

**STUDIES ON THE EFFECT OF
PROGESTERONE SUPPLEMENTATION ON
CONCEPTION RATE FOLLOWING
ARTIFICIAL INSEMINATION IN NORMAL
AND REPEAT BREEDER COWS**

THESIS

BY

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Submitted to



**CSK HIMACHAL PRADESH KRISHI VISHVAVIDYALAYA
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IN

Partial fulfilment of the requirements for the degree

OF

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

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To my Parents

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Dr. Madhumeet Singh
Associate Professor

CERTIFICATE I

This is to certify that the thesis entitled **“Studies on the effect of progesterone supplementation on conception rate following artificial insemination in normal and repeat breeder cows”** 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 Vishvavidyalaya, Palampur is a *bonafide* research work carried out by **Dr. Pravesh Kumar** (Admission No. V-2004-30-16) son of Sh. Hoshiar Singh under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

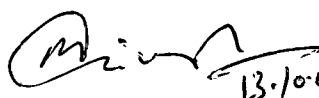
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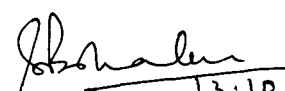
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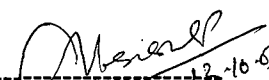
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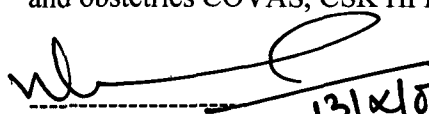
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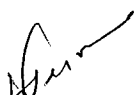
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LIST OF ABBREVIATIONS

µg	-	microgram
µl	-	micro liter
°C	-	Degree Celsius
bTP	-	Bovine trophoblast protein
CIDR	-	Controlled internal drug releasing devices
DCP	-	Digestible crude protein
FAO	-	Food and Agriculture Organization
g/dl	-	Gram per deciliter
g/m	-	Gram per milliliter
GnRH	-	Gonadotrophin releasing hormone
hCG	-	Human chorionic gonadotrophin
hr	-	Hour
I/M	-	Intramuscular
IAEA	-	International Atomic Energy Agency
IU	-	International unit
LH	-	Lutenising hormone
m mol/l	-	milli mole per liter
mg %	-	milligram per cent
mg	-	milligram
mg/dl	-	milligram per deciliter
mg/ml	-	milligram per milliliter
MGA	-	Melengestrol acetate
ng/ml	-	nanogram per milliliter
PRID	-	Progesterone releasing intravaginal devices
r.p.m.	-	Revolutions per minute
TDN	-	Total digestible nutrient

INTRODUCTION

CHAPTER I

INTRODUCTION

The reduced fertility in repeat breeder cows has basically been attributed either to fertilization failure or embryonic mortality consequent to genetic and reproductive tract aberrations, endocrine dysfunctions, infectious invasions and errors in management. However, in the routine infertility work in cattle, some females are frequently observed that have a normal reproductive tract, regular estrous cycle, and no evidence of any disease and yet fail to settle. The exact cause of repeat breeding in many cases remains an enigma and hence termed as unexplained infertility. The cause of failure of conception in such cases is not fully established many a times and as a result, various treatments are used with varied success.

After fertilization, embryos cleave at different rates, sometimes causing the maturity of the embryo to differ from that of the uterus. The uterine environment may be toxic to these embryos that are out of phase, resulting in the death of the embryo. Maternal recognition occurs around days 15-17 of pregnancy. The embryo secretes bovine trophoblast protein-1 (bTP-1) which alters the production of prostaglandins and prevents luteolysis from occurring. If the embryo does not signal its presence adequately to the mother, the mother will continue to cycle as if open (Geisert *et al.*, 1988). Establishment and maintenance of pregnancy and embryo growth in cattle are related to the ability of the corpus luteum (CL) to secrete progesterone (Staples and Hansel, 1961; Estergreen *et al.*, 1968). The progesterone dominance is essential to induce a quiescent state of uterus needed for embryonic survival (Gassner, 1951) and maintenance of pregnancy. Variability in progesterone synthesis may lead to asynchrony between the embryo and uterus (Pope, 1988). Thus, the time and pattern of rise in plasma progesterone concentrations may be an important factor in determining the likelihood of an embryo remaining viable. A

deficiency in the steroid secretion of the CL, especially during early period of gestation could contribute to embryo losses (Staples and Hansel, 1961; Lamming *et al.*, 1989; Thatcher *et al.*, 1994).

There are two major reasons for a lack of progesterone. The first category includes corpora lutea (CLs) that have a short lifespan. Thus, luteolysis occurs before the embryo has time to signal its presence through secreting bTP-1. The second category includes those CLs that have a normal lifespan but secrete low levels of progesterone, which does not suppress the luteolytic affects of the prostaglandins.

As the need for adequate amount of progesterone in early pregnancy is well established and insufficient circulating progesterone is often suggested as a cause of early embryonic mortality, attempts have been made to improve pregnancy rates through the use of progesterone therapy. Experiments have been done to supplement cows with progesterone, which extends the life of short lived CLs or increases the progesterone production. This allows for maternal recognition to take place and less chance of luteolysis occurring (Robinson *et al.*, 1989). Indeed, progesterone supplementation during the luteal phase of the estrous cycle could increase pregnancy rates in cattle (Johnson *et al.*, 1958; Robinson *et al.*, 1989; Macmillan *et al.*, 1991; Sianangama and Rajamahendran, 1992). Doses ranging from 50 to 200 mg with assorted injection regimens either improved (Johnson *et al.*, 1958; Wiltbank *et al.*, 1956) or had no effect (Sreenan and Diskin, 1983; Sreenan *et al.*, 1979) on pregnancy rates.

Inducing formation of an additional CL during the luteal phase of the estrous cycle with a gonadotrophin could be a strategy to increase concentrations of progesterone in plasma during the luteal phase. The rationale for progesterone supplementation in the cow is to allow the embryo to survive to a stage where its effects are strong enough to maintain the CL in a functional state.

Indiscriminate parenteral administration of progesterone in normal as well as repeat breeder cows presented for insemination is becoming a fast growing practice in field conditions of Himachal Pradesh. The present study envisages the effect of this practice on conception in cows.

2. Objectives of investigation: The objectives of this study are;

- a. To study the effect of progesterone supplementation on conception in cows following AI.
- b. To compare the efficacy of progesterone administration in normal and repeat breeder cows.
- c. To record the clinical and biochemical profile in cows following this treatment.

**REVIEW
OF
LITERATURE**

CHAPTER II

REVIEW OF LITERATURE

Repeat breeding:

Among various reproductive problems reducing the reproductive efficiency of dairy animals, repeat breeding is probably the most frustrating one. Repeat breeder syndrome is defined as a condition in which cows or heifers which have regular estrous cycle and appear normal on superficial clinical examination have failed to become pregnant following three or more breeding (Francos, 1979; Barlett *et al.*, 1986).

Repeat breeding syndrome has multiple etiology, including genetic or acquired defects of ova, sperm, or early zygote, infections or inflammatory processes, endocrine dysfunctions, nutritional or managemental deficiencies and other 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 per cent and 29-47 per cent, 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).

It is a problem worldwide to dairymen, with an overall incidence of 10-25 per cent (Hewett, 1968; Barlett *et al.*, 1986). A varied incidence of repeat breeding in cows ranging from 10.1 per cent to 20.6 per cent has been reported (Singh and Pant, 1998; Forbes, 2000; Gustaffson and Emanuelson, 2002). According to a study conducted by Singh and Pant (1998) on 2242 cows, it was observed that the clinical abnormalities were found in 747 (33.3%), managemental problems in 229 (10.2%), anatomical defects in 215 (9.6%) and functional abnormalities in 26 (1.2%) animals. The incidence of repeat breeding also varies with the different breeds. Urade (2001) reported a lower incidence (8.08%) of

repeat breeding in Sahiwal cows and their crossbreds and still lower (4.26%) is reported in Deoni cows and their crossbreds (Narladkar *et al.*, 1994).

Tanabe and Casida (1949) recorded ovulation failure in 4.8 per cent of clinically normal cows. Anovulation and lower survival rate of an embryo, endocrine disorders and increased chromosomal abnormalities are possible causes of lowered fertility in repeat breeder females (Ayalon, 1984). Van Rensberg (1956) recorded 10.04 per cent incidence of ovulation failure. Others have reported very high incidence of anovulation viz; 56.9 per cent (Choudhary *et al.*, 1965) and 31.4 per cent (Sampathkumaran and Iya, 1966). Rao and Kotayya (1980) reported an incidence of 7 per cent for anovulation in repeat breeder cows, whereas according to Khanna and Sharma (1992), 17 per cent of repeat breeding animals exhibit anovulation. Delayed ovulation may also be a cause of repeat breeding. Van Rensberg (1956) found an incidence of 18.04 per cent in functionally infertile cows of Africa. A subsequent study recorded 18 per cent delayed ovulation in same breed of cattle (Van Rensberg and de Vos, 1962). Almost similar incidence of 17.3 per cent, 12 per cent and 17.6 per cent 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 per cent cows. Almost similar incidence of 3.5 per cent was reported by Lemke (1972) in 200 infertile cows.

Tanabe and Casida (1949) studied the nature of reproductive measures in cows of low fertility and observed that 66 per cent of the repeat breeder cows with no clinically detectable abnormality had fertilized, however at 34 days post-breeding, the percentage having embryos had decreased to 23 per cent. Where fertilization rates, following insemination were reported between 89-100 per cent (Kidder *et al.*, 1954), calving rates following a single insemination are generally within the range of 50-60 per cent for both dairy and beef cows (Diskin and Sreenan, 1980).

Embryonic and fetal deaths account for 75 per cent of the total reproductive wastage occurring after each insemination. Embryonic death properly defined relates to the death of already fertilized ova. Most losses have occurred on or probably before day 18. Early embryonic deaths (within 20 days of breeding) seem to account for 75-80 per cent of all embryonic and fetal deaths resulting in substantial loss in production (Sreenan and Diskin, 1983). Early embryonic loss could occur through inadequate uterine environment, as the endometrial secretions essential for stimulating and mediating changes in conceptus growth and differentiation throughout early pregnancy were directed by the steroid environment generated by the ovary. It is now well documented that these uterine secretions are regulated by the ovarian steroids (Wilmot *et al.*, 1986). So, the adequate levels of these steroids are of great importance to maintain a successful pregnancy. Based on another study on 1697 buffaloes, Rao and Kotayya (1980) reported that 14.54 per cent animals showed no anatomical or pathological abnormality but failed to conceive leading to repeat breeding indicating functional disturbances as one of the cause of repeat breeding.

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 days 3, 6 and 7 after estrus (Non-inseminated group). Repeat breeder cow had higher tendency of progesterone receptors than normal cows in inseminated as well as non-inseminated group, which suggested that hormonal imbalance may be related to the repeat breeding problem (Almeida *et al.*, 1986).

Luteal insufficiency:

Corpus Luteum is the main organ secreting progesterone during the early embryonic life of the animal that is necessary for nidation and maintenance of pregnancy. Progesterone is necessary for sustaining pregnancy in the bovine for approximately the

first 163 days (Mc Donald *et al.*, 1953). Pregnancy was maintained by replacement therapy in 8 or 9 cows having CL ablation done at 60th day of pregnancy indicating that progesterone is an essential hormone for maintenance of pregnancy (Mc Donald *et al.*, 1953). So, it was inferred that the conceptus growth is progesterone dependent; an inadequate level of progesterone may results in starvation of the conceptus. A delayed rise in post-ovulatory progesterone or lower luteal phase levels resulted in poor embryo development and little or no interferon secretion on day 16 (Mann *et al.*, 1999). The concentration of interferon-tau in uterine flushes on day 16 is related to circulating concentrations of progesterone between days 4 and 5 (Mann *et al.*, 1998). Therefore, in this context, sufficient progesterone means a concentration high enough to trigger a normal pattern of uterine secretary function by day 4 or 5 after insemination.

Progesterone is the key hormone in controlling reproduction, cyclicity, pregnancy and parturition. During estrous cycle, it induces growth and development of endometrial glands and secretion of uterine milk. Progesterone is necessary to induce a quiescent state in the uterus, which is important for embryonic survival. It also aids in inhibiting uterine motility to facilitate placental attachment. Progesterone could be the mediator of embryonic survival (Dyck and Strain, 1983; Pharazyn, 1992; Jindal *et al.*, 1996). Plasma progesterone concentration during early pregnancy can modify oviductal (Buhi *et al.*, 1990; Murray, 1993) and endometrial (Ashworth and Bazer, 1989; Roberts *et al.*, 1993) development and secretary activity.

An insufficiency of progesterone level arising because of luteal dysfunction has been cited as a cause of infertility and early embryonic death especially during the most critical period of gestation days 10-16. Conditions that are included in luteal dysfunctions include inadequate progesterone production and premature luteolysis. All these conditions

relate to failure of the CL to secrete progesterone in sufficient quantity to maintain the appropriate period of a luteal phase in pregnancy.

Luteal insufficiency or delayed rise of progesterone is considered to be an important cause of early embryonic mortality, leading to repeat breeding syndrome (Kimura *et al.*, 1987) and is one of the reasons for low conception rate in dairy animals (Britt and Holt, 1988). Since progesterone plays an important role for nidation of embryo in the uterus, some workers showed higher progesterone level in fertile cows after insemination than infertile cows (Erb *et al.*, 1976; Hansel *et al.*, 1991; Maurer and Echternkamp, 1982). A beneficial effect of progesterone supplementation in improvement of conception rate in repeat breeder has also been reported (Wiltbank *et al.*, 1956).

Kimura *et al.* (1987) reported that in repeat breeder cows, 62 per cent had an abnormal pattern of milk progesterone levels indicating luteal phase defects. It was observed that among those animals 54 per cent showed delayed rise of progesterone until days 6-11 after insemination, 15 per cent had low levels of progesterone during luteal phase and 31 per cent had a combined pattern of both, suggesting that the delayed formation of corpus luteum with lowered secretion of progesterone during the luteal phase is one of the causes of repeat breeding in dairy cows. Similarly, Kang *et al.* (1994) also found that out of 15 repeat breeder cows, five showed a delayed rise of progesterone until days 7-10, two showed a lower progesterone concentration (0.50 ng/ml) whereas two showed combined pattern of both delayed rise and low concentration of progesterone during luteal phase indicating that luteal dysfunction is one of the important causes of repeat breeding. An endocrine asynchrony between the conceptus and the internal milieu, either at the oviductal or uterine levels may count for the impaired fertility observed in repeat breeder females, confirming the assumption of the multi-causality of the syndrome. Endocrine asynchrony including elevated blood plasma progesterone concentrations at

estrus has been shown to result in impaired fertility and repeat breeding (Gustaffson *et al.*, 1986). It has been inferred that spontaneously occurring asynchrony between the embryo and the maternal environment was prime cause of embryonic mortality. Moreover, higher progesterone concentration and a lower ratio of estrogen and progesterone on days 3-6 were very conducive for the embryo survival (Maurer and Echternkamp, 1982). Thus the possibility exists that progesterone deficiency may be one of the important reasons of repeat breeding. Therefore, various researchers have used progesterone at various time of estrous cycle in various animal species through various routes of administration for embryonic survival.

Biochemical profile of normal and repeat breeder cows:

There has been conflicting opinions regarding role of various nutrients in repeat breeding cows. Under nutrition results in decreased plasma progesterone concentration coupled with reduced gonadotrophin secretion in rats (Howland, 1972). Parker and Blowey (1976) concluded that plasma urea, albumin, glucose, non-esterified fatty acids, acetone, calcium, magnesium and inorganic phosphorus did not show a consistent relationship with potential fertility. Nutritional deficiencies may also result in repeat breeding (Mc Donald *et al.*, 1961; Morrow, 1969; Morrow, 1975; Gerloff and Morrow, 1986; Thatcher, 1986). Meltem and associates (2002) reported some blood biochemical parameters to be non significant between fertile and repeat breeder cows kept under good managerial conditions. Accordingly, a brief account of relationship between certain biochemicals with reproductive function is reviewed below.

a) Total protein:

Effect of protein on reproduction in cattle is compounded by concurrent energy deficiencies (Tassel, 1967). The normal values of total serum protein have been reported to vary from 6.1 to 8.1 g/100 ml with an average value of 7.1 g/100 ml (Payne *et al.*, 1970).

