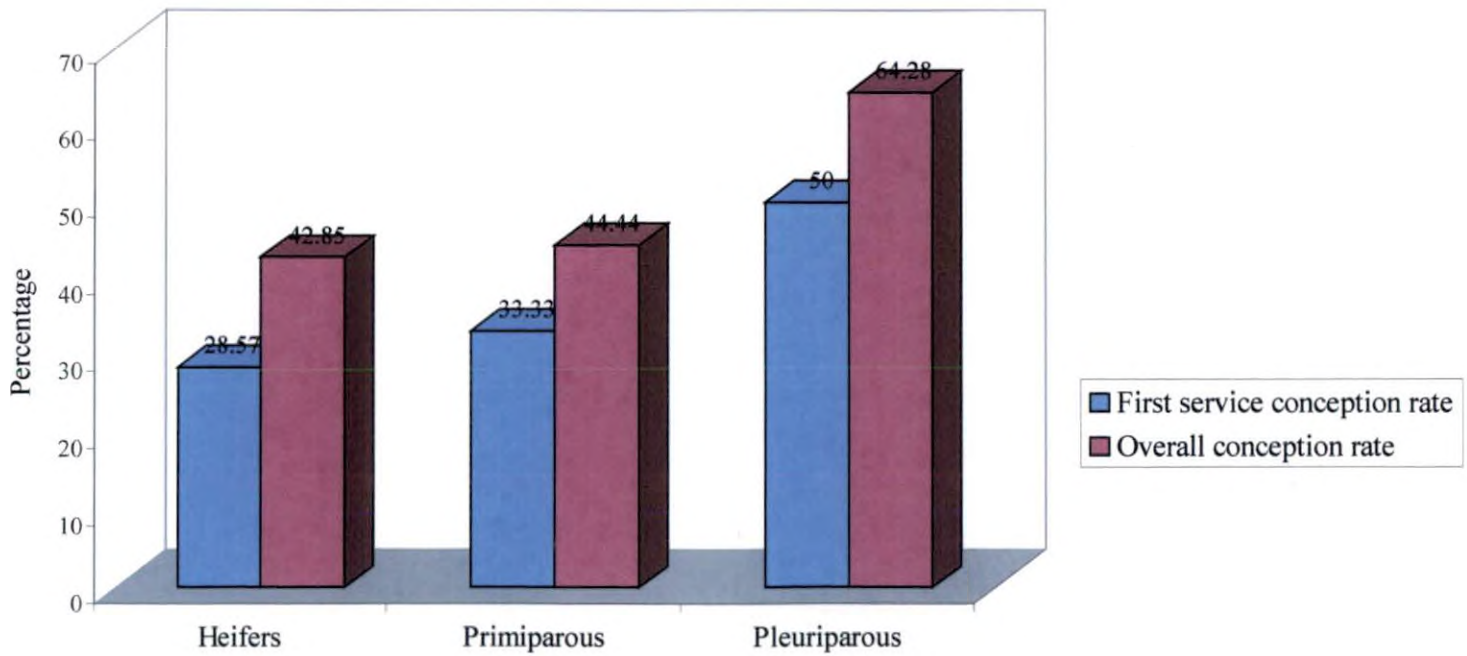

Fig.15 Conception rate in heifers, primiparous and pleuriparous animals after induction of oestrum

