SUPEROVULATORY RESPONSE AND NON-SURGICAL EMBRYO RECOVERY IN RATHI COWS

राठी गायों में बहु अण्डक्षरण एवं शल्य क्रिया रहित भ्रूण एकत्रण

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By
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CERTIFICATE I

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Sanjay Bhojwani

(SANJAY BHOJWANI)

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LIST OF ABBREVIATIONS USED

1. A.I. = Artificial Insemination
2. BSA = Bovine Serum Albumin
3. DPBS = Dulbecco's Phosphate Buffered Saline
4. ET = Embryo Transfer
5. E2 = Oestrogen
6. FCS = Foetal Calf Serum
7. FSH = Follicle Stimulating Hormone
8. GnRH = Gonadotropin Releasing Hormone
9. hMG = Human Menopausal Gonadotropin
10. I.U. = International Units
11. I/M = Intra-Muscular
12. I/U = Intra-Uterine
13. I/V = Intra-Venous
14. LH = Luteinizing Hormone
15. m I.U. = Milli International Units
16. ng = Nanograms
17. nm = Nanometers
18. PMSG = Pregnant Mare Serum Gonadotropin
19. P4 = Progesterone
20. S/C = Sub-Cutaneous
21. µm = Micrometer
22. µg = Micrograms

INTRODUCTION

1. INTRODUCTION

In the recent past, artificial insemination was universally accepted technology for the multiplication of superior germ plasm but still the problems such as reducing the calving interval, late maturity and production of more number of calves of high genetic potential from one cow during the life time remain unsolved. Embryo biotechnology including embryo cryopreservation, *in vitro* fertilization (IVF), embryo micro manipulation, sexing, induction of twinning, cloning and gene transfer has already shown promising results in animal reproduction and productivity. In combination with artificial insemination this technique will certainly be useful to increase the population of the desired genotype of animals with improved potential for milk and draught. Cattle herd improvement for higher productivity can be achieved by means of ETT through faster multiplication of superior germ plasm, exploitation of female gene pool by making available much larger number of progeny from a single high-yielding cow, thus reducing the generation interval and many other areas like genetic manipulation, recombinant DNA technique, etc.

There are, however, a number of limiting factors affecting the widespread use of ETT. The variability of superovulatory response, in terms of quality and quantity of embryos, remains one of the major factors limiting the development of practical superovulation and hence ET programmes.

The success of superovulation is significantly influenced by individual factors viz., availability of feed and fodder, season, temperature, species, breed, age, day of cycle on which treatment is administered, presence or absence of a dominant follicle (Grasso et al., 1989; Huhtinen et al., 1992) and number of superovulatory treatments to which an animal has been subjected. Other aspects such as the type and dosage of gonadotropin, the purity of the products (Murphy et al., 1984) and the administration of GnRH (Savage et al., 1987; Foote et al., 1989) or associated luteolytic agents, all influence the embryo production. Recent reports indicate that the use of prostaglandin F2 alpha during late pre-ovulatory period can enhance or synchronize ovulation rate in superovulated animals (Gallo et al., 1992).

Although the mammalian ovary contains hundreds of thousands of oocytes, the number of progeny a female produces is small. In farm animals, the number of times a female can become pregnant is severely limited by extended duration of gestation. Furthermore, only one or two ova are usually shed per oestrous cycle in non litter-bearing species. The objective of superovulation is to increase the yield of viable ova. The success of ETT programme in domestic animals depends primarily upon the consistent supply of viable embryos. Keeping this in view, the induction of superovulation at a pre-determined time has become imperative. The most common method of induction of superovulation is by stimulating folliculogenesis with the administration of either pituitary gonadotropin- Follicle

stimulating hormone (FSH) or pregnant mare serum gonadotropin (PMSG). Different preparations of gonadotropins and their quantity used are sources of variation in responsiveness.

To induce superovulation, FSH is preferred by most of the embryo transfer centres around the world due to its definite advantages (lower circulating half-life and more precise response). Presently, most of the developing countries like India have to import FSH, which adds tremendously to the cost of ETT. PMSG is known to be another potent source for induction of superovulation, besides it is much cheaper and readily available, but having the disadvantage of a longer circulating half-life (Schams *et al.*, 1978) and a comparatively poor response. Anti-PMSG antisera has also been tried to reduce the over-stimulating effect of PMSG due to its long half-life (Dieleman *et al.*, 1987; Gielen *et al.*, 1990).

It has been established that there are 2 or 3 waves of follicular development per oestrous cycle with only one large follicle becoming dominant per wave (Rajakoski, 1960; Ireland and Roche, 1987). So in the natural course of the events, most of the follicles undergo atresia probably due to the lack of endogenous gonadotropin support. Induction of superovulation is based on the fact that the exogenous gonadotropin support prevents atresia of a large number of follicles and enhances the growth of medium sized follicles. This superovulatory response can further be improved if we prime the animals with smaller doses of the gonadotropins at an early stage of the oestrous cycle and before commencement of superovulation treatment

(Rajamahendran et al., 1987;Petr et. al., 1990). As a consequence an additional pool of the graafian follicles is made available.

Rathi cattle is a native milch breed of Bikaner region of North-West Rajasthan. This breed is very hardy and has many positive characters like heat tolerance, disease resistance, contentedness and adaptability in extreme arid climatic conditions of North-West Rajasthan inspite of impediments like high ambient temperature, scarcity of fodder and water and yet maintains the productive and reproductive status. It has been felt that due to indiscriminate use of crossbreeding programme in the state, pure Rathi population has decreased tremendously in Rajasthan and is almost facing a crisis of extinction. Studies concerning superovulation and embryo recovery have so far not been undertaken in the Rathi breed of cattle. If encouraging results are achieved and the technique of MOET (Multiple Ovulation and Embryo Transfer) is standardized in this breed, it will help in faster proliferation of the superior germ plasm and boost up the production potential of this indigenous breed.

The proposed research work has been planned to study superovulatory response and embryo recovery in the Rathi breed of cattle using PMSG with and without priming.

Most of the early embryo recovery and transfer work was conducted by surgical method which has now been superceded by the non-surgical method.

Embryo evaluation is an important determinant in the success of embryo transfer procedures. Efforts have been made to make the evaluation more objective by the use of laboratory methods such as the dye exclusion test, measures of enzyme activity, glucose uptake tests, and live-dead stains which seem to correlate with morphology and embryo survival following transfer. Several of these methods require complex equipments and/or a lengthy *in vitro* culture period. Hence, to date, microscopical evaluation on a morphological basis remains one of the most subjective methods for embryo evaluation.

The proposed research work was, therefore, designed with the following objectives :-

1. To study the pattern of synchronization of oestrus in Rathi cows using prostaglandins.
2. To carry out superovulation using PMSG, with and without priming, and to study the superovulatory response in Rathi cows.
3. To investigate the effect of priming on the superovulatory response.
4. To study the suitability of the hormonal regimes being applied for superovulation in Rathi cows.
5. To conduct non-surgical recovery of the embryos.
6. To evaluate the embryos for suitability of transfer.
7. To conduct hormonal assay of LH and FSH and determine the endocrine profile (in terms of LH and FSH) in superovulated and control cows.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. ROLE OF PROSTAGLANDINS IN SUPEROVULATION.

2.1.1. SYNCHRONIZATION OF OESTRUS PRIOR TO SUPEROVULATION.

Following the initial report of synchronization of oestrus by intra-uterine infusion of prostaglandin F2 alpha (PGF2α; Rowson *et al.*, 1972), various workers reported the use of exogenous PGF-2 alpha for synchronization of oestrus in cattle (Liehr *et al.*, 1972; Louis *et al.*, 1972).

The use of PGF-2 alpha and its synthetic analogues to shorten the cycle length in cattle was also reported by Lauderdale, 1972; Inskeep, 1973; Tervit *et al.*, 1973; Cooper, 1974; Smith, 1976; Stabenfeldt, *et al.*, 1978; Downey, 1980; Schams and Karg, 1982.

The potent synthetic analogues of prostaglandin are required in a much lower dose because of their greater luteolytic efficacy (Lauderdale, 1972; Cooper, 1974; Schams and Karg, 1982) thus overcoming the cost problem.

PGF-2 alpha, given in two daily doses (0.5-1.0mg) into the uterine horn ipsilateral to the ovary containing the corpus luteum, induces luteolysis with oestrus occurring after 48 to 85 hours of intra-uterine infusion (Shelton, 1973; Heranshaw *et al.*, 1974; Smith, 1974). Although the dose is reduced (5mg I/ut. v/s. 25mg I/M) but

this route of administration defers the advantage of simplicity of treatment and can cause additional problems such as uterine infection (Elsden and Lawson, 1974).

The most convenient method of administration is the intra-muscular route; however, owing to the rapid rate of metabolism of PGF-2 alpha (Piper et al., 1970) high doses are needed to achieve consistent effects.

The prerequisite for prostaglandin administration is the presence of a functional corpus luteum. The failure of animals in the early stages of the oestrous cycle (days 0 to 4) to respond to prostaglandin treatment (Lauderdale, 1972; Rowson et al., 1972; Inskeep, 1973; Cooper, 1974) has led to the proposal of a system of two treatments 11 days apart giving a more rapid and precise response in terms of LH surge and ovulation (Cooper, 1974; Leaver et al., 1975).

In the majority of animals with a functional corpus luteum there is a rapid decrease of plasma progesterone concentration and the animal is detected in oestrus 3 to 4 days later (Roche, 1974 and Oyedipe et al., 1988).

2.1.2. OESTRUS INDUCTION DURING SUPEROVULATION.

PGF-2 alpha or its analogues are routinely administered to embryo donor cows at 48 to 72 hours after the commencement of folliculogenesis (brought about by gonadotropin stimulation) in a superovulatory regimen. The PGF-2 alpha induced luteal regression leads to a marked decrease in serum progesterone which is essential to permit the final stages of follicular development, the onset of oestrus and ovulation (Elsden *et al.*, 1978; Halley *et al.*, 1979).

The use of synchronizing agents has given a greater and more consistent superovulatory response than simply injecting PMSG in the follicular phase of oestrous cycle (Elsden *et al.*, 1974). Prostaglandin is used to synchronize oestrus by injecting it after a period of stimulation with PMSG (Tervit *et al.*, 1973).

Armstrong (1981) recognized prostaglandins of E and F series as the necessary mediators in the ovulatory process of mammalian species.

Gallo *et al.* (1992) observed that ovulation rate can be enhanced or synchronized by the administration of cloprostenol during late preovulatory period in superovulated heifers.

Only 500 to 1000 µg of PGF-2 alpha analogues (Boland et al., 1986; Saumande and Chupin, 1986; Yadav et al., 1986a; Radhakrishnan and Raman, 1987) are required as compared to 25-50 mg of PGF-2 alpha (Booth et al., 1975; Lopez Barbella et al., 1979; Lubbadeh et al., 1980; Hasler et al., 1983; Donaldson, 1984) given by the intra-muscular route.

PGF-2 alpha or its analogues are given either at 48 hours (Rodrigues and Gregory, 1986; Almeida, 1987; Dinar et al., 1987) or at 60-72 hours (Sreenan and Gosling, 1977; Elsden et al., 1978; Yadav et al., 1986a) after the gonadotropin injection.

Animals, pre-treated with gonadotropins, tend to show early (1 day earlier) and more precise onset of oestrus after PGF-2 alpha administration, than those that are not treated (Tervit et al., 1973; Jensen et al., 1982). This is probably due to a larger population of maturing follicles at the time of PGF-2 alpha - induced luteolysis (Tervit et al., 1973).

The onset of superovulatory oestrus was found to occur between 48 and 72 hours after PGF-2 alpha injection in PMSG-treated heifers (Rajamahendran et al., 1976; Saumande, 1980). Alcivar et al. (1984) reported this interval to be 50.4±2.4 hours in PMSG-treated animals v/s. 59.2±2.2 hours for FSH-p - treated animals.

Following the use of PGF-2 alpha analogues, such as 500 µg of cloprostenol, oestrus occurred at 44.2±1.4 hours (Lindsell et al., 1985) and 41.3±1.2 hours (Yadav et al., 1985) after cloprostenol injection in superovulated dairy and beef heifers, respectively. In crossbred cows this interval was recorded as 47.4±0.5 hours from PGF-2 alpha analogue injection to the onset of superovulatory oestrus (Savage et al., 1987).

2.1.3. POST-SUPEROVULATORY OESTRUS INDUCTION;

Following superovulation and embryo recovery, there is often a delay in return to oestrus of the donor which has been attributed to high progesterone concentration resulting from a large number of corpora lutea (Booth et al., 1975; Betteridge et al., 1982). Such a delay in return to oestrus would mean a failure either to collect more embryos during a short span of time by repeated superovulation (Sacher et al., 1987) or to breed further the animal (Jones et al., 1986; Cowen and Sosnik, 1987).

PGF-2 alpha, with its luteolytic effect, leads to early return of donors to oestrus and thus minimizes the days open for such donor cows (Boland et al., 1986; Dinar et al., 1987).

A higher dose of PGF-2 alpha (60mg) has been recommended to induce oestrus in a significantly large number of superovulated cattle as compared to 30mg or 50mg dose levels (Garcia et al, 1983).

A lower percentage (60 per cent) of animals coming to oestrus was observed following administration of PGF-2 alpha analogue (cloprostenol) immediately after embryo recovery (Dinar et al., 1987). However, a double injection of a PGF-2 alpha analogue (fenprostalene) given at 24 hours interval yielded better results than a single injection to induce luteolysis at the time of embryo recovery (Boland et al., 1986).

2.2. FOLLICULAR DYNAMICS.

2.2.1. OVARIAN FOLLICULAR GROWTH AND DEVELOPMENT.

The primordial follicles are established in the ovary during embryonic development. Of the approx. 150,000 primordial follicles present at birth in heifers (Erickson, 1966), less than 100 will mature and ovulate during the life time of an average animal. The vast majority of follicles present at birth degenerate by a process known as atresia. Follicular atresia has been described for several mammalian species including cows (Rajakoski, 1960; Ireland and Roche, 1983).

During the lifetime of the female, primary follicles enter a pool of growing follicles in response to a stimulus that remains undefined. Follicular development to the antral stage may occur independent of gonadotropin action (Dempsey, 1973), but the rate of pre-antral follicle growth is accelerated by gonadotropins.

The development of large follicles on the ovary is a dynamic process. For eg., large follicles appear on the surface of the ovary, regress and are replaced by other large follicles. Why some follicles regress and others go on to ovulate is not understood. Follicles destined to ovulate probably arise from a pool of growing follicles less than 48 hours before ovulation (Hansel and Convey, 1983).

The failure of hormonal treatments to control follicular development and superovulation in domestic animals gives evidence of significant gaps in our understanding of the mechanisms that regulate the development and function of ovulatory follicles. It is generally believed that the ovulatory follicle is "selected" from a pool of similar-sized follicles because it has some advantage over the others and that it ensures its "dominance" over the other follicles in its cohort by, in some way, actively suppressing their further development (Fortune et al., 1988).

Various theories regarding development of follicles in the bovine ovary have been proposed with some workers postulating follicular development in a wave-pattern whereas others regarding it as a continuous process and stating the existence of follicles of all sizes, including large follicles, on each day of the oestrous cycle.

Rajakoski (1960) postulated that there are two "waves" of follicular growth with follicles ≥ 5mm in diameter during oestrous cycles, one occurring between days 3 and 4 of the cycle and producing a large atretic follicle at around day 12 and the second beginning around day 12 to 14 and culminating in ovulation.

On the other hand, Marion et al. (1968) concluded that the growth of follicles is continuous and constant and that follicles develop to > 10mm independent of the stage of the oestrous cycle.

Choudary et al. (1968), Donaldson and Hansel (1968) and Marion and Gier (1971) also presented evidence against waves of follicular growth stating that follicular growth is continuous and independent from the phases of the cycle.

Swanson et al. (1972) agreed with Rajakoski's theory after noticing similar changes in the number and size of follicles in the bovine ovary.

In other studies that employed dye marking of selected large follicles or cauterization of all follicles > 5mm, the authors concluded that growth and replacement of the largest follicles on the ovary is more rapid at the end of the cycle (Dufour et al., 1972; Matton et al., 1981). They found that the preovulatory follicle was the largest follicle present on each pair of ovaries by 3 days prior to oestrus, but before that, the preovulatory follicle was not consistently the largest follicle (Dufour et al., 1972).

Ireland et al. (1979) suggested that few heifers have follicles in the large range between days 1 to 4 but most have follicles in this range after this time, also suggesting a growth wave. (However, only one follicle in the medium or large range but not both are generally observed per pair of ovaries on any day of the cycle. Perhaps, large viable follicles secrete products (i.e. oestrogens) that inhibit growth of new follicles until a large dominating follicle becomes atretic and disappears.)

