STUDIES ON THE EFFECT OF GONADOTROPHIN RELEASING HORMONE ADMINISTRATION ON CONCEPTION RATE FOLLOWING ARTIFICIAL INSEMINATION IN CATTLE

THESIS

BY

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Submitted to

CSK HIMACHAL PRADESH KRISHI VISHVAVIDYALAYA
PALAMPUR-176062 (H.P.) INDIA

IN

Partial fulfilment of the requirements for the degree

OF

MASTER OF VETERINARY SCIENCE
(ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS)

2003

Is there anything I can say

Anything I can give

or do for you?

Because all that I'm

All that I have

I owe to you………

Affectionately dedicated

To

Reverend parents

Who sacrificed their present

to make my future better

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CERTIFICATE I

This is to certify that the thesis entitled **"Studies on the effect of Gonadotrophin Releasing Hormone administration on conception rate following Artificial Insemination in cattle"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Science** in the subject of **Animal Reproduction, Gynaecology and Obstetrics** of CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur is a *bonafide* research work carried out by **Dr. Amit Kumar Sharma** son of Sh. Rajinder Kumar Sharma under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

30.6.03

(Madhumeet Singh)
Chairman,
Advisory Committee

Place: Palampur
Dated: 30 June, 2003

CERTIFICATE II

This is to certify that the thesis entitled **"Studies on the effect of Gonadotrophin Releasing Hormone administration on conception rate following Artificial Insemination in cattle"** submitted by **Dr. Amit Kumar Sharma** son of Sh. Rajinder Kumar Sharma to the Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalya, Palampur, (H.P), India, in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Science** in the subject of **Animal Reproduction, Gynaecology and Obstetrics** has been approved by the Advisory committee after an oral examination of the student in collaboration with an External Examiner.

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ACKNOWLEDGEMENT

I would like to offer my prime heart felt salutation at the lotus feet of the Supreme Being for the unbroken health and vigour, bestowed upon me, all through the life span of my studies in these testing days.

Indeed the words at my command are not enough to put on records my sincere and heartful gratitude to my Major Advisor, Dr. Madhumeet Singh, Associate Professor Department of Animal Reproduction, Gynaecology and Obstetrics, the noble, who taught me, but never bend to accumulate false pride, for his impeccable guidance, constant encouragement, painstaking efforts, immaculate suggestions, analytical vigour and swift execution during the study for which I will remain indebted forever.

In my humble way, may I acknowledge the timely suggestions and inspiring efforts of the members of my Advisory committee, Dr. N.K. Vasishta (Assoc. Professor and Head, Department of Animal Reproduction, Gynaecology and Obstetrics), Dr. Y.P.Thakur (Assoc. Professor Department of Animal Breeding and Genetics), Dr. R.S.Telang (Asstt.Professor, Department of Veterinary Pharmacology and Toxicology), and Dr. Kamlesh Gupta (Professor and Head Department of Animal Breeding and Genetics) CSK HPKV Palampur for their healthy criticism, valuable guidance and help rendered in the finalization of this manuscript.

Esteemed thanks are also extended to the Dean, Post graduate studies and Dean, COVAS and their concerned staff. Also the support provided by the entire teaching fraternity of the college is worth mentioning.

The unconditional help rendered by Sh. Amarnath, Sh. Kamaljit Sharma and Ravi Kumar (supporting staff of Deptt. of Animal Reproduction, Gynaecology and Obstetrics) and Madan, Deepu, Ratan, Bir Singh and Raj Kumar (staff from livestock farm CSK HPKV) is worth mentioning and I am grateful to all of them.

The soothing shades of comraderie, cool sprinkling supports and warm loving wishes of my colleagues Drs. Sandeep Thakur, Sandeep Mishra, Satish Kapoor and Yadwinder would remain glorious memory of my life.

Time stops moving, when I think of acknowledging the nice company and unwavering help of Dil Chahta Hai Drs. Subhash, Vinod, Dinesh, Anil, Promod , Vicky, Singla, Punjabi friends, final year students, every Monalian and Sujanian who had given me moral support *throughout the course of this investigation. Their noble company will remain indebted to me throughout my life.*

I would also like to thank Dr. V.K.Gandotra (Assoc. Professor, Gynaecology) Dr.H.K.Verma (Assoc. Professor, Gynaecology) and Dr G.R. Pangaonkar (Professor and Head, Gynaocology), faculty from PAU Ludhiana, for the plasma progesterone estimation.

Every effort is motivated by the ambition and all ambitions have inspirations behind. I owe this pride place to my parents, who always believed in giving strong educational wins to their son. Words fail to express their benevolence advice, constant encouragement, inspirations and invaluable affection given to me during this period, when I was supposed to share the responsibilities, is indelible. I am also grateful to my magnanimous brother (Deepak) and affectionate bhabhi (Anu) and their kids Sam and Anchita for their moral support and encouragemet in various walks of life.

With personal touch of fervenity I gratefully acknowledge love and blessing of all my uncles and aunts, brothers (Anshu, Babhu,Nishu,Rinku, Tinku and Dr. Saurab), sisters (Anu, Manu, Ditu, Deepi and Surbhi), nani and dadi ji that always gave me the kind of rejuvenation I needed to keep going.

Needless to say errors and omissions are mine.

Palampur
30 June, 2003

Amit Kumar Sharma

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CHAPTER I

INTRODUCTION

Dairy farming is gradually becoming very popular in the hill state of Himachal Pradesh. Dairy management studies have indicated that a 12 to 13 months calving interval is an ideal goal for maintaining profitable dairy herd production. However, low pregnancy rate is widely recognized as one of the major problems facing dairy industry.

In earlier times, natural service of cows with good quality bulls was practiced. With passage of time, artificial insemination (AI) technology has taken over. The main target in dairy industry is to increase the conception rate (CR) but the desired results are not being achieved with this technology. Many factors contribute to poor CR after AI, including improper handling of semen, poor technique and improper timing of insemination. In addition to many such factors, two aspects *viz.* genital infection and hormonal disturbance have a leading role and are probably responsible for fertilization failure and early embryonic mortality. Apart from this, hormonal imbalances are associated with ovulatory defects such as delayed ovulation or prolonged oestrus and which is eventually becoming one of the major causes of the condition in cattle, particularly in crossbreds, as very few cows are observed ovulating within 24 hr of insemination.

In Himachal Pradesh, prolonged oestrus syndrome appears to play a key role in the conception failure, especially in crossbred cows. In this region, crossbred cows have extended oestrus periods ranging from 36 to 72 hours (Singh, 1997). Together with improper timing of insemination in relation to follicle rupture, these factors have emerged as possible cause for poor CR following AI. To achieve satisfactory results, optimal correlation between the time of deposition of semen and moment of ovulation is of utmost importance.

Discovery of hypothalamic regulatory mechanism which acts through stimulating and inhibiting factors on the pituitary glands and the discovery of hypothalamus produced releasing hormones has made it possible to deal with such problems. Various hormones such as GnRH and LH have been recommended to increase CR by bringing the LH surge earlier and thus affecting ovulation. Injections to improve fertility and retaining pregnancy have been used by veterinarians since quite some time now (Lee *et al.*, 1981; Nakao *et al.*, 1983; Valks, 1996; Rayos *et al.*, 1999). This includes administration of gonadotropin releasing hormone (GnRH) analogues and human chorionic gonadotropin (hCG). However, reports on bovine pregnancy rates following these treatments have been inconsistent. As a replacement to hormonal treatment, some workers have reported higher pregnancy rates resulting from increased biostimulation of female during insemination (Randel *et al.,* 1975; Lunstra and Laster, 1982; Custer *et al.,* 1990; Segura and Rodriguej, 1994; Singh *et al.,* 2001).

Gonadotrophin releasing hormone (GnRH) occupies a central role in the reproductive function in mammals. GnRH is a neuropeptide that is released in a synchronized, pulsatile manner from neurons that terminate at the medial basal hypothalamus –median eminence, an area bathed by the hypothalmo-hypophyseal portal vessels. GnRH moves into the portal vessels and is delivered to gonadotrophe cells in the anterior pituitary gland. At gonadotrophe cells, GnRH binds to specific cell-surface receptors, and this triggers a sequence of events that include, GnRH receptor micro-aggregation and internalization, activation of second messenger signal transduction pathways, release of LH and FSH, and de novo synthesis of LH and FSH. The internalization of GnRH receptors after binding by GnRH induces a transient state of insensitivity in gonadotrophe cells to GnRH. Under normal circumstances, new GnRH receptors are synthesized and returned to the surface of gonadotrophe cells, thereby re-instating responsiveness to subsequent stimulation from GnRH (Rekwot *et al.,* 2000)

Another common recommendation for improving low fertility in cows is a double AI. Double insemination at 12 to 24 hr interval is likely to increase the CR as some researchers have postulated this to benefit late ovulating cows (Wilcox and Pfau, 1958; Fleischmann, 1990).

Keeping in view the earlier recommendations and observations, the present study was planned mainly to observe the effect of GnRH analogues administration in dairy cows reared under sub temperate zone of Himachal Pradesh with following objectives;

1. To evaluate the effect of different preparations of GnRH administered at the time of AI on conception in Jersey and crossbred (Jersey X Red Sindhi) cattle
2. To compare the effect of route of administration of hormone on fertility.
3. To compare the effect of double insemination with and without GnRH at 24 hr interval on conception rate (CR) in these categories of cows.
4. To estimate changes in blood progesterone levels following administration of different GnRH analogues.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

GnRH has been tried at the time of A.I. for conception improvement in cattle and reported to increase significantly the overall pregnancy. Many researchers have shown a significant improvement in the first service conception rate of cows administered GnRH at the time of AI in comparison to untreated group (Schels and Mastafawi, 1978; Lee *et al.*, 1981; Nakao *et al.*, 1983). GnRH induces ovulation of a dominant follicle in primiparous dairy cows. Cows treated with GnRH have a LH surge which is maximum 2hr after treatment (McDougall *et al.*, 1995). Osawa and associates (1995) reported that GnRH causes 4-fold increase in plasma LH concentration after 2-2.5 hr. GnRH can be used for ovulation synchronization and timed A.I. programme and the greatest advantage of this is that virtually 100% of the animals can be inseminated within a certain period with a satisfactory pregnancy rate (Nakao, 1998).

About 13 per cent better first service conception rates were observed in GnRH treated cows compared to untreated controls (Goldveck, 1976). Bentele and Humke (1976) also observed an improvement in conception rates after first insemination in GnRH treated cows, but improvement was only 5.77 per cent. Pregnancy rate of 81.65% (8.3% higher than in the control) and improved services per conception (1.39 vs. 1.49) has been reported by Schels and Mostafawi (1978) with the use of GnRH.

Treatment of 292 dairy cows with s/c injection of 2.5 ml of Receptal (10 μg) at the time of insemination resulted in increase in first service pregnancy rate by 9.3 per cent compared to control cows (Moller and Fielden, 1981). Elmer (1983) reported a pregnancy rate of 47.6 per cent in treated versus 35.9 per cent in control cows and Nakao and associates (1983) reported pregnancy rate of 57.2% in cows treated with GnRH analogue compared with 49.7% in control group. An additional insemination and treatment with natural GnRH of cows showed 66.3 per cent conception rate as compared to 46.4 per cent in control cows without additional insemination and hormonal treatment (Berger, 1985). In other researches also, conception rates have been found significantly higher during comparison between GnRH and placebo therapy (Vamerzani *et al.,* 1996; Vamerzani *et al., 1*997). Similarly, cows in which insemination is done just before the middle of oestrus to the end of oestrus and then injected with 2 ml GnRH, the conception rate of 85.7% with 1.3 services per conception has been reported (Rayos *et al.,* 1999). Similarly, GnRH analogue (250 mg Fertagyl) has been found to increase the conception rates in cows given on the day of insemination (70.99% vs. 68.04, $p<0.05$) in comparison to placebo (Valks, 1996). Pandey, (2000) reported that injection of Receptal at the time of A.I. creates physiological changes resulting in increase in the conception rate in cows. Cows inseminated after observed estrus with the use of GnRH analogue had a higher pregnancy ($P< 0.05$) than cows inseminated based on the reading of an activated heat mount detector (Archbald *et al.,* 1993).

Ras and coworkers *(*1991), while comparing administration of different compounds during AI, reported that Receptal and Dirigestran administered on the day of the 1st insemination after calving increased conception rate in herd which was maintained on high quality fodder and yielding on average 4400 kg milk per year by 17.7 and 15%, respectively, and that in herd which was maintained on poor quality fodder and yielding 2750 kg milk per head by 11.3%. The same compounds used in subsequent inseminations increased CR in herd A by 5-8%, but no improvement was observed in herd B. Results in both herds showed seasonal variations. In a similar experiment, Bostedt and associates (1995) found 70.6%, 70.7% and 61.5% conception rates in normal animals and 64.3, 63.2 and 45.4% in delayed ovulating cows, following administration of buserelin, carazolol and control groups, respectively. Iyer and Srikumaran (1992) used Receptal in delayed ovulating animals. Ovulation was judged by presence of CL on the ovaries 10-12 days post AI. The conception rate was 70% in treated group and was significantly higher than control animals.

Weaver and coworkers (1988) concluded that herds with conception rate of 45 per cent or less, benefited from GnRH treatment at first or second insemination while those with conception rate of 60 per cent or more, benefited only at second or later services. Stevenson and associates (1984) reported that conception rate did not improve by hormone treatment at first service but at repeat services it improved by nearly 21% when cows were treated with Gonadotrophin releasing hormone within 30 seconds after insemination. Other researchers have found first service pregnancy rate for the control cows (42.4%) lower ($p < 0.05$) than that for cows treated with GnRH analogue at A.I. (48.8%) or for the combined treatment at AI and at day 12 post AI. (51.5%) (Ryan *et al.*, 1991). Nakao and coworkers (1983) also reported that GnRH injected at insemination was effective, especially in cows at first and third lactations. Pregnancy rates 10 weeks after insemination was 54.1% in the GnRH treated group and 36.9% in the control group (Rao and Naidu, 1987).

Lee and coworkers (1985) suggested that the improved fertility after GnRH treatment at insemination is associated with increased progesterone concentration following ovulation. They also postulated that a LH could enhance recruitment of granulosa cells to become luteal cells to subsequent increase progesterone secretion during the luteal phase of cycle, which in turn improved the pregnancy rate. Efficacy of GnRH-analogue at the time of insemination modulate the occurrence of ovulation and it initiates preovulatory LH surge leading to decreased incidence of delayed ovulation and thus resulting in better synchrony between sperm and ova at the site of fertilization (Lucy and Stevenson, 1986). Grummer and Carroll (1988) hypothesized that the elevated progesterone concentration after GnRH treatment at insemination were associated with an increased delivery of sterol precursor to corpus luteum by an elevation in lipoprotein binding and internalization. However, Deen and coworkers (1994) while studying the effect of administration of GnRH at the time of A.I. on gonadotropin and progesterone profiles of crossbred cows reported that increase in LH and FSH level after treatment varied widely among individual but there was no consistent effect of treatment on the progesterone level.

Stevenson and associates (1990) suggested that injection of GnRH at the time of single insemination (12-16 hrs after onset of oestrus) consistently produced the highest pregnancy rate in all the six herds when compared to treated cows with double insemination and controls with single or double insemination. They further reported that Double AI with or without GnRH administration confers no additional benefit over that from single AI along with GnRH. Similarly, Sharma and coworkers (2002a) suggested that single insemination along with GnRH is sufficient and in the absence of GnRH treatment, double AI gives higher CR as compared to single AI ($p<0.05$) in normal crossbred cows.