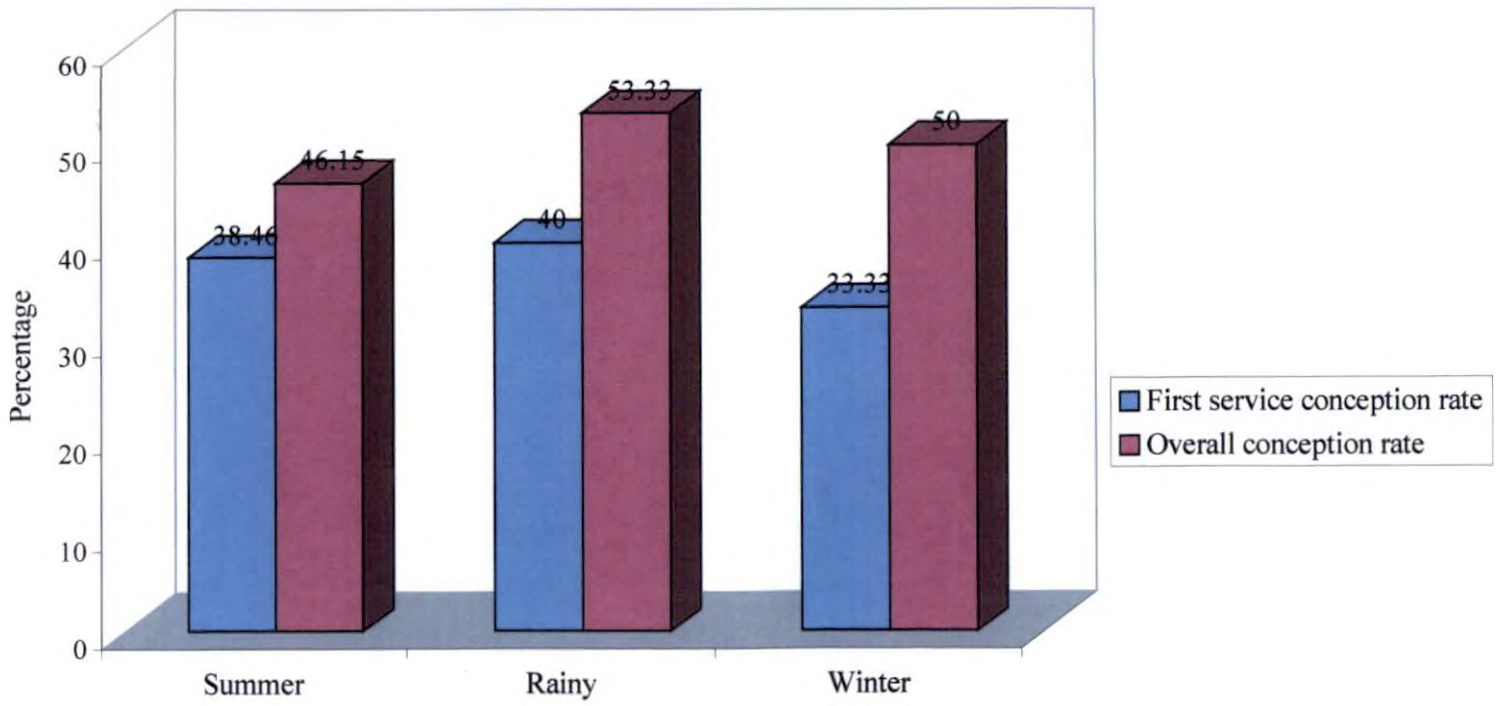

Fig.16 Conception rate during summer, rainy and winter seasons in repeat breeding animals showing prolonged oestrum

Discussion

5. DISCUSSION

The present investigation was undertaken with the objective of evaluating the fertility in repeat breeding cattle with prolonged oestrum after oestrus induction using prostaglandin F_2 alpha analogue, Dinoprost (Lutalyse). The study was aimed to compare the fertility of oestrus induced repeat breeding animals with those repeaters treated with Gonadotrophin Releasing Hormone (GnRH) and human Chorionic Gonadotrophin (hCG).

5.1 OCCURRENCE OF REPEAT BREEDING

The incidence of repeat breeding in the present study was found to be 21.91 per cent among animals presented in clinics for AI and 19.17 per cent among animals belonging to University Livestock Farm, Mannuthy. The incidence was almost similar to the findings of Shukla and Pandit (1989) and Satheshkumar and Punniamurthy (2003). A higher incidence of 34.97 percent repeat breeding was reported by Velayudakumar (2003). However, a lower incidence of 5.59 per cent was reported by Selvaraju *et al.* (2005 a).

5.2 OCCURRENCE OF PROLONGED OESTRUM

Perusal of data on table 1 revealed that 26.26 per cent of repeat breeding cows and heifers exhibited prolongation of oestrual signs. Occurrence was found to be higher in heifers (33.3 per cent) than in cows (25 per cent). Under farm conditions the incidence was high in heifers (25 per cent) than in cows (20.68 per cent) with an overall incidence of 21.62 per cent. The overall incidence reported in the present study was similar to those reported by Khanna and Sharma (1992), Kutty and Ramachandran (2001) and Velayudakumar (2003). However, a lower incidence was reported by Iyer *et al.* (1992). Goley and Kadu (1995) reported a very high incidence of 42.85

per cent anovulation and 14.28 per cent delayed ovulation. In the present study, the occurrence of prolongation of oestrual symptoms was lower under farm conditions than field conditions and this may be due to the better managemental conditions provided in the farm. According to Roberts (1986) infertility or sterility due to nutritional causes were characterized by a failure of conception or early embryonic death. Ovulatory disturbances occur due to endocrine deficiency or imbalance, failure of the development of hormone receptors at the target tissue (Arthur *et al.*, 1996).