Intake of high quality proteins improves conception rate and prevents embryonic loss (Rakha and Igboeli, 1971). In one study total protein in normal and repeat breeding cows at estrus ranged from 6.4 to 10.2 (mean 7.77 ± 0.36) and 5.14 to 10.43 (mean 7.23 ± 0.3) g/ 100 ml, respectively (Enkhia *et al.*, 1982). Increasing levels of dietary crude protein have been associated with decreases in fertility (Ferguson and Chalupa, 1989; Canfield *et al.*, 1990; Elrod *et al.*, 1993). Elrod and associates (1993) identified that a decrease in uterine pH was associated with higher degradable intake of protein and was a possible cause of conception failure. In subsequent work it was suggested that increasing plasma urea nitrogen (PUN) and milk urea nitrogen (MUN) was associated with reduced fertility in a stepwise fashion. Fertility declined in cows with MUN above 16 mg/dl and a further reduction occurred when values were above 20 mg/dl. According to Kumar and Sharma (1991), mean total serum protein in cows at estrus, which later conceived, was 8.45 ± 0.63 g/100 ml, whereas in cows with infertile service, the value was 7.14 ± 0.22 g/100 ml. A significant difference was found in the total plasma protein of normal cycling and delayed pubertal cows, the respective values being 7.45 ± 0.11 and 6.64 ± 0.2 g/100 ml (Shrivastava and Kadu, 1995). In another study, no difference was found in total protein levels between repeat breeding and normal cows; the mean values being 9.31 ± 0.97 and 10.0 ± 0.48 g/100 ml of plasma, respectively (Gandotra *et al.*, 1993). Others have shown that serum albumin concentration, which is dependent upon levels of protein intake, is universally related to the number of services per conception (Rowland *et al.*, 1977). However, contrary reports also exist (Jordan and Swanson, 1979). Lohdi and associates (1998) inferred that lower concentrations of total protein might account for non-cycling, repeat breeding and endometritis in buffaloes. Islam and associates (1994) studied biochemical constituents in normal and repeat breeder cows and reported that serum total protein were significantly higher in normal crossbred cows at day 0 than day 13 of the estrous cycle. Burle and

associates (1995), reported low blood concentrations of total protein in anoestrous cows ($P<0.01$) and the concentrations were significantly higher in cycling cows than in repeat breeders. But Cetn *et al.* (2002) and Chandrahar *et al.* (2003) reported that there is no significant difference of total protein values of fertile and repeat breeder cows. Ahmad *et al.* (2004) found that total protein level differed significantly ($P<0.05$) among cyclic, non cyclic and endometritic cows, being highest in endometritic, followed by non cyclic and lowest in cyclic cows.

b. Cholesterol:

Kample and associates (1990) estimated total serum cholesterol to be 5.42 m mol/l in cows with reproductive disorders as compared to 4.53 m mol/l in healthy cows. Serum cholesterol levels in cows at estrus, which subsequently conceived, was 105.22 ± 2.06 mg/100 ml and was significantly higher than 99.17 ± 1.39 mg/100 ml in the cows that failed to conceive (Kumar and Sharma, 1991). Similarly, Shrivastava and Kadu (1995) detected a significantly higher level of cholesterol in normal cycling cows (109.29 ± 1.85 mg/100 ml) in comparison to delayed pubertal heifers (97.37 ± 2.15 mg/100 ml). Islam and associates (1994) studied biochemical constituents on day 0 (of estrus) and compared with those on day 13 in normal and repeat breeder cows and reported that cholesterol was significantly higher in normal crossbred cows at day 0 than day 13. Burle and associates (1995) reported the concentrations of cholesterol to be significantly higher in cycling cows than in repeat breeders. However, others did not find any significant difference between serum cholesterol values of fertile and repeat breeder cows (Cetn *et al.*, 2002; Chandrahar *et al.* 2003). Their results showed that serum cholesterol of fertile and repeat breeder cows was similar and that this parameter did not seem to be useful for the diagnosis of repeat breeder syndrome. Concentrations of plasma cholesterol were positively associated with expression of estrus at first ovulation, interval from calving to conception, and likelihood

of conception and pregnancy (Westwood *et al.*, 2002). However, Ahmad *et al.* (2004) reported significantly ($P<0.05$) higher values of cholesterol in endometritic cows as compared to cyclic and non-cyclic cows.

c. Glucose

Energy deficiency has been shown to be associated with reproductive failure. McClure (1961) reported that the cows which lost least weight after calving and were in positive energy balance at the time of service had a greater chance of conceiving to first service than those in negative energy balance. Subsequent studies have confirmed this finding (Warnick *et al.*, 1967; King, 1968; Ward, 1968; Moller and Shanon, 1972). In cows blood glucose values less than 30 mg/100 ml were associated with reduced fertility (McClure, 1968).

A comparative study for blood glucose levels in normal and repeat breeder cows at estrus revealed non-significantly lower values in the latter group; the respective mean values being 45.38 ± 3.37 (range 29.7 to 60.0) and 40.41 ± 2.44 (range 27.5 to 59.0) mg/100 ml (Enkhia *et al.*, 1982). Elsewhere, the normal glucose values in cows have been reported to be 45.4 (Payne *et al.*, 1970) and 61.0 mg/100 ml (Llamazares *et al.*, 1988). Also, levels of glucose in cows during fertile and infertile services were found to be similar being 59.0 ± 3.39 and 51.11 ± 2.08 mg/100 ml, respectively (Kumar and Sharma, 1991). Similarly, Cetn and coworkers (2002) and Moghaddam *et al.* (2003) did not find any difference in blood glucose levels in healthy and repeat breeder cows. However, Chandrarah and associates (2003) found significantly lower ($P<0.01$) level of blood glucose in repeat breeder cows and reported that lower glucose concentration can cause repeat breeding syndrome (Rodriguez *et al.*, 2004).

Management of luteal insufficiency:

As the luteal insufficiency is one of the major causes of repeat breeding (Kang *et al.*, 1994), exogenous administration of progesterone during early luteal phase could logically correct the condition thus reducing the incidence of repeat breeding. Parental administration of progesterone in 5-10 days of estrous cycle improved the conception rate significantly (Rosen and Struman, 1989). Walton *et al.* (1990) observed that parental administration of progesterone in cows resulted in increased embryo viability and conception rate. In hormonal control of estrous cycle and maintenance of pregnancy, it is thought that lutenising hormone is necessary for ovulation, and progesterone, which is secreted by corpus luteum, is necessary for nidation and maintenance of pregnancy.

Most of the workers have restricted the use of exogenous progesterone in improving conception rate. Some of the studies indicate that gonadotrophins administration can improve systemic plasma levels of progesterone during early pregnancy, thus compensating for the luteal insufficiency in these animals (Sharma 2001; Sharma *et al.*, 2003), others indicate these treatments to be ineffective (Garverick *et al.*, 1988; Shelton *et al.*, 1990; Barros *et al.*, 1992).

In a study to observe the effect of injection of GnRH on peripheral concentrations of LH and progesterone in repeat-breeder cows, 170 cows returning to service for the 2nd or 3rd time were injected with 20 µg GnRH or 5.0 ml saline solution (controls) on day 12 of the cycle. Treatment with GnRH resulted in a significant increase in plasma LH release from 20 min to 3 hr after treatment up to 97.5 ng/ml; milk progesterone concentration increased up to 6 hr after treatment, when it was 41 per cent higher than before treatment, but 15 days after insemination there was no significant difference between treated cows and controls. Of the treated cows inseminated for the 2nd and 3rd time respectively, 72.4 and 50.0 per cent conceived vs. 59.6 and 40.7 per cent of controls ($P>0.05$). For cows

which did not conceive during the treatment period, the interval between oestrous periods averaged 34 days for treated cows and 28 days for controls (Bostedt and Okyere, 1988).

In a similar study to know the effects of progesterone and human chorionic gonadotrophin administration five days post insemination on plasma and milk concentrations of progesterone and pregnancy rates of normal and repeat breeder dairy cows, it was observed that treatment with a progesterone releasing intravaginal device between days 5 and 12 after estrus elevated plasma progesterone concentrations between days 6 and 8, whereas treatment with injectable progesterone (200 mg) on days 5, 7, 9 and 11 after estrus did not increase plasma progesterone concentrations in comparison with controls. The administration of 1500 IU human chorionic gonadotrophin (hCG) on day 5 after estrus resulted in a sustained increase in plasma progesterone concentrations from day 8 until day 20 when measurements ceased. Pregnancy rates, as a result of artificial insemination (AI) at the pre treatment estrus, were unaffected by any of the treatments and ranged from 57.1 to 75.0 per cent at 45-60 days after AI. In a field trial, of 36 repeat breeder cows treated with 1500 IU hCG on 5.5 days after insemination, 47.2 per cent were pregnant at 60 days, whereas 39.5 per cent of saline treated controls were diagnosed pregnant. Treatment with hCG significantly increased milk progesterone concentrations over controls on days 14 and 20 after insemination (Walton *et al.*, 1990).

According to Diskin and Sreenan (1986) progesterone concentration in the estrous cycle preceding insemination does not seem to affect embryo survival. Following insemination, progesterone concentration increases in pregnant cows, with most evidence suggesting a divergence as early as days 10-12 between pregnant and non-pregnant cows. While it is presumed that this increase arises from a luteotrophic embryo signal, there is no direct evidence to substantiate this before day 16. Progesterone supplementation has increased embryo survival in both normal and repeat breeder cows, though the number of

animals was limited in individual experiments. The administration of hCG during the luteal phase of the oestrous cycle significantly increased the number of corpora lutea and the circulating progesterone concentration, but not embryo survival rate.

Injection of hCG or GnRH agonist on day 5 increased plasma concentrations of progesterone. An injection of hCG given on day 4 or 7 (Breuel *et al.*, 1989), days 2, 3 and 4 (Helmer and Britt, 1987), day 5 (Diaz *et al.*, 1993), and day 6 (Fricke *et al.*, 1993) increased plasma concentrations of progesterone compared with controls. Similarly, Walton *et al.* (1990) reported that injection of hCG on day 5.5 of the estrous cycle increased concentrations of progesterone in milk between day 14 and 20 after insemination.

Pregnant cows have higher concentration of blood progesterone within first 10 days of insemination (Mann *et al.*, 1999). Progesterone is transferred locally from the ovarian/oviductal venous drainage to the uterine artery in cattle (Weems *et al.*, 1988). The local transfer results in higher concentration of progesterone within the uterus ipsilateral to the CL (Pope *et al.*, 1982). Although the local progesterone may be important for uterine function and pregnancy, changes in systemic progesterone may be affecting gonadotrophin secretion. Since progesterone is responsible for maintaining a quiescent favorable environment in the uterus for embryo development, it is logical to consider that supplementation with this hormone in one or the other forms may improve conception rate by improving the progesterone levels in repeat breeding females and thus helps in enhancing the conception rates and ameliorating the problem of repeat breeding (Sharma *et al.*, 2002).

Maintenance of desired progesterone levels during early pregnancy in the animals with luteal insufficiency improve the uterine environment for embryo survival and development (Balakrishanan *et al.*, 1994) and brings about a certain change in uterine

luminal secretions (Devanathan *et al.*, 1999). Role of progesterone in affecting the uterine luminal secretions has been reported in various species as in sheep (Ashworth, 1991), cattle (Thuemmel *et al.*, 1992), pigs (Jindal *et al.*, 1997) and buffaloes (Andurkar *et al.*, 1997).

Intramuscular (I/M) route is considered to be the route of choice by some workers. It is reported that out of 20 animals treated with progesterone intramuscularly, 65 per cent conceived as compared to 20 per cent in control animals without any treatment. The injection was given at the time of service (Herrick, 1977). Wiltbank *et al.* (1956) injected 50 mg progesterone in 36 repeat breeder cows daily for 30 days after insemination and found that 16 cows (44.49%) had normal embryos. Balakrishanan and associates (1994) reported pregnancy rates in recipient cows and heifers treated with progesterone on day of embryo transfer to be 46 per cent and 56 per cent respectively compared to 14.3 and 17 per cent in control cows and heifers, respectively. Others have also reported that conception rate in repeat breeding syndrome can be improved by injection of progesterone (Awagan *et al.*, 2000). But higher conception rates have also been obtained (Johnson *et al.*, 1958; Walton *et al.*, 1990) following progesterone administration.

Panchal *et al.* (1991) reported that I/M administration of hydroxyprogesterone caproate in repeat breeding buffaloes as a single depot on day 4th post insemination, significantly increases pregnancy rates in treatment group as compared to that observed in the control group. Similarly, Singh *et al.* (2002) had also used progesterone 500 mg by I/M route on day 5 after insemination in repeat breeder cows and recorded a conception rate of 60 per cent compared with 50 per cent in normal cows. Srivastava and Kharche (2001) studied the effect of progesterone on conception rate in 48 repeat breeder crossbred cows by administering 250 mg progesterone on 5th, 12th and 19th, days after AI in treatment group. First service conception rate was 39.13 per cent in treated animals while

it was only 8.00 per cent in controls. Thus administration of progesterone in repeat breeder crossbred cattle at first breeding increased the conception rate of treated group by 31.13 per cent over the control for first service conceptions. The overall conception rate of 65.21 per cent for treated group was comparatively higher than overall conception rate of 48 per cent for control group. Similarly, Awasthi *et al.* (2002) tried I/M administration of 500 mg hydroxyprogesterone caproate on days 6-8 after insemination in 20 repeat breeder cows and found that conception rate increased (70%) in treatment group as compared to 20 per cent in control group.

Increased levels of plasma progesterone were also obtained by administration of progesterone (75 mg/day) in repeat breeder cows which in turn was associated with the insemination success (Thuemmel *et al.*, 1992).

Progesterone was also administered orally (Devanathan *et al.*, 1999) in the form of hydroxyprogesterone hexonate tablets (progesterone 500 mg) on day 5 after insemination to crossbred repeat breeder cows that resulted in 53.84 per cent conception rate to first service.

Progesterone in form of Progesterone Releasing Intravaginal Devices (PRID) and Controlled Internal Drug Releasing Devices (CIDR-B) has also been used as slow releasing progesterone devices which improved the conception rate (Lee and Chung, 1989; Cleef *et al.*, 1991; Kerr *et al.*, 1991) in repeat breeder animals. Awagan *et al.* (2000) had used CIDR-B in early and late diestrus and conception rate was 50 per cent and 33.33 per cent, respectively. Overall conception rate in both groups was 41.57 per cent.

Contrarily, CIDR-B along with estradiol 10 mg was used and out of 12 cows, only 3 (25%) were pregnant (Awagan *et al.*, 2000). Almost same results were recorded by Dixit *et al.* (1983). But higher conception rates were reported (Gyawu *et al.*, 1991; Macmillan

and Peterson, 1993) when they treated the normal cows with CIDR-B and estradiol benzoate.

PRID have also been used by Nancy *et al.* (1989) in normal Holstein cows. They found that when PRID inserted on day 5 to day 12 and day 10 to day 17 of estrous cycle, the conception rate was 60 per cent in both groups compared to 30 per cent in untreated control group. No much variation was found when PRID was inserted on day 5 to day 13 and from day 13 to 21 in different animals and conception rate was 44 per cent compared to 42 per cent in untreated control group (Jeffrey and Michael, 1991).

Improved pregnancy rates to 60 per cent over 30 per cent in untreated control cows were also reported by intravaginal implants of progesterone in 28 dairy cows inserted between days 5-12 after AI (Robinson *et al.*, 1989; Stevenson and Mee, 1991). Pregnancy rates were also enhanced by the supplementing norgestomet, progesterone or MGA in the suckled beef cows after timed inseminations (Stevenson *et al.*, 2003). Therefore, it was suggested that progesterone could be effectively used in one form or the other as a remedial measure to improve the plasma progesterone concentrations and thereby the conception rate during early pregnancy especially in cases of repeat breeding due to luteal insufficiency.

Progesterone concentration and it's estimation:

The plasma progesterone assay is a practical aid to diagnose reproductive disorders and to monitor responses of ovarian dysfunction to PGF₂ alpha and GnRH treatment in subestrus 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).