In a comparative study between Buserelin acetate and Gonadorelin in different breeds *viz*. Jersey and Jersey X Red Sindhi crosses, Buserelin acetate proved to be better in both the breeds (Singh *et al.*, 2001b). The conception rate to first service is reported to be higher when the cows are administered with 10μg of GnRH, 12 hr after onset of estrus and insemination is done following the treatment (Selvaraj and Kumar, 2001). Others have compared the relative efficacy of both analogues by different routes of administration and found that intravenous administration of hormone is better in Jersey animals and intra muscular gives better results in the crossbred animals (Sharma *et al.*, 2002b).

However, some researchers have reported no improvement in pregnancy rates at first service accompanied by treatment with GnRH (Mee *et al.,* 1990). Similarly, Archbald and coworkers (1993) and Drew and Peters (1994) also found that injection of GnRH on the day of insemination causes no significant effects on fertility in comparison with untreated control cows. Even in the absence of any effect on conception rate in two separate experiments, GnRH treatment extended the mean inter-estrus intervals by 2 to 5 days (Thatcher *et al.,* 1993; Gonzalez *et al*., 1998). Milvae and coworkers (1984) also reported an increase in estrous cycle length up to 26.2 days. Repeat breeding cows showed an extension in cycle length of 6 days (34 days treatment vs. 28 days control) and an increase in conception rate by 10 per cent after treatment with GnRH on 12th day post insemination (Bostedt and Okyere, 1988). A single injection of GnRH could extend the luteal phase and inter-estrus intervals in non-pregnant cows (Lajili *et al.,* 1991). Contrarily, lengthening of inter-estrus period has been reported by Belluzzi and associates (1991) but in these repeat breeding animals GnRH could not improve the conception rate. Lynch and coworkers (1999) suggested that injection of a GnRH analogue could prevent the reduction in oestrous cycle length and conception rate associated with the administration of progesterone.

GnRH has been used in mid-luteal phase also for conception improvement in cattle. There has been reported 9.4 to 12% improvement in conception when GnRH is administered at mid luteal phase (Robertson *et al*., 1993; Sheldon and Dobson, 1993; Mann and Lamming, 1995) and it acts by causing a fall in plasma estradiol which results in a reduction in the strength of the luteolytic drive, thus improving the chance of an embryo being able to prevent luteal regression (Mann and Lamming, 1995). Mann and Picton (1995) also recorded significant increase in pregnancy rate following treatment with GnRH and reported an accessory ovulation and suggested that GnRH may act through its antiluteolytic mechanism and not by luteotrophic mechanism. According to other view there is improvement in fertility by means of an antiluteolytic effect, through an increase in progesterone concentration, during the crucial period for maternal recognition of pregnancy (Saratsis *et al.*, 1998).

While studying the conception rate following injection of Buserelin on different days in luteal phase, Macmillan and coworkers (1986) reported that treatment did not affect pregnancy rate when injected during days 7-10 post first insemination (64.9% vs. 65.4%) but increased it by 11.5% when given between days 11-13 post first insemination (72.4% vs. 60.9%). The average second insemination pregnancy rate among cows previously injected with 10µg Buserelin during days 11 to 13 post first insemination increased by 15.6% (85.1% vs. 69.5%). They concluded that a single injection of potent analogue of GnRH administered from days 1-13 post first inseminations in lactating dairy cows can affect pregnancy rate. Different doses of Fertirelin acetate have been tried and has been found that 100-200µg doses of Fertirelin improves the pregnancy rate in virgin heifers and suckled cows (Rettmer, *et al.,* 1992). Drew and Peters (1994) used different analogues of GnRH during mid luteal phase on 12th day after insemination and reported mean pregnancy rates of 53.4 and 65.4% for the first insemination and the mean pregnancy rates of 52.9 and 59.4% to repeat inseminations in control and treated cows, respectively. The mean calving to conception intervals were 91.4 and 85.3 days and the incidences of barren cows were 10.2 and 5.2%, respectively.

GnRH analogue has been found to improve the conception rate when injected either at the time of insemination or at mid-cycle prior to insemination (Rao, 1991). Pedroso and coworkers (1992) reported the conception rates to be 36.3 and 45.4% following administration of GnRH analogue at estrus and at mid luteal phase, respectively. It was significantly higher as compared to control (25.4%).

Administration of GnRH at mid luteal phase recruits good quality follicles, ensures ovulation and fertilization and avoiding endocrine and gametes asynchrony at pre-estrus period (Humblot and Thibier, 1981) and it has been suggested that GnRH analogue treated group have much higher recovery rate of embryos (65% vs. 32%, $p<0.05$) than the controls (Thibier *et al.*, 1985).

However some researchers have reported no benefit of GnRH injected during mid luteal phase. Jubb and associates (1990) reported that use of 10 μg Buserelin, 11 to 13 days after insemination, cannot be recommended as a method of improving reproductive performance in dairy herds. The GnRH analogue treated cows had fewer short inter-estrous intervals compared to the control cows, but there were no significant differences in pregnancy rates to either the insemination preceeding or following treatment, in calving to conception interval, or to percent pregnant by the end of mating. Similarly, others have also reported non-significant improvement in pregnancy rate in treated group over the controls (Ellington *et al.,* 1991; Pedro *et al.,* 1994)).

Mijten and associates (1992) did not find any effect of GnRH treatment on day 12 post insemination on conception rate and also no improvement in conception rate to subsequent services was reported. Thun (1994) also reported that routine GnRH treatment 11 days after 1st insemination cannot be justified as the use of hormone has produced adverse affects. Injection of Receptal on day 11-14 after estrus has been reported to increase the serum LH, FSH and progesterone during 6 hr after injection, increases the serum progesterone during several days after injection, alters number and distribution of follicles of different diameters and delays the onset of next dominant follicle and its associated wave of subordinate follicles after injection. As a result of altered follicular dynamics, the luteolytic process is delayed and duration of estrous cycle is extended (Stevenson *et al.,* 1993). Harvey and associates (1994) compared use of GnRH in mid luteal phase in inseminated and un-inseminated cows and suggested that delayed regression of original corpora lutea and the delayed returns to service were evidence of interference with prostaglandin production.

In India, during the last two decades crossbreeding of dairy cattle has played a significant role in white revolution, due to which there is an increase in milk production. But at the same time it is a common observation that the incidence of repeat breeding problem has been increased in indigenous cows due to crossbreeding. The repeat breeding problem leads to long service period and calving interval, which is responsible for low milk and calf production, resulting in economic loss to dairy industry.

Repeat breeder syndrome is defined as a condition in which cows or heifers which have regular oestrous cycle and appear normal on superficial clinical examination have failed to become pregnant following three or more breeding (Francos, 1979; Barlett *et al.*, 1986). It is a problem worldwide to dairymen, with an overall incidence rate of 10 - 25% (Hewett, 1968; Barlett *et al.,* 1986). Repeat breeding is often defined as a syndrome with many etiologies, including genetic or acquired defects of the ova, sperm, or early zygote, infections or inflammatory processes, endocrine dysfunction, nutritional or managemental deficiencies and others factors associated fertilization failure or with early embryonic loss (Gunther, 1981). The incidence of fertilization failure and early embryonic death in repeat breeding were estimated as 29 - 40% and 29 - 47%, respectively (Ayalon, 1978). The most critical period for the embryo survival may be around 5-6 days after insemination, soon after the embryo enters the uterine horn (Ayalon, 1978; Maurer and Chenault, 1983). Luteal dysfunction leading to inadequate progesterone production during the luteal phase after breeding could be a cause of embryonic death, since progesterone plays an important role for nidation of embryo in the uterus. Also, delayed ovulation is becoming an important cause of conception failure in cattle as most of the crossbred cows are exhibiting the prolonged oestrus syndrome (Singh, 1997; Pant, 2002)

Tanabe and Casida (1949) recorded ovulatory failure in 4.8% of clinically normal repeat breeder cows. An incidence of 10.04% was recorded by Van Rensburg (1956) while studying the incidence of ovulation failure. Others have reported very high incidence of anovulation; it was recorded to be 56.9% (Choudhury *et al.*, 1965) and 31.4% (Sampathkumaran and Iya, 1966). Rao and Kotayya (1980) reported an incidence of 7% for anovulation in repeat breeder cows, whereas according to Khanna and Sharma (1992), 17% of repeat breeding animals exhibit anovulation.

Delayed ovulation may also be a cause of functional infertility. According to Drost (1987) the condition is rare in cows. But Van Rensburg (1956) found an incidence of 18.04% in Africa in functionally infertile cows. A subsequent study recorded 18.0% delayed ovulation in same breed of cattle (Van Rensburg and de Vos, 1962). Almost similar incidence of 17.3%, 12% and 17.6% was recorded in repeat breeder cows by Maree (1977), Khanna and Sharma (1992) and Salphale and associates (1992), respectively. Contrarily, Callahan (1969) reported that ovulation was delayed in 3.6% cows. Almost similar incidence of 3.5% was reported by Lemke (1972) in 200 infertile cows.

Bostedt and associates (1977) studied the ovulation in 850 healthy breeding cows by rectal examination of ovaries at 24 hr interval; 72% of cows ovulated within 24 hr. The rate of ovulated follicles rose to 91% after 48 hr. Roine and Saloniemi (1978) investigating the incidence of infertility in dairy cows, observed 5.6% cases of delayed ovulation.

Hancock, (1948) suggested its deleterious effect on fertility by saying that only 36 percent of cows that ovulated on the second day after oestrus conceived. Conception rate of cows inseminated about 10hr after the onset of estrum was about 82 %, at about 20hr after the onset of estrum was about 62 % and by about 30hr after end of estrum the conception rate was about 28 %. This rate rapidly dropped so that by 50-60hr after the onset of estrum very few cows conceived. Insemination of cattle between 8-24hr or especially 7-18hr before ovulation result in the highest rate of conception (Trimberger and Davis, 1943).

GnRH has also been used for the treatment of repeat breeder animals showing delayed ovulation. It increases the pregnancy rate of repeat-breeder cows compared with control group given only a single A.I. (Roussel *et al.,* 1988; Stevenson *et al.,* 1990). Repeat-breeder cows that were treated with GnRH after each of 2 consecutive inseminations resulted in a 53% increase in CR relative to controls (Roussel *et al.,* 1988). The conception rates for the GnRH treated cows were 70% in comparison to 50% in control groups in repeat breeder animals (Rayos, 1995). In another study, 963 repeat breeding dairy cows were treated with 10 μg GnRH at the time of insemination, the conception rate for treatment and control groups were 48.1 and 31.0 per cent, respectively (Bon Durant *et al.*, 1991). Morgan and Lean (1993) found that pregnancy is increased by 12.5% following GnRH injection and this increase is greatest for repeat breeder cows (22.5%). They further reported that at the first service the use of GnRH or its analogues increase the chances of pregnancy by 5.2% and 8.0%, respectively. A 5.9 per cent increase in first insemination pregnancy rate in repeat breeding cows treated with GnRH has been observed by Gonjalez and associates (1994). Rangnekar and coworkers (2002) reported CR of 70% vs. 40% in Fertagyl treated and control repeat breeder cows, respectively. However, Thatcher and associates (1993) suggested that injecting GnRH at the time of 1st insemination in postpartum cows or in repeat breeders had not shown consistent results.

GnRH or it's analogues have been tried at mid luteal phase to treat repeat breeder cattle (Thibier *et al.*, 1985; Bhosrekar *et.al.*, 1986). GnRH @ 20 μg given during mid luteal phase of previous cycle results in improved conception rate i.e. 47.67% vs. 34.37% in control animals (Bhosrekar *et.al.*, 1986). Thibier and coworkers (1985) suggested that injection of GnRH at mid luteal phase enhances both the recovery rate and quality of day 11 embryos in the repeat breeding cows. When 170 repeat breeding cows were injected with GnRH or 5 ml normal saline solution (control) on day 12 after insemination, the conception rates to second and third insemination were 72.4 and 50.4 per cent in treated cows compared to 59.6 and 40.7 per cent in controls (Bostedt and Okyere, 1988).

Scientists have compared the effect of GnRH in normal and repeat breeder cows and have reported variable results. Gonzalez and associates (1998) compared the efficacy of GnRH agonist buserelin after injecting it on day 11-14 of the oestrous cycle in normal and repeat breeder crossbred cows of Venezuela. The conception was reported to be 63.2% and 60.7% in normal and 61.8 and 50.0% in repeat-breeding treated and control, respectively, the differences between treatments being significant for repeat breeders. Treatment had no significant effect on serum progesterone concentration. Vamerzani and coworkers (1999) compared one (along with insemination) and two (one along with insemination and second ten days later at mid luteal phase) injections of GnRH in normal and repeat breeder cows. A single GnRH injection increased pregnancy rate from 45% to 80% and from 40% to 66.66% in normal and repeat breeding cows, respectively. In the repeat breeders given two injection of GnRH, the pregnancy rate was 73.33%.

Osawa and coworkers (1995) compared two different GnRH analogues, fertirelin and buserelin for treatment of follicular cysts. Luteinisation occurred in 75% and 72% cows and conception rates were 74% and 62% following the treatment with two analogues, respectively. They concluded that both these analogues are equally effective in treatment of follicular cysts. Cairoli and coworkers (1994) concluded that buserelin is effective as therapy of cystic ovarian disease whereas Hooijer and associates (1999) suggested that gonadorelin is effective in 66.7% cases of ovarian cysts.

In addition to GnRH injection, treatment with hCG has been used to hasten the time of ovulation in cattle. According to Drost (1987), hCG or GnRH may be administered at the onset of oestrus for the treatment of delayed ovulation. Such cases require administration of hCG 6-8 hr before or at the time of service (Arthur *et al.,* 1982). However, results for hCG have been reported to be disappointing as compared to those for GnRH (Schels and Mastafawi, 1978; Lee *et al.*, 1981; Nakao *et al.,* 1983). Contrarily, Kumar and associates (1994), who reported ovulatory disturbances on account of hormonal imbalance, especially of LH as major cause for failure of fertilization and subsequent poor conception, found hCG (Chorulon) very effective in improving conception in rural cows and buffaloes that failed to conceive after two inseminations. Similarly, conception rate of cows receiving a repeat insemination following hCG treatment is reported to be higher than for the controls (Helmer and Britt 1986). Saini and associates (1987) gave similar results when used hCG in repeat breeder crossbred cows and found that the overall conception was higher than control. Schmidt and coworkers (1996), while studying the effect of GnRH agonists and hCG along with AI at estrus, found conception rate to be 52.9% (54 / 102) and 56.1% (55 / 102), respectively.

hCG treatment 7 days after insemination can be used to produce accessory CL, increase plasma progesterone concentration and reduce the incidence of early embryonic mortality in cattle (Rajamahendran and Sienangama, 1992). GnRH and hCG have been used in cows for improving the fertility in the dairy cows with the retained placenta also (Grguric *et al.,* 2000).

In addition to hormonal treatments, it has been shown that mechanical stimulation of the reproductive tract tends to hasten the LH surge and that clitoral stimulation, hastens ovulation (Randel *et al.,* 1975). Several authors have reported higher pregnancy rates due to increased biostimulation of females during natural mating and AI (Lunstra and Laster, 1982; Custer *et al.,* 1990; Segura and Rodriguej, 1994). It has been shown that manual clitoral stimulation shortens the interval from estrus to ovulation and increases artificial insemination (AI) pregnancy rates in lactating beef cows but not in heifers (Randel *et al.,* 1975). Glauber (1989) reported conception rates to be higher in cows receiving clitoral massage (65.08%) than in the control (52.43%). In a similar study, Singh and coworkers (2001) found significantly higher ($p<0.05$) conception (57.34 %) in clitoral massaged animals as compared to control group (49.34 %). Comparing breed wise improvement in conception rate following clitoral massage, they reported increase of 6.21% (49.17 vs. 55.38 %) and 9.37 % (49.45 vs. 58.82 %) in Jersey and crossbred cows, respectively.