5.3 DURATION AND INTENSITY OF PROLONGED OESTRUM IN REPEAT BREEDING ANIMALS

Duration of prolonged oestrum in repeaters ranged from 32 to 96 h with a mean of 65.65±2.57 h. Thirty five per cent repeat breeding animals exhibited oestrus length between 30-48 h and 45 percent repeat breeding animals exhibited oestrous length between 48-72 h and 20 per cent repeat breeding animals exhibited oestrous length between 72 and 96 h. Velayuda Kumar (2003) also noticed a longer oestrus length of 50.17±3.21 h, 50.08±1.88 h and 48.67±2.13 h in three different groups of repeat breeding animals having ovulatory disturbances. A comparatively lower oestrus duration of 31.5±3.6, 32.14±0.9 h was reported by Gustaffson *et al.* (1986) and Goley and Kadu (1995) in repeat breeding animals.

The intensity of oestrus was found to be high in 31 (77.5 per cent) animals, medium in 8 (20 per cent) animals and low in one (2.5 per cent) animal. Velayudakumar (2003) noticed 44.92, 26.08 and 28.98 per cent of high, medium and low intensity of oestrum in repeat breeders showing ovulatory disturbances.

5.4 OESTRUS RESPONSE AFTER ADMINISTRATION OF PGF_2 ALPHA

Perusal of data in table 4 and fig 7 shows that all the 10 repeat breeding animals in the Groups I, II and III responded to the treatment and the efficacy was 100 per cent. Odde (1990) and Selvaraju *et al.* (2004) also obtained 100 per cent oestrual response on administration of PGF_2 alpha. Leeba (2003) obtained a slightly lowered response of 93.75 per cent. The 100 per cent efficacy in the present study confirms that animals with prolonged oestrum are ovulating and a functional corpus luteum was present on day ten of the cycle.

5.5 TIME TAKEN FOR INDUCTION OF OESTRUS

In the present study, the time taken for induction of oestrus after treatment in Group I, II and III repeat breeding animals were 52.7±2.99 h, 51.7±2.68 h and 52.0±2.68 h respectively. The time taken for induction of oestrus in the present study was comparatively lower than the time taken for induction of oestrus reported by Goley and Kadu (1995), Jacob *et al.* (1995), Leeba (2003) and Selvaraju *et al.* (2004). Lower time taken for induction of oestrum in the present study might be due to use of higher potency PGF_2 alpha analogue, Dinoprost.

5.6 DURATION OF OESTRUS AFTER PROSTAGLANDIN ADMINISTRATION

The mean duration of induced oestrum in Group I, II and III were 68.6±3.75 h, 38.6±3.75 h and 37.4±3.75 h respectively. Analysis of data revealed significant difference in the duration of oestrus between group I and the other two groups (II and III). Duration of oestrus in group I repeat breeding animals was longer than that of group II and III repeat breeding

animals. A lower duration of oestrus in group II and III repeat breeding animals were due to administration of GnRH and hCG repectively which might have hastened the follicular maturation. A lower duration of oestrum was reported by Senthilkumar and Rajasekhar (1998) and Velayudakumar (2003) after administration of GnRH and hCG in repeat breeding animals. In the present study group II and III animals in which GnRH and hCG respectively were administered the duration of oestrus was reduced due to follicular maturation and ovulation at an early period.

5.7 PHYSICAL CHANGES OF THE REPRODUCTIVE TRACT DURING OESTRUS

In the present study the physical changes in the reproductive tract of repeat breeders with prolonged oestrum was found to be more pronounced during natural oestrum than during induced oestrum. However, Velayudakumar (2003) observed that vulval oedema, hyperemia of vaginal mucosa and tonicity of uterine horns to be lesser in repeat breeders with ovulatory disturbances. Jacob *et al.* (1995) reported that the cyclical changes in the reproductive tract were not affected by induction of oestrus with PGF_2 alpha. Physical changes in the reproductive tract were less pronounced during induced oestrum in all the animals in group I, II and III. Similarly in group II and III animals GnRH and hCG administration respectively had not influenced greatly the physical changes of the reproductive tract.