Progesterone concentrations are at their lowest at the time of estrus in normally cycling animals. Basal levels of progesterone during estrus are associated with the

insemination success and increased conception rates. But on the other hand, if the concentrations of progesterone are high at the time of estrus it results in poor conception rates and thus to repeat breeding. Usually after ovulation, luteinization starts and progesterone levels starts rising from day 4 onwards. The corpus luteum on an average matures on day 5 in normally cycling animals. In the normal diestrus phase levels of progesterone rises and reaches a plateau (Kanai and Shimizu, 1984). The plasma progesterone concentrations were higher in normal as compared to repeat breeding cows and buffaloes during different days of the estrous cycle (Agarwal *et al.*, 1982). The peak concentrations of plasma progesterone were found during diestrus (4.43 ± 0.91 ng/ml) and minimum concentrations during estrus phase (0.49 ± 0.09 ng/ml). 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).

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. Gustaffson and associates (1986) reported that progesterone levels were higher ($p < 0.05$) in the repeat breeder heifers than the normal heifers. Bulman 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 days 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. About 7.8 per cent of repeat breeding cows showed erratic or low progesterone concentration (Gonzalez *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 ± 0.01 ng/ml). Higher progesterone profile on day of estrus/insemination can be considered as prognosis of non-fertile cycle (Panchal *et al.*, 1992). Progesterone concentration during estrous cycle prior to breeding may be an important indicator of subsequent fertility of cow (Fonesca *et al.*, 1983).

MATERIALS
AND
METHODS

CHAPTER III

MATERIALS AND METHODS

Altogether 403 cows presented for insemination in livestock farm of CSK Himachal Pradesh Krishivishvavidyalya, Veterinary clinical complex of Dr. G.C. Negi College of Veterinary and Animal Sciences, Palampur and nearby field Veterinary institutions were included in the study. These animals were categorized on the basis of reproductive status (normal or repeat breeder) and number of inseminations to be done (Single or Double).

All the animals for the study were in good health with normal genitalia and no apparent clinical abnormality. These cows were subjected to a thorough clinico-gynaecological examination which consisted of an assessment of general health and careful examination of the genital system. Latter included inspection of perineum, vulva and vagina followed by a careful rectal palpation of cervix, corpus uteri, uterine horns, fallopian tubes and ovaries. In all these cows, the discharge was observed to rule out any turbidity and thus infection in it.

Only those animals that were free from any apparent reproductive disorder and not conceiving were selected for the study as repeat breeder cows.

Feeding and Management:

The animals in the dairy farm 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 the milk production to individuals at the time of milking. The animals were allowed to take water *ad-libitum*. All the animals were vaccinated and dewormed at the appropriate time as per the recommended protocol. The owners of the animals which

were inseminated were advised to follow the standard feeding schedule as well as standard managerial practices time to time.

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, vulvular congestion and mucus discharge. Cows with apparently normal cervico-vaginal mucus discharge, tone of the uterine horn and without any abnormalities of the genital tract were selected for the study.

Artificial Insemination (AI):

The animals of all the groups were randomly selected for single or double insemination. All the animals were inseminated at standing heat with good quality frozen thawed semen. The semen straws were procured from Sperm Station, Palampur. Thawing was done at 40⁰ centigrade for 14 seconds. Vulvar lips were washed with water and dried with cotton. Semen was deposited in body of uterus. In all the double insemination groups, second AI was performed about 24 hours later. Irrespective of single or double insemination, day of first insemination was considered as day 0 for progesterone treatments.

Treatment

In all the treatment group cows, depending on the group, 500 mg hydroxyprogesterone caproate (Duraprogen, Vetcare Division) was injected intramuscularly whereas no treatment was given to the control group cows.

Selection of animals

Overall, 403 cows^{were} taken for this study which included 262 normal and 141 cows with the history of repeat breeding. Schematic distribution of experimental cows in

different insemination groups to study the effect of progesterone supplementation on conception rate has been shown in Table M1.

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

Status of animal	Day of Progesterone administration	Insemination	Cows Inseminated
Normal	0	Single	23
		Double	27
		Pooled	50
	3	Single	19
		Double	35
		Pooled	54
	5	Single	18
		Double	29
		Pooled	47
	Control	Single	48
		Double	63
		Pooled	111
Repeat breeder	0	Single	11
		Double	19
		Pooled	30
	3	Single	13
		Double	19
		Pooled	32
	5	Single	11
		Double	33
		Pooled	44
	Control	Single	16
		Double	19
		Pooled	35

a. Progesterone treated normal cows: The animals included in progesterone treatment group were sub-divided into three groups viz. the progesterone administration simultaneous to AI (group 1), insemination and exogenous administration of progesterone on 3rd day post AI (group 2) and insemination and administration of progesterone on 5th day post AI (group 3). A total of 50, 54 and 47 normal cows were inseminated in groups 1, 2 and 3, respectively. Hydroxyprogesterone caproate (500 mg) was injected intramuscularly in all the animals.

b. Normal control group: Overall, 111 normal cows (normal control, group 4) presented for routine insemination were included in this group. The insemination was done without any treatment.

c. Progesterone treated repeat breeder cows: Repeat breeder cows were sub-divided into three groups viz. the progesterone administration simultaneous to AI (group 5), insemination and exogenous administration of progesterone on 3rd day post AI (group 6) and insemination and administration of progesterone on 5th day post AI (group 7). A total of 30, 32 and 44 normal cows were inseminated in groups 5, 6 and 7, respectively. Hydroxyprogesterone caproate (500 mg) was injected intramuscularly in all the animals.

d. Repeat breeder control group: Overall, 35 repeat breeder cows without any apparent clinical abnormality were inseminated in this group (group 8) without any treatment.

Pregnancy Diagnosis

Pregnancy diagnosis was carried out after 60 days of AI by per rectal examination of animals not returning to estrus.

Measurement of cycle length

The cycle length of 87 randomly selected normal or repeat breeding cows, which returned to estrus after insemination, was evaluated to know the effect of progesterone administration on this parameter. This included 17, 23, 18 and 29 cows returned following

progesterone administration on 0, 3rd or 5th day post AI and control groups, respectively. The day of manifestation of estrus and confirmation by clinico-gynaecological examination was considered as day of estrus.

Blood Collection and preservation

Blood samples from 12 normal (six each from Group 1 and group 4) and 12 repeat breeder cows (six each from Group 5 and group 8) were collected by jugular venipuncture after aseptic precautions. The site was prepared aseptically. About 5 ml blood was collected using the 18/20 gauge needle in heparinised disposable syringe from each animal and was thoroughly mixed. The plasma was separated by centrifugation of the blood @ 3000 r.p.m. for 10 min. and frozen at -20°C in 2 ml capacity micro-centrifuge tubes in duplicate, pending analysis. Repeated thawing of plasma was strictly avoided. Three samples from each cow were collected on days 3, 5 and 7, respectively, post AI.

a. Biochemical estimation

The analysis of total protein, glucose, and cholesterol was done using analyzing kits of Bayer, India on Photometer 5010 pre programmed, automated photometric system (Robert Riele GmbH, Germany)

b. Progesterone estimation (Pre Coated Tube Method)

The kit was assembled by Animal Production unit FAO / IAEA Agriculture and Biotechnology lab, Seibersdorf, Australia 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, camel, horse and swine.

c. 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 100 µl of each of the standards, QC and samples were pipetted into the bottom of the corresponding tube.
5. 1 ml 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 struck sharply downwards on absorbent paper. They were allowed to drain for 2-5 min. and struck 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 done to compare the conception rate in different treatments groups (Chi-square test), variation in cycle length following progesterone administration (“t” test) and blood plasma biochemical and progesterone concentration (“t” test) in normal and repeat breeder cows (Snedecor and Cochran, 1967).

RESULTS

CHAPTER IV

RESULTS

A. EFFECT OF PROGESTERONE SUPPLEMENTATION ON CONCEPTION RATE FOLLOWING ARTIFICIAL INSEMINATION IN NORMAL AND REPEAT BREEDER COWS.

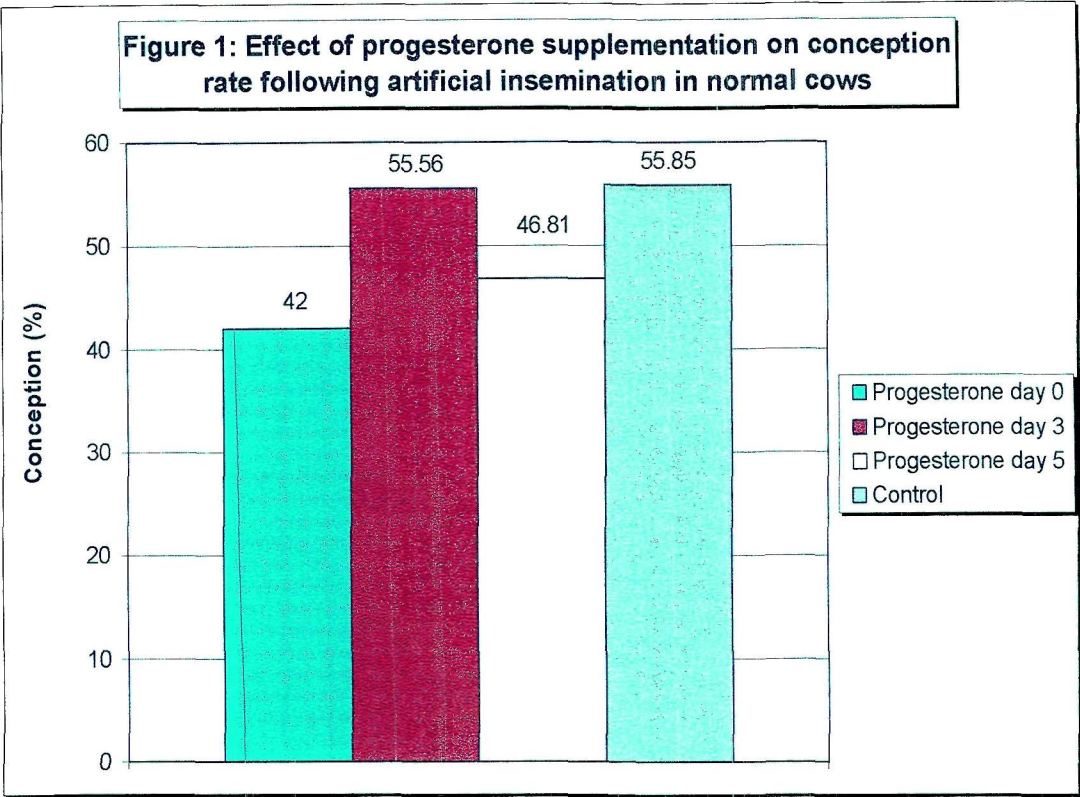
a. Effect of progesterone supplementation on conception rate in normal cows.

Overall effect of exogenous administration of progesterone on conception rate (CR) following artificial insemination in normal cows has been shown in Table 1 and Figure 1.

Table 1: Effect of progesterone supplementation on conception rate following artificial insemination in normal cows.

Day of Progesterone administration	Cows Inseminated	Pregnant	CR (%)
0	50	21	42.00
3	54	30	55.56
5	47	22	46.81
Control	111	62	55.85

In first group, in which 50 cows were administered hydroxyprogesterone caproate intramuscularly simultaneous to AI (day 0), 21 conceived with an overall CR of 42 per cent. In second group, among 54 cows injected progesterone third day post AI, 30 conceived with a CR of 55.56 per cent and in third group, 22 out of 47 inseminated animals were pregnant following intramuscular progesterone administrations on fifth day post insemination. The CR was 46.81 per cent. Whereas, among control normal cows (group 4) 62 conceived out of 111 with an overall conception rate of 55.85 per cent. However, the difference in conception rates between different groups was not statistically significant.



b. Effect of progesterone supplementation on conception rate in repeat breeder cows.

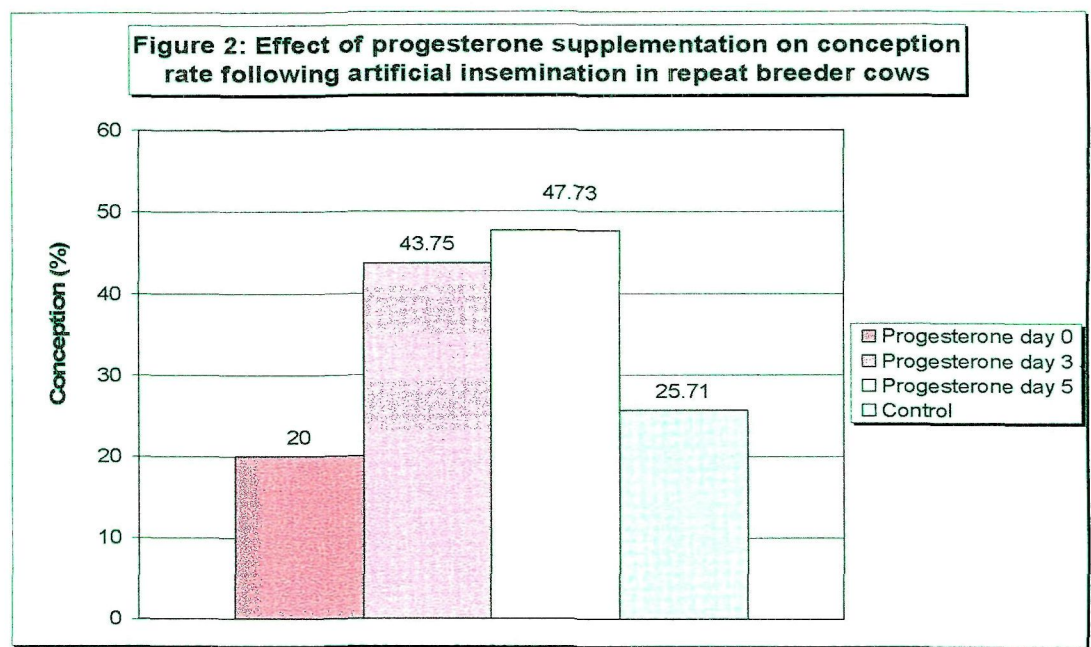
Overall effect of exogenous administration of progesterone on conception rate following artificial insemination in repeat breeder cows has been shown in Table 2 and Figure 2.

Table 2: Effect of progesterone supplementation on conception rate following artificial insemination in repeat breeder cows.

Day of Progesterone administration	Cows Inseminated	Pregnant	CR (%)
0	30	6	20.00 ^a
3	32	14	43.75 ^b
5	44	21	47.73 ^b
Control	35	9	25.71 ^a

Figures with different superscripts within a column differ significantly

In repeat breeder cows where progesterone was administered intramuscularly simultaneous to AI (day 0) in 30 cows (group 5), 6 (20 %) conceived. In sixth group comprising of 32 cows, 14 conceived following intramuscular administration of progesterone on third day post insemination with an overall CR of 43.75 per cent. In group 7, out of 44 cows inseminated, 21 cows conceived with CR of 47.73 per cent following progesterone administration on 5th day post AI. Whereas in control repeat breeder cows (group 8), 9 out of 35 conceived with a conception rate of 25.71 per cent. There was no difference in conception rate between day 0 progesterone administered and control cows. However, conception improved significantly ($P<0.05$) following administration of progesterone on either day 3 or 5 following insemination in comparison to day 0 progesterone administered or control cows.