Other workers suggested that double insemination is better than single insemination, but should be performed only in those cases where signs of estrus persists 24 hr after insemination (Fleischmann, 1990)

PROGESTERONE

Progesterone is produced from the corpus luteum and is indicative of it's function. Its levels are high in progesterone dominance phase and also in pregnant animals in comparison to the non-pregnant animals. The progesterone levels are significantly higher at mid-luteal phase (Kimura *et al.*, 1987) as well as on day 21, 24, 28, 42 and 60 post-insemination (Heap *et al.,* 1976) in pregnant cows as compared with non-pregnant ones. However, some workers suggest higher progesterone concentrations in non-pregnant than pregnant cows and these levels are either due to incomplete luteolysis or premature ovulation (Selvaraj and Kumar, 2001).

Progesterone can be assayed from the milk, serum or plasma samples collected at different intervals. Suitable methods are there to measure progesterone from blood

(Dobson and Fitzpatrick, 1976; Ginther *et al.,* 1976; Faredin *et al.*, 1987) and milk (Lamming and Bulman, 1976; Arnstadt and Cleere, 1981; Antal *et al.*, 1987). Progesterone crosses the mammary gland and appears in milk (Darling *et al.*, 1972). The changes in milk progesterone levels during the reproductive cycle closely follow those of serum. Also, the progesterone levels in milk are closely related to the growth and secretory function of the corpus luteum in the normal cycle and in early pregnancy (Laing and Heap, 1971).

Repeat breeder cows can be investigated by a combination of radioimmunoassay and clinical examination of the ovaries and reproductive tract. Progesterone concentrations have been reported to be higher in repeat breeder cows that did not conceive than in repeat breeder cows that did conceive to AI. (Bage, 1999). Gustafsson and associates (1985) reported that progesterone levels were higher ($p < 0.05$) in the repeat breeder heifers than the normal heifers. Bullman and Lamming, (1978) studied the relationship between milk progesterone levels and the repeat breeding syndrome in dairy cows, irregular or low progesterone profile was noted in 23 per cent of repeat breeder cows. Repeat breeding cows have been reported to have low plasma progesterone profiles during the estrous cycle than the normal cows, the difference being significant on day 8, 13 and 16 after estrus (Dutta *et al.,* 1989). On measuring progesterone concentration in milk fat for 20-22 days after insemination in repeat breeding cows, six of these cows had normal progesterone profiles, while five showed delayed rise in progesterone concentration. Until day 7-10 after insemination, two had lower progesterone concentration and two had combined pattern of delayed rise and low progesterone in milk during luteal phase (Kang *et al.,* 1995). About 7.8 per cent of repeat breeding cows showed erratic or low progesterone concentration (Gonjalej *et al.*, 1994).

Plasma progesterone levels on day of estrus in non-conceived buffaloes (0.60 ± 0.16 ng/ml) have been reported to be almost double the levels found in conceived group (0.29 ± 0.01ng/ml). Higher progesterone profile on day of estrus/insemination can be considered as prognosis of non-fertile cycle (Panchal *et al.*, 1992). Serum progesterone is of practical value in monitoring ovarian activity in cattle and in the identification of acyclic and repeat breeder cows (Rekwot *et al.*, 2000). Progesterone concentration during estrous cycle prior to breeding may be an important indicator of subsequent fertility of cow (Fonesca *et al.*, 1983).

In an experiment progesterone receptors were measured in the cytosol and nuclear fractions of endometrial cells from repeat breeder and normal cows on day 6 post breeding (inseminated group) and on day 3, 6 and 7 after estrus (Non- inseminated group). Repeat breeder cow were found to have higher tendency of progesterone receptors than normal cows in inseminated as well as non- inseminated group, which suggests that hormonal imbalance may be related to the repeat breeding problem (Almeida, *et al.* 1986).

The plasma progesterone assay is a practical aid to diagnose reproductive disorders and to monitor responses of ovarian dysfunction to PGF_2 alpha and GnRH treatment in suboestrus cows (Kang, *et al.*, 1995) and also it is economical and profitable to diagnose pregnancy early after insemination so that cows which fail to conceive may be rebred (Rekwot *et al.*, 2000).

Milvae *et al.*, (1984) demonstrated that injection of a GnRH agonist four times daily on day 9-12 of the cycle increases the luteal phase concentration of plasma progesterone and extends the estrous cycle until a GnRH agonist is injected once between 12 and 14 days post insemination in dairy cows. An acute increase in progesterone has been detected following GnRH administration in both pregnant and non-pregnant cows (Lajili *et al.*, 1991). Bostedt and Okyere (1988) reported that inspite of increased cycle length and initial increase in progesterone at 6 hour of treatment, there was no difference in progesterone concentration on insemination in treated versus control cows.

Repeat breeding heifers had less luteal tissue volume than virgin heifers (Albhin, 1991). GnRH injection between day 11 and 13 post insemination can boost the function of existing corpus luteum (Robertson *et al.*, 1993) and thus increasing the concentration of plasma progesterone. Such an increase in progesterone concentration may be attributed to the luteotrophic response of small luteal cells to LH that is released in response to GnRH (Hansel *et al.*, 1991).

GnRH analogue Buserelin was infused continuously at 80 ng per hr or saline for 7 days. A significant rise in the plasma LH level was observed in the treated animals on day 1 or day 3 after treatment and then the levels declined to pretreatment levels. A similar increase in the plasma FSH profiles was observed in one animal. Clinical examination revealed that 3 of the 4 heifers had follicular activity in response to GnRH infusion, and 2 animals had increased peripheral progesterone levels. One of these animals continued to exhibit periodic progesterone increases, and later ovulated and formed a corpus luteum, while the other heifer reverted to anoestrus (Deen *et al.,* 1996). However, Milvae and associates (1984) noted lower circulating progesterone concentrations in cows immediately following GnRH treatment. In a similar experiment, it was found that inspite of high pregnancy rate, the level of progesterone in plasma were lower in the treatment group (Lucy and Stevenson, 1986). Lewis and coworkers (1990) concluded that it is the pregnancy which affects the milk progesterone profile but GnRH has no effect on progesterone levels. Similarly, Deen and coworkers (1994) reported that there was no consistent effect of treatment on the progesterone level. Similarly, no influence on the subsequent serum progesterone levels at 14th day and 22nd day post insemination has been reported (Shelar *et al.,* 2002).

Alonso and coworkers (1988) studied the progesterone levels at the time of artificial insemination and found that 85% of the animals had progesterone concentrations of < 0.5 ng/ml, which confirmed that they were in oestrus. Of the remaining 15%, 33% showed high progesterone levels and 4 were pregnant, and 22.5% of the animals studied had progesterone levels which were < 1 ng/ml five days after insemination, indicating a hypofunction of the corpus luteum. Hajurka and Chroma (1998) found that the progesterone concentration in the blood on the 8^{th} day after treatment averaged 9.7nmol/litre in ovulating cows, 3.4 nmol/litre in non-ovulating cows and 0.5 nmol/litre in controls. It has been found that the cows with progesterone concentrations exceeding 0.50 ng/ml at the time of insemination and buffaloes with concentrations exceeding 0.90 ng/ml do not become pregnant and therefore it has been suggested that insemination should be performed when progesterone levels are low after oestrus detection (Srivastava and Sahni, 1999). Progesterone concentrations lower than 2 ng/ml milk on the day of insemination, followed by values higher than 2 ng/ml 6 days later, were considered to indicate that the insemination had been performed at the correct time (Janowski, 1988). Kimura and associates (1987) studied the progesterone profile after A.I. and found that 38% had a normal progesterone profile. Progesterone levels in skim milk rose to 1.0 ngm/ml or higher within five days after insemination (0 day = Day of A.I.) and, thereafter, steadily increased to 2 ngm/ml or higher at mid- luteal phase. Another 62% cows had an abnormal pattern of milk progesterone which indicated luteal phase defects after artificial insemination. Eissa and coworkers (1994) recorded that on the day of insemination and for the first 2 days after insemination, skim milk progesterone concentration was less than 0.1 ng/ml in all pregnant cows.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The work was conducted at the livestock farm of CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur from September 2001 to March 2003. Animals were maintained under loose housing system and standard farm conditions. All the animals were in good health with normal genitalia and no apparent clinical abnormalities based upon their history records and/or by rectal examinations. Inseminations were carried out in both heifers as well as pluriparous animals between first and sixth lactation.

Feeding and Management

The animals were fed according to the normal feeding schedule followed at the dairy farm. All the cows were supplied with adequate amount of green fodder depending upon the seasonal availability and concentrate ration containing 70-75% TDN, 16 -18% DCP and 2 % mineral mixture. The concentrate mixture was fed according to milk production to individual animal at the time of milking. The animals were allowed to take water *ad-libidum*. All the animals were vaccinated and dewormed at the appropriate time as per the recommended protocol.

Detection of estrus

The animals were observed for signs of estrus on the basis of behavioral symptoms *viz.* bellowing, restlessness, frequent micturition, mounting, standing to be mounted, vulvar hypertrophy and mucus discharge. Cows with apparently normal cervico-vaginal mucus discharge, tone of the uterine horns and without any abnormality of genital tract were selected for the study following clinico-gynaecological examination. All the animals were inseminated at standing heat with good quality frozen thawed semen procured from bulls maintained in ILIP Semen Processing Laboratory and Regional Bull Centre, Palampur. These bulls were being used for the preparation of frozen semen straws for routine supply in the field.

Treatment

Buserelin acetate (Receptal, Intervet India Ltd.) at the dose rate of 0.0105 mg (2.5 ml) equivalent to 0.01 mg Buserelin and Gonadorelin (Fertagyl, Intervet India Ltd.) at the dose rate of 0.25 mg (2.5 ml) were used for the treatment as GnRH analogues. These hormones were injected simultaneously to first insemination, either through intra muscular (I/M) or intra venous (I/V) routes, depending upon the groups of the animals.

Insemination

Detailed of various insemination groups is shown in Table M1. In all, 448 cows including 216 Jersey and 232 Jersey X Red Sindhi crossbred cows were inseminated during this study. These included 346 normal and 102 repeat breeder cows. Repeat breeder cows selected for this study were having no apparent abnormality except prolonged estrus. Depending upon the groups, either single or double insemination was performed in these cows. In double insemination group, the cows were reinseminated 24 hours after the first one with the semen of same bull.

The animals were divided category wise into two groups *viz.* Jersey (n= 216) and Jersey X Red Sindhi crossbreds (n=232). Jersey cows included normal (n=167) and repeat breeders (n=49). Normal animals were further sub-divided to treatment (n=127) and control groups (n=40). Treatment group comprised of Buserelin acetate (n=61) treated and Gonadorelin (n=66) treated animals. The Buserelin acetate treated animals were sub-divided to I/M (n=40) and I/V (n=21) injected cows which were sub-divided to single (n=20 and n=9) and double insemination (n=20 and n=12) groups, respectively.

Similarly, the cows in Gonadorelin treated group were subdivided to I/M (n=40) and I/V (n=26) injected animals and which were further subdivided to single (n=20 and n=13) and double insemination (n=20 and n=13) groups, respectively. Repeat breeder Jersey cows were inseminated as single (n=11) or double (n=17) insemination regimens following the injection of GnRH analogues injected through I/M route. The animals in control group were also divided into single and double insemination groups (n=11/10 each)

Table M1. Schematic distribution of experimental cows in different insemination groups.

Category	Reproductive Status	Treatment	Route of administration	A.I	Animals Inseminated
Jersey (n=216)	Normal (n=167)	Buserelin acetate (Receptal) (n=61)	Intra muscular	Single	20
				Double	20
			Intra venous	Single	9
				Double	12
		Gonadorelin (Fertagyl) (n=66)	Intra muscular	Single	20
				Double	20
			Intra venous	Single	13
				Double	13
		Control (n=40)		Single	20
				Double	20
	Repeat breeder (n=49)	GnRH analogues pooled	Intra muscular	Single	11
				Double	17
		Control		Single	11
				Double	10
Crossbred (n=232)	Normal (n=179)	Buserelin acetate (Receptal) (n=65)	Intra muscular	Single	20
				Double	20
			Intra venous	Single	13
				Double	12
		Gonadorelin (Fertagyl) (n=74)	Intra muscular	Single	20
				Double	20
			Intra venous	Single	14
				Double	20
		Control (n=40)		Single	20
				Double	20
	Repeat breeder (n=53)	GnRH analogues pooled	Intra muscular	Single	10
				Double	14
		Control		Single	14
				Double	15
Total					**448**

Crossbred animals (n=232) were subdivided to normal (n=179) and repeat breeder animals (n=53). Normal animals were again subdivided to treatment (n=139) and control groups (n=40). Treatment group was constituted by Buserelin acetate (n=65) and Gonadorelin (n=74) treated animals. The Buserelin acetate treated cows were subdivided to I/M (n=40) or I/V (n=25) injected groups which were further subdivided to single (n=20 and n=13) and double insemination (n=20 and n=12) groups, respectively. The animals in Gonadorelin group were subdivided to I/M (n=40) and I/V (n=34) injected cows and were further subdivided to single (n=20 and n=14) and double insemination (n=20 each) groups, respectively. As in Jersey animals, repeat breeder crossbred cows were inseminated as single (n=10) or double (n=14) insemination regimens following the injection of GnRH analogues injected through I/M route only and the animals in control group were divided to single and double insemination groups(n=14,15 each)

Pregnancy diagnosis

Pregnancy diagnosis was carried out 60 days post AI by rectal palpation method in cows not returning to estrus within this duration.

Blood Collection for Progesterone Estimation:

The blood was collected from 21 Jersey (Buserelin acetate treated 9, Gonadorelin treated 6 and Control 6) and 30 crossbred (Buserelin acetate treated 12, Gonadorelin treated 12 and Control 6) cows for progesterone estimation. The blood samples were collected twice on day 0 (day of first insemination) and day 5th from each animal. The blood was collected aseptically by jugular venopuncture. The site was prepared aseptically and blood was collected using the 18/20 gauge needle in 5 ml heparinised syringe. Prior to collection the needle was flushed by using heparin @ 0.1– 0.2 mg/ ml. After collection the plasma was separated by centrifuging the blood sample @3000 r.p.m for 10 min. and frozen at -20°C in 2 ml capacity micro-centrifuge tubes, pending analysis. Repeated thawing of plasma was strictly avoided.

PROCEDURE OF PLASMA PROGESTERONE ASSAY

PROGESTERONE RIA

Pre Coated Tube Method:

The kit was assembled by Animal Production unit FAO / IAEA Agriculture and Biotechnology lab, Seibersdorf, Austria for distribution to FAO / IAEA programme counterparts. Kit was developed for purpose of measuring progesterone in plasma/serum and /or skim milk to aid in monitoring the estrus cycle and/or pregnancy in domestic animals including cattle, buffalo, sheep, goat, camel, horse and swine.

RADIOIMMUNOASSAY PROCEDURE:

1. The blood plasma samples and other assay components were brought to room temperature before starting the assay.
2. Antibody coated tubes were used for labeling standards and samples. Normal plastic tubes (not antibody coated tubes) were used for the Total Count tubes (TC). Two tubes were used for each standard point, TC and Quality control (QC) value.
3. The plasma samples, standards and QC samples were mixed thoroughly prior to assay.
4. The 100µL of each of the standards, QC and samples were pipetted into the bottom of corresponding tube.
5. 1ml of ^{125}I – progesterone was added to each tube within 5 minutes of adding the standards, QC and samples.
6. Tubes were covered with aluminium foil and were incubated at room temperature for 3-4 hours and incubation conditions were kept consistent from assay to assay.
7. After incubation, all tubes were vigorously decanted (except TC which was placed in a separate rack) into an appropriate radioactive waste disposal container.