5.8 INTENSITY OF INDUCED OESTRUS

The percentage of repeat breeding animals showing high, medium and low intensity of oestrum after PGF_2 alpha treatment was 90, 10 and 0; 20, 50 and 30 and 50, 30 and 20 in group I, II and III respectively. In the present study more number of animals showed higher intensity of oestrus in group I

as compared to groups II and III. Goley and Kadu (1995) also observed intense oestrus in 78.57 per cent repeat breeder cows during induced oestrum where as medium and weak oestrus in 14.28 and 7.14 per cent respectively. Leeba (2003) reported 57.14, 28.57 and 14.2 per cent of high, medium and low intensity of oestrus during induced oestrus where as Jacob *et al.* (1995) reported 66.66, 19.04 and 14.24 per cent of intense, medium and weak oestrus after prostaglandin treatment. In the present study, a lower intensity of oestrus was observed in group II and III repeat breeding animals in which GnRH and hCG were administered. This was probably due to early maturation and ovulation of follicle resulting in cessation of oestrum. However Selvaraju *et al.* (2004) have reported that administration of hCG at various stages following oestrus induction did not alter the intensity of oestrual signs in repeat breeder cows.

5.9 COMPARISION OF DURATION OF OESTRUM IN OESTRUS INDUCED GROUPS

Mean duration of oestrum in group I, II and III repeat breeding animals showing prolongation of oestrual signs were 65.8±4.59 h, 66.8±5.6 h and 58.2±5.71 h in natural oestrum and 68.6±3.75 h, 38.6±3.75 h and 3.4±3.75 h in induced oestrum. Analysis of data showed that in group I animals in which prostaglandin alone was administered, the mean duration of oestrus during natural oestrum and induced oestrum was not significantly different. However, the duration of induced oestrum was significantly lower as compared to duration of natural oestrum in group II ($t=7.33$) and III ($t=3.32$) repeat breeding animals. This was due to administration of GnRH and hCG in group II and III respectively, thereby hastening the follicular maturation and ovulation at an earlier time. Jacob *et al.* (1995) and Leeba (2003) observed a longer duration of oestrum in prostaglandin induced animals. However, Nair and Madhavan (1984) reported that the duration of

induced oestrum did not show any marked variation from the natural oestrus in crossbred cows supporting the present study.

5.10 CONCEPTION RATE DURING INDUCED OESTRUM

The conception rate during induced oestrum in group I, II, III and control group were 50, 40, 40 and 30 per cent respectively (Table 11 and Fig. 14). The conception rate was maximum in group I prostaglandin treated repeat breeding animals compared to all other groups. Improved conception rate during prostaglandin induced oestrum in apparently healthy animals were reported by Duetsher *et al.* (1982), Plunkett *et al.* (1984) and Leeba (2003). Similarly, in repeat breeding animals, improved conception rate was reported by Selvaraju *et al.* (2005 b). However, Strelow (1993) reported that conception rate in induced animals did not differ from that of untreated controls. In the present study, there was improvement in conception rate during induced oestrum as compared to control group. This is in agreement with the findings of Leeba (2003) who reported a first service conception rate of 50 and 42.86 per cent in heifers and cows with a history of metestral bleeding induced with Dinoprost. It could be inferred that prostaglandin administration had improved the uterine milieu and altered the endocrine status of repeaters leading to improvement in fertility.

In group II and III in which GnRH and hCG was administered during induced oestrus, a lower conception rate of 40 and 40 per cent respectively was obtained as compared to group I repeat breeding animals. A higher conception rate was reported by Lee *et al.* (1983), Roussel (1988), Mee *et al.* (1993), Goley and Kadu (1995), Selvaraj and Kumar (2001) and Shelar *et al.* (2002) in repeat breeding cattle following administration of GnRH. Iyer and Sreekumaran (1992) and Velayudakumar (2003) also obtained a higher conception rate when GnRH was administered to repeat breeding cattle

having ovulatory disturbances. Similarly, Lucy and Stevenson (1986), Rosenberg *et al.* (1991) and Doleiel *et al.* (2002) reported improved conception rate in apparently healthy animals. However, Archbald *et al.* (1993) reported that treatment with GnRH prior to insemination did not improve the conception rate in repeat breeding dairy cows. Anderson and Malmo (1985), Mee *et al.* (1990) and Ryan *et al.* (1994) reported a similar report of failure to increase conception rate on administration of GnRH in apparently healthy animals.