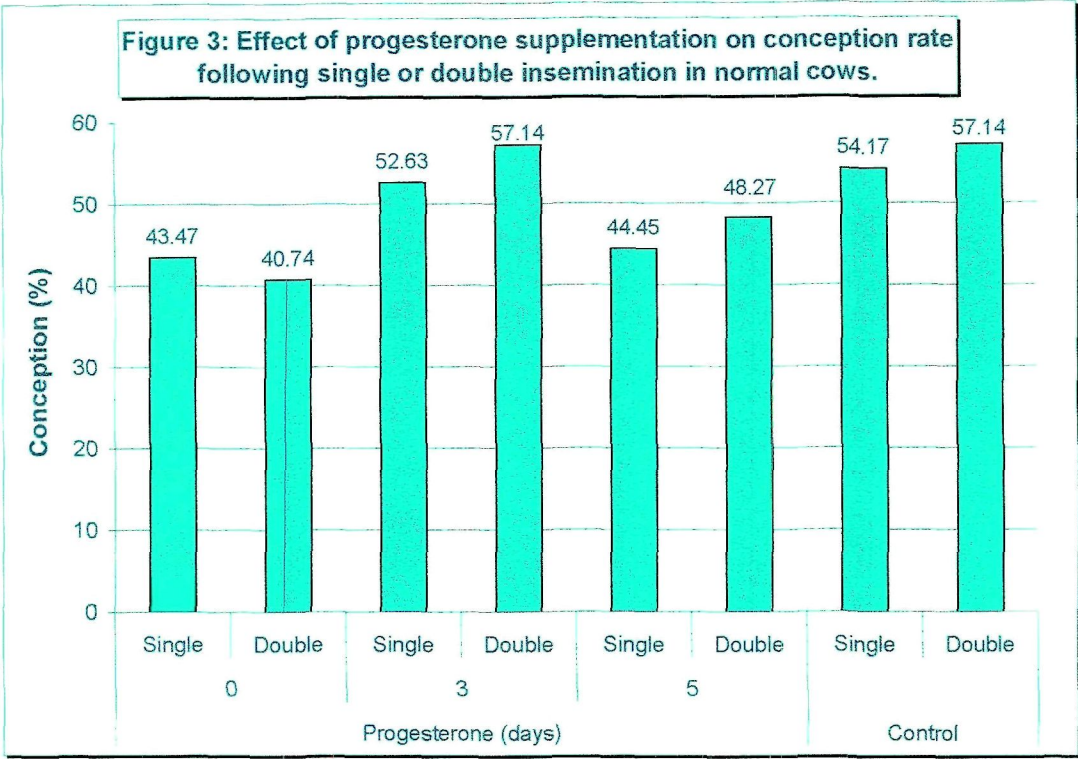
c. Effect of progesterone supplementation on conception rate following single or double insemination in normal cows.

Overall effect of exogenous administration of progesterone on conception rate following single or double insemination in normal cows has been shown in Table 3 and Figure 3.

Table 3: Effect of progesterone supplementation on conception rate following single or double insemination in normal cows.

Day of Progesterone administration	Insemination	Cows Inseminated	Pregnant	CR (%)
0	Single	23	10	43.47
	Double	27	11	40.74
3	Single	19	10	52.63
	Double	35	20	57.14
5	Single	18	8	44.45
	Double	29	14	48.27
Control	Single	48	26	54.17
	Double	63	36	57.14

Insemination of 23 and 27 cows in group 1 with the administration of hydroxyprogesterone caproate intramuscularly simultaneous to AI, resulted in conception of 10 and 11 animals with a CR of 43.47 and 40.74 per cent following single or double insemination, respectively. Similarly, following the insemination of 19 and 35 cows, injected progesterone third day post AI (group 2), 10 (52.63 %) and 20 (57.14 %) animals conceived following single or double insemination, respectively. In group 3, of the 18 animals, 8 (44.45 %) conceived following single and out of 29 cows 14 (48.27 %) conceived following double AI after the administration of progesterone 5th day post insemination in normal cows. In control group, 26 and 36 animals conceived out of 48 and 63 with conception rates of 54.17 and 57.14 per cent with single and double AI, respectively. However, the difference in groups was not significant.



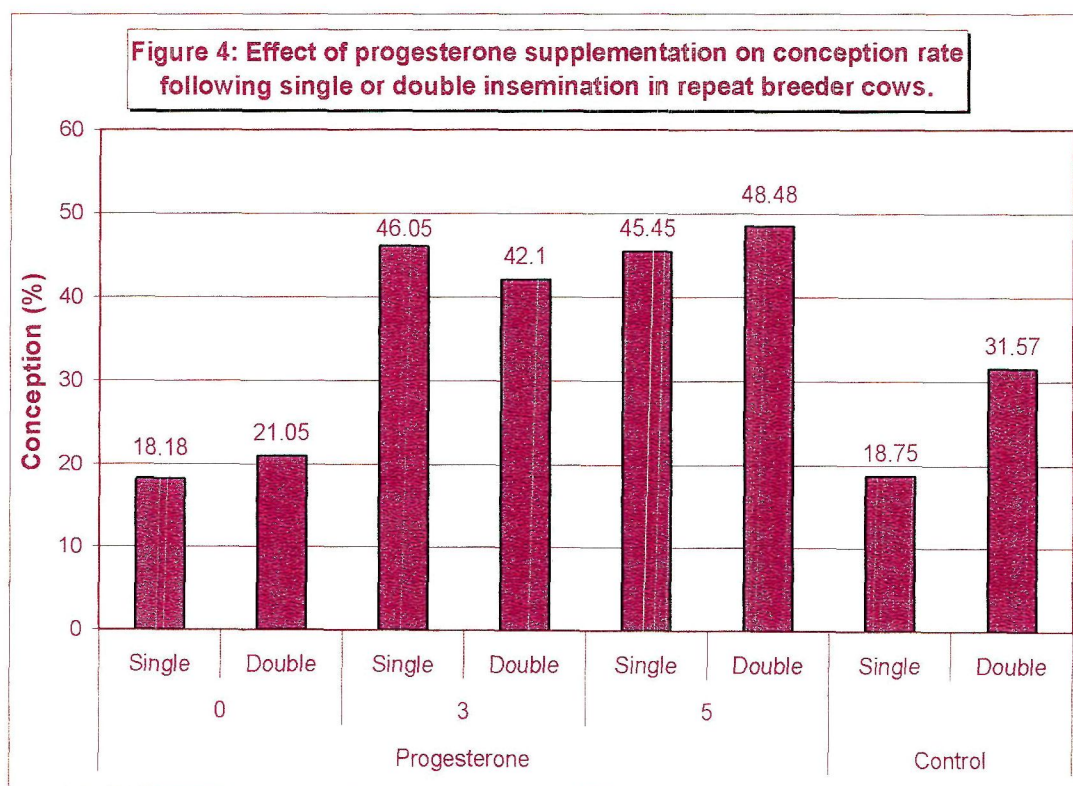
d. Effect of progesterone supplementation on conception rate following single or double insemination in repeat breeder cows.

Overall effect of exogenous administration of progesterone on conception rate following single or double insemination in repeat breeder cows has been shown in Table 4 and Figure 4.

Table 4: Effect of progesterone supplementation on conception rate following single or double insemination in repeat breeder cows.

Day of Progesterone administration	Insemination	Cows Inseminated	Pregnant	CR (%)
0	Single	11	2	18.18
	Double	19	4	21.05
3	Single	13	6	46.05
	Double	19	8	42.10
5	Single	11	5	45.45
	Double	33	16	48.48
Control	Single	16	3	18.75
	Double	19	6	31.57

After the insemination of 11 and 19 repeat breeder cows (group 5) along with administration of progesterone intramuscularly simultaneous to AI, 2 and 4 conceived with a CR of 18.18 and 21.05 per cent following single or double insemination, respectively. Similarly, following the insemination of 13 and 19 cows in each of the two groups injected progesterone third day post AI (group 6), 6 and 8 animals conceived and the CR was 46.05 and 42.10 per cent following single and double insemination, respectively, whereas following the insemination of 11 and 33 cows, injected progesterone fifth day post AI (group 7), 5 and 16 animals conceived with a CR of 45.45 and 48.48 per cent following single or double insemination, respectively. In repeat breeder control group 3 and 6 cows conceived out of 16 and 19 cows with a conception rate of 18.75 and 31.57 per cent with single or double insemination, respectively.



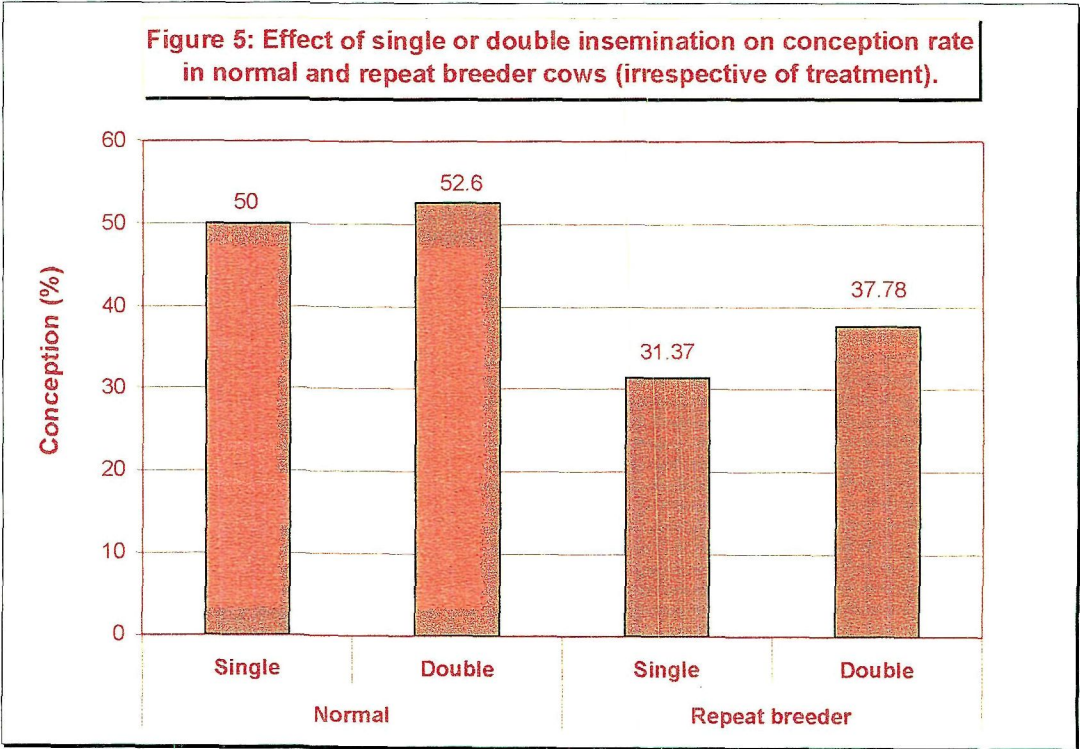
e. Overall effect of single or double insemination on conception rate in normal and repeat breeder cows.

Irrespective of treatment, the overall effect of single or double insemination on conception rate in normal and repeat breeder cows has been shown in Table 5 and Figure 5.

Table 5: Effect of single or double insemination on conception rate in normal and repeat breeder cows (irrespective of treatment).

Status of animal	Insemination	Cows Inseminated	Pregnant	CR (%)
Normal	Single	108	54	50.00
	Double	154	81	52.60
Repeat breeder	Single	51	16	31.37
	Double	90	34	37.78

Overall, 108 and 154 normal cows were inseminated and conception rate was 54 (50 %) and 81 (52.60 %) with single and double insemination, respectively. Whereas in repeat breeders over all,conception rate was 31.37 per cent and 37.78 per cent in single and double insemination groups, respectively.



f. Effect of progesterone supplementation on conception rate in normal cows inseminated at university dairy farm and field Veterinary institutions.

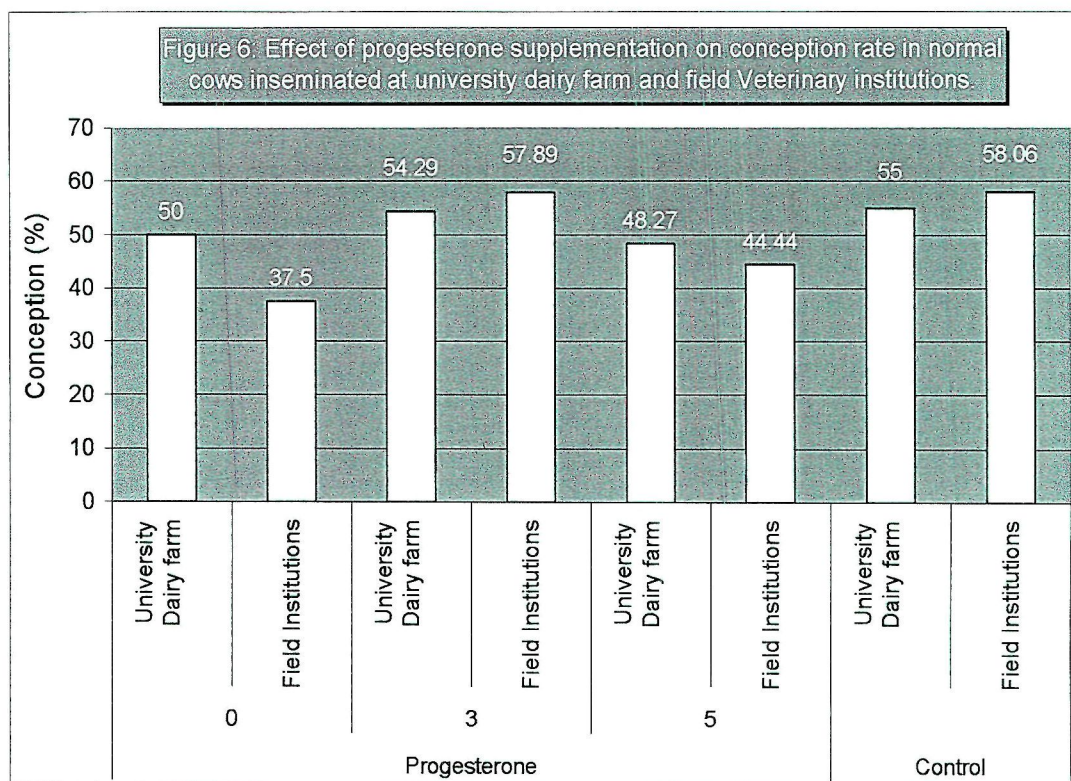
Effect of progesterone supplementation on conception rate in normal cows inseminated at university dairy farm and field Veterinary institutions has been shown in Table 6 and Figure 6.

Table 6: Effect of progesterone supplementation on conception rate in normal cows inseminated at university dairy farm and field Veterinary institutions.

Day of Progesterone administration	Inseminated at	Cows Inseminated	Pregnant	CR (%)
0	University Dairy farm	18	9	50.00
	Field Institutions	32	12	37.50
3	University Dairy farm	35	19	54.29
	Field Institutions	19	11	57.89
5	University Dairy farm	29	14	48.27
	Field Institutions	18	8	44.44
Control	University Dairy farm	80	44	55.00
	Field Institutions	31	18	58.06
Overall	University Dairy farm	162	86	53.08
	Field Institutions	100	49	49.00

Insemination of 18 and 32 cows along with the administration of hydroxyprogesterone caproate intramuscularly simultaneous to AI, resulted in conception of 9 and 12 animals with a CR of 50 and 37.50 per cent in university dairy farm and field Veterinary institutions, respectively. Similarly, following the insemination of 35 and 19 cows, receiving progesterone third day post AI, 19 (54.29 %) and 11 (57.89 %) animals conceived following inseminations at university dairy farm and field Veterinary institutions, respectively. Of the 29 animals, 14 (48.27 %) conceived in university dairy

farm and out of 18, 8 (44.44 %) conceived amongst inseminated at field Veterinary institutions after the administration of progesterone 5th day post insemination in normal cows. In control group, 44 and 18 out of 80 and 31 cows conceived with a conception rate of 55.00 and 58.06 per cent in university dairy farm and field Veterinary institutions, respectively. Overall, irrespective of the treatment 86 and 49 cows conceived out of 162 and 100 with a conception rate of 53.08 and 49.00 per cent in university dairy farm and field Veterinary institutions, respectively.



g. Effect of progesterone supplementation on conception rate in repeat breeder cows inseminated at university dairy farm and field Veterinary institutions.