Holding the rack upside down, the tubes were striked sharply downwards on absorbent paper. They were allowed to drain for 2-5 min and striked sharply downwards to remove residual droplets.

8. The radioactivity was counted in all the tubes for a fixed time (50 sec or 1 min) by using gamma counter.

Statistical analysis

Statistical analysis was carried out by chi square test and 't' test as described by Snedecor and Cochran (1967).

RESULTS

CHAPTER IV

RESULTS

A. Conception following GnRH administration

Comparative efficacy of GnRH analogues, Buserelin acetate (Receptal) and Gonadorelin (Fertagyl), injected through intramuscular and intravenous route on conception in Jersey (n=127) and crossbred (n=139) cows following single or double insemination has been shown in table 1 and figure 1.

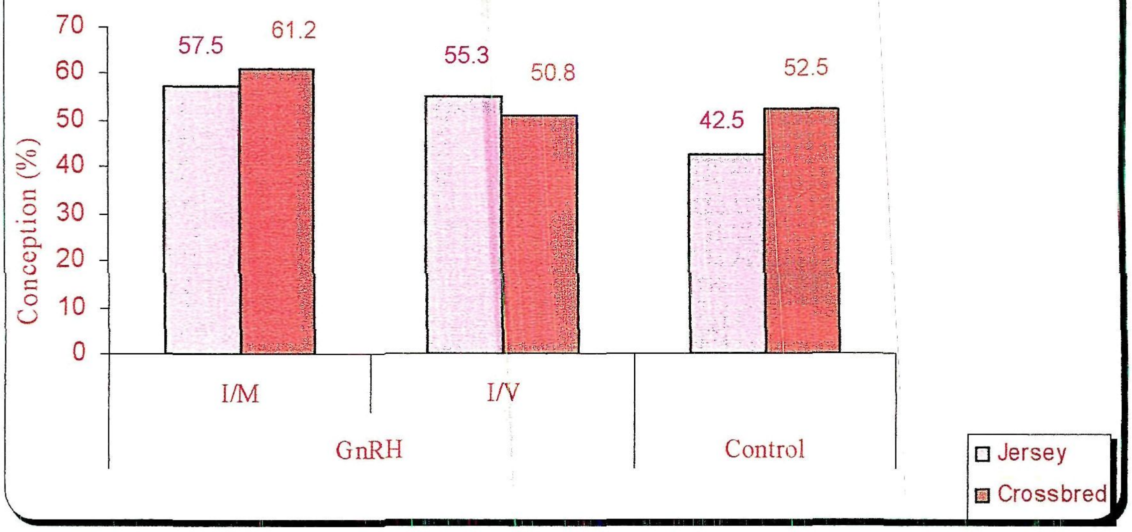
The conception rate (CR) in treatment group following Buserelin acetate injection through intra-muscular or intra-venous routes in Jersey cows was 60.0% (24/40) and 61.9% (13/21), respectively. Similarly, CR in treatment group when Gonadorelin was injected through intra-muscular and intra-venous routes in Jersey animals was 55.0% (22/40) and 50% (13/26), respectively. CR was 57.5% (46/80) and 55.3% (26/47) in intra-muscular and intra-venous injected groups, respectively, when the data were pooled. There was no difference in CR following injection of GnRH by either of the routes. CR in control group was 42.5% (17/40) and this also did not differ significantly from either of the treatment groups.

The CR in treatment group following Buserelin acetate injection through intra-muscular or intra-venous routes in crossbred animals was 62.5% (25/40) and 48.0% (12/25), respectively. Similarly, CR in treatment group when Gonadorelin was injected through intra-muscular or intra-venous routes in crossbred animals was 60.0% (24/40) and 52.9% (18/34), respectively. The pooled CR was 61.2% (49/80), 50.8% (30/59) and 52.5% (21/40) in intra-muscular, intra-venous and control groups, respectively. CR was better when GnRH analogues were injected by intra-muscular route as compared to intra venous administration or control groups.

Table 1: Comparative efficacy of GnRH analogues injected through intra-muscular (I/M) or intra-venous (I/V) routes on conception in Jersey and crossbred cows.

Category	Treatment	Route of administration	A.I	Animals Inseminated	Conceived	Conception (%)
Jersey (n=167)	Buserelin acetate (Receptal)	I/M	Single	20	13	65.0
			Double	20	11	55.0
			Pooled	**40**	**24**	**60.0**
		I/V	Single	9	6	66.6
			Double	12	7	58.3
			Pooled	**21**	**13**	**61.9**
	Gonadorelin (Fertagyl)	I/M	Single	20	12	60.0
			Double	20	10	50.0
			Pooled	**40**	**22**	**55.0**
		I/V	Single	13	6	46.1
			Double	13	7	53.8
			Pooled	**26**	**13**	**50.0**
	GnRH analogues pooled	**I/M**	Single	40	25	62.5
			Double	40	21	52.5
			Pooled	**80**	**46**	**57.5**
		I/V	Single	22	12	54.5
			Double	25	14	56.0
			Pooled	**47**	**26**	**55.3**
	Control		Single	20	8	40.0
			Double	20	9	45.0
			Pooled	**40**	**17**	**42.5**
Crossbred (n=179)	Buserelin acetate (Receptal)	I/M	Single	20	13	65.0
			Double	20	12	60.0
			Pooled	**40**	**25**	**62.5**
		I/V	Single	13	6	46.1
			Double	12	6	50.0
			Pooled	**25**	**12**	**48.0**
	Gonadorelin (Fertagyl)	I/M	Single	20	12	60.0
			Double	20	12	60.0
			Pooled	**40**	**24**	**60.0**
		I/V	Single	14	8	57.1
			Double	20	10	50.0
			Pooled	**34**	**18**	**52.9**
	GnRH analogues pooled	**I/M**	Single	40	25	62.5
			Double	40	24	60.0
			Pooled	**80**	**49**	**61.2**
		I/V	Single	27	14	51.8
			Double	32	16	50.0
			Pooled	**59**	**30**	**50.8**
	Control		Single	20	9	45.0
			Double	20	12	60.0
			Pooled	**40**	**21**	**52.5**

Figure 1 : Comparative efficacy of GnRH analogues injected through intra-muscular (I/M) or intra-venous (I/V) routes on conception in normal Jersey and crossbred cows



The effect of GnRH analogues, Buserelin acetate (Receptal) and Gonadorelin (Fertagyl), injected through intra-muscular route on conception in Jersey (n=167) and crossbred (n=179) cows following single or double insemination has been shown in Table 2 and Figure 2.

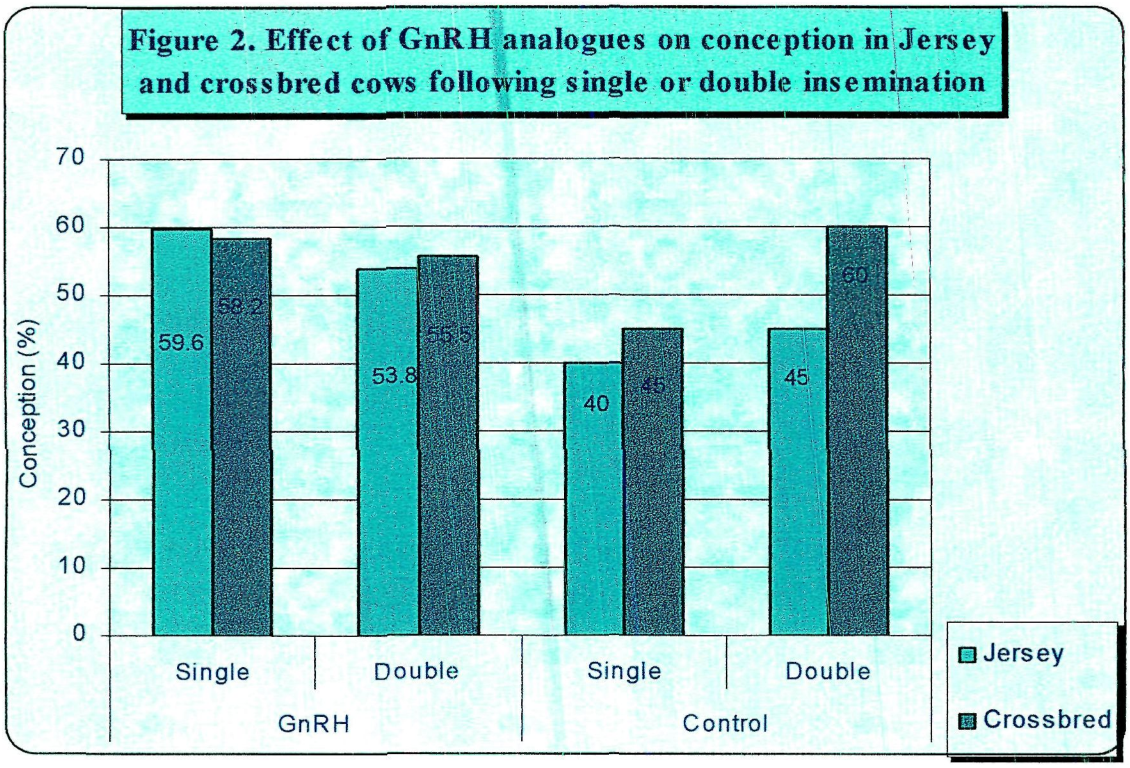
After insemination of 29 and 32 Jersey cows in two Buserelin acetate (Receptal) treated groups, 19 and 18 animals conceived with a conception rate (CR) of 65.5% and 56.2% following single or double insemination, respectively. Similarly, following the insemination of 33 Jersey cows in each of the two Gonadorelin (Fertagyl), treated groups, 18 and 17 animals conceived and the CR was 54.5% and 51.5% following single and double insemination, respectively. However, the CR was 40% and 45% following single or double insemination, respectively, in control group.

The difference of 9.3% in CR between single (65.5%) or double (56.2%) insemination following Buserelin administration and similarly, the difference between 54.5% and 51.5% to single or double AI following Gonadorelin (Fertagyl) administration was not significant.

Table 2: Effect of GnRH analogues on conception in Jersey and crossbred cows following single or double insemination.

Category	Treatment	A.I	Animals Inseminated	Conceived	Conception (%)
Jersey (n=167)	Buserelin acetate (Receptal)	Single	29	19	65.5
		Double	32	18	56.2
	Gonadorelin (Fertagyl)	Single	33	18	54.5
		Double	33	17	51.5
	GnRH analogues pooled	**Single**	**62**	**37**	**59.6**
		Double	**65**	**35**	**53.8**
	Control	**Single**	**20**	**8**	**40.0**
		Double	**20**	**9**	**45.0**
Crossbred (n=179)	Buserelin acetate (Receptal)	Single	33	19	57.5
		Double	32	18	56.2
	Gonadorelin (Fertagyl)	Single	34	20	58.8
		Double	40	22	55.0
	GnRH analogues pooled	**Single**	**67**	**39**	**58.2**
		Double	**72**	**40**	**55.5**
	Control	**Single**	**20**	**9**	**45.0**
		Double	**20**	**12**	**60.0**

The difference of 11% between Buserelin and Gonadorelin, following single (65.5% vs. 54.5%) and 4.7% (56.2% vs. 51.5%) in double insemination, respectively was not significant. Among 62 and 65 Jersey cows inseminated following single and double insemination, irrespective of GnRH used, 37 (59.6%) and 35 (53.8%), respectively, conceived. The difference was non significant.



Similarly, following the insemination of 33 and 32 crossbred cows in two Buserelin acetate (Receptal) treated groups, 19 and 18 animals conceived with a CR of 57.5% and 56.2% following single or double insemination, respectively. Following the insemination of 34 and 40 crossbred cows in two Gonadorelin (Fertagyl), treated groups, 20 and 22 animals conceived with a CR of 58.8% and 55.0% with single or double insemination, respectively. In control group, after insemination of 20 animals each in single or double insemination group, 9 (45%) and 12 (60%) cows conceived, respectively. The difference between single (57.5%) or double (56.2%) insemination following Buserelin administration was not significant. Similarly, the difference between single (58.8%) or double (55.0%) insemination following Gonadorelin (Fertagyl) administration was also not significant. From the pooled data of 67 and 72 crossbred cows inseminated by single and double insemination irrespective of GnRH analogue used, 39 (58.2%) and 40 (55.5%) conceived, respectively. The difference was non significant.

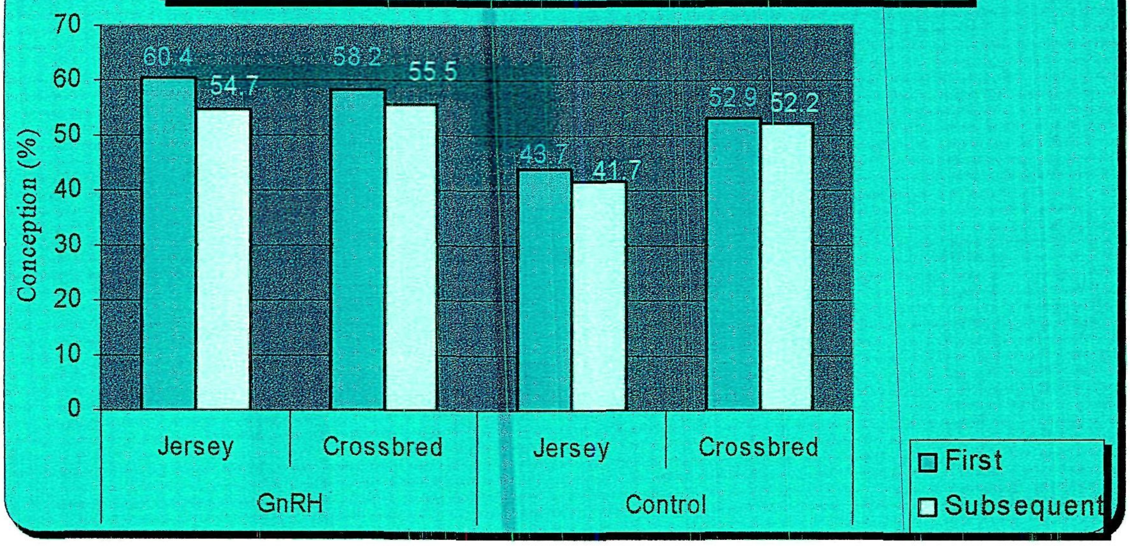
Effect of GnRH analogues injected during first or subsequent (second + third) estruses on conception in normal Jersey (n=167) and crossbred cows (n=179) has been shown in table 3 and figure 3.

Table 3: Effect of GnRH analogues injected during first or subsequent (second + third) estruses on conception in normal Jersey and crossbred cows.

Category	Treatment	Insemination	Animals Inseminated	Conceived	Conception (%)
Jersey (n=167)	Buserelin acetate (Receptal)	First	23	15	65.2
		subsequent	39	22	56.4
	Gonadorelin (Fertagyl)	First	20	11	55.0
		subsequent	45	24	53.3
	GnRH pooled	First	43	26	60.4
		subsequent	84	46	54.7
	Control	First	16	7	43.7
		subsequent	24	10	41.7
Crossbred (n=179)	Buserelin acetate (Receptal)	First	34	21	61.7
		subsequent	33	19	57.5
	Gonadorelin (Fertagyl)	First	32	18	56.2
		subsequent	38	22	57.8
	GnRH pooled	First	67	39	58.2
		subsequent	72	40	55.5
	Control	First	17	9	52.9
		subsequent	23	12	52.2

The animals which were presented for insemination for the first time after parturition were categorized as first estrus cows and those presented for second or third time after parturition were grouped as subsequent estrus cows. Among Jersey cows, 23 and 39 animals were inseminated in first and subsequent estruses using Buserelin acetate and out of them 15 (65.2%) and 22 (56.4%) conceived, respectively. Similarly, when gonadorelin was injected in 20 and 45 Jersey animals in first and subsequent estruses, 11 (55%) and 24 (53.3%) cows conceived, respectively. From pooled data of treatment groups CR of 60.4% (26/43) and 54.7% (46/84) in first and subsequent estrus group, respectively, was obtained. In control group the CR was 43.7% and 41.7% following insemination in first or subsequent estrus.