The observations of Matton et al. (1981) that as the cycle advances, there is an increase in the rate of replacement of large follicles and an enhancement in the growth rate of medium-sized follicles, could not be explained on the basis of the circulating gonadotropin levels since the levels of FSH and LH do not appear to differ during the early and late stages until day 18. However, the results did not support the concept of two

waves of follicular growth proposed by Rajakoski (1960), but they did support the concept of an intraovarian inhibitory action of the largest follicle on smaller follicles.

Matton et al. (1981) indicated that in cattle follicular growth and atresia of follicles > 6mm diameter were more rapid after day 13 than before day 8 of the cycle. Most large follicles persist on the ovarian surface for at least 5 days between day 3 and 13 of the oestrous cycle. After day 13, most of these large follicles disappeared within a 5 days period and were replaced by new preovulatory small follicles.

Ireland and Roche (1983) determined the development of three large oestrogen-active follicles over the course of the cycle at about 7 day intervals, with only the last follicle successfully ovulating.

Ireland and Roche (1987) hypothesized that during bovine oestrous cycles there are 3 successive phases in which one follicle is selected, becomes dominant, and either ovulates or becomes atretic, depending on plasma progesterone concentrations during its dominance phase.

Savio et al. (1988) concluded that follicular dynamics during the oestrous cycle of the majority of heifers was characterized by the growth of 2 or 3 dominant follicles, that, after variable patterns of growth, reached maximum sizes at comparable stages of the oestrous cycle.

Fortune *et al.* (1988) also found that there are three waves of follicular growth during the oestrous cycle, with the third wave culminating in ovulation. Each wave consists of the development of one follicle to a large size, while other follicles in the cohort fail to enlarge or grow only slightly and gradually regress.

Fortune *et al.* (1991), Roche and Boland (1991) and Taylor and Rajamahendran (1991), with the help of ultrasound imaging, have shown that cattle exhibit 2 or 3 waves of follicular development during an oestrous cycle. Long cycles generally have 3 dominant follicles, the 2nd dominant follicle is the ovulatory follicle.

Thus we see that histological examination, by early investigators, of ovaries obtained at slaughter generated conflicting ideas about the pattern of development of large bovine follicles.

It is now well established from histologic observations, follicle dye-marking techniques, hormone measurements and more recently ultrasonographic examination of ovaries, that follicle growth occurs in waves throughout the oestrous cycle. During each of these waves, several follicles begin to grow with one "dominant" follicle outstripping and suppressing the further growth of the other (subordinate) follicles in its cohort. Only in the last wave of the cycle does the dominant follicle normally ovulate. (Armstrong, 1993).

2.2.2. DAY OF INITIATION OF SUPEROVULATORY TREATMENT

Rajakoski (1960) has reported that the majority of bovine ovarian follicles are in various stages of atresia and that atresia was most rampant during mid-cycle.

Savio *et al*. (1988) analyzed the rates of growth and atresia and suggested that the rates of growth and atresia were slowest during mid-cycle. Their results suggest that the most difficult time to stimulate follicular growth is at mid-cycle, and this observation may have relevance to superovulatory treatments initiated at this time.

Hence, to prevent the follicular atresia and accelerate the growth of medium-sized follicles, superovulatory procedures are initiated with the administration of a gonadotropin during the mid-luteal phase of the cycle (day 8 to 14), followed by an injection of PGF-2 alpha 48 hours later, to induce luteal regression resulting in oestrus and ovulation (Betteridge, 1977; Elsden *et al*., 1978; Monniaux *et al*., 1983; Donaldson, 1984; Agarwal *et al*., 1992).

It is already established that the follicular dynamics of bovine oestrous cycle is characterized by 2 or 3 waves of follicular development, with one large follicle becoming dominant per wave. These waves occur at about 7 day intervals over the course of the cycle with only the last follicle successfully ovulating (Ireland and Roche,

1987; Fortune et al., 1988; Roche and Boland, 1991; Fortune et al., 1991). The dominant follicles are generally present on day 6-7, 14-16, and in the follicular phase of the cycle (Sirois and Fortune, 1988).

2.2.3. INFLUENCE OF DOMINANT FOLLICLE OVER SUPEROVULATORY RESPONSE

Thus, we see that when superovulatory treatment is initiated during the mid-luteal phase of the oestrous cycle (i.e. day 8-14), there is an absence of a dominant follicle on the ovaries.

Pierson and Ginther (1988) suggested that the presence of a large dominant follicle may have a negative effect on the superovulatory response, since the occurrence of a large dominant follicle was also accompanied by selection against other follicles.

Grasso et al. (1989) reported a decreased ovulatory response when superovulation was initiated in the presence of a dominant follicle.

Goulding et al. (1990) observed that the superovulatory treatment initiated on day 2 resulted in a decreased number of ovulations and embryos recovered as compared to day 10 of the oestrous cycle.

Guilbault et al. (1991a) suggested that the presence of a dominant follicle before superovulation may decrease the superovulatory response and/or alter the maturation of the follicles during treatment, especially when emergence of the dominant largest follicle occurs within 3 days of the start of treatment.

Driancourt (1991) and Ko et al. (1991) concluded that a dominant follicle causes regression of its subordinate follicles, and during its growing phase suppresses the emergence of the next wave. Probably, the largest recruited follicle inhibited the FSH support to the other follicles through negative feedback actions of oestradiol and inhibin, or it might be due to a direct suppressive effect exerted through an unknown paracrine mechanism (Driancourt, 1991).

Huhtinen et al. (1992) also related the relatively good responses to superovulation on day 8 to 14 of the oestrous cycle to the absence of a dominant follicle at the time of initiation of gonadotropin treatment.

Gray et al. (1992) found no difference in the yield of transferable or total embryos when treatment with FSH was begun during the period of morphological regression of the dominant follicle compared to control cows in which FSH treatment was begun on day 10 without regard for absence or presence of a dominant follicle.

2.3. GONADOTROPIN PRIMING

Looking at the "wave-pattern" of follicular growth and maturation in the bovine oestrous cycles, Fortune et al. (1988) hoped that these observations might generate ideas about ways to improve superovulation. For eg., perhaps treatment with a gonadotropin (like FSH) during the first wave could increase the number of follicles > 4 mm present during the second wave and application of a superovulating treatment at the beginning of the 2nd wave might then result in a better synchronized group of ovulating follicles.

Cows have both pre-and post-ovulatory peaks of circulating FSH (Dobson, 1978). This has led to several attempts to experimentally modify the post-ovulatory FSH levels (by the administration of FSH post-ovulation) in hopes of increasing the follicular recruitment.

There is conflicting evidence in the literature on the effects of early cycle gonadotropin in cattle. Some reports show evidence of a positive effect of gonadotropin priming (Rajamahendran et al., 1987; Ware et al., 1988; Petr et al., 1990; Touati et al., 1991). In other studies, similar priming treatments either had no significant effect on superovulatory response (Rieger et al., 1988; Gray et al., 1992) or resulted in a significant reduction in ovulation rate and embryo recovery (Grasso et al., 1989; Lussier and Carruthers, 1989; Guilbault et al., 1989; Guilbault et al., 1991b).

Monniaux *et al*. (1983) proposed the use of FSH early in the cycle to stimulate the development of follicles by reducing the rate of atresia.

Moor *et al*. (1984) hypothesized that priming the ovary with gonadotropin to prevent atresia of pre-antral follicles and to stimulate antral follicle development before superovulation may be a beneficial procedure in terms of increasing the ovulatory response.

2.3.1. POSITIVE EFFECT OF PRIMING

Rajamahendran *et al*. (1987) primed one group of donor cows with 2.5 mg of FSH on days 3 and 4 of the oestrous cycle. Superovulation was then carried out in all animals using FSH on days 9-13 of the oestrous cycle. The mean number of excellent and good embryos recovered was greater for the primed animals. It was concluded that administration of low doses of FSH at the beginning of the oestrous cycle increases ovulation rate, number of ova and excellent and good quality embryos recovered.

Ware *et al*. (1988) primed heifers with 10 mg FSH-p on day 2 or 3 of the oestrous cycle. The primed heifers had a higher number of ovulations and a higher number of fertilized ova indicating that there was no significant deterioration in ovum quality due to priming. The results showed that FSH-p priming improved superovulatory efficiency in cattle.

Petr. et al. (1990) primed one group of cow with 200I.U. PMSG on day 4 and superovulated all cows with 2800 I.U. PMSG between days 8 and 12. The PMSG- primed cows had a higher average number of corpora lutea (17.8 v/s. 7.2) and a higher per cent of recovered ova (70.2 v/s. 60.5). The per cent of good quality embryos recovered, though, was not significantly different between the two groups (70.7 and 79.3).

Touati et al. (1991) also reported significantly increased superovulation rates in cows given priming dose of FSH at the beginning of the cycle.

2.3.2. NO EFFECT OF PRIMING

Rieger et al. (1988) observed that pre-treatment with 10mg FSH-p on day 2 of the oestrous cycle had no effect on the total superovulatory response or on the number of transferable embryos collected at day 7 of gestation. It was suggested that FSH - priming early in the cycle may be advantageous in promoting superovulation only when the superovulatory response of the population of animals is otherwise weak.

Gray et al. (1992) also found no beneficial effect upon priming the cows with 5mg FSH-P on day 2 and 3 of the oestrous cycle and concluded that priming was not an effective means for improving the superovulatory response in cattle.

2.3.3. NEGATIVE EFFECT OF PRIMING

The studies of Grasso *et al*. (1989) showed that FSH-priming resulted in a significant reduction in ovulation rate and embryo recovery and hence a decreased superovulatory response.

Lussier and Carruthers (1989) injected cows twice daily with FSH-P from day 1 to 6 and then initiated superovulation and found that pre-treatment with FSH-P decreased the superovulatory response. It was suggested that the mechanism for this would appear to be associated with reduced endogenous FSH prior to the start of superovulation.

Guilbault *et al*. (1989) concluded that priming the heifers with 10mg FSH-P on day 3 of the oestrous cycle decreased the superovulatory response and this was associated with the presence of a higher number of follicles ≥ 7mm prior to initiation of the superovulation treatment.

Guilbault *et al*. (1989) suggested that priming with FSH-P at the beginning of the oestrous cycle altered the profile of endogenous FSH concentrations prior and during the superovulation treatment and this reflected, to some extent, the alterations in development of large follicles. Presence of large follicles and/or their effect on endogenous FSH concentrations may be associated with the reduced superovulatory response in FSH-primed heifers.

Guilbault et al. (1991b) again observed decreased superovulatory responses when priming was done with 10mg FSH on day 3 of the oestrous cycle. It was concluded that assessment of follicular status prior to superovulation may be important for the improvement of superovulatory response in cattle.

DISCUSSION

The failure of FSH priming to increase ovarian response to a subsequent superovulatory treatment may have been due to inappropriate time interval between the priming and superovulatory treatment (Armstrong, 1993).

Priming with FSH has been reported to cause a delay in the development of the dominant follicle (Grasso et al., 1989). In addition, large follicle has been associated with a reduced superovulatory response (Grasso et al., 1989).

In the study of Gray et al. (1992), in which no effect of FSH priming was observed, the dominant follicle in the FSH primed animals reached maximum diameter 1.5 days earlier than in controls, and may have lost its dominating capabilities by the time the superovulatory treatment was begun.

In contrast, in the studies of Grasso et al. (1989), in which FSH priming decreased the superovulatory response, the development of the dominant follicle was

delayed by 2 days as a result of FSH priming. The delayed regression of the dominant follicle probably resulted in suppression of subordinate follicles present when the superovulatory treatment was begun, resulting in fewer follicles responding to the superovulatory treatment.

Another obvious explanation is the probable variation in LH content in the product used. Unfortunately, the FSH used to prime cattle also contains LH, and this has been implicated as a cause of early ovulations (Callesen et al., 1987). Touati et al. (1991) collected significantly more embryos as well as transferable embryos when the FSH priming product was relatively free of LH. Any beneficial effects of FSH priming would appear to be contingent upon the use of an FSH product relatively free of LH (Touati et al., 1991).

Gray et al. (1992) suggested several possible explanations for the differences between results of priming in various studies:

1. The FSH dose for superovulation varied from 28 to 38mg.
2. The initial day for the superovulatory treatment varied from 8 to day 16.
3. The priming dose of FSH varied from 2.5mg on day 3 and 4 to 10mg on either day 2 or day 3.

(Rajamahendran et al., 1987; Ware et al., 1988; Rieger et al., 1988; Grasso et al., 1989).

2.4. SUPEROVULATION

Superovulation can be defined as the increased ovulatory response, above the number that would be expected to occur naturally, generated in an animal by the administration of exogenous gonadotropic hormones. Its objective is to increase the number of ova released by an animal, and thereby the potential number of embryos. In monotocous animal like cattle, three or more than three ovulations are considered as superovulation (Betteridge, 1980).

Superovulation is a key element of embryo transfer in cattle since it increases the number of follicles to reach maturity. But despite the fact that over 40 years have elapsed since the birth of the first calf by embryo transfer, superovulation still remains a major limiting factor in the contributions that embryo transfer can make to the genetic improvement of cattle. The unpredictability of response causes severe logistic problems which contribute to the high cost of embryo production in conventional embryo transfer programmes (Armstrong, 1993).

The superovulatory response is extremely variable and is influenced by the genetic make-up, the number of antral follicles capable of responding to gonadotropins, the stimulatory agent used, its biological activity, mode of injection and the time of oestrous cycle at which superovulatory treatment is begun (Mapletoft, 1984; Boland *et al.*, 1991).

The ovarian status of the donor at the time of hormone treatment appears to be a major determinant of the superovulatory response (Monniaux et al., 1983) and it has been the subject of much recent research aimed at improving superovulation success.

The conclusion seems inescapable that animal variability is at least as important a determinant of superovulatory response as is the particular hormone product used, provided that an effective (optimal) dose is used (Armstrong, 1993). Other sources of variability are the stage of the oestrous cycle when treatment is begun, gonadotropin priming, presence or absence of a dominant follicle, success of manipulation of the follicular wave system to modify the status of the dominant follicle and to increase the recruitment from pre-antral follicle pool (Armstrong, 1993).

For superovulation, either follicle stimulating hormone (FSH) or pregnant mare serum gonadotropin (PMSG) is administered. Injection of either gonadotropin appears to be effective in reducing atresia of smaller follicles (Boland et al., 1991).

PMSG is a glycoprotein molecule which exhibits both FSH and LH-like activity. The high n-acetyl-neuraminic acid content of the PMSG molecule confers upon it a half-life of about 5 days (Bevers and Dieleman, 1987) which greatly exceeds that of LH or FSH (30 minutes and 110 minutes for LH and FSH respectively) (Moor et al., 1984).

The long half-life of PMSG in cows leads to residual PMSG after the primary superovulatory stimulation which is supposed to adversely affect the number of transferable embryos (Bevers and Dieleman, 1987; Dieleman et al., 1993).

There is variability in both FSH and LH activity of PMSG not only among pregnant mares, but also between bleedings in the same mare taken at different times during gestation (Gonzalez et al., 1978).

Experiments in the cow suggest that PMSG preparations with high FSH activity relative to LH produced higher superovulatory response than when FSH/LH ratio was low (Humphrey et al., 1979).

Commercial FSH has been shown to vary between lots in both FSH and LH activity (Chupin et al., 1984). Data of Murphy et al. (1984) show a reduced ovulatory response as the ratio of LH/FSH increased. [Probably the elevated LH can disrupt the balance of androgen and oestrogen production necessary for prevention of atresia in follicles. Alternatively, LH may induce down-regulation of its receptors on theca and/or granulosa cells (Murphy et al., 1984)].

However, if there is too much LH present, either premature ovulation or luteinization of FSH-stimulated follicles can occur. Both FSH and PMSG can cause premature ovulation (Callesen et al., 1986 and 1987) which may be

responsible for the failure of prostaglandin F2 alpha to induce complete luteolysis and may be associated with a failure of an oestradiol and consequently failure of LH surges (Callesen et al., 1986).

Human menopausal gonadotropin (hMG) has been occasionally used for the purpose of superovulation, as also certain pituitary extracts as well as bovine follicular fluid (BFF).

2.4.1. SUPEROVULATION USING PMSG

Hafez et al. (1963) superovulated heifers with 3000 or 5000 I.U. PMSG in one, two, or three doses, administered on day 12,14,16, or 18 of oestrous cycle, with or without enucleation of C.L.; 2000 I.U. HCG were given 3,4,5,6, or 7 days after the first PMS injection. Response was greater in animals that underwent C.L. enucleation. No advantage was obtained by initiating PMS treatment earlier than the 16th day after oestrus nor by dividing 3000 I.U. of the gonadotropin in 2 or 3 daily doses. The total number of follicles developed was higher at 5000 I.U. but there was a substantial increase in per cent of luteinized and haemorrhagic follicles. The higher dose of PMS (5000 I.U.) did not adversely affect the proportion of ova that were either recovered or fertilized.