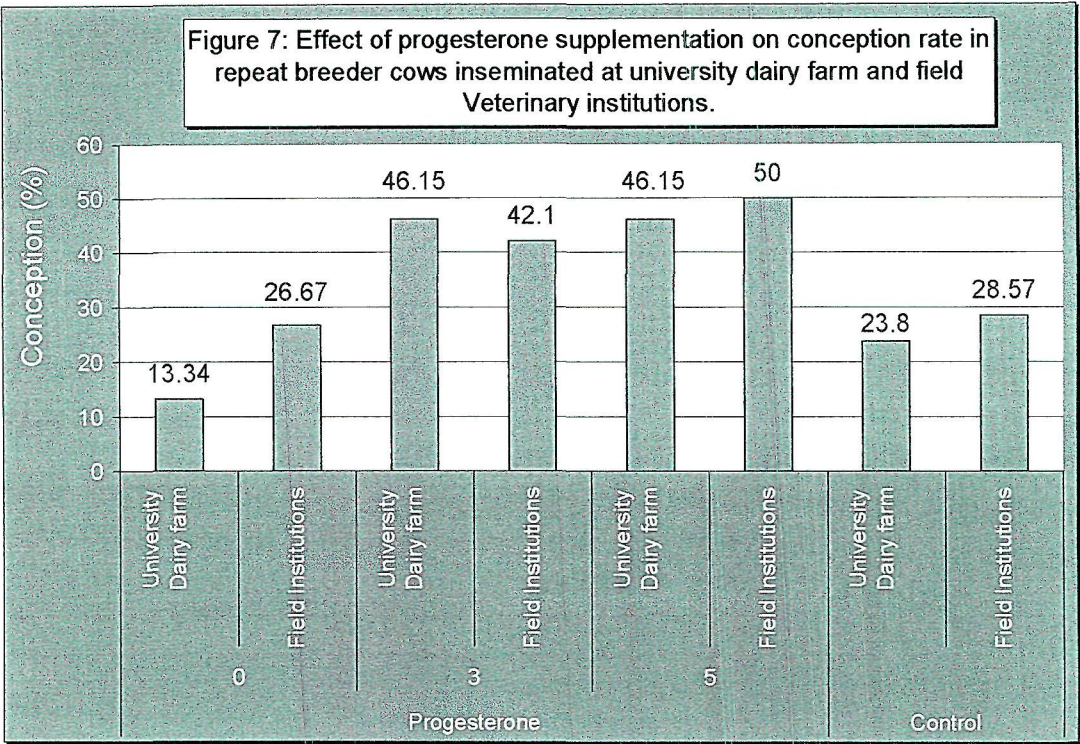
Effect of progesterone supplementation on conception rate in repeat breeder cows inseminated at university dairy farm and field Veterinary institutions has been shown in Table 7 and Figure 7.

Table 7: Effect of progesterone supplementation on conception rate in repeat breeder cows inseminated at university dairy farm and field Veterinary institutions.

Day of Progesterone administration	Inseminated at	Cows Inseminated	Pregnant	CR (%)
0	University Dairy farm	15	2	13.34
	Field Institutions	15	4	26.67
3	University Dairy farm	13	6	46.15
	Field Institutions	19	8	42.10
5	University Dairy farm	26	12	46.15
	Field Institutions	18	9	50.00
Control	University Dairy farm	21	5	23.80
	Field Institutions	14	4	28.57
Overall	University Dairy farm	75	25	33.34
	Field Institutions	66	25	37.88

Following the insemination of 15 repeat breeder cows each at university dairy farm and field Veterinary institutions along with administration of progesterone intramuscularly simultaneous to AI, 2 and 4 conceived with a CR of 13.34 and 26.67 per cent, respectively. Similarly, following the insemination of 13 and 19 cows in each of the two groups injected progesterone 3rd day post AI, 6 and 8 animals conceived and the CR was 46.15 and 42.10 per cent in university dairy farm and field Veterinary institutions, respectively. Whereas, following the insemination of 26 and 18 cows, administered progesterone 5th day post AI, 12 and 9 animals conceived with a CR of 46.15 and 50 per cent at university dairy farm and field Veterinary institutions, respectively. In repeat breeder control group, 5 and 4 cows conceived out of 21 and 14 cows inseminated, with a conception rate of 23.80 and 28.57 per cent in university dairy farm and field Veterinary

institutions, respectively. Overall, irrespective of the treatment 25 cows each conceived at university dairy farm and field Veterinary institutions, out of 75 and 66 inseminated with a conception rate of 33.34 and 37.88 per cent, respectively.



B. EFFECT OF PROGESTERONE SUPPLEMENTATION ON ESTROUS CYCLE LENGTH OF COWS.

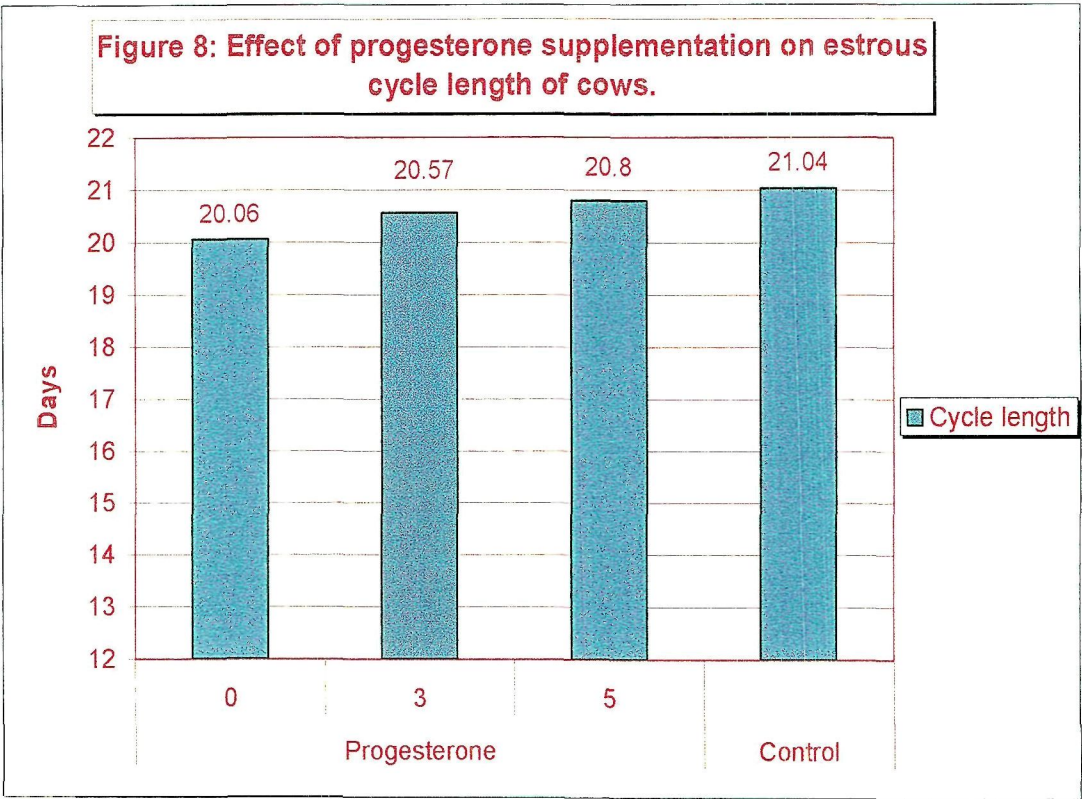
Effect of progesterone supplementation on different days on subsequent estrous cycle length of cows has been shown in Table 8 and Figure 8.

Table 8: Effect of progesterone supplementation on estrous cycle length of cows.

Day of Progesterone administration	Cycle length
0 (n= 17)	20.06 ± 0.36 ^a
3 (n= 23)	20.57 ± 0.54 ^{ab}
5 (n= 18)	20.80 ± 0.34 ^{ab}
Control (n= 29)	21.04 ± 0.20 ^b

Figures with different superscripts within a column differ significantly

Following administration of progesterone simultaneous to AI, the average estrous cycle length of treated cows was recorded as 20.06 ± 0.36 days and after administration of progesterone on day 3 of cycle, the mean estrous cycle length of cows was 20.57 ± 0.54 days. The mean estrous cycle length was 20.80 ± 0.34 days following the administration of progesterone on day 5, whereas in control group the average estrous cycle length was 21.04 ± 0.20 days.



C. EFFECT OF PROGESTERONE SUPPLEMENTATION ON BLOOD PLASMA BIOCHEMICALS AND PROGESTERONE PROFILE IN NORMAL AND REPEAT BREEDER COWS.

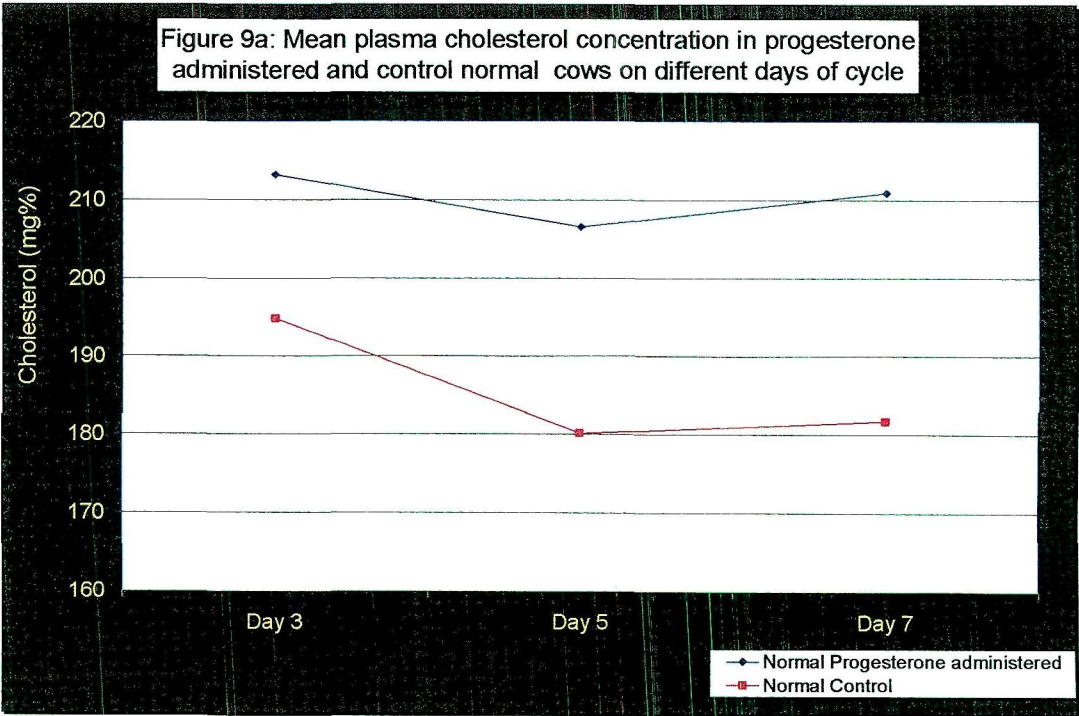
a. Effect of progesterone supplementation on blood plasma cholesterol in normal and repeat breeder cows

Effect of progesterone supplementation on blood plasma cholesterol in normal and repeat breeder cows has been shown in Table 9 and Figures 9 a and 9 b.

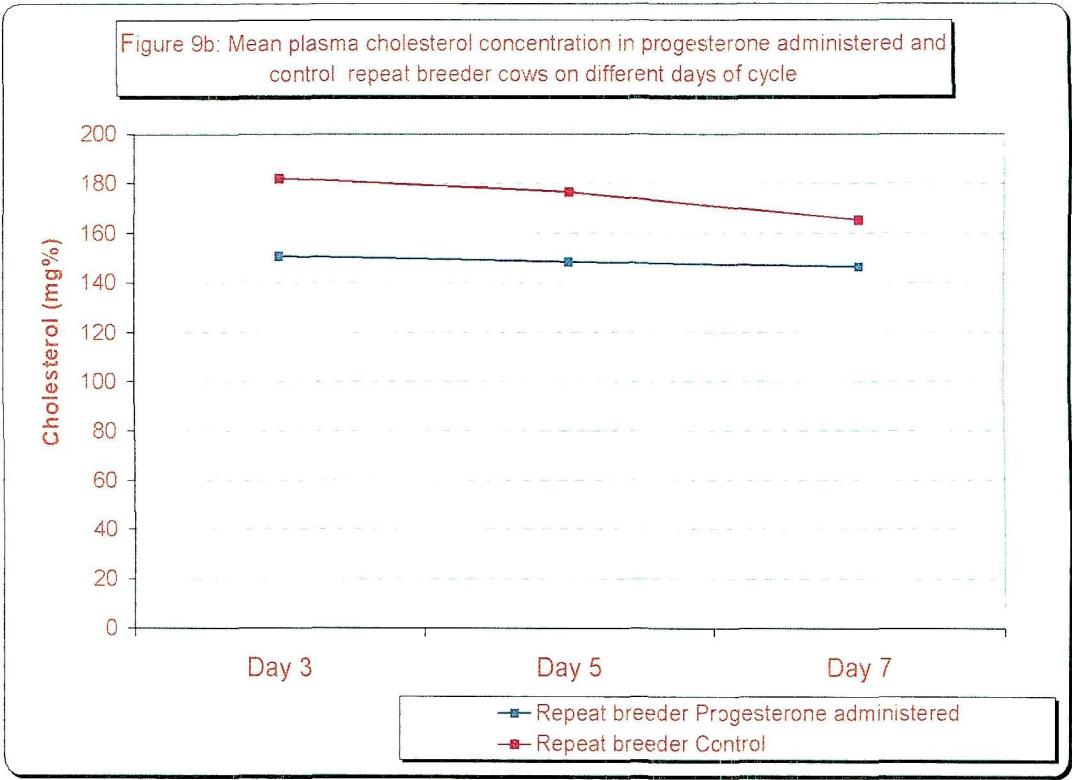
Table 9: Mean plasma cholesterol concentration in progesterone administered and control normal and repeat breeder cows on different days of cycle.

Reproductive Status	Group	Cholesterol (mg %)		
		Day 3	Day 5	Day 7
Normal (n=12)	Progesterone Administration (n=6)	213.20±10.70	206.58±20.05	210.95±18.10
	Control (n=6)	194.82±16.94	180.12±14.07	181.72±16.65
Repeat breeder (n=12)	Progesterone Administration (n=6)	150.90±12.64	148.55±03.95	146.55±08.71
	Control (n=6)	182.23±24.52	176.55±19.45	165.50±09.59

In normal progesterone treated cows the average cholesterol concentrations were 213.20±10.70, 206.58±20.05 and 210.95±18.10 mg per cent on days 3, 5 and 7, respectively, after progesterone administration simultaneous to AI. Whereas, in normal control cows, the average comparative values were, 194.82±16.94, 180.12±14.07 and 181.72±16.65 mg per cent on days 3, 5 and 7 of cycle, respectively.



In repeat breeder cows, the average cholesterol concentration was 150.90 ± 12.64 mg per cent on day 3, 148.55 ± 03.95 mg per cent on day 5 and 146.55 ± 08.71 mg per cent on day 7 of estrous cycle, following administration of progesterone simultaneous to AI. Whereas, in control repeat breeder cows the average concentrations were 182.23 ± 24.52 , 176.55 ± 19.45 and 165.50 ± 9.59 mg per cent on days 3, 5 and 7 of cycle, respectively.



However, the difference in cholesterol concentration on respective days between progesterone administered and control cows was not significant in normal as well as repeat breeder animals.

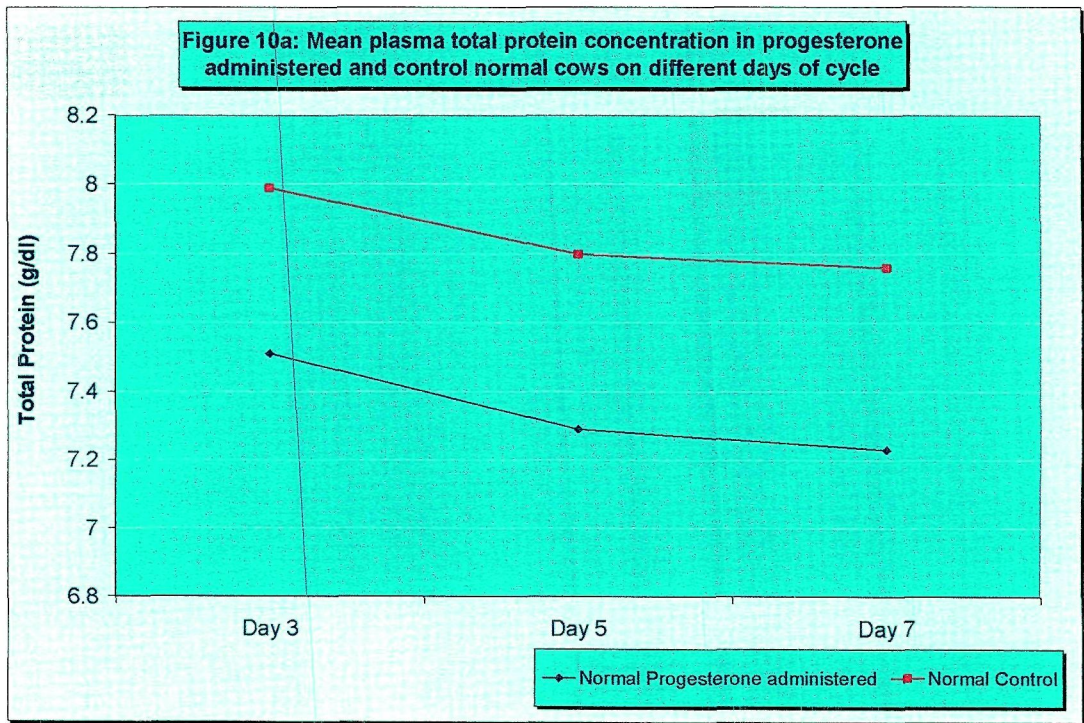
b. Effect of progesterone supplementation on total protein concentration in blood plasma of normal and repeat breeder cows

Effect of progesterone supplementation on total protein concentration in blood plasma of normal and repeat breeder cows has been shown in Table 10 and Figures 10 a and 10 b.