Figure 3 : Effect of GnRH analogues injected during first or subsequent (second + third) post partum estruses on conception in normal Jersey and crossbred cows



Among crossbred cows, out of 34 and 33 animals inseminated in first and subsequent estruses using Buserelin acetate 21 and 19 conceived giving CR of 61.7% and 57.5%, respectively. Similarly, when Gonadorelin was injected in 32 and 38 crossbred cows in first and subsequent estruses, 18 and 22 conceived giving CR of 56.2% and 57.8%, respectively. The difference in CR of both treatments was not significant. From the pooled data the CR was 58.2% and 55.5%, respectively in first and subsequent estrus. In control animals, the CR was 52.9% (9/17) and 52.2% (12/23) in first or subsequent estrus insemination, respectively.

Effect of GnRH analogues injected during different lactations on conception in normal Jersey (n=167) and crossbred (n=179) cows has been shown in table 4 and figure 4.

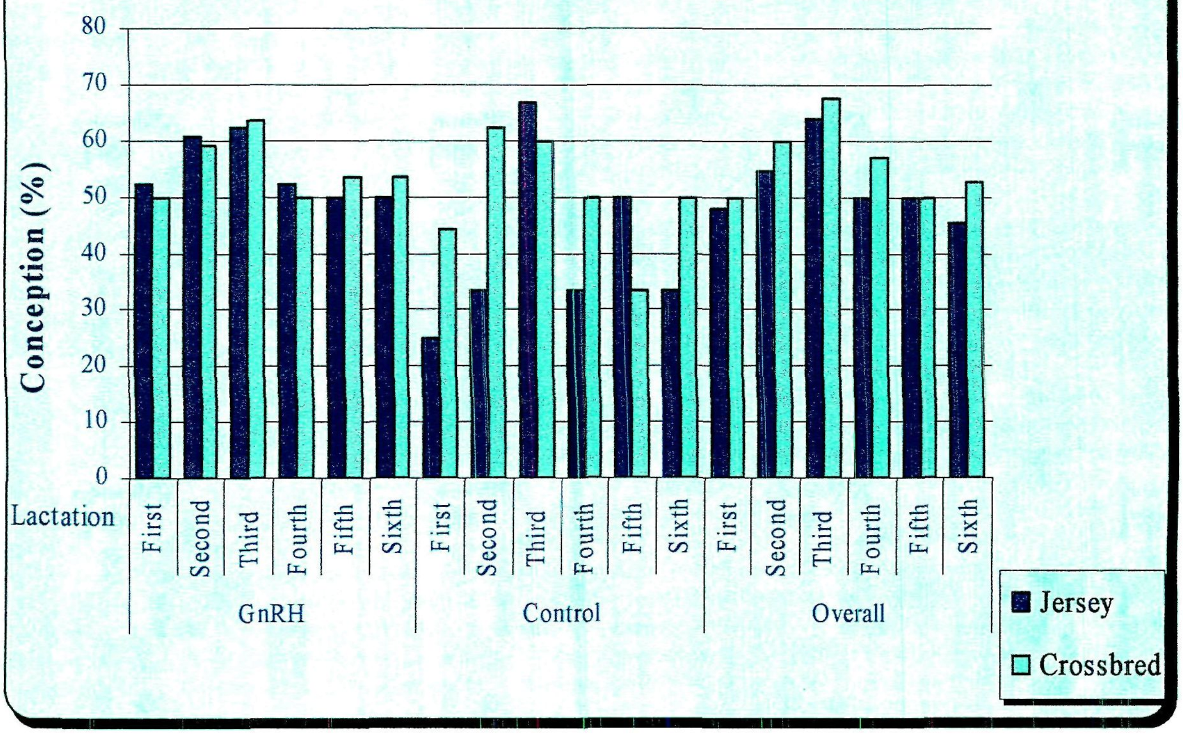
Table 4: Effect of GnRH analogues injected during different lactations on conception in normal Jersey and crossbred cows.

Category	Treatment	Lactation	Animals Inseminated	Conceived	Conception (%)
Jersey (n=167)	GnRH	First	21	11	52.3
		Second	33	20	60.6
		Third	24	15	62.5
		Fourth	21	12	52.3
		Fifth	10	5	50.0
		Sixth	18	9	50.0
	Control	First	4	1	25.0
		Second	9	3	33.3
		Third	12	8	66.6
		Fourth	9	3	33.3
		Fifth	2	1	50.0
		Sixth	4	1	33.3
	Pooled	**First**	**25**	**12**	**48.0**
		Second	**42**	**23**	**54.7**
		Third	**36**	**23**	**63.8**
		Fourth	**30**	**15**	**50.0**
		Fifth	**12**	**6**	**50.0**
		Sixth	**22**	**10**	**45.4**
Crossbred (n=179)	GnRH pooled	First	29	15	50.0
		Second	22	13	59.0
		Third	33	21	63.6
		Fourth	10	6	50.0
		Fifth	15	8	53.3
		Sixth	30	16	53.3
	Control	First	9	4	44.4
		Second	8	5	62.5
		Third	10	6	60.0
		Fourth	4	2	50.0
		Fifth	3	1	33.3
		Sixth	6	3	50.0
	Pooled	**First**	**38**	**19**	**50.0**
		Second	**30**	**18**	**60.0**
		Third	**43**	**27**	**67.5**
		Fourth	**14**	**8**	**57.1**
		Fifth	**18**	**9**	**50.0**
		Sixth	**36**	**19**	**52.7**

Jersey animals (n=167) were divided into different lactation-order wise groups varying from first to sixth. The CR was 52.3, 60.6, 62.5, 52.3, 50 and 50% in treatment group and 25.0, 33.3, 66.6, 33.3, 50 and 33.3% in control group following insemination in first to sixth lactations, respectively. Irrespective of treatment, pooled CR was 48.0, 54.7, 63.8, 50.0, 50.0 and 45.4% respectively, following insemination in first to sixth lactation. There was a steady rise in CR from first to third lactation in both the groups which declined subsequently.

Among crossbred cows (n=179) the CR were 50.0, 59.0, 63.6, 50.0, 53.3 and 50% following insemination in first to sixth lactation in treatment group, respectively. Similarly, CR was 44.4, 62.5, 60.0, 50.0, 33.3 and 50.0%, respectively, following insemination in first six lactation in control group. Irrespective of treatment pooled CR was 50.0, 60.0, 67.5, 57.1, 50.0 and 52.7% in first six lactations, respectively. There CR improved till third lactation and declined subsequently as in Jersey cows.

Figure 4 : Effect of GnRH analogues injected during different lactations on conception in normal Jersey and crossbred cows



Effect of GnRH analogues on conception in Jersey and crossbred repeat breeder cows have been shown in table 5 and figure 5.

Table 5: Effect of GnRH analogues on conception in Jersey and crossbred repeat breeder cows.

Category	Treatment	A.I	No. of animals Inseminated	Conceived	Conception (%)
Jersey (n=49)	GnRH	Single	11	6	54.5
		Double	17	8	47.0
	Control	Single	11	4	36.3
		Double	10	4	40.0
Crossbred (n=53)	GnRH	Single	10	5	50.0
		Double	14	6	42.8
	Control	Single	14	6	42.8
		Double	15	8	53.3

For this study, 49 Jersey and 53 crossbred cows were selected and were further subdivided in treatment and control groups. In Jersey cows, after insemination of 11 and 17 animals following single or double AI using GnRH analogues, 6 and 8 conceived, giving CR of 54.5 and 47 percent, respectively. In control group, 4 animals each conceived out of 11 and 10 cows giving CR of 36.3 and 40 per cent respectively.

In crossbred repeat breeder animals, after insemination of 10 and 14 animals following single or double AI, 5 and 6 cows conceived, respectively, giving CR of 50.0 and 42.8 percent following the use of GnRH analogues. Similarly, in control animals 6 and 8 cows conceived out of 14 and 15 animals inseminated giving CR of 42.8 and 53.3 per cent, respectively.

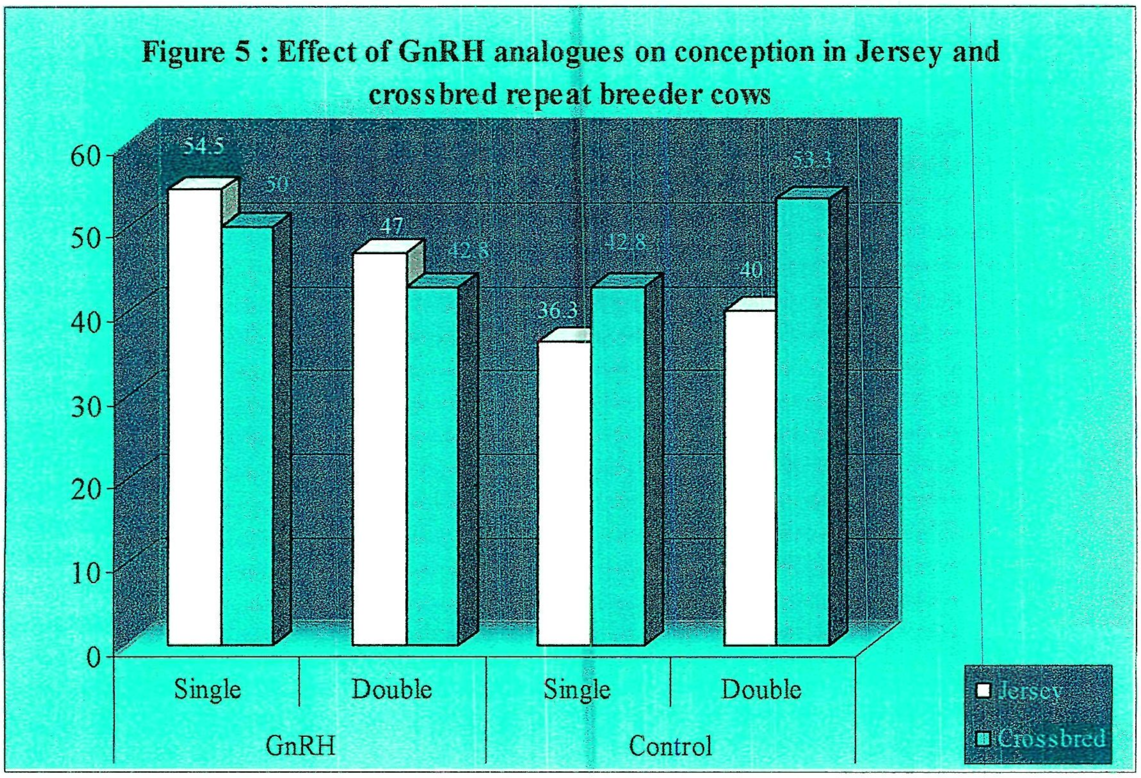


Figure 5 : Effect of GnRH analogues on conception in Jersey and crossbred repeat breeder cows

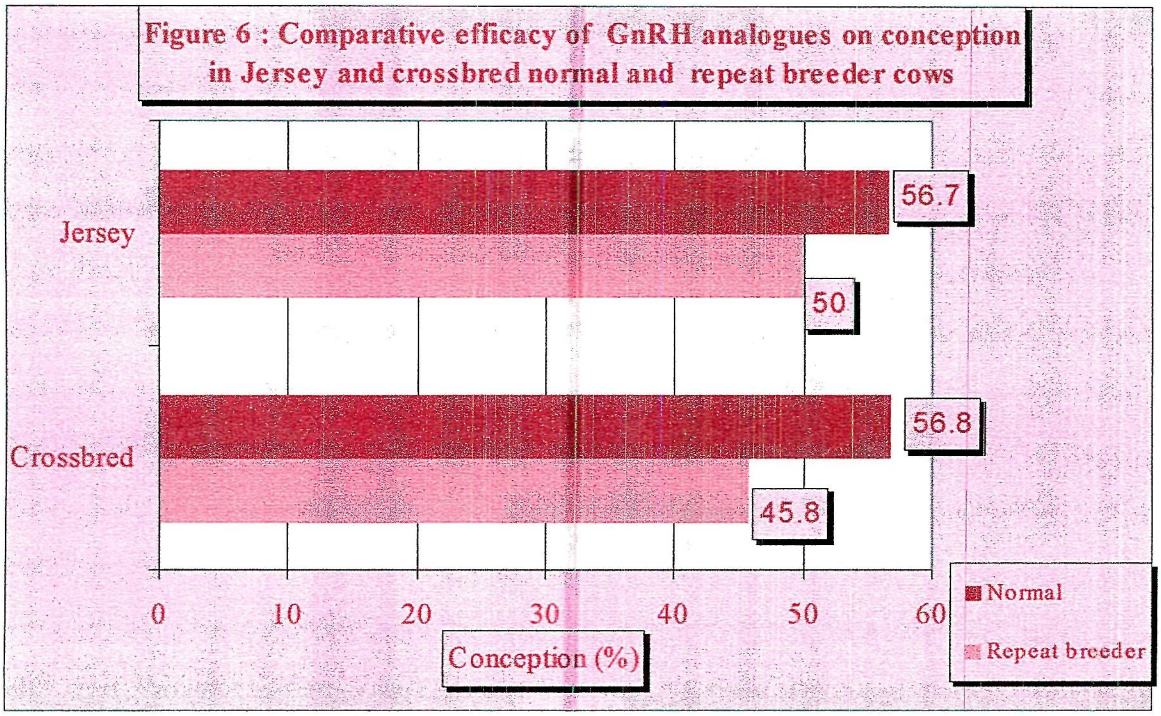
Comparative efficacy of GnRH analogues on conception in Jersey and crossbred normal and repeat breeder cows has been shown in table 6 and figure 6.

Table 6: Comparative efficacy of GnRH analogues on conception in Jersey and crossbred normal and repeat breeder cows.

Category	Reproductive status	A.I	No. of animals Inseminated	Conceived	Conception (%)
Jersey (n=155)	Normal	Single	62	37	59.6
		Double	65	35	53.8
		Pooled	**127**	**72**	**56.7**
	Repeat breeder	Single	11	6	54.5
		Double	17	8	47.01
		Pooled	**28**	**14**	**50.0**
Crossbred (n=163)	Normal	Single	67	39	58.2
		Double	72	40	55.5
		Pooled	**139**	**79**	**56.8**
	Repeat breeder	Single	10	5	50.0
		Double	14	6	42.8
		Pooled	**24**	**11**	**45.8**

After insemination of 62 and 65 normal Jersey animals following single and double insemination, 37 (59.6%) and 35 (53.8%) conceived while in repeat breeder animals 6 (54.5%) and 8 (47.01%) conceived out of 11 and 17 cows following single or double insemination, respectively. There was no significant difference in single and double insemination groups, so on pooling data in normal and repeat breeder animals the CR was 56.5% v/s 50.0% (72/127 v/s 14/28), respectively, in normal and repeat breeder animals.

Following insemination of 67 and 72 normal crossbred animals subsequent to single or double insemination 39 (58.2%) and 40 (55.5%) cows conceived while in repeat breeder animals 5 (50.0%) and 6 (42.8%) conceived out of 10 and 14 following single insemination and double insemination. There was no significant difference in single and double insemination groups, so on pooling data in normal and repeat breeder animals the CR was 56.8 v/s 45.8% (79/139 v/s 11/24) respectively, in normal and repeat breeder animals.