Schwartz and Shelby (1969) induced multiple ovulations in heifers using (Gp.I) 1500 I.U. PMS on day 5 and 2000 I.U. PMS on day 16; and (Gp.II) 1500 I.U. PMS on day 16. The animals received 1000 I.U. HCG I/V at the first sign of oestrus and A.I. was performed. The average number of ovlations per heifer for each treatment group were (Gp.I) 6.56 and (Gp.II) 4.72 respectively.

Becker and Pinheiro (1986) compared the response of Nelore cows to superovulation using either FSH-P or PMSG. PMSG, given in doses of 1000 and 2000 I.U., proved to be more effective in inducing superovulation since it was administered as a single dose. They suggested that avoiding stress in handling is essential for a good response to hormonal stimulation by Zebu cattle.

Saumande and Chupin (1986) observed that while a large dose of PMSG (over 3000 I.U.) increases the number of ovulations, the quality of embryo decreases, therefore the number of transferable embryos is not improved. The inhibition observed with the largest doses reflects the absence of the preovulatory LH discharge or its effect.

Schmitz (1987) superovulated cows on day 10 of the cycle using FSH/LH and PMSG (2500 I.U.). 500 µg cloprostenol was given 48 hours after beginning the hormone treatment. After induction of oestrus, inseminations were carried out at 12 hours intervals until the end of oestrus. Embryo recovery took place 7 days

after A.I. The average number of C.L. was significantly higher for FSH/LH treatment than for PMSG (9.3±3.6 v/s. 6.8±3.9), while the embryo recovery rate was the same (53 per cent). The average number of embryos recovered was 4.9±5.0 v/s. 3.6±4.4. Embryo quality was not significantly affected by hormone or sequence of treatment.

Elecko *et al*. (1988) treated cows with 2000 or 3000 I.U. PMSG and 0.5 mg cloprostenol 48 hours later. Insemination was carried out 72,84 and 96 hours after treatment. Embryos were recovered by flushing 7 days after A.I.. No embryos were recovered from cows that had been given 2000 I.U. PMSG, and 8 embryos were recovered from 8 cows each given 3000 I.U. PMSG. Of these, 4 embryos were judged as suitable for transfer. Weight of ovaries averaged 17.3 and 40.7g for cows given the two doses of PMSG respectively, the difference being significant.

Kim *et al*. (1988a) injected heifers with 1000 or 2000 I.U. PMSG, daily for 3 days. In the two groups, the number of C.L. averaged 3.2 and 9.2, number of embryos recovered 2.3 and 6.0 and embryo recovery rate (i.e. number of embryos per number of C.L.) 73.6 and 70.1 per cent respectively.

Caral *et al*. (1989b) superovulated cows and heifers using 2000-2500 I.U. PMSG on days 8-14 of the oestrous cycle (Gp.1) or immediately after the presence of

a C.L. determined by means of rectal palpation (Gp.2), followed by an injection of 500 µg PGF-2 alpha in both groups. The number of embryos recovered averaged 2.6 and 1.5 respectively for cows in groups 1 and 2 v/s. 5.0 and 4.3 for heifers; the per cent of transferable embryos was 62 and 52.9 respectively for cows in groups 1 and 2 v/s. 80.0 and 79.7 for heifers.

Caral et al. (1989a) superovulated 25 heifers by means of an injection of 1500-2000 I.U. PMSG on day 8-12 of natural oestrus, followed by 500 µg PGF-2 alpha 48 hours later. The number of C.L. and embryos per female averaged 7.4 and 4.6 respectively v/s. 7.9 and 4.7 respectively for 25 heifers receiving the same treatment during an induced oestrus.

Chung et al. (1989) superovulated cows with 2000 I.U. PMSG, followed 48 hours later by 25mg PGF-2 alpha. The number of C.L. on day 6-9 of the cycle averaged 8.8 and the rate of normal embryo recovery was 60.4 per cent.

Fuente et al. (1989) injected cows with 40-48mg FSH or 1500-2500 I.U. PMSG, followed by 0.5mg of a PGF-2 alpha analogue 48 hours later. All females were inseminated with frozen semen 12+24 hours after the onset of oestrus. The number of embryos averaged 7.8±5.9 and 3.3±2.5 respectively of which 5.4±5.0 and 2.1±1.0 were viable, and the overall production of embryos of treated females in the two groups averaged 6.8±6.2 and 2.4±2.0 ($P < 0.001$).

Jordt and Lorenzini (1989) observed that in cows superovulated with PMSG, a total of 4 embryos and an average of 4.1±0.3 ova per donor resulted; whereas cows superovulated with FSH produced an average of 3.7±0.4 embryos.

Kadu *et al.* (1989) conducted a trial in which superovulation was induced with FSH and PMSG + HCG in crossbred cows. Embryos were collected on 7th day after oestrus. The mean number of C.L. was 12±1. The mean 8.33 embryos were collected, of which 0.66 were morphologically normal and transferable and 7.66 were unfertilized.

Budevich *et al.* (1990) conducted superovulation using 2500 I.U. PMSG (Folligon) followed by 0.5 mg cloprostenol 48 hours later, and a triple insemination, with a 12 hours interval between inseminations. Of the treated cows, 30.4 per cent did not give a superovulatory response (averaging 0-2 ovulations). The production of transferable embryos per donor averaged 3.9.

O'Farrell and Hartigan (1990) superovulated normal and repeat breeding cows using 2400-3000 I.U. PMSG and non-surgical embryo recovery was attempted after double insemination. An average of 2.07 and 1.65 of embryos from normal and repeat breeding cows respectively ($P < 0.01$) were considered viable.

Rodrigues et al. (1990) collected embryos from Nelore, Indo-Brazilian and Gir cows treated with a single dose of 2500 I.U. PMSG or 25-40 mg FSH+progesterone+PGF-2 alpha. Cows were inseminated 3 times, 10,20 and 30 hours after first standing oestrus. The number of viable embryos collected per cow on day 7 after oestrus averaged 4.11, 5.8 and 3.09 for the three breeds respectively.

Horiuchi et al. (1991) superovulated 4 Japanese Black cows by injection of 2000 I.U. PMSG followed 72 hours later by 750 µg PGF-2 alpha and 200 µg LHRH. The number of C.L. averaged 22.5±5.3 (16-29), the number of ova recovered 20.5±5.0 (15-27), and number of embryos recovered 10.5±5.8 (5-16).

Manickam et al. (1991) injected 11 cows with 2000 I.U. PMSG and 25 mg PGF-2 alpha on days 10-13 of the oestrous cycle. Nine cows had > 4 C.L. and 1-4 embryos were recovered non-surgically from 4 cows.

Reichenbach (1991) obtained DNA embryos suitable for gene microinjection using 2000, 2500 or 3000 I.U. PMSG for superovulating heifers and cows. Treatment with 3000 I.U. PMSG produced the highest ovulation rate, but higher fertilization rates were obtained with 2500 I.U..

Subramaniam et al. (1991a) treated 31 crossbred cows with PMSG and PGF-2 alpha for superovulation. From the 19 cows responding to treatment, 45 embryos were recovered non-surgically.

Sergeev et al. (1991) superovulated cows using FSH (p FSH from USA, Follitropin from USSR, Grofolon from USSR and Follicotropin form Czechoslovakia) or PMSG (Folligon). All cows were synchronized using the same cloprostenol preparation. For the 5 treatments, the per cent that superovulated was 85.9, 78.3, 100, 80 and 86.7 per cent respectively, the number of ovulations per donor 11.5, 8.9, 9.3, 7.7 and 11.2, the per cent of donors from which embryos were recovered 85.6, 77.6, 83.3, 81.2 and 84.6, the number of embryos per donor 7.2, 6.2, 7.6, 5.7 and 6.7 and the number of good quality embryos 5.4, 3.1, 2.6, 4.5 and 3.3.

Thomas et al. (1991) carried out superovulation in 10 (normally cycling) crossbred culls with 2000-2500 I.U. PMSG on day 10 of oestrous cycle, and with 0.625-0.75 mg PGF-2 alpha 48 hours later. Cows were inseminated thrice, 3,12 and 24 hours after oestrus. 12 embryos were recovered from 3 cows by uterine flushing 7 days after A.I.. Embryos were not recovered from the remaining cows.

Mishra et al. (1992) reported superovulation in Holstein Friesian (HF) and Jersey crosses with 3000 and 2500 I.U. PMSG (Folligon) respectively. 30mg lupristiol (Prosolvin) was injected intra-muscularly at 48 and 60 hours after PMSG injection in 2 equal doses. Donors were inseminated thrice at 12 hours intervals following onset of oestrus. Non-surgical embryo collection, evaluation,

freezing, thawing and transfers were made. (Jersey crosses showed better performance than HF in terms of superovulatory response, total and viable embryos).

Pawshe et al. (1992) superovulated cows and heifers on day 11 of oestrus with FSH-P (32mg) or PMSG (@ 2500 I.U.). 50mg Dinofertin intra-muscularly was given in both the groups. 1200 I.U. HCG was given intra-muscularly at the time of A.I.. No significant difference was observed in the onset or length of oestrus in either group. All experimental animals showed intense oestrus. The number of C.L. and anovulatory follicles was 10.57±1.90 and 2.70±0.42 in FSH-P group, and 10.42±2.37 and 4.0±0.30 in PMSG group respectively. The average number of C.L. and anovulatory follicles in cows was significantly higher ($P<0.01$) than in heifers.

2.4.2. SUPEROVULATION USING FSH

FSH is a glycoprotein molecule composed of two subunits - alpha and beta. The beta subunit is species specific and is responsible for the biological activity (Papkoff, 1977). FSH has less sialic acid content than PMSG which accounts for its shorter biological half-life, about 110 minutes to approx. 4 to 5 hours (Laster, 1972; Schams et al., 1978; Moor et al., 1984). Hence, a relatively continuous application of the hormone, such as once or twice daily injection for 4 to 5 days, is required for maintaining sufficient concentration of the hormone in the body to produce superovulation in bovine (Betteridge, 1977).

Commercial FSH is infact a mixture of FSH and LH and these products vary between preparations and between lots of a given preparation in both FSH and LH activity (Chupin et al., 1984; Murphy et al. 1984; Shea et al., 1984). Excess of LH causes premature ovulation (Moor et al., 1984; Callesen et al., 1987) so that the oocyte is not capable of being fertilized at the normal time (Moor et al., 1984) and this problem cannot be overcome even by multiple inseminations (Donaldson, 1985) leading to reduced recovery of transferable embryos (Donaldson and Ward, 1986).

To reduce the frequency of premature oocyte activation, use of purer FSH preparations has been advocated (Murphy et al., 1984; Donaldson and Ward, 1985; Foote and Ellington, 1988).

A FSH to LH ratio of 5:1 caused only a non-significant decline in the number of ovulations (Murphy et al., 1984) whereas a ratio of 10:1 gave an optimum superovulatory response (Chupin et al., 1984).

Although an increased dose of FSH-P leads to increased recruitment and development of the small antral follicles, it causes reduced embryo recovery possibly due to overstimulation of ovaries (Donaldson, 1984). So, in order to maximize the superovulatory response, a dose of FSH-P ranging from 32 to 50mg has been used (Donaldson, 1984; Kweon et al., 1987; Rajamahendran et al., 1987;

Totey et al., 1988b; Hutter and Cabodevila, 1990; Hutter et al., 1990).

FSH-P has been used as a single injection per day (Smith et al., 1973; Lubbadeh et al., 1980; Becker and Pinheiro, 1986) or double injection of morning and evening (Halley et al., 1979; Walton and Stubbings, 1986; Rieger et al., 1988; Goulding et al., 1991) or three injections per day (Monniaux et al., 1983).

Various dose schedules have been employed using FSH-P in a superovulatory regimen, viz., an increasing dose schedule (Critser et al., 1980), a constant dose schedule (Becker and Pinheiro, 1986; Walton and Stubbings, 1986; Khilkevich and Ovchinnikov, 1988; Kuchukhidze, 1988; Eckery et al., 1990; Stubbings et al., 1990) or a decreasing dose schedule (Savage et al., 1987; Boland et al., 1988; Guilbault et al., 1989; Mapletoft et al., 1990; Slimane and Quali, 1991; Pawshe et al., 1992; Totey et al., 1992). A decreasing dose schedule increased ovulation rate and the number of good quality embryos in comparison to constant or increasing dose (Monniaux et al., 1983).

The FSH-P dose schedule has been used for 3 days (Walton and Stubbings, 1986; Rajamahendran et al., 1987; Kojima et al., 1990; Gray et al., 1992) or 4 days (Totey et al., 1988a; Eckery et al., 1990; Slimane and Quali, 1991; Pawshe et al., 1992) or 5 days (Afanasev, 1988;

Kuchukhidze, 1988; Ramakrishna and Ramachandraiah, 1989; Hutter et al., 1990; Breuel et al., 1991). The per cent of transferable embryos was significantly higher in cows given 5 days treatment than the 4 days treatment group (Donaldson, 1984). The 3 days and 4 days treatments varied only in the yield of viable embryos (Walton and Stubbings, 1986).

Seidel et al. (1968) found no differences between FSH and PMSG whereas Becker and Pinheiro (1986) found PMSG to be more effective in a superovulation programme than FSH.

Schmitz (1987) and Slimane and Quali (1991) observed that ovulation rate was higher for FSH than for PMSG while the embryo recovery rate was the same.

Critser et al. (1980), Sergeev et al. (1991) and Pawshe et al. (1992) found no difference between superovulatory treatments with FSH or PMSG, in terms of ovulatory response and embryo recovery.

A higher ovulatory response followed by a higher number of quality embryos may be obtained following the use of FSH, compared to PMSG (Elsden et al., 1978; Aslanov, 1989; Fuente et al., 1989; Refsdal et al., 1989; Riha, 1989; Becker et al., 1990; Unal, 1990; Goulding et al., 1991; Rommel and Rehbock, 1991; Sarvaiya et al., 1992).

2.4.3. USE OF PMSG-ANTISERA

For superovulation, PMSG has been the most universally used gonadotropin since it is readily available, cheap and convenient to administer requiring only a single injection (Seidel et al., 1968). Due to the long half-life of PMSG (about 5 days: Schams et al., 1978), superovulation with PMSG induces a second wave of follicles after ovulation of the first (Booth et al., 1975; Saumande, 1980) causing high concentrations of oestradiol in the peripheral blood during early embryonic development (Bouters et al., 1983).

The increased post-ovulatory oestrogen secretion may result from either the rescue of follicles in early atresia (Monniaux et al., 1984) or the growth and maturation of preantral or small antral follicles (Wise et al., 1986) or both mechanisms (Monniaux et al., 1984).

This increase in circulating concentrations of oestrogen may reduce gamete transport and embryonic survival (Booth et al., 1975; Betteridge, 1977).

To overcome the problem of ovarian hyperstimulation caused by the residual PMSG, various workers have used anti-PMSG or neutra-PMSG at various stages of the superovulatory regimen. This has led to variable results:

(a) No beneficial effect of anti- PMSG was reported by Greve et al., 1988; Kim et al., 1988b; Zeitoun et al., 1988; Callesen et al., 1989; Alfuraiji et al., 1990; Callesen et al., 1990; Bruijn and Booman, 1991; Zeitoun et al., 1991.

(b) Positive effect of anti- PMSG was reported by Dieleman et al., 1987; Wang et al., 1987; Wang et al., 1988; Dieleman et al., 1989; Gielen et al., 1990.

Administration of polyclonal anti-PMSG sera, produced in turkeys (Dhondt et al., 1978), sheep (Moyaert et al., 1985; Wang et al., 1987) or goats (Kummer et al., 1980) to PMSG- treated cows at oestrus shortened the duration of oestrus (Dhondt et al., 1978; Moyaert et al., 1985), increased ovulation rate (Dhondt et al., 1978; Kummer et al., 1980; Wang et al., 1987), increased fertilization rate (Dhondt et al., 1978; Moyaert et al., 1985) and decreased the number of large anovulatory follicles (Kummer et al., 1980; Moyaert et al., 1985; Wang et al., 1987).

Similar results were obtained with a monoclonal antibody to PMSG (Moyaert et al., 1985; Kim et al., 1987; Saumande and Chupin, 1987; Wang et al., 1988; Dieleman et al., 1989).

However, administration of anti-PMSG at 12 or 24 hours after oestrus did not increase ovulation rate or embryo (ova) collection but increased per cent of transferable embryos (Saumande et al., 1984).