A higher conception rate in hCG treated repeat breeding animals was reported by Mehta *et al.* (1986), Goley and Kadu (1995), Srivatsava and Aahlawat (1998), Selvaraju *et al.* (2003) and Selvaraju *et al.* (2004). However, Swanson and Young (1990) observed that hCG administration at the time of insemination failed to improve conception rate. In the present study the conception rate in the animals treated with GnRH and hCG were lower than those treated with PGF_2 alpha alone. It could be inferred that GnRH and hCG administration had not improved the conception rate. Mialot *et al.* (1999) recommended AI at the observed oestrum in PGF_2 alpha induced animals when the oestrus detection was conducted most satisfactorily. However, herds in which oestrus detection was poor, they recommended the sequence of GnRH+PGF_2 alpha protocol and fixed time AI. In the present study prostaglandin administration might have resulted in an optimum LH surge favouring follicular maturation and ovulation in group I animals and hence a higher conception rate was obtained. However, in animals treated with GnRH and hCG probably higher LH response was produced which might have resulted in early maturation of follicle and liberation of immature ova resulting in failure of conception.

5.11 OVERALL CONCEPTION RATE

Overall conception rate for three consecutive oestrus in group I, II, III and control group were 60, 60, 50 and 40 percentage respectively (Table 11). Overall conception rate in PGF_2 alpha treated and PGF_2 alpha+GnRH treated repeat breeding animals showing prolonged oestrum were similar showing that GnRH treatment has not improved the conception rate as compared to PGF_2 alpha treated repeat breeding animals. In group III in which PGF_2 alpha+hCG was administered the overall conception rate was lesser than group I and II confirming that hCG administration had not improved the conception rate. Prostaglandin administration had a favourable influence on fertility in all the three experimental groups as compared to control group. Overall conception rate was not significantly improved in treatment with GnRH and hCG as reported by Archbald *et al.* (1993) and Swanson and Young (1990). In the present study, also overall conception rate was not improved in GnRH and hCG treated animals supporting the findings of Archbald *et al.* (1993) and Swanson and Young (1990). The above findings reveal that conception rate can be improved in repeat breeding animals showing prolonged oestrum by prostaglandin administration alone followed by insemination in the induced oestrus.

In the present study, among oestrus induced repeat breeding animals showing prolonged oestrum, a higher conception rate was observed in pleuriparous animals (64.28 per cent) followed by primiparous (44.44) and heifers (42.85). An increased conception rate in primiparous animals was observed by Nakao *et al.* (1983), Rosenberg *et al.* (1991) and Stevenson *et al.* (2003). However, Ajitkumar *et al.* (1995) observed that parity of cows did not influence the conception rate. In the present study the conception rate was higher in pleuriparous animals compared to primiparous and heifers, these findings disagree with findings of earlier workers.

The conception rate was slightly higher during rainy (53.33 per cent) season followed by winter (50 per cent) and summer (46.15 per cent) season. The conception rate during summer season was less as compared to other two seasons. Lowered conception rate during summer season due to heat stress was reported by Stevenson *et al.* (1984). However, Ajitkumar *et al.* (1995) opined that season of the year did not influence the conception rate.

From the present study it could be confirmed that inducing oestrus by prostaglandin alone was highly effective in the treatment of repeat breeding animals showing prolonged oestrum and a reasonable conception rate could be obtained. Administration of GnRH and hCG during induced oestrus was not beneficial in improving the conception rate in repeat breeding animals showing prolonged oestrum. However, more studies are required to establish the relationship between the hormonal profile of repeaters with prolonged heat and then treatment with prostaglandins.

6. SUMMARY

The present study was undertaken to evaluate the fertility in repeat breeding cattle with prolonged oestrum after oestrus induction using prostaglandin F_2 alpha analogue, Dinoprost (Lutalyse). The study was aimed to compare the fertility of oestrus induced repeat breeding animals showing prolonged oestrum with those treated with Gonadotrophin Releasing Hormone (GnRH) and human Chorionic Gonadotrophin (hCG).