B. Plasma progesterone profile following GnRH administration

Mean plasma progesterone concentration (ng/ml) in Jersey and crossbred cows at day 0 (day of estrus) and day 5 post AI following treatment with different GnRH analogues has been shown in table 7 and figures 7a, 7b and 7c.

Table 7: Mean plasma progesterone concentration (ng/ml) in Jersey and crossbred cows at day 0 and 5 post AI following treatment with different GnRH analogues.

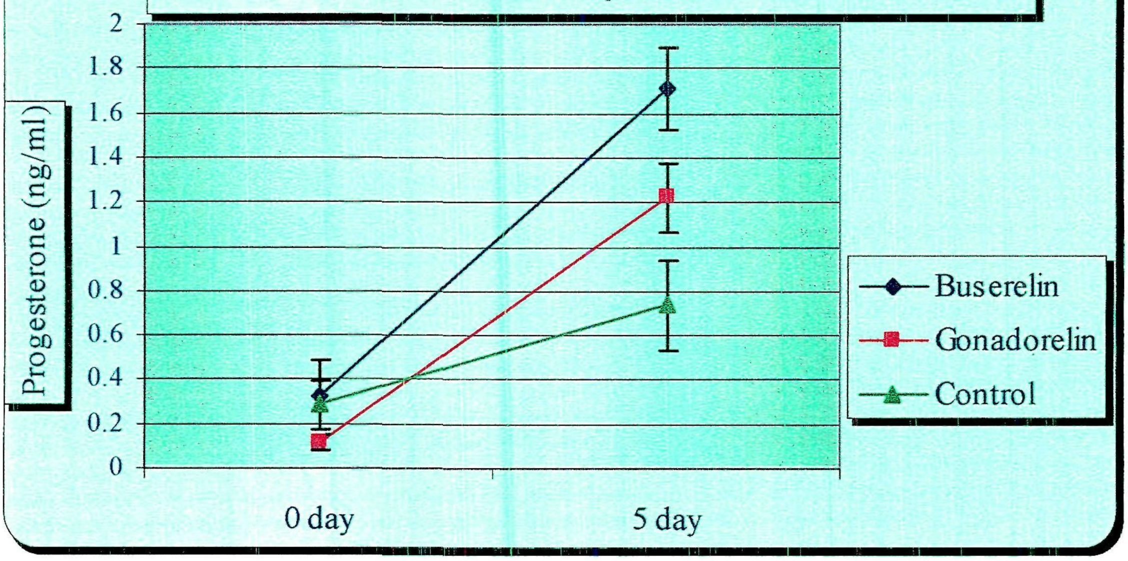
Category	Treatment	0 day	5th day
Jersey (n=21)	Buserelin acetate (Receptal, n=9)	0.327±0.155	1.710±0.181**
	Gonadorelin (Fertagyl, n=6)	0.113±0.037	1.220±0.157*
	GnRH pooled (n=15)	0.245±0.098	1.470±0.131**
	Control (n=6)	0.285±.112	0.738±.201
Crossbred (n=30)	Buserelin acetate (Receptal, n=12)	0.114±0.039	1.459±0.260**
	Gonadorelin (Fertagyl, n=12)	0.169±0.046	1.440±0.175**
	GnRH pooled (n=24)	0.162±.033	1.447±0.169**
	Control (n=6)	0.146±0.031	0.505±0.153

*Significant ($P < 0.05$), ** Highly significant ($P < 0.01$) between days 0 and 5

The mean plasma progesterone concentration on day 0 and 5th day in Buserelin acetate (Receptal) treated Jersey (n=9) animals was 0.327±0.155 and 1.710±0.181 ng/ml, respectively. There was significant rise in progesterone level from 0 to 5th day post insemination ($p< 0.01$). Similarly, the mean (±SE) plasma progesterone concentration on day 0 and 5th day in Gonadorelin (Fertagyl) treated Jersey (n=6) animals was 0.113±0.037 and 1.220±0.157 ng/ml, respectively. There was significant rise in progesterone level from 0 to 5th day post insemination ($p< 0.05$). When the data of mean progesterone were pooled, concentrations were 0.245±0.098 and 1.470±0.131 ng/ml on 0 and 5 day post insemination, respectively. Rise in progesterone values between 0 and 5 day post insemination was highly significant ($p < 0.01$).

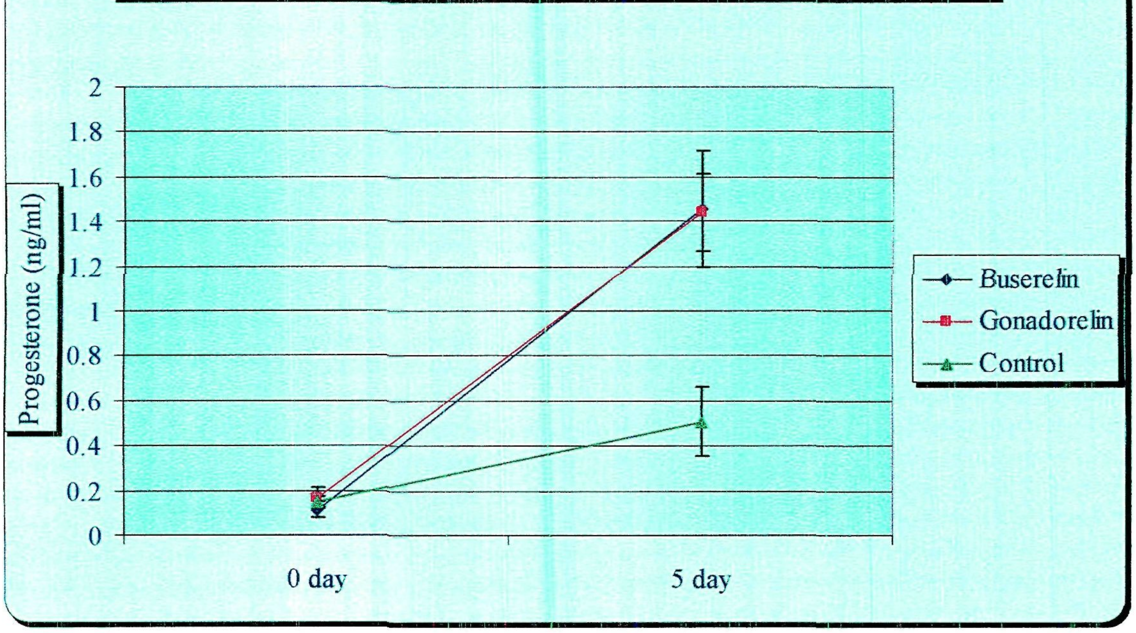
In control Jersey (n=6) animals mean progesterone was 0.285±0.112 and 0.738±0.201 ng/ml on 0 and 5 day post insemination, respectively. The rise in progesterone levels was statistically non significant. There was no difference in day O progesterone concentration between treatment and control groups (0.245±0.098 and 0.285±0.112), however, on day 5 the difference was statistically significant (1.470±0.131 and 0.738±0.201, $p < 0.05$).

Figure7a : Mean plasma progesterone concentration at day 0 and 5 post AI following treatment with different GnRH analogues in Jersey cows



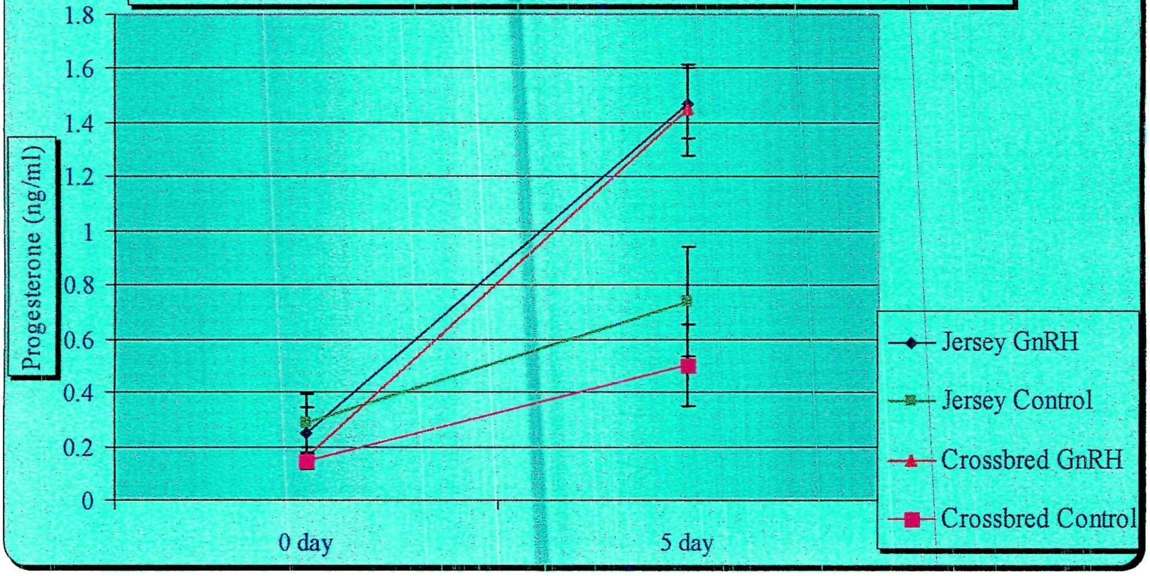
The mean plasma progesterone concentration on day 0 (day of insemination) and 5th day in Buserelin acetate (Receptal) treated crossbred animals (n=12) was 0.114±0.039 and 1.459±0.260 ng/ml, respectively. There was significant rise in progesterone level from 0 to 5th day post insemination ($p < 0.01$). Similarly, the mean progesterone levels on day 0 and 5th day in Gonadorelin (Fertagyl) treated crossbred animals (n=12) was 0.169±0.046 and 1.440±0.175 ng/ml, respectively and the rise in progesterone level from 0 to 5th day post insemination was highly significant ($p < 0.01$).

Figure 7b : Mean plasma progesterone concentration at day 0 and 5 post AI following treatment with different GnRH analogues in crossbred cows



There was no difference in progesterone concentration in the two treatment groups, so the data were pooled (n=24) and the final mean values of progesterone were 0.162±0.033 and 1.447±0.169 ng/ml on 0 and 5 day post insemination, respectively. There was significant rise in progesterone values between 0 and 5 day post insemination ($p< 0.01$). In control crossbred animals (n=6) in which no treatment was given the mean progesterone concentration was 0.146±0.031 and 0.505±0.153 ng/ml on 0 and 5 day post insemination, respectively but the rise in progesterone was statistically non significant. There was no difference in day O progesterone concentration (0.162±0.033 and 0.146±0.031) between treatment and control groups; however, on day 5 the difference was statistically significant (1.447±0.169 and 0.505±0.153) in crossbred animals ($p< 0.01$).

Figure 7c : Mean plasma progesterone concentration in Jersey and crossbred cows at day 0 and 5 post AI following treatment with GnRH analogues



Mean plasma progesterone concentrations (ng/ml) in pregnant and non pregnant Jersey (n=21) and crossbred (n=30) cows at day 0 and 5 post AI following treatment has been shown in table 8 and Figure 8.

Table 8: Mean plasma progesterone concentration (ng/ml) in pregnant and non pregnant Jersey and crossbred cows at day 0 and 5 post AI.

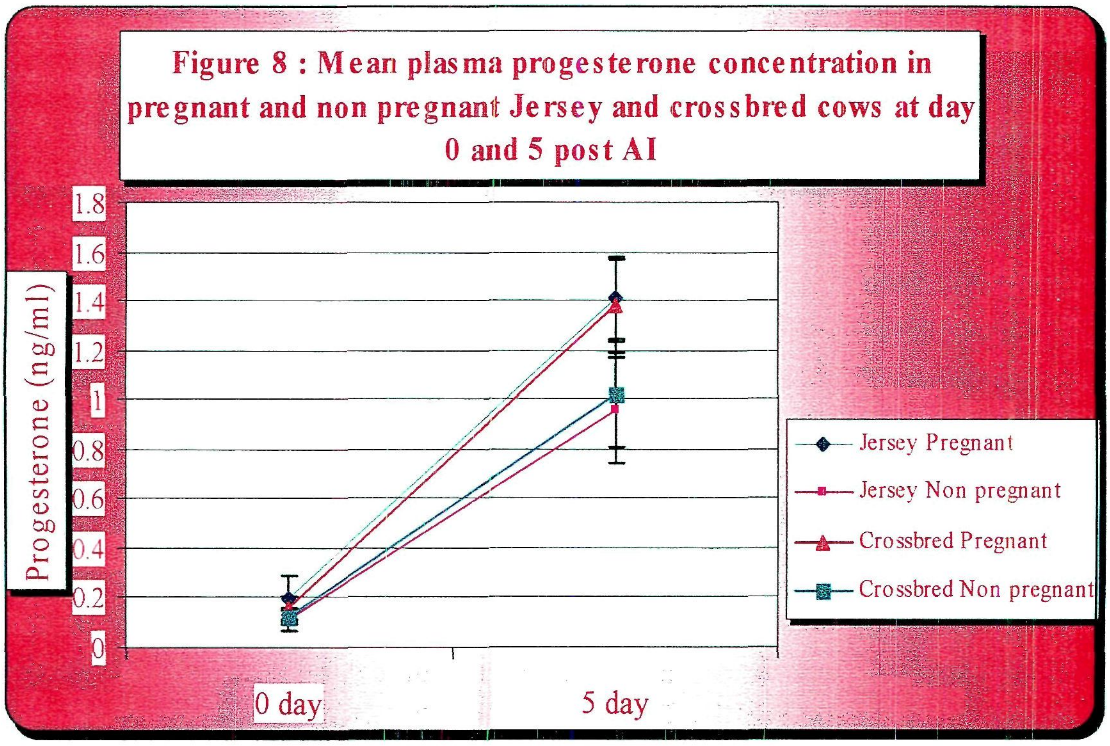
Category	Status	0 day	5 day
Jersey (n=21)	Pregnant (n=14)	0.194±0.096	1.408±0.162**
	Non pregnant (n=7)	0.107±0.039	0.953±0.215*
Crossbred (n=30)	Pregnant (n=15)	0.158±0.046	1.382±0.196**
	Non pregnant (n=15)	0.125±0.035	1.024±0.213**

* Significant (P < 0.05), ** Highly significant (P < 0.01) between days 0 and 5

The mean plasma progesterone concentration in pregnant (n=14) and non pregnant (n=7) Jersey animals were 0.194±0.096 and 0.107±0.039 ng/ml on day 0 and 1.408±0.162

and 0.953±0.215 ng/ml on day 5, respectively. The rise in progesterone concentration from day 0 to 5 in pregnant animals was highly significant (p< 0.01 and significant (p<0.05) in non pregnant cows. However, there was no difference in progesterone concentration between pregnant and non pregnant Jersey cows both on day 0 or 5.

The mean plasma progesterone concentration in pregnant (n=15) and non pregnant (n=15) crossbred animals were 0.158±0.046 and 0.125±0.035 ng/ml on day 0 and 1.382±0.196 and 1.024±0.213 ng/ml on day 5 post treatment, respectively. The rise in progesterone concentration from day 0 to 5 in pregnant as well as non pregnant animals was highly significant (p< 0.01). However, there was no difference in progesterone concentration between pregnant and non pregnant crossbred cows either on day 0 or 5 post AI.



DISCUSSION

CHAPTER V

DISCUSSION

GnRH has been successfully used in various species of animals like bitches (Schmidt, 1996), gilts (Konig *et al.,* 1990), rabbits (Gabor and Zoldag, 1989) and mares (Kilicarslan *et al.,* 1996 and Ataman, *et al*., 2000) and cattle (Nikolovo *et al.,* 1986) for the induction of ovulation. Many studies have shown improvement in the conception rate of cows administered GnRH at the time of AI. (Lee *et al*., 1981; Nakao *et al*., 1983).