The variability of the effects observed by the use of anti-PMSG is due mainly to the time at which anti-PMSG is administered. If it is administered before the onset of the preovulatory LH surge, it exerts an adverse effect over the superovulatory response (Kim et al., 1987; Callesen et al., 1990); whereas neutralization of PMSG shortly after the preovulatory LH peak synchronizes final follicular maturation and shortens the period of multiple ovulations, resulting in a two-fold increase of the ovulation rate (Dieleman et al., 1987; Dieleman and Bevers, 1987).

Dieleman et al. (1993) stated that the outcome of applying anti-PMSG depends upon:

(a) The timing of the injection of anti-PMSG, and

(b) The variability in primary response of the cows.

It was concluded that PMSG anti-PMSG administration in cattle is a simple superovulatory treatment especially when used for repeated superovulation. Its efficiency could be improved by determining or controlling the preovulatory LH peak (Dieleman et al., 1993).

2.4.4. INCORPORATION OF GnRH OR ITS ANALOGUE IN A SUPEROVULATORY REGIMEN

It has been found that some superovulated dairy cows present abnormal LH surges, and that this has a detrimental effect on both ovulation and fertilization rates (Greve et al., 1984a).

Superovulation alters the endocrine profile (Saumande, 1980; Yadav et al., 1986a) and probably shifts qualitatively and quantitatively intrafollicular events (Moor et al., 1984; Callesen et al., 1986; Hyttel et al., 1986).

Gonadotropin releasing hormone (GnRH) plays an important role in regulating release of gonadotropins, particularly through its own pulsatile release (Peters et al., 1985).

Nawito et al. (1977) reported that a GnRH analogue, Buserelin, was a highly potent releasing factor for gonadotropins, which tended to prolong the LH surge.

Griffin and Randel (1978) observed a lack of adequate gonadotropic stimulus from the pituitary gland of Brahman cattle which implied that the pituitary of the Brahman cow is less able to respond to hypothalamic releasing hormones.

Becker and Pinheiro (1986) noticed a large per cent of degenerated embryos in superovulated Nelore cattle and suggested that this was possibly due to endocrine imbalances in the periovulatory period leading to asynchronous ovulations. They also found 11.9 per cent luteinized follicular cysts.

LH release is pulsatile. The pulsatile administration of Buserelin might be more effective (Voss et al., 1989) than GnRH which is a pulse generator in vivo (Peters et al., 1985).

Roussel et al. (1988) suggested that a possible effect of GnRH is on the time of ovulation, which may affect the LH surge and thus aid in the development of more viable corpora lutea which may in turn increase the progesterone levels thereby increasing early embryonic survival rates (Lee et al., 1983; Stevenson et al., 1984; Phatak et al., 1986).

These results indicate that GnRH could have a beneficial effect in Zebu cattle.

Many studies have been conducted to evaluate the utilization of GnRH as part of a superovulatory regimen for cattle, with GnRH or one of its analogues being administered at or close to the time of insemination. This has led to variable results.

(a) A beneficial effect of GnRH or its analogue was reported by Savage and Mapletoft, 1984; Wubishet et al., 1986; Savage et al., 1987; Foote et al., 1989.

(b) No beneficial effect of GnRH or its analogue was reported by Delgado et al., 1989; Voss et al., 1989; Posadas et al., 1991.

(a) Positive effect:

Savage and Mapletoft (1984) found that injection of GnRH at the end of a superovulatory regimen in crossbred cows resulted in a significant increase in the total number of embryos recovered and in a tendency for increased number of fertilized ova and transferable embryos.

Wubishet et al. (1986) found increases in the fertilization rate and in the number of developing embryos when GnRH was used at the end of a superovulatory regimen in cattle.

Savage et al. (1987) administered GnRH following PGF-2 alpha in a superovulation programme which resulted in a small but significant increase in ova-embryos recovered.

Foote et al. (1989) also reported an increase in the yield of embryos when they used Buserelin, a GnRH analogue, in a superovulatory regimen.

(b) No Effect

Delgado et al. (1989) reported that GnRH treatment did not affect the number of palpable corpora lutea, follicles, total number of ova or transferable embryos. Cows given GnRH had fewer palpable corpora lutea than control cows but this difference was not seen in heifers. Also, GnRH seemed to depress

ovulation rate in donors not previously superovulated, but this effect was not observed with subsequent superovulation.

Voss *et al.* (1989) observed no significant increase in the embryo recovery rate upon administration of Buserelin, a GnRH analogue, in a superovulatory regimen.

Posadas *et al.* (1991) evaluated the use of GnRH as part of a superovulatory regimen for Zebu cattle and said that incorporation of GnRH in superovulatory treatment for Zebu cattle does not improve the results of such treatment. They concluded that the endocrine imbalances found by Greve *et al.* (1984a) in superovulated *Bos taurus* cows, or those suggested by the ovarian findings of Becker and Pinheiro (1986), cannot be easily resolved by the addition of ovulatory drugs.

2.4.5. USE OF HUMAN MENOPAUSAL GONADOTROPIN (hMG)

Attempts have been made to control the negative effects of PMSG (viz. aberrations in follicular development and ovarian hyperstimulation) by administering anti-PMSG antibodies or by combining another gonadotropin such as hMG into the treatment.

hMG is a highly purified standardized biological product which, following administration to animals, shows the disappearance of hLH and hFSH as best described by an equation composed of three exponential terms (Lauria et al., 1982).

The very short half-life relative to the first component (13.8 to 20 minutes for hLH and 21.3 to 24.5 minutes for hFSH) probably reflects distribution in the circulation. The second (130 to 164 minutes for hLH and 170 to 200 for hFSH) and third components (875 to 1020 minutes for hLH and 1075 to 1090 minutes for hFSH) can equate to the half-life in cattle (Lauria et al., 1982).

Lauria et al. (1982) used hMG in small numbers of animals and showed a response similar to the use of PMSG. However, a dose response trial using hMG gave an equivalent yield of embryos to that using pFSH, albeit with small numbers of donors (Mc Gowan et al., 1985). There was a considerable number of unovulated follicles present at embryo recovery in the groups treated with the highest dose level of hMG.

Bono et al. (1991) compared the effects of different superovulation regimens using PMSG and hMG, administered either single or in combination. The treatment with PMSG or hMG both produced a large increase of oestrogen concentration (an intense steroidogenic response). The PMSG + hMG treatment led to a better

controlled steroidogenic response although less intense steroidogenesis was observed. The PMSG+hMG treatment was characterized by a lower ova production and lower variation and resulted in a higher per cent of transferable ova.

Furthermore, PMSG treatment revealed increases in ovary volume to the size of an orange, irregularities in return to normal cyclic activity and a number of cases of ovarian cysts. Hence, PMSG/hMG treatment was recommended as an alternate to the very expensive treatment using hMG alone (Bono et al., 1991).

2.5 ESTIMATION OF OVARIAN RESPONSE

2.5.1. DETERMINATION OF THE NUMBER OF LUTEAL AND FOLLICULAR STRUCTURES.

Superovulatory response in cows is generally estimated by studying the number of corpora lutea (C.L.) and large unovulated follicles on day 7 or day 8 of the cycle by rectal palpation of the ovary (Plasse et al., 1968; Seidel et al., 1968; Becker and Pinheiro, 1986; Walton and Stubbings, 1986; Rajamahendran et al., 1987; Savage et al., 1987; Rieger et al., 1988; Totey et al., 1988a; Mehmood et al., 1991; Posadas et al., 1991). Less frequently, endoscopy (Wishart and Snowball, 1973; Bernard et al., 1983) or direct observation via laparotomy (Shea et al., 1984; Hay et al., 1990) has also been used.

Corpora lutea have been reported to be more difficult to detect by palpation per rectum in Bos indicus females than in Bos taurus females (Plasse et al., 1968).

In most cases, ovarian responses are assured by rectal palpation, but the technique is subject to error and can only be considered an estimate (Guay and Bedoya, 1981; Monniaux et al., 1983) since many structures do not exhibit prominences on the external surface of the ovaries or even simulate a superimposition (Becker and Pinheiro, 1986).

The precision of the estimation of the number of C.L.by rectal palpation decreases when ovulation rate exceeds 4-5 per ovary leading, therefore, to an underestimation of the luteal numbers (Barnes et al., 1982; Walton and Stubbings, 1986; Rajamahendran et al., 1987) and is totally inadequate when more than 10 C.L. are present on the ovaries (Monniaux et al., 1983).

However, Savage et al. (1987) found that the mean number of C.L palpable per rectum was highly correlated with mean number of C.L. at post-mortem, mean number of embryos collected and mean number of fertilized and transferable embryos.

2.5.2. HORMONE ASSAY

Different hormonal assays have been attempted by various scientists in order to have an estimate of the ovarian response (as shown below) :

Sr. No.	Hormone assayed	Reference
1	Only E2	Alfuraiji *et al*., 1990
		Callesen *et al*., 1990
2	Only P4	Foote *et al*., 1989
		Bolzoni *et al*., 1991
3	E2 and P4	Khilkevich & Ovchinnikov, 1988
		Mehmood *et al*., 1991
		Savio *et al*., 1991
4	Only LH	Kanitz *et al*., 1990
5	LH and P4	Sprague *et al*., 1971
		Walton and Stubbings, 1986
		Yadav *et al*., 1986a
6	LH, E2 and P4	Voss *et al*., 1986
		Bevers and Dieleman, 1987
		Callesen *et al*., 1987
		Dieleman *et al*., 1987
		Armas *et al*., 1988
		Goto *et al*., 1988
7	LH, FSH, E2 & P4	Kaneko *et al*., 1989
		Ezoe *et al*., 1991
		Kaneko *et al*., 1991(a)
		Kaneko *et al*., 1991(b)

It is well known that the hypothalamic and pituitary hormones are released in bursts (Gonzalez-Padilla *et al*., 1975). The LH and FSH are released in episodes or pulses with an hourly frequency occuring

throughout the ovarian cycle (Schallenberger et al., 1984; Walters and Schallenberger, 1984; Walters et al., 1984).

Rahe et al. (1980) indicated that the LH surge is composed of extremely large pulses of high frequency (upto 1 pulse per 15 to 20 minutes).

Yadav et al. (1986a) also reported that LH release was pulsatile during all periods of oestrous cycle and that the pulse frequency varied from 1 to 2 pulses per hour.

Plasma oestradiol-17β concentrations are low for most of the oestrous cycle, but rise during the preovulatory period reaching a peak at, or just prior to, the onset of oestrus (Peters, 1985).

The preovulatory rise in oestradiol concentrations stimulates the LH and FSH preovulatory surges. The highest oestradiol concentrations were present 6 to 8 hours before the onset of the LH surge (Walters and Schallenberger, 1984).

In the cow, sampling of blood from the posterior vena cava, a source close to ovarian venous outflow, showed a close relationship between LH and oestradiol-17β pulsatile release (Schallenberger et al., 1984; Walters and Schallenberger, 1984; Walters et al., 1984).

LH and oestradiol-17ß are temporally related (Dieleman et al., 1986; Helmer & Britt, 1987) because oestradiol-17ß induces the preovulatory surge of LH and the rise in LH causes the decline in oestradiol-17ß secretion from the dominant follicle (Dieleman et al., 1986).

Concentration of progesterone rises from about day 4 of the cycle to a peak between days 8 and 16, then decreasing to baseline before the next oestrus and ovulation. Progesterone is also secreted in a pulsatile manner, pulses coinciding during the luteal phase with those of FSH (Walters et al., 1984) rather than with LH.

Pulses of progesterone occur as a direct result of FSH pulses and pulses of oestradiol occur as a result of LH stimulation (Schallenberger et al., 1984; Walters and Schallenberger, 1984; Walters et al., 1984).

Bevers and Dieleman (1987) observed no LH surge in 16.7% of the superovulated heifers. This failure of the LH surge after superovulation did not appear to be caused by significantly different plasma concentrations of cortisol, prolactin or PMSG, compared with those of cows responding with an LH surge.

Kaneko et al. (1989) observed that in 2 of the 4 superovulated cows, the LH peaks were suppressed to 67 and 68% of the normal levels.

There was no correlation between plasma progesterone content at the onset of superovulation and ovulation rate (determined by rectal palpation) or the number of viable embryos flushed (Walton and Stubbings, 1986).

The oestradiol concentration during the preovulatory LH surge and the number of preovulatory follicles were correlated (0.73; $P<0.01$) (Bevers and Dieleman, 1987).

Peak concentrations of oestradiol-17β and progesterone were significantly correlated with the number of corpora lutea at slaughter (0.76 and 0.71 respectively), the concentration of oestradiol-17β was correlated with that of LH (0.78) and LH concentration was significantly correlated with the number of ovulations (0.84) (Armas *et al.*, 1988).

The number of normal embryos recovered was correlated with the plasma oestradiol concentration and plasma LH concentration at oestrus (0.56 and 0.66 respectively, both $P<0.001$) and with the plasma progesterone concentration on the day of embryo collection (0.41, $P<0.05$) (Goto *et al.*, 1988).

Significant correlations were demonstrated between plasma concentrations of oestradiol-17β and the ovulation rate (number of corpora lutea). In heifers with preovulatory oestradiol-17β surges, both the ovarian

response (number of corpora lutea and follicles) and the quality of ova recovered (number of transferable embryos) was clearly better compared to heifers without this surge (Callesen et al., 1990).

There was no correlation between the concentration of oestradiol-17ß at oestrus and ovulation rate (Ezoe et al., 1991).

The number of embryos collected was significantly correlated with plasma progesterone (-0.56) and oestradiol (0.80) levels on the day of oestrus. The increase in progesterone concentration from day 0 to day 7 was a significant indicator of embryo yield. The rise of oestradiol during the follicular phase indicated follicular development, and therefore embryo yield (Mehmood et al., 1991).

Plasma progesterone concentrations were highly correlated with the number of corpora lutea palpated (0.92; $P<0.01$) and with the number of ova and embryos recovered (0.88; $P<0.01$) (Wubishet et al., 1991).

Relationships between hormone characteristics and number of transferable embryos recoverd from superovulated donor cows

Hormone characteristic	Relationship with transferable embryos	Reference
Increase in P4 during superovulation treatment period	Negative Negative	Saumande & Batra, 1985 Tamboura *et al*, 1985
Concentration of P4 after PGF and before ovulation	Negative Negative	Jensen *et al*., 1982 Greve *et al*., 1984b
Concentration of P4 for 2d following induced oestrus	Negative None	Jensen *et al*., 1982 Saumande *et al*., 1985
Concentration of P4 from d3 to d7 after induced oestrus	None Positive	Jensen *et al*., 1982 Saumande *et al*., 1985
Peak E2 at induced oestrus	None None	Saumande & Batra, 1985 Savage *et al*., 1987
Duration of LH surge	Positive Positive	Donaldson, 1985 Kweon *et al*., 1987
Magnitude of LH surge	Positive Positive	Donaldson, 1985 Callesen *et al*., 1987
Endogenous FSH at start of FSH treatment	Positive	Kweon *et al*., 1987
Endogenous FSH between PGF and AI	Positive	Kweon *et al*., 1987

S.No.	Interval from Inj. PG to LH peak (hours)	Reference
1.	59±2h	Walters et al., 1984
2.	48h	Walton & Stubbings, 1986
3.	PMSG treated = 37h FSH treated = 52h Controls = 82h	Yadav et al., 1986a
4.	43.3±1.69h	Yadav et al., 1986b
5.	42.7±1.1 (SEM)h	Dieleman et al., 1987
6.	PMSG treated = 38.6±6.4h FSH treated = 46.8±5.9h	Kweon et al., 1987
7.	43.9±1.5h	Bevers & Dieleman, 1987
8.	44.2 to 48.5h	Kanitz et al., 1990

S.No.	Interval from Oestrus to LH peak (hours)	Reference
1.	Average = 2h (0-8h)	Sprague et al., 1971
2.	2h	Yadav et al., 1986b
3.	1.96±0.54h	Bevers & Dieleman, 1987
4.	2h	Yadav, 1988

S.No.	Interval from Inj. PG to onset of oestrus (hours)	Reference
1.	41.3±1.25h	Yadav et al., 1986b
2.	Pituitary extract treated = 55.6h PMSG treated = 44.6h Controls = 72.0h	Yigezu et al., 1989
3.	39.7 to 42.0h	Kanitz et al., 1990
4.	24 to 48h	Mehmood et al., 1991

S.No.	Interval from LH peak to ovulation (hours)	Reference
1.	Average = 29h (0-52h)	Sprague et al., 1971
2.	22 h	Yadav et al., 1986b
3.	24 to 30h	Dieleman & Bevers, 1987
4.	22 to 30h	Dieleman et al., 1987
5.	22h	Yadav, 1988
6.	PMSG treated Cows = 23.5h PMSG treated Heifers = 20 to 22h FSH treated Cows & heifers= 19 to 23h	Kanitz et al., 1990

Collection and handling of samples

The pulsatile secretion of the reproductive hormones is reflected in episodic peaks of the hormones in blood. Thus, the concentration of hormone in a single blood sample may not be very representative of the actual amount of hormone being secreted during a particular physiological state (Britt and Holt, 1988).