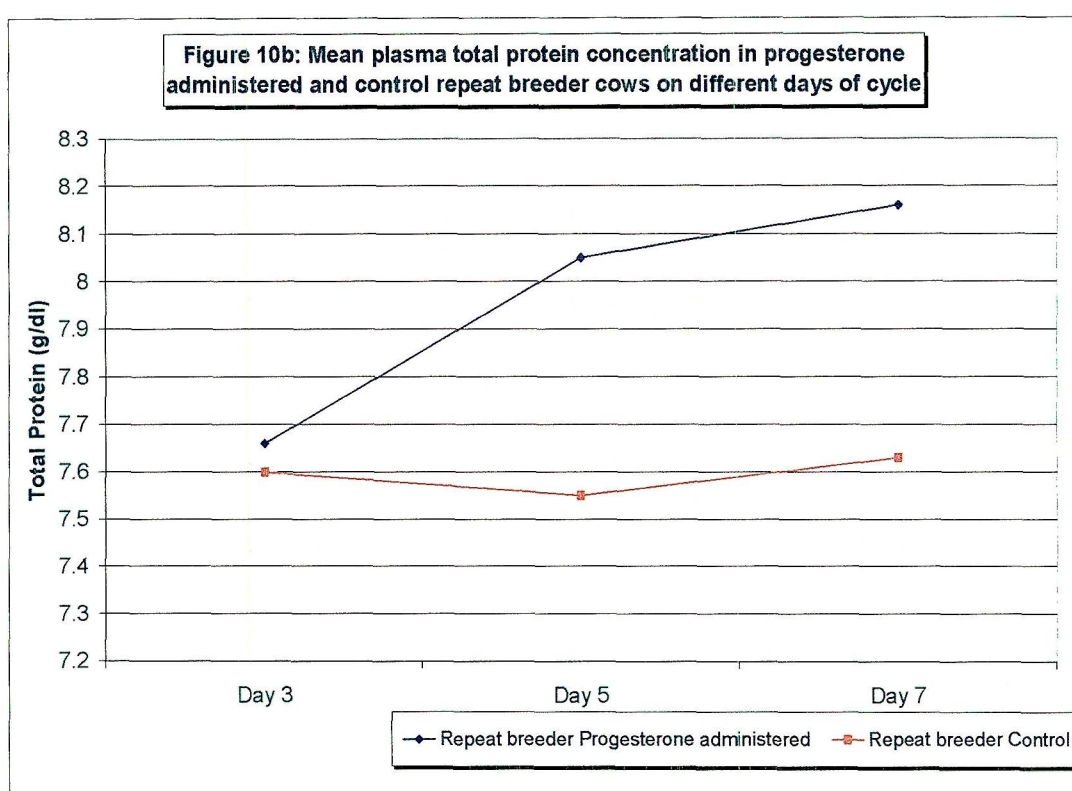
Table 10: Mean plasma total protein concentration in progesterone administered and control normal and repeat breeder cows on different days of cycle.

Reproductive Status	Group	Total Protein (g/dl)		
		Day 3	Day 5	Day 7
Normal (n=12)	Progesterone Administration (n=6)	7.51±0.36	7.29±0.37	7.23±0.39
	Control (n=6)	7.99±0.26	7.80±0.32	7.76±0.28
Repeat breeder (n=12)	Progesterone Administration (n=6)	7.66±0.49	8.05±0.43	8.16±0.41
	Control (n=6)	7.60±0.30	7.55±0.34	7.63±0.33

In normal progesterone treated cows the average total protein concentrations were 7.51±0.36, 7.29±0.37 and 7.23±0.39 g/dl on days 3, 5 and 7, respectively, after administration of progesterone simultaneous to AI. In control normal cows, where no treatment was given, the average protein concentrations were 7.99±0.26, 7.80±0.32 and 7.76±0.28 g/dl on days 3, 5 and 7 of estrous cycle, respectively.



In repeat breeder cows the average total protein concentration was 7.66 ± 0.49 g/dl on day 3, 8.05 ± 0.43 g/dl on day 5 and 8.16 ± 0.41 g/dl on day 7 of estrous cycle, following administration of progesterone simultaneous to AI. Whereas, in control repeat breeder cows the average total protein concentrations were 7.60 ± 0.30 , 7.55 ± 0.34 and 7.63 ± 0.33 g/dl on day 3, 5 and 7 of estrous cycle, respectively.



However, the difference in total protein concentration on respective days between progesterone administered and control cows was not significant in normal as well as repeat breeder animals.

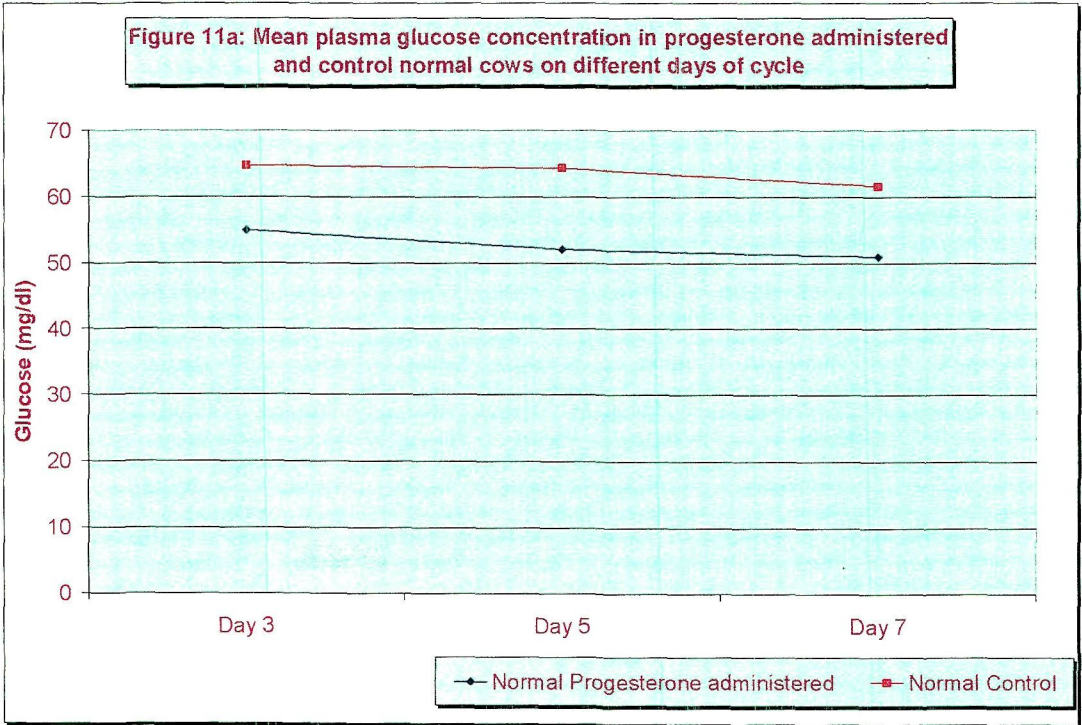
c. Effect of progesterone supplementation on blood plasma glucose in normal and repeat breeder cows.

Effect of progesterone supplementation on blood plasma glucose in normal and repeat breeder cows has been shown in Table 11 and Figures 11 a and 11 b.

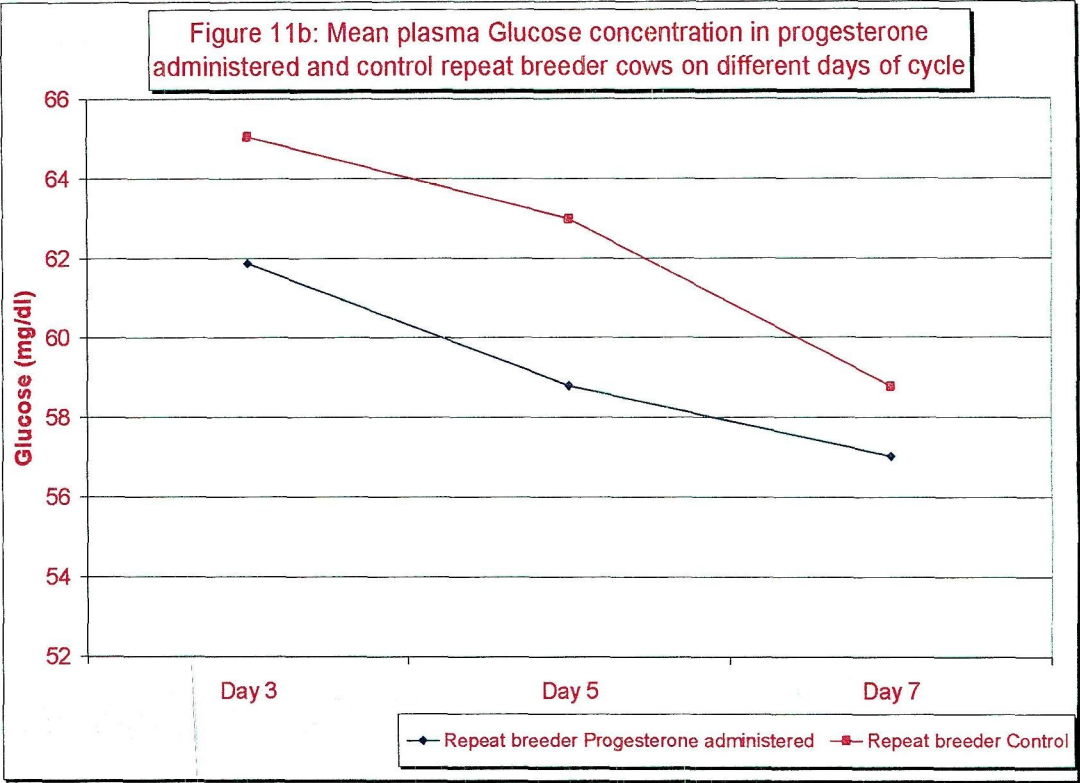
Table 11: Mean plasma glucose concentration in progesterone administered and control normal and repeat breeder cows on different days of cycle.

Reproductive Status	Group	Glucose (mg/dl)		
		Day 3	Day 5	Day 7
Normal (n=12)	Progesterone Administration (n=6)	55.04±6.40	52.13±4.87	51.03±4.12
	Control (n=6)	64.80±3.29	64.37±4.27	61.64±5.13
Repeat breeder (n=12)	Progesterone Administration (n=6)	61.89±5.72	58.79±3.39	57.02±2.30
	Control (n=6)	65.07±4.07	63.01±3.22	58.78±3.76

In normal progesterone treated cows the mean glucose concentrations were 55.04±6.40, 52.13±4.87 and 51.03±4.12 mg/dl on day 3, 5 and 7, respectively, following progesterone administration simultaneous to AI. In normal control cows, where no treatment was given, these concentrations were 64.80±3.29, 64.37±4.27 and 61.64±5.13 mg/dl on days 3, 5 and 7 of estrous cycle, respectively.



In repeat breeder cows, after administration of progesterone on day of AI, the mean glucose concentration was 61.89 ± 5.72 mg/dl on day 3, 58.79 ± 3.39 mg/dl on day 5 and 57.02 ± 2.30 mg/dl on day 7 of estrous cycle, whereas in control repeat breeder cows the average glucose concentrations were 65.07 ± 4.07 , 63.01 ± 3.22 and 58.78 ± 3.76 mg/dl on days 3, 5 and 7 of estrous cycle, respectively.



However, the difference in blood plasma glucose concentration on days 3, 5 or 7 between progesterone administered and control cows was not significant in normal as well as repeat breeder animals.

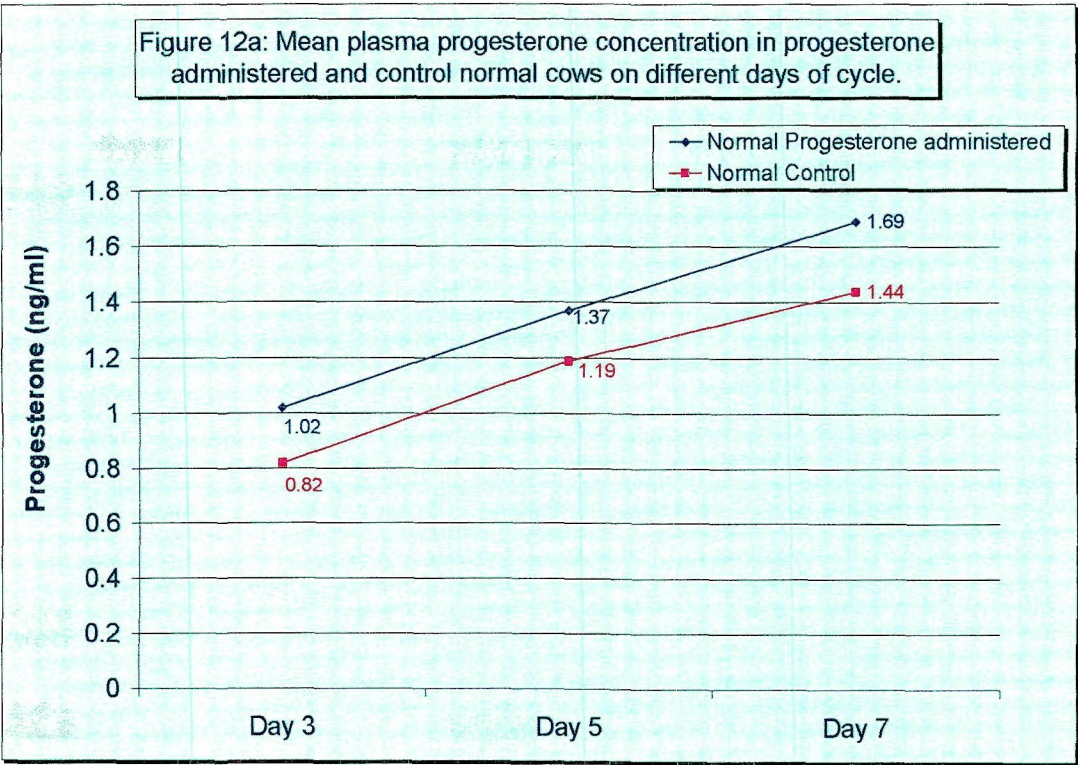
d. Effect of progesterone supplementation on blood plasma progesterone in normal and repeat breeder cows.

Effect of progesterone supplementation on blood plasma progesterone in normal and repeat breeder cows has been shown in Table 12 and Figures 12 a and 12 b.

Table 12: Mean plasma progesterone concentration in progesterone administered and control normal and repeat breeder cows on different days of cycle.