In the present study, Buserelin acetate (Receptal) and Gonadorelin (Fertagyl), both GnRH analogues, were injected at the time of AI to evaluate their effect on conception in Jersey and crossbred cows. Buserelin acetate was numerically but non-significantly better as compared to Gonadorelin in Jersey cattle. However, in crossbred cows there was no difference in the effect between the two GnRH analogues. Both preparations were almost equally effective with Buserelin acetate (Receptal) having little edge over Gonadorelin (Fertagyl) in Jersey cows. Chenault (1990) also reported no differences in pregnancy rates of a large number of cows when he compared two different analogues (Fertirelin acetate and Buserelin). No other reference was available in the literature comparing these two analogues.

The GnRH analogues were injected at the time of insemination either through intra-muscular or intra-venous routes in Jersey and crossbred cows (Table 1). In Jersey animals the conception rates were 57.5, 55.3 and 42.5% in intra-muscular, intra-venous and control groups respectively. There was an improvement of 15% and 12.8% over control animals when GnRH was injected through intra-muscular or intra-venous routes, respectively. There was a marginal increase in conception (2.2%) when GnRH was injected through intra-muscular route as compared to intra-venous administration.

Others studies have also reported improvement in conception following administration of GnRH prior to insemination (Schels and Mastafawi, 1978; Lee *et al.*, 1981; Nakao *et al.*, 1983; Thibier *et.al* 1985; Bhosrekar, *et.al* 1986), at the time of A.I. (Nakao, *et.al* 1983; Boul and Keraby, 1986) or at mid cycle post A.I. (Macmillan *et al.*, 1986; Rao 1991; Drew and Peters, 1994). GnRH induces ovulation of a dominant follicle in primiparous dairy cows. Cows treated with GnRH have a LH surge which is maximum 2hr after treatment (McDougall *et al.,* 1995). Osawa and associates (1995) reported that GnRH causes 4-fold increase in plasma LH concentration after 2-2.5 hr. GnRH can be used for ovulation synchronization and timed A.I. programme and the greatest advantage of this is that virtually 100% of the animals can be inseminated within a certain period with a satisfactory pregnancy rate (Nakao, 1998). There has been reported a tendency for increased conception when cows are given GnRH and inseminated in early estrus. Administration of GnRH soon after estrus consistently resulted in higher conception rates at all services than when GnRH treatment is given at later period of estrus (Stevenson *et al.*, 1984).

The improvement in pregnancy rate after treatment with GnRH at A.I may be related to insufficient GnRH release from the hypothalamus (Ryan *et al.,*1991). The treatment with GnRH during estrus may be affecting time of ovulation, fertilization rates, corpus luteum development, progesterone secretion and embryonic survival. The GnRH is acting possibly through its effects on release of follicle stimulating hormone (FSH) and luteinising hormone (LH), but its direct effect on the reproductive tract cannot be ignored, because GnRH like molecules (gonadocrinins) have been isolated from ovarian follicle (Ying *et al.,* 1981). If GnRH is acting through LH secretion, perhaps late ovulating repeat breeding cows (Dekruif, 1978) benefit from GnRH treatment by hastened ovulation and improved fertilization rates. Roche (1977) demonstrated that GnRH given 48 hr after a luteolytic dose of prostaglandin F2 alpha hastened ovulation in beef heifers by as much as 18 hr compared with untreated heifers with GnRH. The secretion of LH may also improve potential development of CL and increase progesterone secretion as demonstrated by increased progesterone concentration after administration of hCG on days 2, 3 and 4 of estrous cycle (Helmer and Britt, 1983). Maurer and Echternkamp (1982) found higher survival in cows with higher preovulatory surges of LH and earlier post ovulatory rises of luteal progesterone. Those effects could be mediated by exogenous GnRH induced LH secretion at insemination. Because the preovulatory surge of LH normally occurs about 6 hr after the onset of estrus (Schams *et al.*, 1977) treatment by GnRH at insemination may have induced a secondary surge of LH before or after the preovulatory surge of LH. That added increment of LH may be beneficial to events associated with conception. Further work needs to be done to elucidate the effects of GnRH on hormone secretion at estrus and its mechanism for improving conception.

Lee and coworkers (1985) suggested that the improved fertility after GnRH treatment at insemination is associated with increased progesterone concentration following ovulation. They also postulated that LH could enhance recruitment of granulosa cells to become luteal cells to subsequent increase progesterone secretion during the luteal phase of cycle, which in turn improved the pregnancy rate. Efficacy of GnRH-analogue at the time of insemination modulate the occurrence of ovulation and it initiates preovulatory LH surge leading to decreased incidence of delayed ovulation and thus resulting in better synchrony between sperm and ova at the site of fertilization (Lucy and Stevenson, 1986).

However, some researchers have reported no improvement in pregnancy rates following treatment with GnRH (Mee *et al.*, 1990; Archbald *et al.*, 1993; Drew and Peters, 1994) *in comparison to untreated control cows.*

In crossbred animals the conception rates were 61.2, 50.8 and 52.5 % in intra-muscular, intra-venous and control groups, respectively. There was an improvement of 8.7% in conception following intra-muscular administration of hormone when compared with control group. Interestingly, there was a difference of 10.4% between intra-muscular and intra-venous route of administration in crossbred animals, the former being better. Conception in animals administered GnRH through intra-venous route was even lower (1.7%) than control cows.

Stress due to violent struggling during intra-venous administration in crossbred cows might be the reason for low conception. It has been suggested that stressors reduce fertility by interfering with the mechanisms that regulate the precise timing of events within the follicular phase. Acute stressors imposed at precisely defined times have been investigated for effects on different parts of the reproductive control mechanism (Dobson and Smith, 2000). Activation of the hypothalamus-pituitary-adrenal axis by stressors reduces the pulsatility of GnRH/LH by actions at both the hypothalamus and pituitary gland, ultimately depriving the ovarian follicle of adequate LH support. This leads to reduced estradiol production by slower growing follicles. Such a hypothesis is supported by the marked decrease in estradiol secretion observed after reducing the exogenous LH pulses driving follicular growth in an ovarian autotransplant model (Dobson *et al.*, 1999a).

The combination of above effects in LH pulsatility at hypothalamic and pituitary levels no doubt contributes to the delay and reduced magnitude of the LH surge observed after transport or insulin administration in the follicular phase just prior to the expected LH surge (Dobson *et al.*, 1999b). This effect on the LH surge control mechanism could be exerted directly via an influence of GnRH on production of its own receptors, or indirectly by the induced reduction in estradiol which in turn will alter the balance of systems controlling LH surge release.

It can be concluded from this observation that GnRH improves conception in normal cows. Whereas, in Jersey animals either of the routes can be followed for GnRH administration, in crossbred animals only intra-muscular route should be preferred. No reference could be found in literature where the comparison of different route of administration of GnRH has been done.

In the present study, GnRH was administered at estrus along with single or double (24 hours apart) inseminations. In this, among 62 and 65 Jersey cows inseminated following single or double insemination, 37 (59.6%) and 35 (53.8%) conceived, respectively. Similarly, out of 67 and 72 crossbred cows inseminated following single and double insemination group, 39 (58.2%) and 40 (55.5%) conceived, respectively. In crossbred control animals with double insemination 12 (60%) animals conceived out of 20 cows inseminated (table 2).

In a similar study by Stevenson and associates (1990) it was suggested that injection of GnRH at the time of single insemination (16-18 hrs after onset of estrus) consistently produced the highest pregnancy rate when compared to treated cows with double insemination. They further reported that double AI with or without GnRH administration confers no additional benefit over that from single AI along with GnRH. However, in present study double insemination without hormone in crossbred cows increased the conception. Our findings are in agreement with those of Fleischmann (1990) and Wilcox and Pfau (1958) who suggested that double insemination is better than single insemination, but should be performed only in those cases where signs of estrus persists 24 hr after insemination. Sharma and coworkers (2002a) reported that single insemination along with GnRH is sufficient and in the absence of GnRH treatment, double AI gives higher CR as compared to single AI ($p < 0.05$) in normal crossbred cows.

It can be concluded from this observation that if GnRH is being injected along with AI in normal animals, single insemination is sufficient and double insemination has no additional advantage. If no hormone has to be injected then double insemination at 24 hr interval should be preferred, particularly in crossbred animals.

During this trial, effect of GnRH analogues injected during first or subsequent estruses on conception in normal Jersey and crossbred cows was also studied. In Jersey animals, CR was 60.4 and 54.7 % in first and subsequent estrus following GnRH administrating, respectively. In control animals the CR was 43.7 and 41.7%, respectively, in first and subsequent estrus. In crossbred cows, the CR was 58.2% and 55.5% in GnRH treated and 52.9% and 52.2% in control cows in first and subsequent estrus, respectively.

There was non significant improvement in CR when GnRH was used in first service as compared to subsequent services post partum. These findings corroborate with the observations of other researchers (Gunzler *et.al.*, 1976; Goldveck, 1976; Schels and Mostafawi, 1978; Mori and Takahashi, 1978) who reported an increase of 9-14% in the first insemination pregnancy rate in cows following treatment with GnRH. Grunert (1976) stated that GnRH treatment at first insemination was more successful in cows in the earlier postpartum period and in those with high milk yield, since high milk production was generally believed to cause the delayed ovulation. Goldveck (1976) reported a very clear cut result indicating that cows inseminated early in the post partum period (4-6 week post partum) and cows having a higher milk yield (30 kg per day or more) and younger cows (3 year old or younger) responded more often with an increased conception rate to GnRH treatment.

Our study failed to elucidate further the reasons for variability in pregnancy rates at first service after GnRH treatment. It is possible that geographical location, breed type and nutritional management might have profound effects on GnRH responses. It appears that more positive results are associated with the use of GnRH analogues (Mee *et al.,* 1990). Although GnRH and its analogues elicit a surge like release of LH during the preovulatory phase (Lucy and Stevenson, 1986) and analogues produced greater release of LH than native GnRH during the luteal phase (Chenault *et al.,* 1989), it does not appear that the activity of GnRH or its analogues was a factor associated with the variability of GnRH on pregnancy rates from first service.

Ryan and associates (1991) concluded that first service pregnancy rate for the control cows (42.4%) was lower ($p < 0.05$) than that for cows treated with GnRH analogue at A.I. (48.8%) or for the combined treatment at AI and at day 12 post AI. (51.5%). The conception rate to first service is reported to be higher when the cows are administered 10μg of GnRH, 12 hr after onset of estrus and insemination is done following the treatment (Selvaraj and Kumar, 2001).

Stevenson and associates (1984) reported that conception rate did not improve by hormone treatment at first service but at repeat services there was an improvement of nearly 21% when cows were treated with Gonadotrophin releasing hormone within 30 seconds after insemination. GnRH stimulates the release of luteotrophic gonadotropin, which triggers the ovulation after a fixed time lag and, thus, contact between live ovum and vigorous sperm takes place at an optimum moment for fertilization (Schels and Mastafawi, 1978). Weaver and coworkers (1988) concluded that herds with conception rate of 45 per cent or less, benefited from GnRH treatment at first or second insemination while those with conception rate of 60 per cent or more, benefited only at second or later services. However, some researchers have reported no improvement in pregnancy rates at first service accompanied by treatment with GnRH (Mee *et al.,* 1990; Archbald *et al.,* 1993; Drew and Peters, 1994) on fertility in comparison with untreated control cows.

Various researchers have studied the effect of number of lactations on conception rate after using GnRH (Nakao *et al.,* 1983; Mee *et al*., 1990). In present study the CR was 48.0, 54.7, 63.8, 50.0, 50.0 and 45.4 % in Jersey animals and 50.0, 60.0, 67.5, 57.1, 50.0 and 52.7 % in Jersey X Jersey Red Sindhi crossbred animals following insemination in first to sixth lactation, respectively (Table 4). The CR was less in first lactation and increased in second and third in both the breeds. It again decreased in subsequent lactations. It is apparent from this observation that irrespective of whether treatment has been given or not, comparatively higher CR can be expected during second or third lactation. Similar studies have been done by Nakao and associates (1983) who reported that GnRH injected at first and third lactation insemination improved CR but Mee and coworkers (1990) reported higher conception rate for first lactation than subsequent lactations.

GnRH has been used for improving the conception rate in repeat breeder animals (Roussel *et al.,* 1988; Rayos, 1995). In Jersey animals, CR of 54.5 and 47.0% was achieved following GnRH administration after single or double insemination, respectively. In repeat breeding control cows, CR was 36.3 and 40%, following single or double insemination, respectively (table 5). There was an improvement of 18.2 and 7 %, respectively, between treated and untreated cows following single and double insemination. In crossbred animals CR after GnRH analogues administration was 50.0 and 42.8% following single or double insemination, respectively. In control cows CR was 42.8 and 53.3%, following single or double insemination, respectively. There was an improvement of 7.2% following GnRH administration and single insemination. Double insemination in control group in crossbred animals yielded higher CR (10.5%) in comparison to treated cows with double AI.

Two of the most consistent causes of repeat breeders compared with normal heifers and cows are reduced rate of fertilization and embryonic survival (Ayalon, 1978; Tanebe *et al.,* 1985). The effects of GnRH treatment at A.I. on rates of fertilization and embryonic mortality are unknown, but may be related to timing of ovulation and progesterone secretion by the corpus luteum. It has been shown that the amount of LH released in the preovulatory LH surge was less and its timing relative to the onset of estrus was delayed in repeat breeding heifers compared with normal heifers, suggesting a delay in ovulation (Gustafsson *et al.,* 1985).

As in normal animals of both the breeds, single insemination was found to be better in repeat breeder cows also, when performed along with GnRH. Double insemination did not have any additional advantage when GnRH was injected along. However, in the absence of GnRH in repeat breeder crossbred cows, simple double insemination gave better results.

Our findings are in accordance with other researchers also (Roussel *et al.,* 1988) who reported 53% increase in CR as compared to control animals. Rayos (1995) reported 20% improvement in CR in repeat breeder cows following GnRH administration in comparison to control group. In another study, the conception rate for treatment and control groups were 48.1 and 31.0 %, respectively (Bon Durant *et al.,* 1991). Rangnekar and coworkers (2002) reported CR of 70% v/s 40% in Fertagyl treated and control repeat breeder cows, respectively. GnRH injection during estrus may also have a normalizing effect on cows suffering from delayed ovulation or anovulatory estrous cycles by initiating a sufficient discharge of LH from the pituitary to cause ovarian response and eventual ovulation (Schels and Mostafawi, 1978). However, Thatcher and associates (1993) suggested that injecting GnRH at the time of 1st insemination in postpartum cows or in repeat breeders had not shown consistent results.

In present study, in Jersey animals CR of 56.5 and 50.0% was achieved in normal and repeat breeder cows, respectively. There was 6.5% more conception in normal animals in comparison to the repeat breeder cows. Similar results were obtained in crossbred animals where the CR of 56.8 and 45.8% (11% higher) was achieved in normal and repeat breeder animals, respectively. Although single insemination along with GnRH treatment or double insemination without any hormone treatment improved conception in repeat breeder cows, improvement was not as in normal animals probably because of unseen secondary etiologies.

Similar findings have been reported by Vamerzani and coworkers (1999) who compared one (along with insemination) and two (one along with insemination and second ten days later at mid luteal phase) injections of GnRH in normal and repeat breeder cows. A single GnRH injection increased pregnancy rate from 45% to 80% and from 40% to 66.66% in normal and repeat breeding cows, respectively. There was an additive effect of two times GnRH therapy in repeat breeding cows. Gonzalez and associates (1998) compared the efficacy of GnRH agonist Buserelin after injecting it on day 11-14 of the estrous cycle in normal and repeat breeder crossbred cows of Venezuela. The conception was reported to be 63.2% and 60.7% in normal and 61.8% and 50.0% in repeat-breeding treated and control, respectively.