Some hormonal events are so brief that hourly (or more frequent) sampling is required to estimate hormone profiles accurately. For example, the preovulatory LH surge lasts about 8 hours (Helmer and Britt, 1987) and it is necessary to collect samples frequently to characterize the magnitude of secretion of the LH surge (Britt and Holt, 1988).

Blood was sampled every 4 hours from 20 to 72 hours after the first prostaglandin injection to analyze for luteinizing hormone (LH), oestrogen and progesterone (Voss et al., 1986).

Blood samples from heifers were taken at 2 hours intervals from 24 hours after cloprostenol (prostaglandin analogue) treatment until slaughter (between 48 to 100 hours after the first cloprostenol treatment) and the plasma was assayed for LH concentrations (Yadav et al., 1986b).

Peripheral blood was sampled every hour from 30 hours after prostaglandin to analyze for luteinizing hormone (LH) (Dieleman and Bevers, 1987; Dieleman et al., 1989).

Blood was collected for luteinizing hormone (LH) assay at 2 hours intervals on the 5th day after the first follicle stimulating hormone (FSH) injection (Wubishet et al., 1991).

S.No.	Levels of FSH & LH in cattle (ng/ml)	Reference
1.	LH Basal level = 2.5 At oestrus = 61	Sprague et al., 1971
2.	LH surge = 7 to 32	Walters et al., 1984
3.	LH surge	Yadav et al., 1986a
	FSH treated = 24.2±1.02 PMSG treated = 17.1±2.04 Control = 16.7±1.24	
4.	LH peak = 90.2±21.14	Yadav et al., 1986b
5.	LH peak	Kweon et al., 1987
	PMSG treated = 55.9±23.2 FSH treated = 49.9±11.3	
	FSH peak	
	PMSG treated = 67.4±29.2 FSH teated = 85.2±23.1	
6.	LH peak = 79.7±20.1	Goto et al., 1988
7.	FSH & LH concentration	Kaneko et al., 1989
	Before superovulation = 0.5 to 2.5	
	After superovulation = 25 to 50	
8.	LH peak = 44.7±12.4 = 46.1±12.4	Palasz et al., 1989
9.	Preovulatory FSH peak	Kaneko et al., 1991b
	Natural ovulating = 84.9 Superovulated = 59.8	

S.No.	Duration of LH surge (hours)	Reference
1.	6 to 10h	Walters et al., 1984
2.	3.75 to 10.25h	Yadav et al., 1986a
3.	PMSG treated = 12.4±2.5h FSH treated = 12.5±1.8h	Kweon et al., 1987

2.6. EMBRYO COLLECTION AND EVALUATION

2.6.1. EMBRYO COLLECTION

Bovine embryos move from the oviduct to the uterus 4 to 5 days after oestrus (3 to 4 days after ovulation), although in superovulated cows a few remain in the oviduct through day 7 (Newcomb et al., 1976). A high percentage of embryos can usually be recovered non-surgically from the uterus 6 or more days after the beginning of oestrus (Elsden et al., 1976). The uterine embryos are preferred because they result in higher pregnancy rates (Newcomb and Rowson, 1975).

Since bovine embryos form no intimate attachment to the uterus before day 18, they can be recovered non-surgically until this time, although they are increasingly prone to damage after day 14. It appears that a larger number of normal embryos can be obtained non-surgically 6to 8 days after oestrus than at other times (Seidel, 1981).

Previously, embryos were collected by surgical means around day 4 of the oestrus. Rowson et al. (1969) first demonstrated the mid-ventral laparotomy technique for surgical recovery of embryos. The major disadvantage of the surgical procedures is the virtually unavoidable induction of peri-ovarian adhesions which can reduce subsequent fertility, the risks of anaesthetic-related mortality and other surgical complications.

The development and improvement in non-surgical methods of embryo recovery (Brand et al., 1978) provided an opportunity to obtain embryos repeatedly from high producing cows without being detrimental to long-term fertility of the donor cows. But its demerit is that the embryos which are still in the oviduct at the time of recovery cannot be collected.

Mostly, the non-surgical recoveries were made on day 6th (Sreenan, 1975; Brand et al., 1978; Trounson et al., 1978; Wright, 1981; Goncalves, 1987; Monty and Racowsky, 1988), or day 7th (Schmitz, 1987; Kuchukhidze, 1988; Foote et al., 1989; Kadu et al., 1989; Mapletoft et al., 1990; Rodrigues et al., 1990; Slimane and Quali, 1991; Thomas et al., 1991) or day 8th (Trounson et al., 1978; Wright, 1981; Monty and Racowsky, 1988; Garcia-Winder et al., 1989) of the oestrous cycle. Non-surgical recovery as early as day 5th (Horiuchi et al., 1991) or as late as day 9th (Trounson et al., 1978) of the oestrous cycle has also been attempted.

Landsverk *et al*. (1991) superovulated cows between D8 and D12 with FSH-P or Folltropin and performed flushings on D7 and D8 after oestrus. The first flushing yielded 2.4±0.6 total ova and 1.8±0.5 transferable embryos. The corresponding results for 2nd flushing were 5.6±1.5 and 3.9±1.1. They concluded that it was favourable to repeat the flushing in cows in which a discrepancy existed between the number of ova expected and those obtained after one flushing.

Subramaniam *et al*. (1991b) observed similar results while performing "Repeat Flushing and Embryo Recovery" (RFER) in superovulated crossbred (*Bos indicus* x *Bos taurus*) donor cows. The flushing was performed using modified Dulbecco's PBS (phosphate buffer saline) with 2 per cent FCS (foetal calf serum) on D6 after estimating the number of C.L. by rectal palpation. Thirty-two embryos from PMSG-treated cows and 38 from FSH-treated cows were recovered in the first attempt while 15 and 17 from these two groups, respectively were recovered in the repeat flushing. Repeat flushing recovery was significant ($P<0.01$). The recovery enhancement on total embryos recovered was 31.9 and 30.9 per cent respectively for PMSG and FSH-treated cows.

Hay *et al*.(1990) compared the efficacy of two non-surgical methods of embryo recovery from the uterus. In the first method each uterine horn was independently

flushed with physiological saline solution (PSS) through a Foley catheter. In the second method both uterine horns were simultaneously flushed with PSS by passing the catheter into the uterine body. The difference between the recovery rates (40 percent v/s. 31 percent) of the two flushing methods was not significant (P>0.05).

2.6.2. EMBRYO EVALUATION

Embryo evaluation is an important determinant to the success of embryo transfer procedures. Gross morphological evaluation of embryos is useful in predicting pregnancy rates for groups of embryos but is of limited value in determining survival of individual embryos (Lindner and Wright, 1983).

A great deal of variability exists in morphological development and embryo quality within and among donors. Embryo recovery in the superovulated cow commonly results in a range of embryonic cell stages differing in estimated developmental ages from 24 to 48 hours (Lindner and Wright, 1983).

Morphological evaluation has been widely used to delineate embryo quality (Shea *et al.*, 1976; Elsden *et al.*, 1978; Schneider *et al.*, 1980; Shea, 1981; Wright, 1981). Embryo evaluation involves the identification of embryonic cell stage of development and an assessment of quality based on morphological characteristics (Mapletoft, 1984). Parameters commonly used to evaluate embryo

quality include shape, colour, number and compactness of cells, size of the perivitelline space, number of extruded and degenerated cells, and the number and size of vesicles.

Systems classifying embryos into good, fair and poor categories appear to be the simplest and most reliable. Transferable embryos are the ones classified as excellent, good or fair (Lindner and Wright, 1983; Lindsell et al., 1986).

Embryos are classified and evaluated by morphological examination at 50-100 X magnification (Shea, 1981; Lindner and Wright,1983). The overall diameter of the bovine embryo is 150-190 µm including a zona pellucida of thickness 12-15 µm. The diameter of the embryo remains virtually unchanged from the one-cell stage until blastocyst expansion. Early cleavage-stage embryos are commonly referred to by the number of cells present, such as the zygote, two-cell, etc, upto the 16-cell stage (Lindner and Wright, 1983).

Other methods of embryo evaluation include in vitro culture (Mapletoft, 1980), dye exclusion tests (Kardymowicz, 1972), measures of enzyme activity (Schilling et al., 1979b) glucose uptake (Renard et al., 1980) and live-dead stains (Schilling et al., 1979a). These methods require complex equipment and a lengthy in vitro culture period thus limiting their use. To date, microscopic evaluation of embryos remains a very subjective assessment.

MATERIAL AND METHODS

3. MATERIAL AND METHODS

The present investigation was conducted on healthy Rathi cattle during the months of June to November, 1993. The experimental animals (n=12) were selected from the college dairy farm at the C.V.A.S. campus, Bikaner (Rajasthan). The animals were in the age group of 6 to 10 years of age (2 to 4 parity), free from gynaecological trouble (as determined by rectal palpation), cycling regularly and free from infection (as determined by microbiological investigation of the oestrual discharge). All the animals were maintained under standard feeding and uniform farm managemental conditions.

The experimental cows were studied in different groups:

1. Control animals (n=2)
2. Unprimed animals, superovulated with PMSG (n=5)
3. Primed animals (primed with PMSG), subsequently superovulated with PMSG (n=5)

The animals were subjected to two different superovulatory regimes (with and without priming) to study the comparative efficacy of the following two regimes:

I st Regime

Cows with well developed C.L.

Inj. PGF2α, 25 mg, I/m
(Lutalyse; Upjohn)

5/2 – 3d

Oestrus

10d

Inj. PMSG, 3000 I.U., I/m
(Folligon; Intervet)

2d (48 hours)

Inj. PGF2α, 25mg I/m

24-36 hours

Oestrus
A.I.-A.M., P.M., A.M.; or P.M., A.M., P.M.

7d

Embryo Recovery

1d

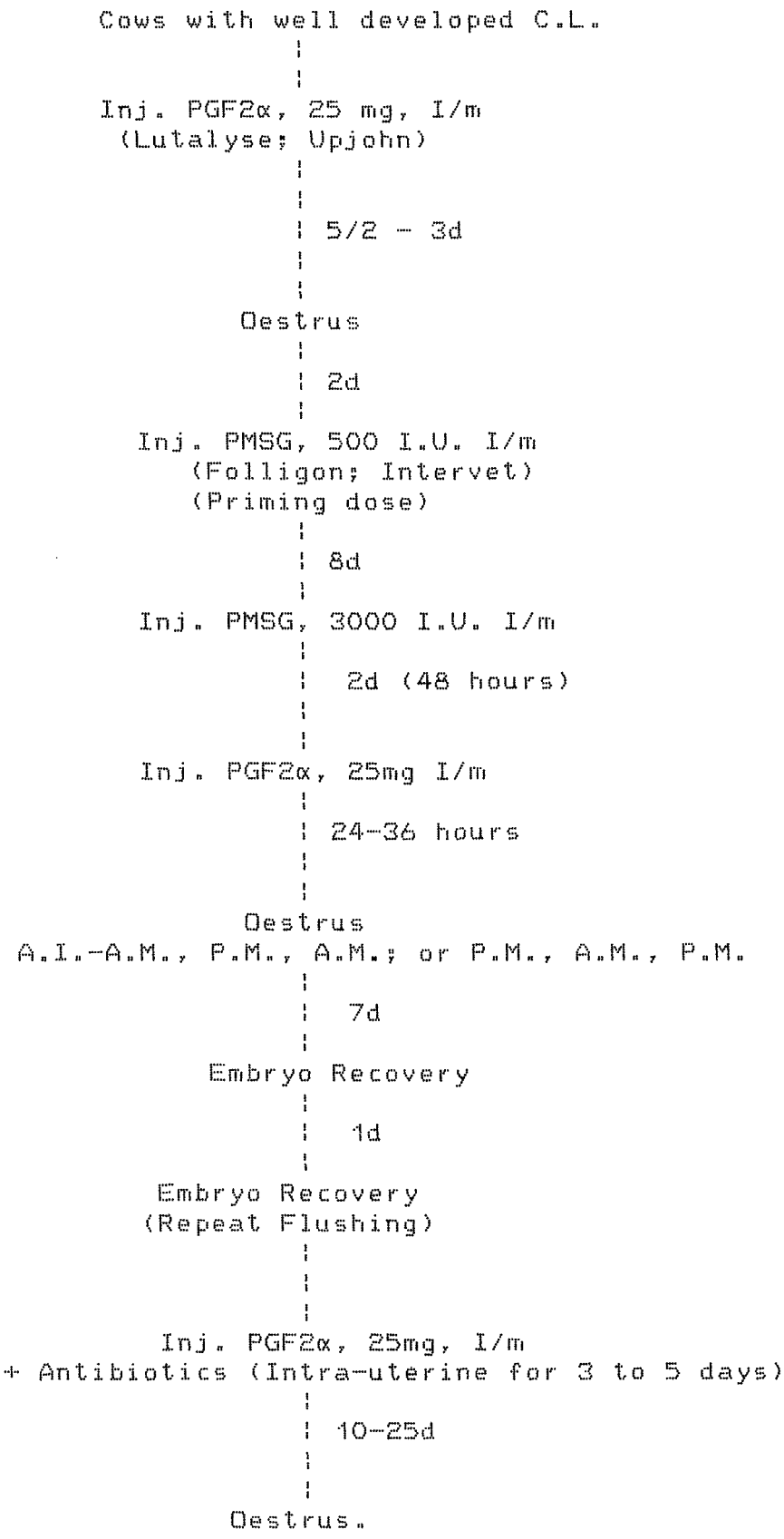
Embryo Recovery
(Repeat flushing)

Inj. PGF2α, 25mg, I/m
+ Antibiotics (Intra-uterine for 3 to 5 days)

10-25d

Oestrus.

II nd Regime


Cows with well developed C.L.
Inj. PGF2α, 25 mg, I/m
(Lutalyse; Upjohn)
5/2 - 3d
Oestrus
2d
Inj. PMSG, 500 I.U. I/m
(Folligon; Intervet)
(Priming dose)
8d
Inj. PMSG, 3000 I.U. I/m
2d (48 hours)
Inj. PGF2α, 25mg I/m
24-36 hours
Oestrus
A.I.-A.M., P.M., A.M.; or P.M., A.M., P.M.
7d
Embryo Recovery
1d
Embryo Recovery
(Repeat Flushing)
Inj. PGF2α, 25mg, I/m
+ Antibiotics (Intra-uterine for 3 to 5 days)
10-25d
Oestrus.

3.1. SUPEROVULATION

Rathi cows were superovulated by the administration of commercially available preparation of pregnant mare serum gonadotropin (PMSG) (Folligon, Intervet Int., Boxmeer, Holland) on the 10th day of the oestrous cycle, followed 48 hours later by an intra-muscular injection of PGF-2 alpha (Lutalyse; Upjohn, Agvet Division, Unichem Laboratories). In the primed group, priming was carried out by 500 I.U. PMSG (Folligon, Intervet, Holland) on the 2nd day of the oestrous cycle. No gonadotropin was given to the cows of control group. The cows were inseminated thrice at approx. 12 hours interval, the first insemination commencing approx. 12 hours after the onset of oestrus. Two straws of semen were used at each A.I.. Embryos were recovered non-surgically at day 7 and 8 after A.I.. Immediately following 2nd flush, antibiotics were infused into the uterus to reduce the possibility of uterine infection. Injection of PG (prostaglandin) was also administered intramuscularly to help the donor return to normal hormonal status quickly and also to kill the embryos that may have been left behind.

Preparation of solution

PMSG (Folligon) - Each 1000 I.U. of freeze-dried powder of PMSG was reconstituted with 5ml of the solvent.

3.2. ATTRIBUTES

The proposed plan of work included the following attributes:

i) Interval from PGF2α injection to the onset of oestrus.

ii) Interval from PGF2α injection (given after superovulation with PMSG) to the onset of oestrus.

iii) Interval from PGF2α injection (given after non-surgical embryo recovery) to the onset of oestrus.

iv) Number of ovulations in the two different superovulatory regimes (i.e. number of palpable corpora lutea on the ovaries).

v) Number of transferable embryos recovered.

vi) Stages of the embryos-Morula, Blastocyst, Hatched blastocyst and unfertilized or degenerated ova.

vii) Quality of the embryos-Excellent, Good, Fair, Poor.