Reproductive Status	Group	Progesterone (ng/ml)		
		Day 3	Day 5	Day 7
Normal	Progesterone administration	1.02±0.08	1.37±0.09	1.69±0.16
	Control	0.82±0.07	1.19±0.06	1.44±0.09
Repeat breeder	Progesterone administration	0.91±0.07	1.17±0.10	1.52±0.09
	Control	0.78±0.12	1.11±0.09	1.48±0.12

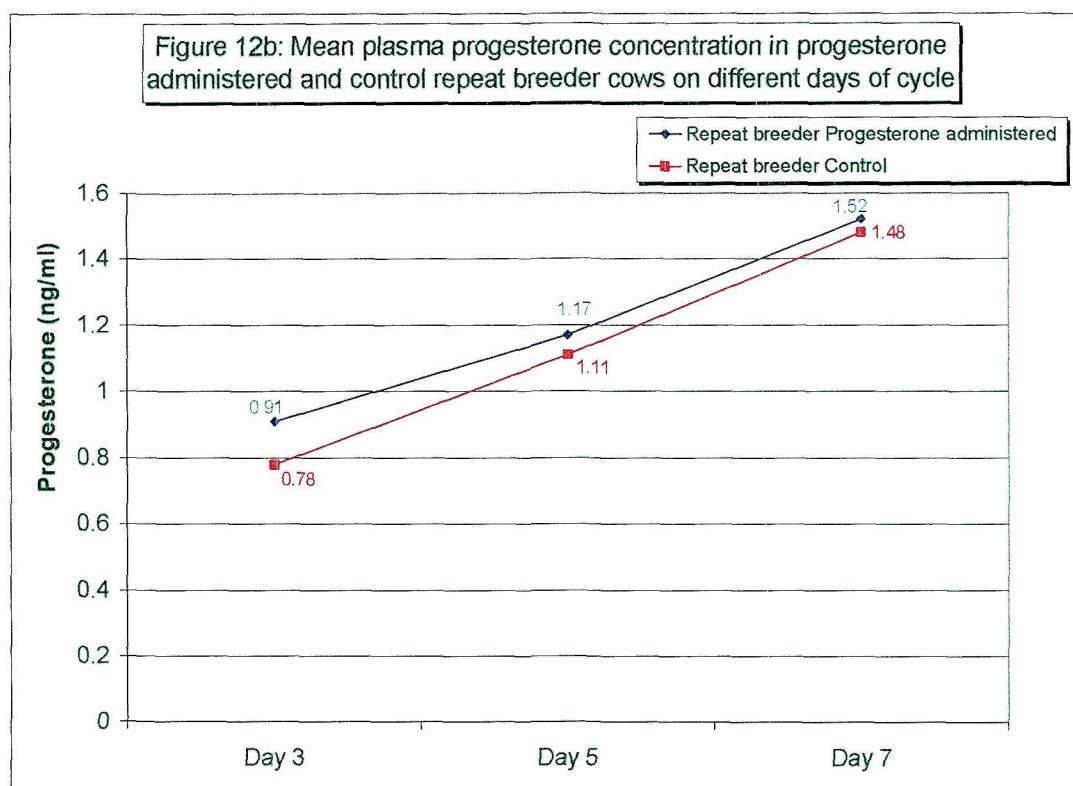
In normal progesterone treated cows the average progesterone concentrations were 1.02±0.08, 1.37±0.09 and 1.69±0.16 ng/ml on days 3, 5 and 7, respectively following progesterone administration simultaneous to AI. In normal control animals, where no treatment was given, the average progesterone concentrations were 0.82±0.07, 1.19±0.06 and 1.44±0.09 ng/ml on days 3, 5 and 7 of estrous cycle, respectively.



In repeat breeder cows the mean progesterone concentration was 0.91±0.07 ng/ml on day 3, 1.17±0.10 ng/ml on day 5 and 1.52±0.09 ng/ml on day 7 of estrous cycle,

following administration of progesterone simultaneous to AI. Whereas, in control repeat breeder cows the average progesterone concentrations were 0.78 ± 0.12 , 1.11 ± 0.09 and 1.48 ± 0.12 ng/ml on days 3, 5 and 7 of estrous cycle, respectively.

Mean plasma progesterone concentration was higher in progesterone administered normal as well as repeat breeder cows on days 3, 5 or 7 of estrous cycle when compared to control cows not administered any treatment on respective days.



However, the difference in progesterone concentration on 3, 5 or 7 days between progesterone administered and control cows was not significant in both normal as well as repeat breeder animals. Also, the difference in progesterone concentration on days 3, 5 or 7 between normal and repeat breeding cows was not significant.

DISCUSSION

CHAPTER V

DISCUSSION

Repeat breeding syndrome is a major source of economic wastage in dairy herds, contributing to lower dairy profits via wastage of semen and insemination costs, increasing intervals to conception, veterinary treatments (including calving induction in seasonal calving areas), increased culling and replacement costs and lost genetic gain through *increased generation intervals* (Bartlett *et al.*, 1986). There are increased dry period and calving intervals in repeat breeders and moreover such animals require more number of services per conception with longer service periods resulting in very heavy economic losses (Pandey *et al.*, 1994).

Amongst repeat breeding cattle, some females are frequently observed that have no evidence of any reproductive disorder but still fail to conceive. Various treatments are used in such animals with varied success. Progesterone is a popular treatment in these cows amongst field veterinary staff. The present study was conducted to record the effect of this practice on conception in normal and repeat breeder cows.

Overall, 403 cows were selected for this study that included 262 normal cows and 141 with the history of repeat breeding.

Among the normal cows, in which hydroxyprogesterone caproate was administered intramuscularly simultaneous to AI, the overall CR was 42.00 per cent. Similarly, among cows injected progesterone third or fifth day post AI, CR was 55.56 and 46.81 per cent, respectively. Whereas, in control normal cows where no progesterone was administered along with AI, overall conception rate was 55.85 per cent. Though, the difference in conception rates between different groups was not significant statistically, low conception was recorded in normal cows receiving progesterone simultaneous to AI.

Kumar and associates (2005) reported almost similar results (40.0%) after administration of progesterone on day of AI in normal cows. The conception rates were 50.6 and 36.00 per cent in cows in which progesterone was administrated on days 3 and 5, respectively. However, higher conception rates have been reported (Nancy *et al.*, 1989) who found that when PRID was inserted on day 5 to 12 and day 10 to day 17 of estrous cycle in normal Holstein cows, the conception rate was 60.0 per cent in both groups compared to 30.0 per cent conception rate in untreated control group. Not much variation was found when PRID was inserted either on day 5 to day 13 or from days 13 to 21 in different animals and conception rate was 44 per cent compared to 42 per cent in untreated control group (Jeffrey and Michael, 1991). A low CR (10%) has been reported by Singh *et al.* (2002) following progesterone administration simultaneous to AI in normal animals, as compared to the cows not given any treatment (50.0 %).

In our study, injecting progesterone simultaneous to insemination in otherwise normal cows reduced conception rate. These results are in accordance with Lynch *et al.*, (1999) who administered progesterone to 22 normal heifers on day 2 of estrus and obtained 45.4 per cent CR and this was less ($p < 0.05$) than the 73.9 per cent obtained with their 23 untreated contemporaries.

Administration of exogenous progesterone on day 3rd or 5th post insemination had no beneficial effect on improving conception in normal cows. Similarly, Sreenan and Diskin (1983) also did not find any beneficial effect of progesterone supplementation in improving conception rate in dairy cows.

Progesterone concentrations are at their lowest at the time of estrus in normally cycling animals. Basal levels of progesterone during estrus are associated with the insemination success and increased conception rates. But on the other hand, if the concentrations of progesterone are high at estrus they result in poor conception rates and

thus repeat breeding. Usually after ovulation, luteinization starts and progesterone levels starts rising from day 4 onwards. The corpus luteum on an average matures on day 5 in normally cycling animals. In the normal diestrus phase level of progesterone rises and reaches a plateau (Kanai and Shimizu, 1984).

The time and pattern of rise in plasma progesterone concentrations may be an important factor in determining the likelihood of an embryo remaining viable. There are suggestions that abnormally high levels of progesterone are detrimental for embryo (Farin and Farin, 1995). Ginther (1970) reported that progesterone, if given early between days 1-8 of cycle, can induce luteolysis.

Sub optimal crosstalk between the conceptus and the endometrial epithelial cells leads to secretion of PGF₂ alpha and resumption of ovulatory cycles with cessation of pregnancy (Thatcher *et al.*, 1986; Mann and Lamming, 2001; Thatcher *et al.*, 2001). Progesterone secretion by the CL is essential for orchestrating the histotrophic environment for nourishment of the conceptus. Indeed progesterone and estradiol act as systemic regulators leading to local oviductal and endometrial timed events and they program the uterus to regress the CL if there is sub-optimal communication between conceptus and uterus via secretion of PGF₂ alpha (Robinson *et al.*, 2001). An important component of the luteolytic mechanism is the peri-estrus induction of endometrial estradiol and oxytocin receptors. Exposure to progesterone reestablishes the lipid pool of endometrial phospholipids necessary for subsequent PGF₂ alpha synthesis. With prolonged progesterone exposure there is a down regulation of progesterone receptors such that there is an up-regulation of endometrial estradiol receptors that are important for induction of oxytocin receptors (Robinson *et al.*, 2001). However, progesterone also inhibits luteolysis by decreasing sensitivity to oxytocin by binding to oxytocin receptors and blocking the second messenger system (Grazzini *et al.*, 1998). Nevertheless, the most

important effect of progesterone in blocking luteolysis is by enhancing conceptus development which, in turn, stimulates secretion of interferon-tau (Mann and Lamming, 2001).

In repeat breeder cows where progesterone was administered intramuscularly simultaneous to AI, 20 per cent conceived. The intramuscular administration of progesterone on third day post insemination resulted in CR of 43.75 per cent. Similarly, 47.73 per cent CR was achieved following progesterone administration on 5th day post AI. In control repeat breeder cows the conception rate was 25.71 per cent. There was no difference in conception rate between day 0 progesterone administered and control cows. However, conception improved significantly ($P<0.05$) following administration of progesterone on either day 3 or 5 following insemination as compared to day 0 progesterone administered or control cows.

In the cow the sole source of progesterone at this time is the corpus luteum. The rationale for progesterone supplementation in the cow is to allow the embryo to survive to a stage where its effects are strong enough to maintain the corpus luteum in a functional state. Some of the earlier studies suggested progesterone supplementation as a possible therapy for subfertile or repeat breeding cows and indicated trends towards increased pregnancies ranging from about 10 to 60 per cent in progesterone supplemented cows (Wiltbank *et al.*, 1956; Johnson *et al.*, 1958).

The progesterone supplementation has been shown to improve conception rates in cows when administered early in pregnancy, if treatment is initiated no earlier than 3 days following insemination (Wiltbank *et al.*, 1956; Robinson *et al.*, 1989). Early studies were conducted by Wiltbank and associates (1956) in which cows diagnosed as repeat breeders were injected with progesterone daily from 3 days after estrus until or day 34. The treatment led to slightly positive but non significant results. Macmillan and coworkers

(1991) treated inseminated lactating cows with CIDR devices. The treatments that significantly increase conception rates from 65.7 per cent in control cows to 79.2 per cent in treated cows were initiated 6 to 8 days after insemination, and lasted for either 6 or 12 days. In a study by Robinson and coworkers (1989), lactating cows treated with PRID devices for 7 days beginning on day 5 or day 10 after insemination had a 60 per cent conception rate, compared to 30 per cent of untreated controls. On the contrary, progesterone supplementation of heifers through the use of CIDR devices for days 1 through 8 after insemination (new CIDR) or days 2 through 9 after insemination (used CIDR) significantly reduced the conception rate (Van Cleeff *et al.*, 1989). In experiment 1, however, the use of the CIDR device for days 7 through 13 after insemination didn't significantly alter the conception rate. However, only a limited number of animals were used. Walton *et al.* (1990) also failed to stimulate the pregnancy rate in Holstein cows that received progesterone releasing intravaginal device on days 8-12 post insemination.

The studies by Macmillan and coworkers (1991), using CIDR devices, and by Robinson and coworkers (1989), using PRID devices, resulted in significantly positive results. Rosen and Struman (1989) observed that parenteral administration of progesterone after 5-10 days of estrus had improved the conception rate significantly in repeat breeder cows. Devanathan and associates (1999) used 500 mg progesterone 5th day post AI and achieved the conception rate of 53.84 percent in repeat breeder cows. Similarly, 65.21 per cent cows conceived when 250 mg progesterone was administered at 5th, 12th and 19th, day after AI in repeat breeder cows (Srivastava and Kharche, 2001). In another study, the conception rate in repeat breeding cows was found to be 60 per cent after administering progesterone 5th day post AI (Singh *et al.*, 2002). Awasthi and associates (2002) also reported 70 per cent conception rate by using progesterone 6-8 days post insemination.

The increase in fertility in response to progesterone supplementation in these studies suggests that, of the presumably multiple causes of early embryonic mortality in lactating cows, some are due to progesterone deficiency.

Providing progesterone supplementation during the early stages of intrauterine embryonic development may induce a uterine environment more conducive to embryonic survival in such cows, and thereby rescue some of the delayed embryos. It is possible that a significant portion of reduced fertility in the cow population is attributable to inadequate maternal luteal function and consequent inadequacies in the progesterone dependent uterine environment (Henricks *et al.*, 1971; Lukaszewska and Hansel, 1980; Hansel, 1981; Wiebold, 1988; Lamming *et al.*, 1989). Several reports advocate the beneficial effects of progesterone supplementation for maintenance of early pregnancy in cows (Wiltbank *et al.*, 1956). Early embryonic loss could occur through inadequate uterine environment. The higher conception rate in animals by using progesterone may be due to improved uterine environment for embryo survival and development (Balakrishanan *et al.*, 1994). Exogenous administration of progesterone brings about certain favourable changes in the uterine luminal fluid (Devanathan and Pattabiraman, 1997). Endometrial secretions, essential for stimulating and mediating changes in conceptus growth and differentiation throughout early pregnancy was directed by the steroid environment generated by the ovary (Wilmot *et al.*, 1986). Higher progesterone concentration and a lower ratio of estradiol to progesterone on days 3 and 6 were very conducive for the growth of embryo (Maurer and Echternkamp, 1982). Progesterone could be the mediator of embryonic survival (Dyck and Strain, 1983; Pharazyn, 1992; Jindal *et al.*, 1996). Plasma progesterone concentration during early pregnancy can modify oviductal (Buhi *et al.*, 1990; Murray, 1993) and endometrial (Ashworth and Bazer, 1989; Roberts *et al.*, 1993) development and secretory activity.

During early pregnancy the bovine embryo must produce a protein called interferon-tau which inhibits the development of the luteolytic mechanism (Mann and Lamming, 2001). Failure to inhibit luteolysis is the major cause of pregnancy loss in cows. The embryo must produce sufficient quantities of interferon-tau by about day 16 to prevent luteolysis. Its ability to achieve this is largely dependent on the pattern of maternal progesterone production (Mann *et al.*, 1998). A late rise in progesterone after ovulation, or poor progesterone secretion during the luteal phase results in the development of poor embryos capable of producing little or no interferon-tau at the critical time. The embryo inhibits luteolysis by preventing development of oxytocin receptors on the luminal epithelium of the uterine endometrium and thus oxytocin-induced secretion of PGF₂ alpha and by the induction of a prostaglandin synthesis inhibitor within the endometrium. In sheep it has been hypothesized that interferon-tau acts to inhibit endometrial oestrogen receptors and thus oestrogen-induced up-regulation of oxytocin receptors. In cows, the embryo inhibits the development of oxytocin receptors and the initiation of luteolysis without causing any change in uterine estrogen receptors. Thus in the cow, the mechanism by which interferon-tau inhibits oxytocin receptor development remains to be determined (Mann *et al.*, 1999).

Not only are progesterone concentrations important for pregnancy, but a delay in post-ovulatory rise in progesterone compromises conceptus development and its ability to secrete interferon-tau, which results in reduced conception rates (Darwash and Lamming, 1998). Although no pre-established progesterone concentration determines a successful pregnancy (Mann and Lamming, 1999), progesterone concentrations on days 4 and 5 after mating were correlated with uterine luminal concentrations of interferon-tau on day 16 (Wathes *et al.*, 2003).