In present study the levels of plasma progesterone were evaluated on the day of A.I. and on day 5 post insemination in treatment and control groups (table 7). There was a significant increase in progesterone level from day 0 to day 5 following Buserelin acetate ($P<0.01$) and Gonadorelin ($P<0.05$) treatments. There was also a significant difference ($P<0.01$) in progesterone concentration on day 5 in both the breeds between treatment and control groups. In Jersey animals mean values of progesterone after administration of GnRH analogues were 0.245 ± 0.098 and 1.470 ± 0.131 ng/ml on day 0 and 5 day post insemination, respectively. Rise in progesterone values between day 0 and 5 day post insemination were highly significant ($p < 0.01$). In control Jersey animals in which no treatment was given the mean progesterone was 0.285±0.112 and 0.738±0.201 ng/ml on day 0 and 5 post insemination, respectively, but the rise in progesterone was not statistically significant. There was no difference in progesterone concentration on day 0 but on day 5 the difference was significant ($p < 0.05$) between treatment and control group.

In crossbred animals mean values of progesterone were 0.162±0.033 and 1.447±0.169 ng/ml, respectively, on day 0 and 5 following administration of GnRH analogues. There was significant rise in progesterone concentration between day 0 and 5 ($p < 0.01$). In control crossbred animals in which no treatment was given the mean progesterone was 0.146±0.031 and 0.505±0.153 ng/ml, respectively. On comparing levels of progesterone between treatment and control group, there was no difference on day 0 but on day 5 the difference was highly significant ($p < 0.01$). As GnRH induced earlier ovulation, CL was formed earlier and thus progesterone level was higher on day 5 as compared to untreated controls.

Similar studies have been done by Mee and associates (1993) where they suggested that GnRH induces an additional LH surge to enhance active luteinization of granulosa and thecal cells to ensure adequate production of progesterone in developing CL. GnRH may have acted on the developing CL to promote conversion of small luteal cells to large luteal cells, which are responsible for about 85% of the progesterone secretion at luteal phase (Niswender *et al.*, 1985). Alonso and coworkers (1988) who evaluated progesterone levels at the time of artificial insemination and found that 85% of the animals had progesterone concentrations of < 0.5 ng/ml, which confirmed that they were in oestrus. Cows with progesterone concentrations exceeding 0.50 ng/ml at the time of insemination and buffaloes with concentrations exceeding 0.90 ng/ml do not become pregnant and therefore it has been suggested that insemination should be performed when progesterone levels are low after oestrus detection (Srivastava and Sahni, 1999).

There was a significant increase in progesterone level in pregnant as well as non pregnant normal Jersey and crossbred cows from day 0 to day 5 (table 8). Pregnant animals had higher levels of progesterone than non pregnant animals on day 5, but difference was non significant. These findings are in accordance with those of Deen and associates (1994) who suggested that GnRH treated pregnant animals had low progesterone concentration (1010.2±165.9 pg/ml) on day 4 as compared to GnRH treated non-pregnant animals (1346.6±628.7 pg/ml). Kumar and Selvaraj, (2003) reported lower progesterone level at estrus in pregnant (0.15±0.03 ngm/ml) than non pregnant (0.29±0.04 ngm/ml) repeat breeder cows. Higher progesterone profile on day of estrus/insemination can be considered as prognosis of non-fertile cycle (Panchal *et al.*, 1992).

SUMMARY

CHAPTER VI

SUMMARY

The study was conducted at the livestock farm of CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur. A total of 448 Jersey and Jersey X Red Sindhi crossbred cows were inseminated during this study that included 346 normal and 102 repeat breeder cows.

Buserelin acetate (Receptal, Intervet India Ltd.) at the dose rate of 0.0105 mg (2.5 ml) equivalent to 0.01 mg Buserelin and Gonadorelin (Fertagyl, Intervet India Ltd.) at the dose rate of 0.25 mg (2.5 ml) were used for the treatment as GnRH analogues. These hormones were injected simultaneously with first insemination, either through intra muscular (I/M) or intra venous (I/V) routes, depending upon the groups of the animals.

The animals were divided breed wise into two groups *viz.* Jersey (n= 216) and Jersey X Red Sindhi crossbreds (n=232). Jersey cows were subdivided to normal (n=167) and repeat breeder animals (n=49). Normal animals were further sub-divided to treatment (n=127) and control groups (n=40). Treatment group comprised of Buserelin acetate (n=61) treated and Gonadorelin (n=66) treated animals. The Buserelin acetate treated animals were sub-divided to I/M (n=40) and I/V (n=21) injected cows which were sub-divided to single (n=20 and n=9) and double insemination (n=20 and n=12) groups, respectively.

Similarly, the cows in Gonadorelin treated group were subdivided to I/M (n=40) and I/V (n=26) injected animals and which were further subdivided to single (n=20 and n=13) and double insemination (n=20 and n=13) groups, respectively. Repeat breeder Jersey cows were inseminated as single (n=11) or double (n=17) insemination regimens following the injection of GnRH analogues injected through I/M route. The animals in control group were also divided to single and double insemination groups (n=11 & 10 each)

Crossbred animals (n=232) were subdivided to normal (n=179) and repeat breeder animals (n=53). Normal animals were again subdivided to treatment (n=139) and control groups (n=40). Treatment group was constituted by Buserelin acetate (n=65) and Gonadorelin (n=74) treated animals. The Buserelin acetate treated cows were subdivided to I/M (n=40) or I/V (n=25) injected groups which were further subdivided to single (n=20 and n=13) and double insemination (n=20 and n=12) groups, respectively. The animals in Gonadorelin group were subdivided to I/M (n=40) and I/V (n=34) injected cows and which were further subdivided to single (n=20 and n=14) and double insemination (n=20 each) groups, respectively. As in Jersey animals, repeat breeder crossbred cows were inseminated as single (n=10) or double (n=14) insemination regimens following the injection of GnRH analogues injected through I/M route only and the animals in control group were divided to single and double insemination groups (n=20 each).

Pregnancy diagnosis was carried out 60 days post AI by rectal palpation method in cows not returning to estrus within this duration.

The blood was collected from 21 Jersey (Buserelin acetate treated n=9, Gonadorelin treated, n=6 and Control n=6) and 30 crossbred (Buserelin acetate treated n=12, Gonadorelin treated, n=12 and Control n=6) cows for progesterone estimation. The blood samples were collected twice from each animal. First sample was collected on day 0 and second on day 5.

Conception rate (CR) in treatment group following Buserelin acetate injection through I/M or I/V routes in Jersey animals was 60.0% and 61.9%, respectively. Similarly, CR in treatment group when Gonadorelin was injected through I/M and I/V routes in Jersey animals was 55.0% and 50%, respectively. On pooling the data, the CR was 57.5% and 55.3% in I/M and I/V injected groups, respectively. CR in control group was 42.5%. CR in treatment group following Buserelin acetate injection through I/M or I/V routes in crossbred animals was 62.5% and 48.0%, respectively. Similarly, CR in treatment group when Gonadorelin was injected through I/M or I/V routes in crossbred animals was 60.0% and 52.9%, respectively. On pooling the data, the CR was 61.2%, 50.8% and 52.5% in I/M, I/V and control groups, respectively. CR was better when GnRH analogues were injected by I/M route as compared to I/V administration or control groups. In Jersey cows, GnRH increased conception following AI when given by either of the routes (I/M or I/V) but there was no effect of route of administration of hormone on conception. However, in crossbred cows I/M administration of GnRH gave much better results as compared to I/V route or control animals. There was no difference in conception between the animals administered GnRH by I/V route or control in crossbred animals. After inseminating Jersey cows in two Buserelin acetate treated groups CR of 65.5% and 56.2% was achieved following single or double insemination, respectively. Similarly, following the insemination of two Gonadorelin treated groups, CR was 54.5% and 51.5% following single and double insemination, respectively. In control group, 40% and 45% cows conceived, respectively. After pooling the data CR was 59.6% and 53.8% following single and double insemination, respectively. Similarly, following the insemination of crossbred cows in two Buserelin acetate treated groups CR was 57.5% and 56.2% and with two Gonadorelin treated groups CR was 58.8% and 55.0% with single or double insemination, respectively. In control group, 45% and 60% cows conceived, respectively. After pooling the data 58.2% and 55.5% CR was achieved following single or double insemination, respectively. Single insemination was found to be better in both the breeds, when performed along with GnRH. Double insemination did not give any additional advantage and rather decreased the CR when GnRH was injected. However, in normal crossbred cows, simple double insemination gave better results even without GnRH.

Among Jersey cows, inseminated in first and subsequent estruses using Buserelin acetate, CR was 65.2% and 56.4%, respectively. Similarly, when gonadorelin was injected in first and subsequent estruses, CR was of 55% and 53.3%, respectively. In control cows the CR was 43.7% and 41.7% following insemination in first or subsequent estrus. Among crossbred cows, CR was 61.7% and 57.5% following Buserelin administration and 56.2% and 57.8% following gonadorelin administration in first and subsequent estruses, respectively. In control animals, the CR was 52.9% and 52.2% in the two groups, respectively.

The effect of number of lactation (1^{st} to 5^{th}) on conception after administration of GnRH as well as control group was also evaluated in Jersey and crossbred cows. The CR was 52.3, 60.6, 62.5, 52.3, 50 and 50% in treatment group and 25.0, 33.3, 66.6, 33.3, 50 and 33.3% in control group following insemination in first to sixth lactations, respectively. Whereas, crossbred cows the CR were 50.0, 59.0, 63.6, 50.0, 53.3 and 50% following insemination in first to sixth lactation in treatment group, respectively. Similarly, CR was 44.4, 62.5, 60.0, 50.0, 33.3 and 50.0%, respectively, following insemination in the first six lactation in control group. There was a rise in CR from first to third lactation which decreased subsequently.

In repeat breeder Jersey cows, CR was 54.5 and 47.0% in treatment group and 36.3 and 40% in control group, respectively, following single or double insemination. In crossbred repeat breeder animals, CR was 50.0 and 42.8% following the use of GnRH analogues and 42.8 and 53.3% in control cows following single or double insemination, respectively. Single insemination was found to be better in repeat breeder cows when performed along with GnRH as in normal animals of both the breeds. Double insemination did not have any additional advantage when GnRH was injected. However, in repeat breeder crossbred cows, simple double insemination gave better results even without

GnRH. After inseminating normal Jersey animals the CR was 59.6% and 53.8% whereas, in repeat breeder animals 54.5% and 47.01% cows conceived following single and double insemination, respectively. On pooling data in normal and repeat breeder animals the CR was 56.5% and 50.0%, respectively. Following insemination of crossbred animals subsequent to single or double insemination 58.2% and 55.5% cows conceived while in repeat breeder animals ·50.0% and 42.8%o conceived, respectively. On pooling data in normal and repeat breeder animals the CR was 56.8 and 45.8% respectively. Following GnRH treatment, improvement in CR was more in normal than in repeat breeder cows.

The mean plasma progesterone concentration on day 0 and 5 in Buserelin acetate treated Jersey animals was 0.327±0.155 and 1.710±0.181 ng/ml. There was significant rise in progesterone level from 0 to 5th day post insemination ($p< 0.01$). Similarly the mean on day 0 and 5th day in Gonadorelin treated Jersey animals was 0.113±0.037and 1.220±0.157 ng/ml, respectively. There was significant rise in progesterone level from 0 to 5th day post insemination ($p< 0.05$). There was no difference in progesterone concentration in two treatment groups, so the data were pooled and the mean progesterone concentration were 0.245±0.098 and 1.470±0.131 ng/ml on 0 and 5 day post insemination, respectively. Rise in progesterone values between 0 and 5 day post insemination were highly significant ($p< 0.01$). In control Jersey animals in which no treatment was given the mean progesterone was 0.285±0.112 and 0.738±0.201 ng/ml on 0 and 5 day post insemination, respectively. The rise in progesterone was statistically non significant. There was no difference in day O progesterone concentration (0.245±0.098 and 0.285±0.112) between treatment and control groups; however, on day 5 the difference was statistically significant (1.470±0.131 and 0.738±0.201, $p< 0.05$). The mean plasma progesterone concentration on day 0 and 5 in Buserelin acetate treated crossbred animals was 0.114±0.039 and 1.459±0.260 ng/ml, respectively. There was significant rise in progesterone level from 0 to 5th day post insemination ($p < 0.01$). Similarly, the mean on day 0 and 5th day in Gonadorelin treated crossbred animals was 0.169±0.046 and 1.440±0.175 ng/ml, respectively and the rise in progesterone level from 0 to 5th day post insemination was highly significant ($p < 0.01$).

There was no difference in progesterone concentration in the two treatment groups, so the data were pooled and the final mean values of progesterone were 0.162±0.033 and 1.447±0.169 ng/ml on 0 and 5 day post insemination, respectively. There was significant rise in progesterone values between 0 and 5 day post insemination ($p < 0.01$). In control crossbred animals in where treatment was given the mean progesterone concentration was 0.146±0.031 and 0.505±0.153 ng/ml on 0 and 5 day post insemination, respectively but the rise in progesterone was statistically non significant. There was no difference in day O progesterone concentration (0.162±0.033 and 0.146±0.031) between treatment and control groups; however, on day 5 the difference was statistically significant (1.447±0.169 and 0.505±0.153) in crossbred animals ($p < 0.01$). As the GnRH causes ovulation to occur earlier, CL is formed earlier in such cows and thus progesterone level is higher on day 5 as compared to untreated controls and probably responsible for higher conception rate.

The mean progesterone concentration in pregnant and non pregnant Jersey animals were 0.194±0.096 and 0.107±0.039 ng/ml on day 0 and 1.408±0.162 and 0.953±0.215 ng/ml on day 5, respectively. The rise in progesterone concentration from day 0 to 5 in pregnant animals was highly significant ($p < 0.01$). Similarly, in non pregnant cows also the rise was significant ($p < 0.05$). However, there was no difference in progesterone concentration between pregnant and non pregnant Jersey cows either on day 0 or 5. The mean plasma progesterone concentration in pregnant and non pregnant crossbred animals were 0.158±0.046 and 0.125±0.035 ng/ml on day 0 and 1.382±0.196 and 1.024±0.213 ng/ml on day 5 post treatment, respectively. The rise in progesterone concentration from day 0 to 5 in pregnant as well as non pregnant animals was highly significant ($p < 0.01$).

However, there was no difference in progesterone concentration between pregnant and non pregnant crossbred cows on day 0 or 5.

Conclusions drawn from this study are;

1. Both preparations are almost equally effective with Busereline acetate (Receptal) having little edge over Gonadorelin (Fertagyl) in Jersey cows.
2. In Jersey cows either of the two routes (I/M or I/V) of administration of hormone can be adopted but in crossbred cows only I/M route should be preferred.
3. If GnRH is to be injected along with AI in normal animals, single insemination is sufficient. In case no hormone is to be injected, double insemination at 24 hr. interval is advantageous.
4. No difference in the CR is there whether GnRH is given during first or subsequent post-partum estruses. Higher CR can be expected during second or third lactation both in treated as well as control animals.
5. In prolonged estrus repeat breeder animals single insemination along with GnRH is sufficient. In the absence of hormonal treatment in such animals, double insemination at 24 hr. interval gives better results, particularly in crossbred cows. Although single insemination along with GnRH or double insemination without any hormone improves conception in repeat breeder cows, improvement can not be expected as in normal animals probably because of unseen secondary etiologies.
6. As the GnRH induces ovulation earlier, CL is formed earlier in such cows and thus progesterone level is higher as compared to untreated controls on day 5 post AI.

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