3.3. MEDIA

Modified Dulbecco's Phosphate-buffered saline (DPBS) containing 0.1 per cent Bovine Serum Albumin (BSA) Fraction V (Hi Media, Bombay, India) was used for flushing. The same medium was supplemented with 1.5 per cent BSA for preparation of holding media. DPBS (Ca, Mg, Free) was procured as a dry media (Hi media, Bombay, India) and dissolved in 800ml triple glass distilled water. Sodium pyruvate (0.036 g/l) (Glaxo, Bombay, India), Glucose (1 g/l) (E. Merck, Bombay, India), Streptomycin (100 µg/ml) and Penicillin G (100 I.U./ml) (IDPL, India) were added to it.

Separately $CaCl_2$ (0.10 g/l) (E. Merck, Bombay, India) and $MgCl_2 . 6 H_2O$ (0.10g/l) (Glaxo, Bombay, India) were dissolved in 100ml triple glass distilled water each. The three solutions were mixed after cooling to 4 ^{o}C temperature. The pH of the medium was adjusted to 7.2. Medium was sterilized by filtration through cellulose acetate (Millipore) filter of 0.22µ pore size and stored at 4 ^{o}C (Table1).

Table 3.1 : Modified Dulbecco's Phosphate Buffered Saline.

CONSTITUENT	WEIGHT(GM)	VOLUME TRIPLE DISTILLED WATER (ML)
NaCl	8.00	
KCl	0.20	
Na_2HPO_4	1.15	
KH_2PO_4	0.20	
Bovine Serum Albumin (Fraction V)	1.00	800(Solution 1)
Glucose	1.00	
Na pyruvate	0.036	
Penicillin G	100 I.U./ml	
Streptomycin	100 µg/ml	
$CaCl_2$	0.10	100(Solution 2)
$MgCl_2 .6H_2O$	0.10	100 (Solution3)

Preparation

1. Make up solutions 1,2 and 3 separately; cool to 4 ^{o}C and then mix.
2. Adjust pH to 7.1 to 7.2 with $NaHCO_3$ or HCl if necessary.
3. Confirm that osmolality is about 290 m Osm/Kg.
4. Sterilize by filtration through cellulose acetate (Millipore) filters, pore size 0.22µ.
5. Store at 20 ^{o}C for upto 3 months.

3.4. NON-SURGICAL EMBRYO RECOVERY

Recovery attempts were carried out on days 7 and 8 after A.I.. Donor animals were fasted for food and water for approx. 1 day prior to an attempt. The rectum was emptied of faeces and an epidural block of 5ml of a 2 per cent solution of procaine hydrochloride was given. The vulva and the surrounding area were washed with soap and water and a cervical dilator was introduced and guided manually per rectum through the cervix to the common body. A 3-way Foley's catheter with the stillete as a stiffner, was introduced through the cervix. When the cuff was situated about the middle of the uterine horn, the stillete was withdrawn from the catheter.

The cuff was inflated by injecting 5-10 ml of air. After clamping the outlet, the medium was introduced in 50ml aliquots. The clamp on the outlet tube was released when 75-100ml of the medium had been introduced into the uterine horn. Flushings were collected in sterile 1 lt. measuring cylinders. The process of filling the horn and releasing the outlet clamp was continued until 500ml of the medium had been used. The cuff was then deflated and the apparatus withdrawn from the uterus. The catheter, with the stillete re-inserted, was then pushed into the opposite horn which was flushed in the same manner.

The measuring cylinders containing the flushings were allowed to stand for 45 minutes at 37°C. The supernatant was siphoned into separate measuring cylinders until 50 to 100 ml was left in the original cylinder. Then the contents were mixed with a swirling action and poured into ova collection dishes (searching dishes) and examined for embryos.

3.5. EMBRYO EVALUATION

After collection, the embryos were isolated under a stereoscopic microscope (with built-in illumination) at 20 X magnification. A fine siliconized Pasteur pipette was used to transfer the embryos to fresh holding medium (modified DPBS with 1.5 per cent BSA) in another dish for morphologic examination at 40 X magnification.

Quality of individual embryos was determined by the criteria suggested by Lindner and Wright (1983):

1. Excellent-An ideal embryo, spherical, symmetrical with cells of uniform size, colour and texture.
2. Good- Trivial imperfections such as a few extruded blastomeres, irregular shape, few vesicles.
3. Fair- Definite but not severe problems, presence of extruded blastomeres, vesiculation, few degenerated cells.
4. Poor- Severe problems, numerous extruded blastomeres, degenerated cells, cells of varying sizes, large numerous vesicles but a viable appearing embryo mass.

3.6. ESTIMATION OF OVARIAN PARAMETERS

An attempt was made to determine the ovarian length, width and thickness in all animals by rectal palpation (following the procedure adopted by Randhawa, 1980, in buffalo heifers) at three different stages : -

(a) At normal oestrus,
(b) At superovulatory oestrus,
(c) At flushing.

A three-way interaction ANOVA (Analysis of Variance) was applied separately for ovarian length, width and thickness to analyze the effect of :-

(i) Priming,
(ii) The site (location) of ovaries (right or left),
(iii) The stage at which the ovarian size was determined,
(iv) The interactions of the above three,

on the ovarian parameters.

The data of Randhawa (1980) revealed highly significant ($P<0.05$) correlations between the estimated ovarian measurements and the actual measurements (obtained immediately after slaughter) in buffalo heifers.

3.7. HORMONE ASSAY

An attempt was made to study the pattern of secretion of LH and FSH in normal (control) and superovulated cows. For this purpose blood from control and superovulated cows was collected to perform LH and FSH assay.

3.7.1. Blood sampling

(a) Superovulated cows: Samples of blood, 6-8ml in amount each time, were collected from the jugular vein. Initially, blood was collected every 4 hours after PGF-2 alpha injection (given after superovulation with PMSG) for a period of 24 hours (i.e. 6 samples at 4 hours interval). After that blood samples were collected every 2 hours for a period of 48 hours (i.e. 24 samples at 2 hours interval). So the sampling stopped approx. when the heat symptoms started subsiding. So in all, 30 samples from each cow were assayed.

(b) Control cows: Blood sampling was started after 48 hours of PG injection (because the untreated control cows were supposed to take a longer time to come to oestrus than the superovulated cows, hence late sampling was done), initially at 4 hours interval for a period of 24 hours (6 samples) and then at 2 hours interval for a period of 12 hours (6 samples). So 12 samples in all were collected, first 6 at 4 hours interval and last 6 at 2 hours interval.

Blood samples were immediately centrifuged and the plasma thus separated was transferred to storage vials and stored at - 20 °C until assayed.

Kits used

Hormone assay (LH and FSH) was performed using the LH Enzyme Immunoassay Test Kit (Medix Biotech. Inc., USA) and the FSH Enzyme Immunoassay Test kit (Medix Biotech. Inc., USA). All the samples were run in duplicate.

Due to the lack of availability of Kits, LH assay was performed over 6 cows (3 cows each from unprimed and primed group) plus the 2 control cows, while the FSH assay could be performed only over 3 cows of unprimed group plus the 2 control cows.

3.7.2. FSH ENZYME IMMUNOASSAY

(a) Principle of the test

The Medix FSH quantitative test is based on the principle of a solid enzyme-linked immunosorbent assay. The assay system utilizes mouse monoclonal anti-FSH for solid phase (microtiter wells) immobilization and sheep anti-alpha FSH in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the FSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45 minute

incubation at room temperature, the wells are washed with water to remove unbound labelled antibodies. A solution of 3,3',5,5' Tetramethylbenzidene (TMB) is added and incubated for 15 minutes resulting in the development of a blue colour. The colour development is stopped with the addition of 2N HCl. and the resulting yellow colour is measured spectrophotometrically at 450nm. The concentration of Follicle Stimulating Hormone is directly proportional to the colour intensity of the test sample.

(b) Reagents

(1) Antibody- coated wells.

Microtiter wells coated with mouse monoclonal anti-FSH.

(2) Enzyme conjugate reagent.

Contains sheep polyclonal anti-alpha FSH conjugated to horseradish peroxidase.

(3) Reference standard set.

Contains 0,2.5,5,10,25,50,100 and 200 mI.U./ml (WHO, 2nd IRP HMG) human FSH in bovine serum with preservatives. Lyophilized.

(4) Colour Reagent A.

Contains hydrogenperoxide in acetate buffer.

(5) Colour Reagent B.

Contains 3,3',5,5'-Tetramethylbenzidine stabilized in bufffer solution.

(6) 2N HCl.

Contains dilute hydrochloric acid.

(c) Assay procedure:

(1) Secure the desired number of coated wells in the holder.

(2) Dispense 50 µl of standards, samples, and controls into appropriate wells. Gently mix for 30 seconds.

(3) Dispense two drops (100µl) of enzyme conjugate reagent into each well.

(4) Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.

(5) Incubate at room temperature for 45 minutes. Prepare TMB solution up to three hours before use.

(6) Remove the incubation mixture by flicking the plate contents into a suitable waste container.

(7) Rinse and flick the microtiter wells 5 times with running tap or distilled water.

(8) Strike the wells sharply on absorbent paper to remove residual water droplets.

(9) Dispense 200 µl TMB Substrate Reagent into each well. Gently mix for 5 seconds.

(10) Incubate at room temperature in the dark for 15 minutes.

(11) Stop reaction by adding one drop (50µl) of 2N HCl to each well. Gently mix for 5 seconds.

(12) Read O.D. (optical density) at 450nm with a microtiter well reader.

3.7.3. LH ENZYME IMMUNOASSAY

(a) Principle of the test

The medix LH quantitative test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes sheep polyclonal anti-LH for solid phase (microtiter wells) immobilization, and a mouse monoclonal anti-LH in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45 minute incubation at room temperature, the wells are washed with water to remove unbound labelled antibodies. A solution of 3,3',5,5'- Tetramethylbenzidine (TMB) is added and incubated for 15 minutes, resulting in the development of a blue colour. The colour development is stopped with the solution of 2N HCl, and the resulting yellow colour is measured spectrophotometrically at 450 nm. The concentration of luteinizing hormone is directly proportional to the colour intensity of the test sample.

(b) Reagents

(1) Antibody- coated wells.

Microtiter wells coated with sheep polyclonal anti-l

(2) Enzyme Conjugate Reagent.

Contains mouse monoclonal anti-LH conjugated to horseradish peroxidase.

(3) Reference standard set.

Contains 0,2.5,5,10,25,50,100 and 200 mI.U./ml (WHO. Ist IRP, 68/40) human LH in bovine serum with preservatives. Lyophilized.

(4) Colour Reagent A.

Contains hydrogenperoxide in acetate buffer.

(5) Colour Reagent B.

Contains 3,3',5,5'- Tetramethylbenzidine stabilized in buffer.

(6) 2N HCl.

Contains dilute hydrochloric acid.

(c) Assay procedure:

(1) Secure the desired number of coated wells in the holder.

(2) Dispense 50 µl of standards, samples, and controls into appropriate wells. Gently mix for 30 seconds.

(3) Dispense two drops (100µl) of enzyme conjugate reagent into each well.

(4) Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.

(5) Incubate at room temperature for 45 minutes.

Prepare TMB solution up to three hours before use.

(6) Remove the incubation mixture by flicking the plate contents into a suitable waste container.

(7) Rinse and flick the microtiter wells 5 times with running tap or distilled water.

(8) Strike the wells sharply on absorbent paper to remove residual water droplets.

(9) Dispense 200 µl TMB Substrate Reagent into each well. Gently mix for 5 seconds.

(10) Incubate at room temperature in the dark for 15 minutes.

(11) Stop reaction by adding one drop (50µl) of 2N HCl to each well. Gently mix for 5 seconds.

(12) Read O.D. (optical density) at 450nm with a microtiter well reader.

A mini ELISA reader (Metertech Inc., USA) was used to determine the optical density (O.D) (absorbance) of the samples. Since each sample was run in duplicate, the mean O.D. for each sample was calculated as

$$\text{Mean O.D.} = \frac{\text{O.D.I} + \text{O.D. II}}{2}$$

A standard curve, each for LH and FSH, was plotted after determining the O.D. of the standards with the help of the standards supplied with the kits.

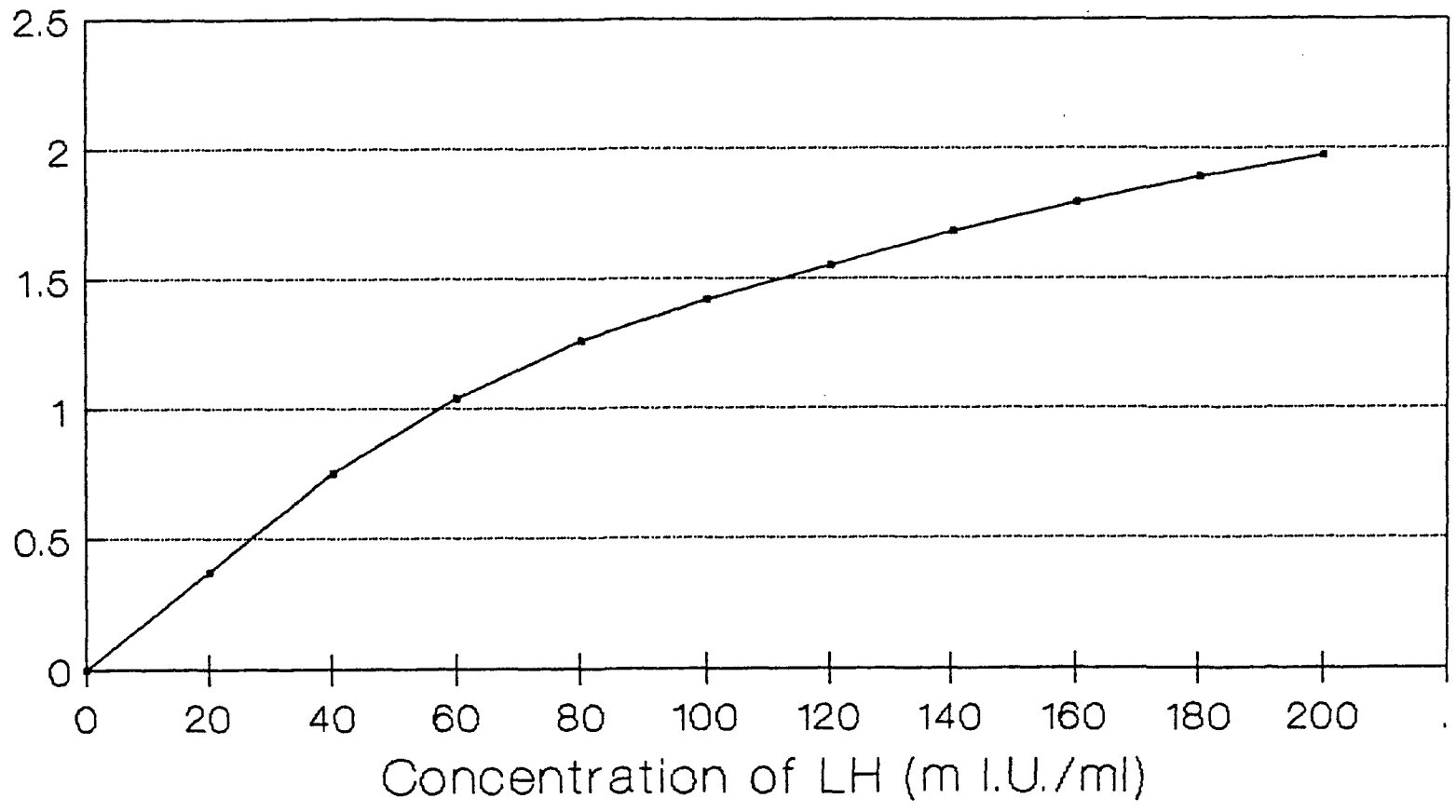
Subsequently, with the help of the standard curve, the concentration of LH and FSH could be known for each sample after determining the mean O.D. for each sample.

On completion of the assay, a graph was plotted for each cow to study the endocrine profile and the pattern of LH and FSH secretion in control cows and superovulated cows.

Table 3.2. : LH assay - table for standard graph.