In our study, supplementation of progesterone during early luteal phase (day 3rd or 5th) improved conception in repeat breeder cows without any apparent clinical abnormality. Because progesterone plays a major role in stimulating the production of several endometrial proteins and growth factors (Geisert *et al.*, 1992), supplemental progesterone during the first 4 days after AI increased morphological development and biosynthetic activity of day 14 conceptuses (Garrett *et al.*, 1998). Mann and Lamming (1999) demonstrated that supplemental progesterone was beneficial to fertility increasing conception rates when administered prior to day 6 after AI in lactating dairy cows. Collectively, these data indicate that progesterone availability in the early diestrus phase may benefit conception rates and embryonic survival. Walton *et al.* (1990) observed that parental administration of progesterone in cows resulted in increased embryo viability and conception rate.

Furthermore, the benefits of supplemental progesterone were more clearly evident when utilized in lactating cows of lower fertility, i.e. cows with conception rates of less than 50 per cent. Therefore, timing of administration of supplemental progesterone is critical (days 4–5 of gestation) probably because it alters the secretory activity of the endometrium, thus influencing embryonic growth (Garrett *et al.*, 1998; Geisert *et al.*, 1992). In embryo transfer technology progesterone treatment to recipient cows during 1-5 days of estrous cycle promoted the survival rate of transferred frozen embryos and improved pregnancy rate. Variability in progesterone synthesis may lead to asynchrony between the embryo and uterus (Pope, 1988).

Overall, conception rate was 50.00 and 52.60 per cent with single and double insemination, respectively, in normal cows. Whereas in repeat breeders over all conception rate was 31.37 per cent and 37.78 per cent in single and double insemination

groups, respectively. Overall conception was non-significantly better when cows were inseminated twice at an interval of 24 hours as compared to single AI.

Our findings are in agreement with those of Fleischmann (1990) and Wilcox and Pfau (1958) who suggested that double insemination was better than single insemination, but should be performed only in those cases where signs of estrus persist 24 hour after insemination. Sharma and coworkers (2002) also suggested that, double AI yields higher CR as compared to single AI ($P < 0.05$) in normal crossbred cows.

Following administration of progesterone simultaneous to AI, the average estrous cycle length of treated cows was recorded as 20.06 ± 0.36 days and in control group it was 21.04 ± 0.20 days. Exogenous progesterone administration on day 0 of estrus significantly reduced length of subsequent estrous cycle. This observation is in accordance with other researchers who reported that progesterone supplementation immediately after AI should be avoided because it advances the uterus and increases the occurrence of short cycles (Van Cleeff *et al.*, 1996; Garrett *et al.*, 1998).

Lynch and associates (1999) administered progesterone per vaginum to heifers for 10 days from day 2 (Estrus = Day 0). Other heifers served as an untreated control. The average inter-estrus interval for those heifers treated with progesterone was 17.0 days and was less ($p < 0.05$) than the average intervals for those in the control group (20.0 days). They concluded that initiating the chronic administration of progesterone to cattle produces shortened estrous cycles containing one or two wave-like sequences of ovarian follicle development.

In the study, no significant effect of exogenous administration of progesterone on blood cholesterol, total protein and glucose levels on days 3, 5 or 7 in either normal or repeat breeder animals was evident. However, no relevant literature could be available to interpret the effect of administration of progesterone on these biochemicals in literature.

Mean progesterone concentration was non significantly higher in progesterone administered normal as well as repeat breeder cows on days 3, 5 or 7 of estrous cycle when compared to control cows not administered any treatment on respective days. The difference in progesterone concentration on days 3, 5 or 7 between normal and repeat breeding cows was not significant. Lamming and coworkers (1989) reported that inseminated cows which failed to conceive had lower plasma and milk progesterone concentrations from days 10 to 16, compared to the pregnant cows. In these studies, there were no detectable differences in plasma or milk progesterone before day 10 between inseminated cows which conceived and those which did not.

Providing progesterone supplementation during the early stages of intrauterine embryonic development in luteal insufficient cows may induce a uterine environment more conducive to embryonic survival, and thereby rescue some of these embryos. However, administration of exogenous progesterone has no beneficial effect on improving conception in normal cows, rather it may prove to be detrimental in certain cases.

SUMMARY

CHAPTER VI

SUMMARY

Altogether 403 cows (262 normal and 141 cows with the history of repeat breeding) presented for insemination in livestock farm of CSK Himachal Pradesh Krishivishvavidyalya, Veterinary clinical complex of Dr. G.C.Negi College of Veterinary and Animal Sciences, Palampur and nearby field Veterinary institutions were included in the study. Only those animals that were free from any apparent reproductive disorder and not conceiving were selected for the study as repeat breeder cows.

The animals of all the groups were randomly selected for single or double insemination. In all the double insemination groups, second AI was performed about 24 hours later. Irrespective of single or double insemination, day of first insemination was considered as day 0 for progesterone treatments. In all the treatment group cows, depending on the group, 500 mg hydroxyprogesterone caproate (Duraprogen, Vetcare Division) was injected intramuscularly whereas no treatment was given to the control group cows.

The animals included in progesterone treatment group were sub-divided into three groups viz. the progesterone administration simultaneous to AI (group 1, n=50), insemination and exogenous administration of progesterone on 3rd day post AI (group 2, n=54) and insemination and administration of progesterone on 5th day post AI (group 3, n=47). Whereas, 111 normal cows inseminated without progesterone (group 4) were treated as normal control. Repeat breeder cows were sub-divided into three groups viz. the progesterone administration simultaneous to AI (group 5, n=30), insemination and exogenous administration of progesterone on 3rd day post AI (group 6, n=32) and insemination and administration of progesterone on 5th day post AI (group 7, n=44).

Whereas, 35 repeat breeder cows without any apparent clinical abnormality and without any treatment constituted the control group.

Pregnancy diagnosis was carried out after 60 days of AI by per rectal examination of animals not returning to estrus.

The cycle length of 87 randomly selected normal or repeat breeding cows, which returned to estrus after insemination, was evaluated to know the effect of progesterone administration on this parameter. This included 17, 23, 18 and 29 cows returned following progesterone administration on 0, 3, 5 days post AI and control groups, respectively.

Blood samples from 12 repeat breeder and 12 normal cows (three samples each on days 3, 5 and 7) were collected post AI for the analysis of total protein, glucose, cholesterol and progesterone.

The CR in normal cows receiving progesterone treatment on days 0, 3 and 5 were 42.0, 55.56 and 46.81 percent, respectively. In normal control cows, it was 55.85 per cent. The differences in CR between different groups were not statistically significant.

In repeat breeder cows receiving progesterone treatment on days 0, 3 and 5 post insemination, CR were 20.0, 43.75 and 47.73 per cent, respectively. In control repeat breeder cows, 25.71 per cent cows conceived. There was no difference in conception rate between day 0 progesterone administered and control cows. However, conception improved significantly ($P < 0.05$) following administration of progesterone on either day 3 or 5 following insemination in comparison to day 0 progesterone administered or control cows.

Insemination of 23 and 27 cows in group 1 with the administration of hydroxyprogesterone caproate intramuscularly simultaneous to AI, resulted in conception of 10 and 11 animals with a CR of 43.47 and 40.74 per cent following single or double insemination, respectively. Similarly, following the insemination of 19 and 35 cows,

injected progesterone third day post AI (group 2), 10 (52.63 %) and 20 (57.14 %) animals conceived following single or double insemination, respectively. In group 3, of the 18 animals, 8 (44.45 %) conceived following single and out of 29 cows 14 (48.27 %) conceived following double AI after the administration of progesterone 5th day post insemination in normal cows. In control group, 26 and 36 animals conceived out of 48 and 63 with conception rates of 54.17 and 57.14 per cent in single and double AI, respectively. However, the difference in groups was not significant.

After the insemination of 11 and 19 repeat breeder cows (group 5) along with administration of progesterone intramuscularly simultaneous to AI, 2 and 4 conceived with a CR of 18.18 and 21.05 per cent following single or double insemination, respectively. Similarly, following the insemination of 13 and 19 cows in each of the two groups injected progesterone third day post AI (group 6), 6 and 8 animals conceived and the CR was 46.05 and 42.10 per cent following single and double insemination, respectively, whereas following the insemination of 11 and 33 cows, injected progesterone fifth day post AI (group 7), 5 and 16 animals conceived with a CR of 45.45 and 48.48 per cent following single or double insemination, respectively. In repeat breeder control group 3 and 6 cows conceived out of 16 and 19 cows with a conception rate of 18.75 and 31.57 per cent with single or double insemination, respectively.

Overall, 108 and 154 normal cows were inseminated and conception rate was 54 (50 %) and 81 (52.60 %) with single and double insemination, respectively. Whereas, in repeat breeders over all conception rate was 31.37 per cent and 37.78 per cent in single and double insemination groups, respectively.

Insemination of 18 and 32 cows along with the administration of hydroxyprogesterone caproate intramuscularly simultaneous to AI, resulted in conception of 9 and 12 animals with a CR of 50 and 37.50 per cent in university dairy farm and field

Veterinary institutions, respectively. Similarly, following the insemination of 35 and 19 cows, receiving progesterone third day post AI, 19 (54.29 %) and 11 (57.89 %) animals conceived following inseminations at university dairy farm and field Veterinary institutions, respectively. Of the 29 animals, 14 (48.27 %) conceived in university dairy farm and out of 18, 8 (44.44 %) conceived amongst inseminated at field Veterinary institutions after the administration of progesterone 5th day post insemination in normal cows. In control group 44 and 18 out of 80 and 31 cows conceived with a conception rate of 55.00 and 58.06 per cent in university dairy farm and field Veterinary institutions, respectively. Overall, irrespective of the treatment 86 and 49 cows conceived out of 162 and 100 with a conception rate of 53.08 and 49.00 per cent in university dairy farm and field Veterinary institutions, respectively.

Following the insemination of 15 repeat breeder cows each at university dairy farm and field Veterinary institutions along with administration of progesterone intramuscularly simultaneous to AI, 2 and 4 conceived with a CR of 13.34 and 26.67 per cent, respectively. Similarly, following the insemination of 13 and 19 cows in each of the two groups injected progesterone 3rd day post AI, 6 and 8 animals conceived and the CR was 46.15 and 42.10 per cent in university dairy farm and field Veterinary institutions, respectively. Whereas, following the insemination of 26 and 18 cows, administered progesterone 5th day post AI, 12 and 9 animals conceived with a CR of 46.15 and 50 per cent at university dairy farm and field Veterinary institutions, respectively. In repeat breeder control group, 5 and 4 cows conceived out of 21 and 14 cows inseminated, with a conception rate of 23.80 and 28.57 per cent in university dairy farm and field Veterinary institutions, respectively. Overall, irrespective of the treatment 25 cows each conceived at university dairy farm and field Veterinary institutions, out of 75 and 66 inseminated with a conception rate of 33.34 and 37.88 per cent, respectively.

Following administration of progesterone simultaneous to AI, the average estrous cycle length of treated cows was recorded as 20.06 ± 0.36 days and it was 20.57 ± 0.54 and 20.80 ± 0.34 days in cows receiving progesterone treatment on days 3 or 5 post AI, respectively. Whereas, in control group, the average estrous cycle length was 21.04 ± 0.20 days.

In normal progesterone treated cows the average cholesterol concentrations were 213.20 ± 10.70 , 206.58 ± 20.05 and 210.95 ± 18.10 mg per cent on days 3, 5 and 7, respectively, after progesterone administration simultaneous to AI. Whereas, in normal control cows, the average comparative values were, 194.82 ± 16.94 , 180.12 ± 14.07 and 181.72 ± 16.65 mg per cent on days 3, 5 and 7 of cycle, respectively.

In repeat breeder cows, the average cholesterol concentration was 150.90 ± 12.64 mg per cent on day 3, 148.55 ± 03.95 mg per cent on day 5 and 146.55 ± 08.71 mg per cent on day 7 of estrous cycle, following administration of progesterone simultaneous to AI. Whereas, in control repeat breeder cows the average concentrations were 182.23 ± 24.52 , 176.55 ± 19.45 and 165.50 ± 9.59 mg per cent on days 3, 5 and 7 of cycle, respectively.

However, the difference in cholesterol concentration on respective days between progesterone administered and control cows was not significant in normal as well as repeat breeder cows.

In normal progesterone treated cows the average total protein concentrations were 7.51 ± 0.36 , 7.29 ± 0.37 and 7.23 ± 0.39 g/dl on days 3, 5 and 7, respectively, after administration of progesterone simultaneous to AI. In control normal cows, the average protein concentrations were 7.99 ± 0.26 , 7.80 ± 0.32 and 7.76 ± 0.28 g/dl on days 3, 5 and 7 of estrous cycle, respectively.

In repeat breeder cows the average total protein concentration was 7.66 ± 0.49 g/dl on day 3, 8.05 ± 0.43 g/dl on day 5 and 8.16 ± 0.41 g/dl on day 7 of estrous cycle, following

administration of progesterone simultaneous to AI. Whereas, in control repeat breeder cows the average total protein concentrations were 7.60 ± 0.30 , 7.55 ± 0.34 and 7.63 ± 0.33 g/dl on days 3, 5 and 7 of estrous cycle, respectively.

However, the difference in total protein concentration on respective days between progesterone administered and control cows was not significant in normal as well as repeat breeder cows.

In normal progesterone treated cows the mean glucose concentrations were 55.04 ± 6.40 , 52.13 ± 4.87 and 51.03 ± 4.12 mg/dl on days 3, 5 and 7, respectively, following progesterone administration simultaneous to AI. In normal control cows, these concentrations were 64.80 ± 3.29 , 64.37 ± 4.27 and 61.64 ± 5.13 mg/dl on days 3, 5 and 7 of estrous cycle, respectively.

In repeat breeder cows, after administration of progesterone on day of AI, the mean glucose concentration was 61.89 ± 5.72 mg/dl on day 3, 58.79 ± 3.39 mg/dl on day 5 and 57.02 ± 2.30 mg/dl on day 7 of estrous cycle, whereas in control repeat breeder cows the average glucose concentrations were 65.07 ± 4.07 , 63.01 ± 3.22 and 58.78 ± 3.76 mg/dl on days 3, 5 and 7 of estrous cycle, respectively.

However, the difference in blood plasma glucose concentration on days 3, 5 or 7 between progesterone administered and control cows was not significant in normal as well as repeat breeder cows.

In normal progesterone treated cows the average progesterone concentrations were 1.02 ± 0.08 , 1.37 ± 0.09 and 1.69 ± 0.16 ng/ml on days 3, 5 and 7, respectively following progesterone administration simultaneous to AI. In normal control animals, the average progesterone concentrations were 0.82 ± 0.07 , 1.19 ± 0.06 and 1.44 ± 0.09 ng/ml on days 3, 5 and 7 of estrous cycle, respectively.

In repeat breeder cows the mean progesterone concentration was 0.91 ± 0.07 ng/ml on day 3, 1.17 ± 0.10 ng/ml on day 5 and 1.52 ± 0.09 ng/ml on day 7 of estrous cycle, following administration of progesterone simultaneous to AI. Whereas, in control repeat breeder cows the average progesterone concentrations were 0.78 ± 0.12 , 1.11 ± 0.09 and 1.48 ± 0.12 ng/ml on day 3, 5 and 7 of estrous cycle, respectively.

CONCLUSIONS

1. In normal cows, injecting progesterone simultaneous to insemination reduced conception rate.
2. Exogenous administration of progesterone had no beneficial effect in improving conception in normal cows when given on day 3rd or 5th post insemination.
3. In repeat breeder cows without any apparent clinical abnormality, supplementation of progesterone on day 3rd or 5th post insemination improved conception.
4. There was no beneficial effect of administering exogenous progesterone on day 0 even in repeat breeder cows.
5. Overall conception was non-significantly better when cows were inseminated twice at an interval of 24 hours as compared to single AI.
6. The conception rate was better in normal cows inseminated at university dairy farm as compared to field Veterinary institutions. Whereas, in repeat breeder cows the conception was better in animals inseminated at field Veterinary institutions. However, the difference was non significant.
7. Exogenous progesterone administration on day 0 of estrus reduced length of subsequent estrous cycle.
8. There was no effect of exogenous administration of progesterone on blood cholesterol, total protein and glucose levels on days 3, 5 or 7 in either normal or repeat breeder animals.
9. The mean plasma progesterone levels were higher on days 3, 5 or 7 in progesterone treated cows in comparison to those not given any treatment both in control and repeat breeder animals. However, the difference was non significant.

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