The material for the present study consisted of crossbred cows and heifers belonging to University Livestock Farm, Mannuthy and those presented at Artificial Insemination Centre, attached to the Department of Animal Reproduction, College of Veterinary and Animal Sciences, Mannuthy, Thrissur during the period from August 2004 to July 2005. Repeat breeding animals with a history of prolonged oestrum were randomly selected and allotted into four groups of ten animals each. Repeat breeding animals showing prolonged oestrum in group I were induced using 25 mg dinoprost, a PGF_2 alpha analogue (Lutalyse) on tenth day of cycle followed by artificial insemination twice at 24 h interval on detecting proper signs of oestrum. Repeat breeding animals showing prolonged oestrum in group II and III were induced using 25 mg dinoprost, a PGF_2 alpha analogue (Lutalyse) on tenth day of cycle followed by administration of GnRH and hCG respectively 48 h after PGF_2 alpha administration. These animals were inseminated twice at 24 h interval on detection of proper signs of oestrum. Animals in group IV were subjected to insemination during natural oestrum.

Out of 817 crossbred cows and heifers presented for AI in clinics 179 (21.91 per cent) animals were screened as repeat breeders, which includes 17.31 and 22.99 per cent heifers and cows, respectively. Out of 179 repeat

breeders, prolonged oestrum was exhibited by 47 (26.26 per cent) animals, which comprises of 38 cows (25 per cent) and 9 heifers (33.3 per cent). Among the 193 farm animals investigated, incidence of repeat breeding was 19.17 per cent, which includes 15.68 and 20.42 per cent heifers and cows respectively. Among 37 repeat breeding farm animals it was found that prolonged oestrum occurred in six cows (20.68 per cent) and two heifers (25 per cent) with an overall occurrence of 21.62 per cent.

The duration of prolonged oestrum ranged from 32 to 96 hrs with a mean of 65.65 ± 2.57 h. Out of the 40 repeat breeding animals showing prolonged oestrum selected for the study, 14 (35 per cent) animals exhibited oestrus length between 30-48 h, 18 (45 per cent) animals between 48-72 h, and 8 (20 per cent) animals between 72 and 96 h. The intensity of oestrum was found to be high in 31 (77.5 per cent), medium in eight (20 per cent) and low in one (2.5 per cent) animal.

All the 30 repeat breeding animals (10 each) responded to the treatment by exhibiting oestrus signs and hence the efficacy was 100 per cent. The time taken for induction of oestrus in group I, II and III repeat breeding animals were 52.7 ± 2.99 h, 51.7 ± 2.68 h and 52.0 ± 2.68 h respectively. Analysis of data showed that there was no significant difference on time taken for induction of oestrus between the three groups. The duration of oestrus in groups I, II and III were 68.6 ± 3.75 h, 38.6 ± 3.75 h and 37.4 ± 3.75 h respectively. Analysis of data revealed that there was a significant difference in the duration of oestrus between group I and the other two groups (II and III). Physical changes in the reproductive tract of repeat breeders with prolonged oestrum was found to be more pronounced during natural oestrum than during induced oestrum.

In group I, out of ten oestrus induced repeat breeding animals, 9 (90 per cent) showed high intensity of oestrus and one (ten per cent) showed medium intensity of oestrus. In group II, of the ten repeat breeding animals induced, two (20 per cent) showed high intensity of oestrus, five (50 per cent) showed medium intensity and three (30 per cent) showed low intensity of oestrus. Out of the ten oestrus induced repeat breeding animals in group III, five (50 per cent) showed high intensity of oestrus, three (30 per cent) showed medium intensity and two (20 per cent) showed low intensity of oestrum.

The mean duration of oestrum in group I, II and III repeat breeding animals were 65.8 ± 4.59, 66.8 ± 5.6 and 58.2 ± 5.71 h in natural oestrum and 68.6 ± 3.75, 38.6 ± 3.75 and 37.4 ± 3.75 h in induced oestrum. Analysis of data showed that there was no significant difference between repeat breeding animals in group I on duration of oestrum before and after induction ($t = 0.83$). But there was a significant difference among group II ($t = 7.33$) and group III ($t = 3.32$) repeat breeding animals on duration of oestrum before and after induction.