Standard	Mean Absorbance (Optical density)	Concentration m I.U./ml
0	0.000	0
2.5	0.040	2.5
5	0.092	5
10	0.185	10
25	0.560	25
50	0.900	50
100	1.422	100
200	1.966	200

Graph depicting standard curve for LH

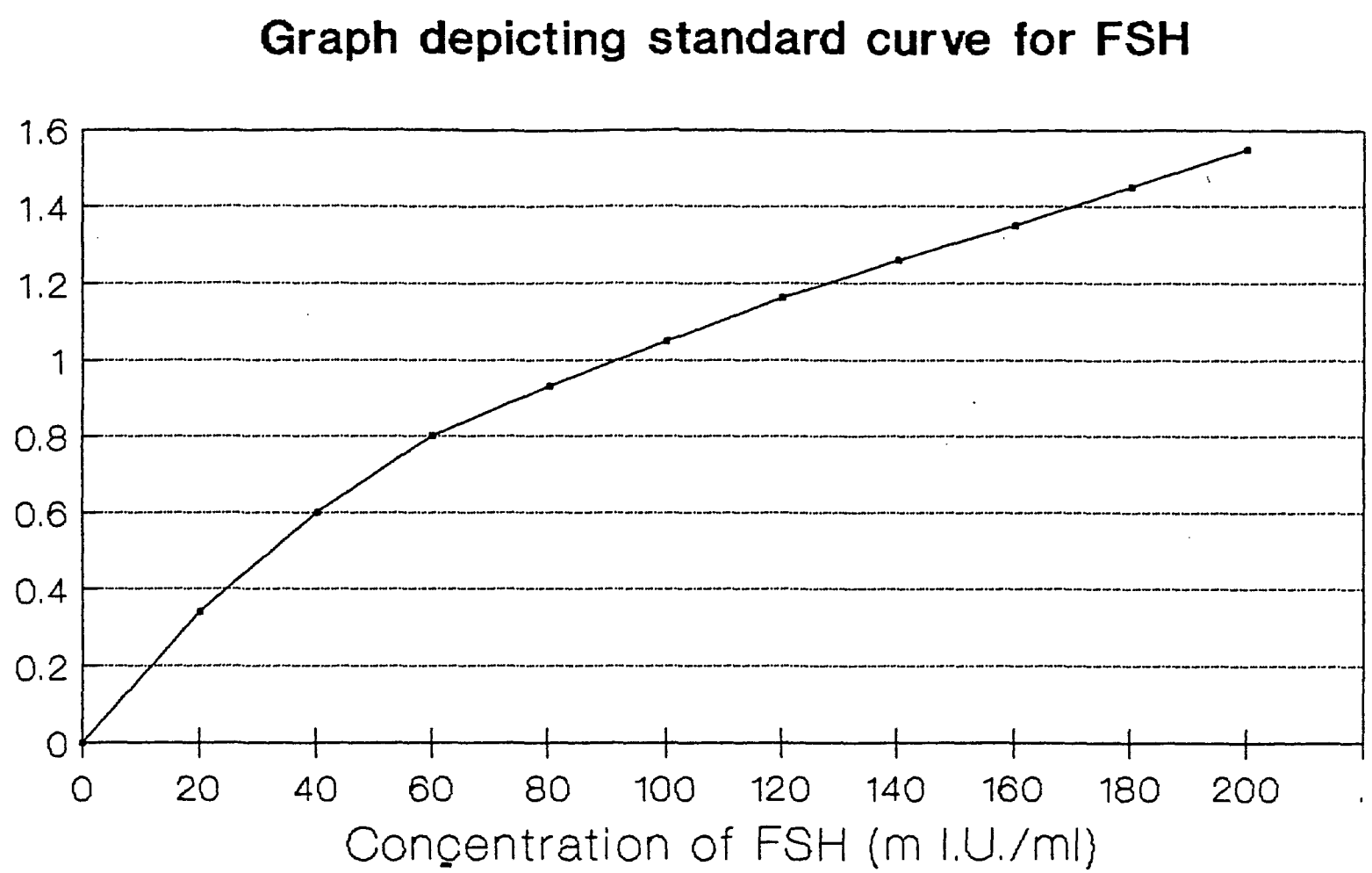


Y axis = Absorbance (Optical density)

Fig. No. 1.

Table 3.3. : FSH assay - table for standard graph.

Standard	Mean Absorbance (Optical density)	Concentration m I.U./ml
0	0.000	0
2.5	0.121	2.5
5	0.150	5
10	0.202	10
25	0.427	25
50	0.644	50
100	1.050	100
200	1.550	200



Y axis = Absorbance (Optical density)

Fig. No. 2.

3.8. STATISTICAL ANALYSIS

The mean and S.E. (Standard Error) were calculated by using conventional methods as suggested by Snedecor and Cocharan (1967). Different mean comparisons were carried out by the Student's t-test (Snedecor and Cocharan, 1967).

For different ovarian measurements, analysis of variance (ANOVA) was carried out by using following mathemathical model :-

$$Y_{ijkl} = \mu + T_i + L_j + S_k + e_{ijkl}$$

Y_{ijkl} is the measurement of l^{th} animal belonging to i^{th} treatment of the j^{th} side at the k^{th} stage.

μ is the overall population mean.

T_i is the effect of i^{th} treatment (i=1,2).

L_j is the effect of j^{th} side of ovary (j=1,2).

S_k is the effect of k^{th} stage of cycle (k=1,2,3).

e_{ijkl} is the error term associated with each observation and assumed to be NID (o,σ^2_e) which make the analysis valid.

Note : NID (o,σ^2_e) = Normally and independently distributed with zero mean and equal variance.

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

A total of 12 Rathi cattle of different parities (2 to 4) and ages (6 to 10 years) were used for this study. The experiment was designed to study the superovulatory response using PMSG, with and without priming.

4.1. OESTRUS SYNCHRONIZATION/INDUCTION

Oestrus was synchronized prior to superovulation in all animals, irrespective of their treatment regimen. The mean interval from prostaglandin injection to the onset of oestrus was calculated separately for the unprimed and the primed group (table 1).

Table 4.1. : Interval from PGF-2 alpha injection to the onset of oestrus.

	Cow No.	Interval (hours)
UNPRIMED	1	36
	2	72
	3	72
	4	72
	5	48
PRIMED	6	72
	7	48
	8	96
	9	60
	10	70

Oestrus was induced at a mean interval of 60±7.589 hours in unprimed group and 69.6±7.960 hours in the primed group after the injection of PGF-2 alpha. No significant differences were observed in the two groups (P>0.05).

Control cow no.	Time from PG injection to oestrus (hours)
1	72
2	80

The findings are in accordance with those of Roche (1974) and Oyedipe et al. (1988) who reported that animals with a functional C.L. were detected in oestrus 3 to 4 days after PGF-2α injection.

Table 4.2. : Interval from PGF-2 alpha injection (given after superovulation with PMSG) to the onset of superovulatory oestrus.

	Cow No.	Interval (hours)
UNPRIMED	1	46
	2	26
	3	24
	4	28
	5	36
PRIMED	6	36
	7	48
	8	48
	9	24
	10	48

The superovulatory oestrus occurred at a mean interval of 32±4.05 hours in the unprimed group and 40.8±4.8 hours in the primed group after PGF-2 alpha injection given post-superovulation. Although the mean interval was greater in the primed group, the difference remained non-significant (P>0.05).

The results are in accordance with the following scientists who reported this interval to be 24 to 48 hours (Mehmood et al., 1991), 39.7 to 42 hours (Kanitz et al., 1990) and 41.3±1.25 hours (Yadav et al., 1986b) in superovulated cows.

Table 4.3. : Interval (days) from PGF-2 alpha injection (given after embryo recovery i.e. post-superovulatory injection of PGF-2 alpha) to the onset of oestrus.

	Cow No.	Interval (days)
UNPRIMED	1	15
	2	17
	3	17
	4	19
	5	16
PRIMED	6	14
	7	21
	8	19
	9	24
	10	18

Post-superovulatory induction of oestrus was induced following repeat flushing. The animals in the unprimed group exhibited oestrus at a mean interval of 16.8±0.663 days while in the primed group this interval was 19.2±1.655 days, the difference between the two groups being non-significant (P>0.05).

The results are in accordance with those of Halbert et al. (1989) who reported an interval of 18.7±4.0 days from PGF-2α (Lutalyse) injection to the first post-superovulatory oestrus.

4.2. SUPEROVULATION RESPONSE

The superovulatory response was studied by palpation of the number of graafian follicles formed at superovulatory oestrus and the number of corpora lutea (ovulation rate) felt at the time of embryo recovery (the day of flushing).

Table 4.4. : Number of G.F. (felt by rectal palpation) at superovulatory oestrus.

	Cow No.	No. of G.F. R.O.	L.O.	Total
UNPRIMED	1	2	4	6
	2	2	3	5
	3	3	3	6
	4	4	3	7
	5	5	5	10
PRIMED	6	5	4	9
	7	5	3	8
	8	2	4	6
	9	4	5	9
	10	5	4	9

Note : R.O.=Right ovary, L.O.=Left ovary, G.F.=Graafian follicles

In the unprimed group, a mean number of 6.8±0.860 G.F. could be felt by rectal palpation as compared to a mean number of 8.2±0.583 G.F. in the primed group. The difference was again found to be non-significant ($P>0.05$) between the two groups.

Table 4.5. : Number of C.L. or ovulation rate (determined by rectal palpation) on the day of flushing.

	Cow No.	No. of C.L. R.O.	L.O.	Total
UNPRIMED	1	2	5	7
	2	5	4	9
	3	5	5	10
	4	5	6	11
	5	Cystic	Cystic	-
PRIMED	6	8	7	15
	7	7	5	12
	8	4	5	9
	9	Cystic	Cystic	-
	10	Cystic	Cystic	-

Note : R.O. = Right ovary, L.O.=Left ovary, C.L.=Corpora lutea, Cystic=Ovaries exhibited anovulatory follicular cysts.

The mean ovulation rate was 9.25±0.854 and 12±1.732 for the unprimed and the primed groups, respectively. Although the ovulation rate was higher for the primed group, the difference observed was non-significant (P>0.05).

It was concluded that priming had no significant effect on the ovulation rate. The results are in accordance with Rieger et al., 1988 and Gray et al., 1992 who also observed no beneficial effect of priming.

4.3. ESTIMATION OF OVARIAN PARAMETERS

Table 4.6. : Table showing the estimation of ovarian parameters at three different stages.

Donor Cow No.	OVARIAN SIZE																	
	At Normal oestrus						At Superovulatory oestrus						At Flushing					
	R. Ovary			L. Ovary			R. Ovary			L. Ovary			R. Ovary			L. Ovary		
	L (cm)	W (cm)	T (cm)	L (cm)	W (cm)	T (cm)	L (cm)	W (cm)	T (cm)	L (cm)	W (cm)	T (cm)	L (cm)	W (cm)	T (cm)	L (cm)	W (cm)	T (cm)
1	3	2	1	2.5	2	1	5	4	2.5	6	4.5	3	5.5	4	2.5	7	6	3
2	3.5	2	1.5	2.5	2	1	4.5	4	2	5	4	2.5	5	4	2	5.5	4.5	2.5
3	3.5	2.8	1.5	2.5	2	1.2	6.5	5	2.5	6	4.5	2.5	8	6.5	3	7	6	2.8
4	3	2.5	2	2.5	2.5	1.8	6	4.5	2.5	5	4	2	8	6	3	7.5	6	3
5	3.5	2	1.5	3	2	1	5.5	4	2.5	5.5	4	2	7	5.5	2.5	6.5	4	2.5
6	3.5	2.5	1.5	2.5	2	1.5	6.5	4.5	3	5.5	4.5	3	7.5	6	2.5	7.5	6.5	2.5
7	3	2.5	1	3	2	1	5.5	4.5	2.5	5.5	4	2.5	6	5	2.5	6	4	2.5
8	2.5	1.5	1.5	3	2.5	1.8	3.5	2.5	2	5	5	2.5	4.5	3	2	6	4.5	2.5
9	4	2.5	2	2.5	2	1	6.5	4	3	5	3.5	2.5	7	5	3	5.5	4	2.5
10	3.5	2	1.2	3	2	1.2	6	4	2.5	5.5	4	3	6.5	4.5	3	7	5	3

Note : L=Length

W=Width

T=Thickness

Table 4.7. : Mean±S.E. for ovarian length (cm).

Main effect	Classification	No.	Mean±S.E.
Overall mean	–	60	5.00±0.21
Treatment	Unprimed (U)	30	5.05±0.32
	Primed (P)	30	4.95±0.29
Site	Right (R)	30	5.12±0.30
	Left (L)	30	4.88±0.31
Stage	Normal oestrus (N)	20	3.00±0.10[c]
	Superovulatory oestrus (S)	20	5.47±0.16[b]
	Flushing (F)	20	6.52±0.22[a]
Treatment x Site	UxR	15	5.17±0.44
	PxR	15	5.07±0.42
	UxL	15	4.93±0.48
	PxL	15	4.63±0.49
Treatment x Stage	UxN	10	2.95±0.14
	PxN	10	3.10±0.16
	UxS	10	5.50±0.20
	PxS	10	5.45±0.27
	UxF	10	6.70±0.33
	PxF	10	6.35±0.30
Site x Stage	RxN	10	3.30±0.13
	LxN	10	2.70±0.08
	RxS	10	5.55±0.31
	LxS	10	5.40±0.12
	RxF	10	6.50±0.39
	LxF	10	6.55±0.24

Note : Figures with any one same superscript within a sub-class did not differ significantly (P>0.05) from each other.

Table 4.8. : Analysis of variance for ovarian length (cm).

Source of variation	d.f.	M.S.	F value
Treatment	1	0.15	0.24
Site	1	0.8166	1.30
Stage	2	65.510	104.12 *
Treatment x Site	1	0.01	0.01
Treatment x Stage	2	0.2625	0.42
Site x Stage	2	0.5542	0.88
Treatment x Site x Stage	2	0.0725	0.11
Error	48	0.6291	

Note : * = Significant at P<0.01

OVARIAN LENGTH

The ANOVA for ovarian length as shown in table 4.8 reveals a highly significant ($P<0.01$) effect of the stage of the cycle and no effect of treatment and site of the ovary. The all possible interactions between the three main effects (treatment, site and stage) also showed no significant effect over the ovarian length.

The overall mean ovarian length was 5.0±0.21 cm. The ovarian length did not vary significantly between the unprimed and primed group (5.05±0.32 cm and 4.95±0.29 cm., respectively) nor did it vary between the right and left ovaries (5.12±0.30 and 4.88±0.31 cm., respectively).

However, there was a significant effect of the stage (at which the ovarian length was determined) on the ovarian length : 3.0±0.10 cm, 5.47±0.16 cm and 6.52±0.22 cm at normal oestrus, superovulatory oestrus and at flushing, respectively.

An increasing trend of ovarian length was observed as the stage advanced, probably due to gonadotropin stimulation.

Table 4.9. : Mean±S.E. for ovarian width (cm).

Main effect	Classification	No.	Mean±S.E.
Overall mean	-	60	3.79±0.175
Treatment	Unprimed (U)	30	3.89±0.263
	Primed (P)	30	3.68±0.233
Site	Right (R)	30	3.79±0.242
	Left (L)	30	3.78±0.256
Stage	Normal oestrus (N)	20	2.21±0.062[c]
	Superovulatory oestrus (S)	20	4.15±0.121[b]
	Flushing (F)	20	5.00±0.226[a]
Treatment x Site	UxR	15	3.92±0.374
	PxR	15	3.67±0.319
	UxL	15	3.87±0.382
	PxL	15	3.70±0.351
Treatment x Stage	UxN	10	2.18±0.095
	PxN	10	2.25±0.083
	UxS	10	4.25±0.111
	PxS	10	4.05±0.217
	UxF	10	5.25±0.318
	PxF	10	4.75±0.318
Site x Stage	RxN	10	2.33±0.094
	LxN	10	2.10±0.067
	RxS	10	4.10±0.208
	LxS	10	4.20±0.133
	RxF	10	4.95±0.345
	LxF	10	5.05±0.311

Note : Figures with any one same superscript within a sub-class did not differ significantly (P>0.05) from each other.

Table 4.10. : Analysis of variance for ovarian width (cm).

Source of variation	d.f.	M.S.	F value
Treatment	1	0.6615	1.30
Site	1	0.0015	0.003
Stage	2	40.7432	79.98 *
Treatment x Site	1	0.0282	0.05
Treatment x Stage	2	0.4065	0.80
Site x Stage	2	0.1815	0.36
Treatment x Site x Stage	2	0.0982	0.19
Error	48	0.5094	

Note : * = Significant at P<0.01

OVARIAN WIDTH

The ANOVA for ovarian width as shown in table 4.10 reveals a highly significant ($P<0.01$) effect of the stage of the cycle and no effect of treatment and site of the ovary. The all possible interactions between the three main effects (treatment, site and stage) also showed no significant effect over the ovarian width.

The overall mean ovarian width was 3.79±0.175 cm. The ovarian width did not vary significantly between the unprimed and primed group (3.89±0.263 cm and 3.68±0.233 cm, respectively) nor did it vary between the right and left ovaries (3.79±0.242 and 3.78±0.256 cm, respectively).