In group I, five (50 per cent) repeat breeding animals showing prolonged oestrum conceived to induced oestrum and overall conception rate for three consecutive oestrus was 60 per cent. In group II, four (40 per cent) repeat breeding animals showing prolonged oestrum required single insemination and two (20 per cent) required three inseminations to conceive. The overall conception rate for three consecutive oestrus was 60 per cent. In group III, four (40 per cent) repeat breeding animals showing prolonged oestrum conceived to first insemination and overall conception rate for three consecutive oestrus was 50 per cent. In group IV, three (30 per cent) repeat breeding animals showing prolonged oestrum conceived to first insemination and overall conception rate was 40 per cent. The overall conception rate for

three consecutive oestrus in heifers, primipara and pluripara induced with Dinoprost were 42.85, 44.44 and 64.28 per cent respectively. The overall conception rate for three consecutive oestrus in repeat breeding animals showing prolonged oestrum during summer, rainy and winter seasons were 46.15, 53.33 and 50.00 respectively.

From the present study it could be confirmed that inducing oestrus by prostaglandin alone was highly effective in the treatment of repeat breeding animals showing prolonged oestrum and a reasonable conception rate could be obtained. Administration of GnRH and hCG during induced oestrus was not beneficial in improving the conception rate in repeat breeding animals showing prolonged oestrum. Hence it can be recommended that induction of oestrus using prostaglandin could be employed for enhancing the conception rate in repeat breeding animals with the history of prolonged oestrum.

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FERTLITY TRIALS ON INDUCED OESTRUM IN REPEAT BREEDING CATTLE WITH PROLONGED OESTRUM

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

Master of Veterinary Science

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ABSTRACT

The objective of the present study is to evaluate the fertility in repeat breeding cattle with prolonged oestrum after oestrus induction and subsequent treatment with GnRH and hCG. Detailed data of cross bred cows and heifers belonging to University Livestock Farm, Mannuthy and those presented at Artificial Insemination Centre, attached to the Department of Animal Reproduction, College of Veterinary and Animal Sciences, Mannuthy were collected. Repeat breeding animals with a history of prolonged oestrum were randomly selected and allotted into four groups of ten animals in each. Group I repeat breeding animals were subjected to induction of oestrus using 25 mg dinoprost, a PGF_2 alpha analogue (Lutalyse) and insemination was done twice at an interval of 24 h on detection of proper signs of oestrum. In group II, oestrus induction was done using 25 mg Dinoprost on tenth day of cycle and 10 µg Buserelin a GnRH analogue (Receptal) was administered 48 h after PGF_2 alpha administration. In group III, oestrus was induced using 25 mg Dinoprost on tenth day of cycle and 1500 IU hCG (Chorulon) was administered 48 h after PGF_2 alpha administration. Both group II and III repeat breeding animals were inseminated twice at an interval of 24 h on detection of proper signs of oestrum. Group IV repeat breeding animals were subjected to insemination during natural oestrum.

A total of 817 animals were screened of which 21.91 per cent animals were repeat breeders, out of which 26.26 per cent animals showed prolongation of oestrual signs. Among the 193 farm animals investigated, 19.17 per cent were repeaters out of which 21.62 per cent of animals showed prolonged oestrum. The duration of prolonged oestrum ranged from 32 to 96 h with a mean of 65.65 ± 2.57 h. Out of 40 repeat breeding animals

showing prolonged oestrum selected for the study, 35 per cent animals exhibited oestrus length between 30-48 h, 45 per cent between 48-72 h, and 20 per cent between 72 and 96 h. The intensity of oestrum was found to be higher in 77.5 per cent, medium in 20 per cent and low in 2.5 per cent animals.

All the 30 repeat breeding animals in group I, II, III subjected to PGF_2 alpha administration responded to the treatment by exhibiting oestrus signs, and hence the efficacy was 100 per cent. The time taken for induction of oestrus in group I, II and III were 52.7 ± 2.99 h, 51.7 ± 2.68 h and 52.0 ± 2.68 h respectively. The duration of oestrus in groups I, II and III were 68.6 ± 3.75 h, 38.6 ± 3.75 h and 37.4 ± 3.75 h respectively. Physical changes in the reproductive tract of repeat breeding animals showing prolonged oestrum were more pronounced during natural oestrum than during induced oestrum.

The conception rate during induced oestrum in group I, II, III and control group were 50, 40, 40 and 30 per cent respectively. Overall conception rate for three consecutive oestrus in group I, II, III and control group were 60, 60, 50 and 40 percentage respectively. It can be recommended that induction of oestrus using prostaglandin could be employed for enhancing the conception rate in repeat breeding animals with the history of prolonged oestrum.