However, there was a significant effect of the stage (at which the ovarian width was determined) on the ovarian width : 2.21±0.062 cm, 4.15±0.121 cm and 5.00±0.226 cm at normal oestrus, superovulatory oestrus and at flushing respectively.

An increasing trend of ovarian width was observed as the stage advanced, probably due to gonadotropin stimulation.

Table 4.11. : Mean±S.E. for ovarian thickness (cm).

Main effect	Classification	No.	Mean±S.E.
Overall mean	–	60	2.18±0.086
Treatment	Unprimed (U)	30	2.14±0.121
	Primed (P)	30	2.21±0.124
Site	Right (R)	30	2.19±0.113
	Left (L)	30	2.16±0.131
Stage	Normal oestrus (N)	20	1.37±0.077 c
	Superovulatory oestrus (S)	20	2.52±0.077 b
	Flushing (F)	20	2.64±0.071 a
Treatment x Site	UxR	15	2.17±0.152
	PxR	15	2.22±0.174
	UxL	15	2.12±0.195
	PxL	15	2.20±0.182
Treatment x Stage	UxN	10	1.35±0.115
	PxN	10	1.38±0.109
	UxS	10	2.40±0.100
	PxS	10	2.65±0.107
	UxF	10	2.68±0.106
	PxF	10	2.60±0.100
Site x Stage	RxN	10	1.47±0.108
	LxN	10	1.26±0.104
	RxS	10	2.50±0.105
	LxS	10	2.55±0.117
	RxF	10	2.60±0.125
	LxF	10	2.68±0.076

Note : Figures with any one same superscript within a sub-class did not differ significantly (P>0.05) from each other.

Table 4.12. : Analysis of variance for ovarian thickness (cm).

Source of variation	d.f.	M.S.	F value
Treatment	1	0.063375	0.53
Site	1	0.0120	0.10
Stage	2	19.8152	82.62*
Treatment x Site	1	0.0034	0.03
Treatment x Stage	2	0.2843	1.18
Site x Stage	2	0.2636	1.10
Treatment x Site x Stage	2	0.0692	0.23
Error	48	5.7559	

Note : * = Significant at P<0.01

OVARIAN THICKNESS

The ANOVA for ovarian thickness as shown in table 4.12 reveals a highly significant ($P<0.01$) effect of the stage of the cycle and no effect of treatment and site of the ovary. The all possible interactions between the three main effects (treatment, site and stage) also showed no significant effect over the ovarian thickness.

The overall mean ovarian thickness was 2.18±0.086 cm. The ovarian thickness did not vary significantly between the unprimed and primed group (2.14±0.121 cm and 2.21±0.124 cm, respectively) nor did it vary between the right and left ovaries (2.19±0.113 and 2.16±0.131 cm, respectively).

However, there was a significant effect of the stage (at which the ovarian thickness was determined) on the ovarian thickness : 1.37±0.077 cm, 2.52±0.077 cm and 2.64±0.071 cm at normal oestrus, superovulatory oestrus and at flushing respectively.

An increasing trend of ovarian thickness was observed as the stage advanced, probably due to gonadotropin stimulation.

4.3. EMBRYO RECOVERY

A total of 11 embryos were recovered from the donor cows, out of which only 7 were viable (transferable) while the other 4 were degenerated embryos. Three of the ten superovulated cows exhibited bilateral anovulatory follicular cysts, the cystic ovaries of these three cows were diagnosed at the time of flushing.

Table 4.13 : Embryo recovery from various cows.

Cow No.	No. of Embryos Recovered	Developmental Stage of the Embryo	Quality of the Embryo
1	2	Both Blastocysts	Both Good
2	1	Morula	Excellent
3	2	1 Morula, 1 Degenerated	Good, Poor
4	1	Degenerated	Poor
5	NIL (Cystic cow)	-	-
6	2	Both Degenerated	Both Poor
7	2	Both Morula	Both Good
8	1	Blastocyst	Excellent
9	NIL (Cystic cow)	-	-
10	NIL (Cystic cow)	-	-

Post-superovulatory PGF-2 alpha was given (after repeat flushing) to all the cows irrespective of the fact whether any recovery of embryos occurred or not or whether the cow went cystic or did not plus antibiotics (Oxytetracycline boluses) were infused intra-uterine for three days.

Since the embryo recovery in this experiment was very low, the effect of priming, if any, on embryo recovery rate could not be judged properly. However, study of other parameters revealed that priming had no beneficial effect on the number of follicles formed, the number of corpora lutea (ovulation rate) or the post-superovulatory return to oestrus in Rathi cattle. So, overall it can be stated that priming had no beneficial effect on the superovulatory response. The results are in accordance with the results of Rieger et al., 1988 and Gray et al., 1992 who also observed no beneficial effect of priming.

A constant problem with current methods for the superovulation of cattle is the inability to predict the number of viable embryos that will be recovered following treatment (Monniaux et al., 1983; Moor et al., 1984). The superovulatory responses may be limited by a genetic component or by the availability of growing follicles (Monniaux et al., 1983).

Reasons for low embryo recovery

The differences in ovarian response with respect to the number of corpora lutea and the embryos collected can be explained in various ways : -

(1) Lack of egg uptake by the infundibulum due to the intense ovarian reaction : Ovary size might be such that the fimbriae are incapable of investing the ovary sufficiently to allow complete recovery of ova (Hafez et al.,1963; Becker and Pinheiro, 1986; Bono et al., 1991).

(2) Premature or delayed ovulation (asynchronous ovulation) (Newcomb *et al.*, 1976; Becker and Pinheiro, 1986; Zeitoun *et al.*, 1991).

(3) Delayed egg or embryo descent because of imbalance in the gonadal steroids (Becker and Pinheiro, 1986).

(4) Accelerated egg or embryo descent into the uterus because of the same ovarian hormone imbalance (Newcomb *et al.*, 1976; Becker and Pinheiro, 1986; Hawk, 1988).

(5) Deviant systemic endocrine profiles affecting early embryonic development (Hafez *et al.*, 1963; Newcomb *et al.*, 1976; Elsden *et al.*, 1978; Saumande, 1980; Foote and Ellington, 1988; Zeitoun *et al.*, 1991; Armstrong, 1993).

(6) Suboptimal sperm transport leading to low fertilization rates or complete absence of fertilization (Hafez *et al.*, 1963; Hawk, 1988; Hunter, 1988; Hyttel *et al.*, 1991; Armstrong, 1993).

Anovulatory follicles have also been reported by Silvas et al,1981 and Kuttner ,1978 after PMSG treatment for superovulation .

It was suggested by Sreenan (1978) that these follicles persisted due to their failure to bind sufficient quantity of LH during pre-ovulatory surge.

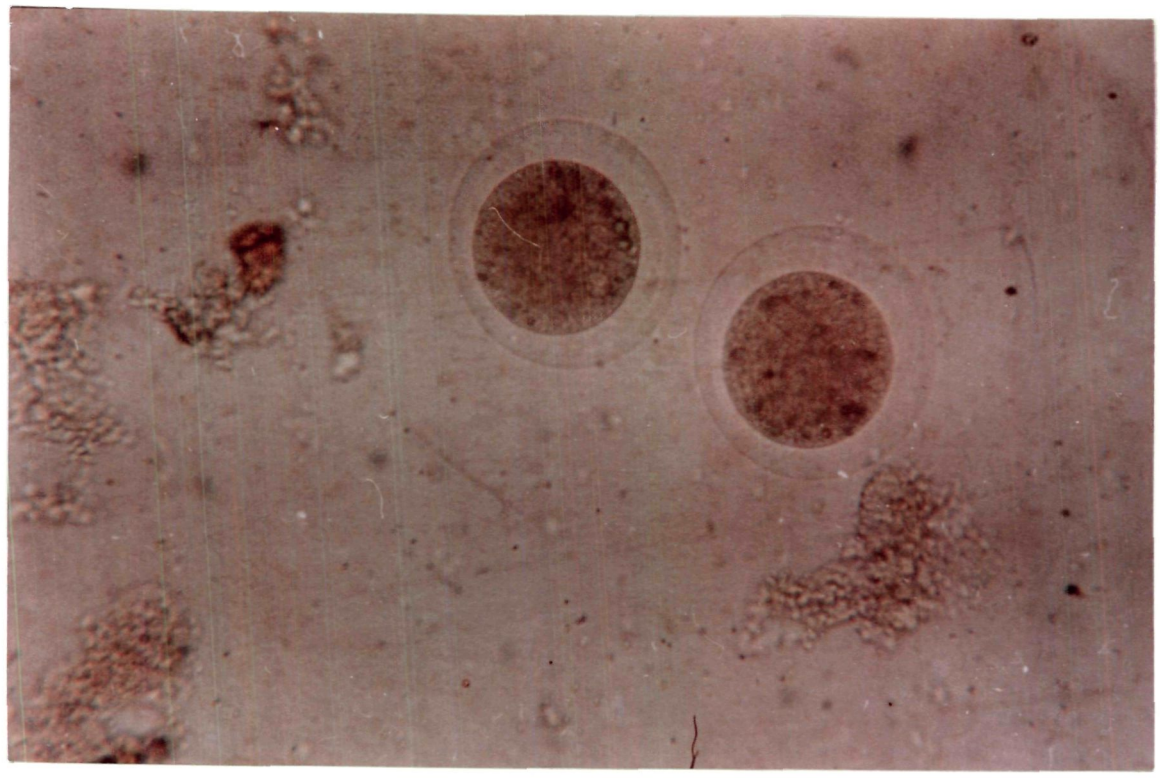
Becker and Pinheiro (1986) also found 11.9 per cent luteinized follicular cysts and concluded that lack of egg uptake by the infundibulum had occurred in cases of increased ovulation, with excessive increase in volume of the ovary.

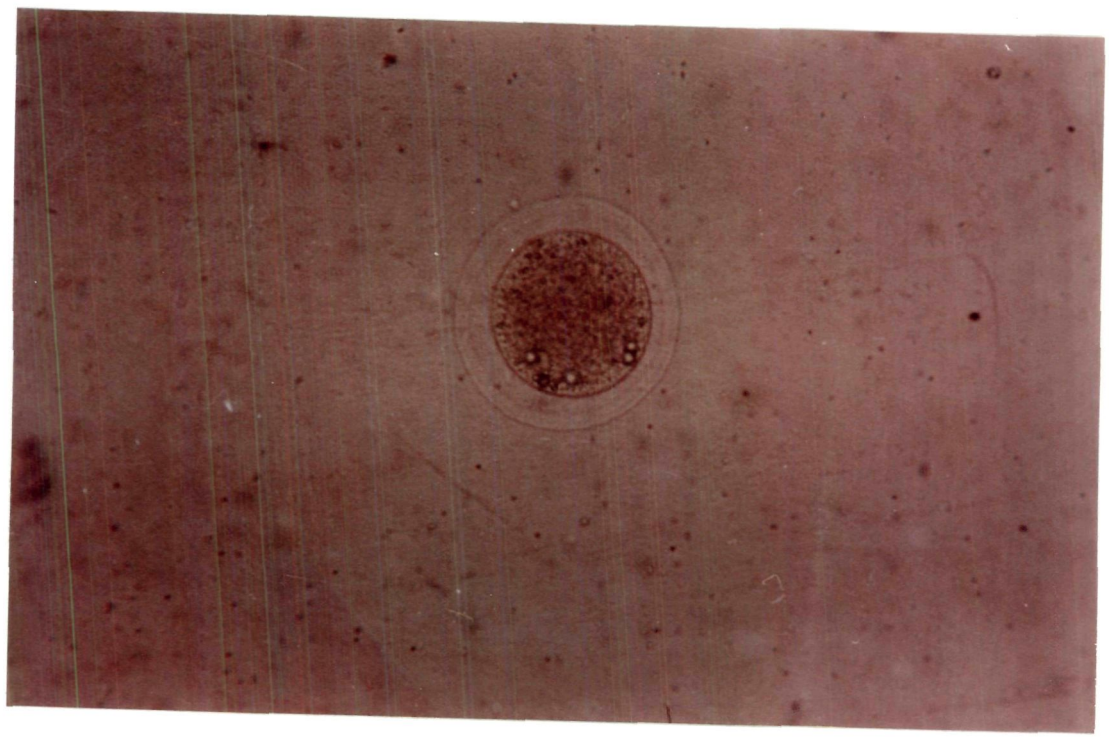
Bhattacharya et al. (1989) also reported development of anovulatory follicles following superovulation with PMSG in crossbred cows. These follicles disappeared spontaneously and were not palpable on rectal examination after a week's time.

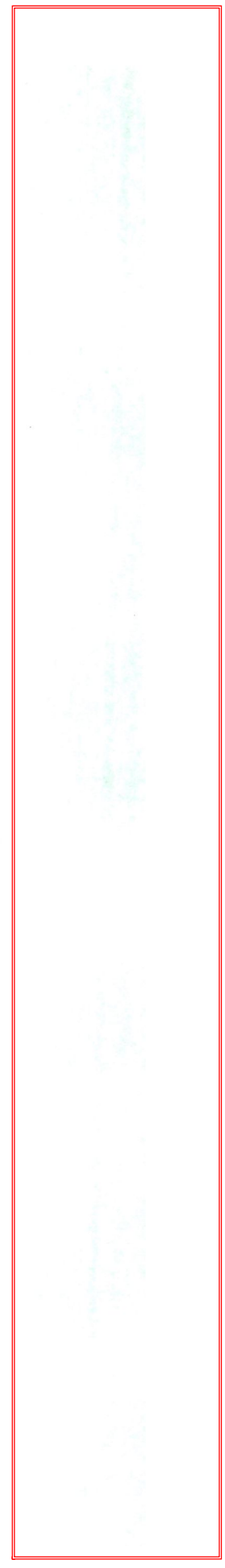
The use of PMSG has been associated with high numbers of unovulated follicles because of its long half-life in blood (Boland et al., 1991; Zeitoun et al., 1991).

The growth and maturation of small follicles stimulated by PMSG continued after the pre-ovulatory LH surge and resulted in the post-ovulatory oestrogen rise (Moyaert et al., 1985) and the increased number of large anovulatory follicles.

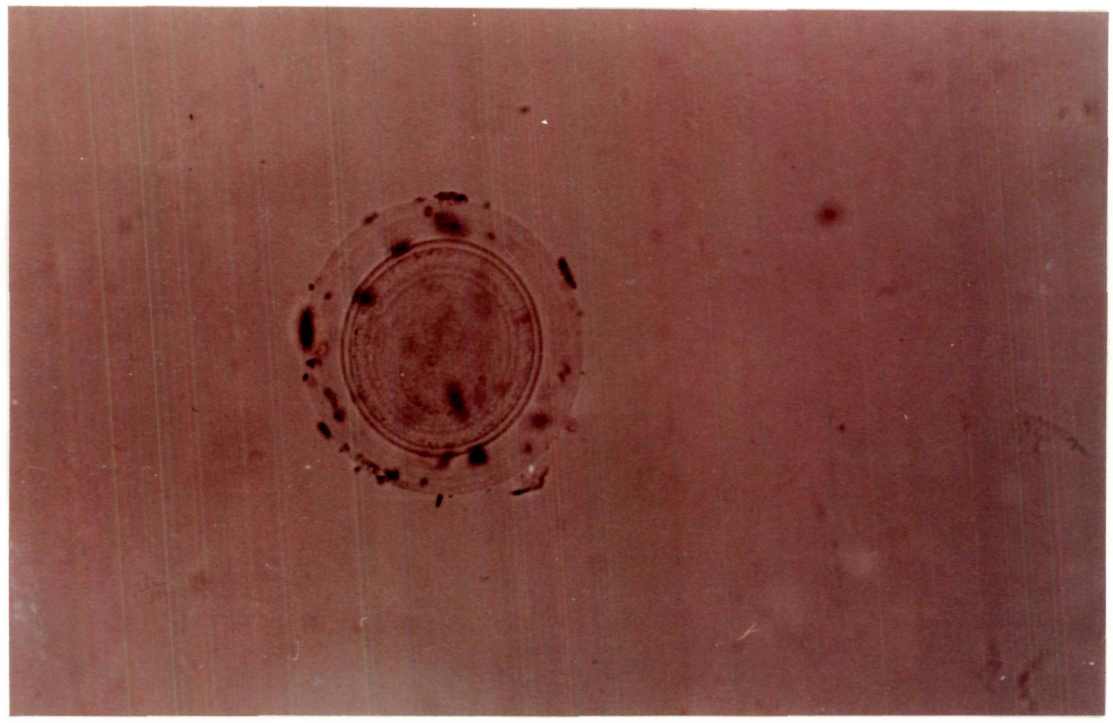
PMSG treatment revealed increases in ovary volume to the size of an orange with a number of cases of ovarian cysts and irregularities in return to normal cyclic activity (Bono et al., 1991).





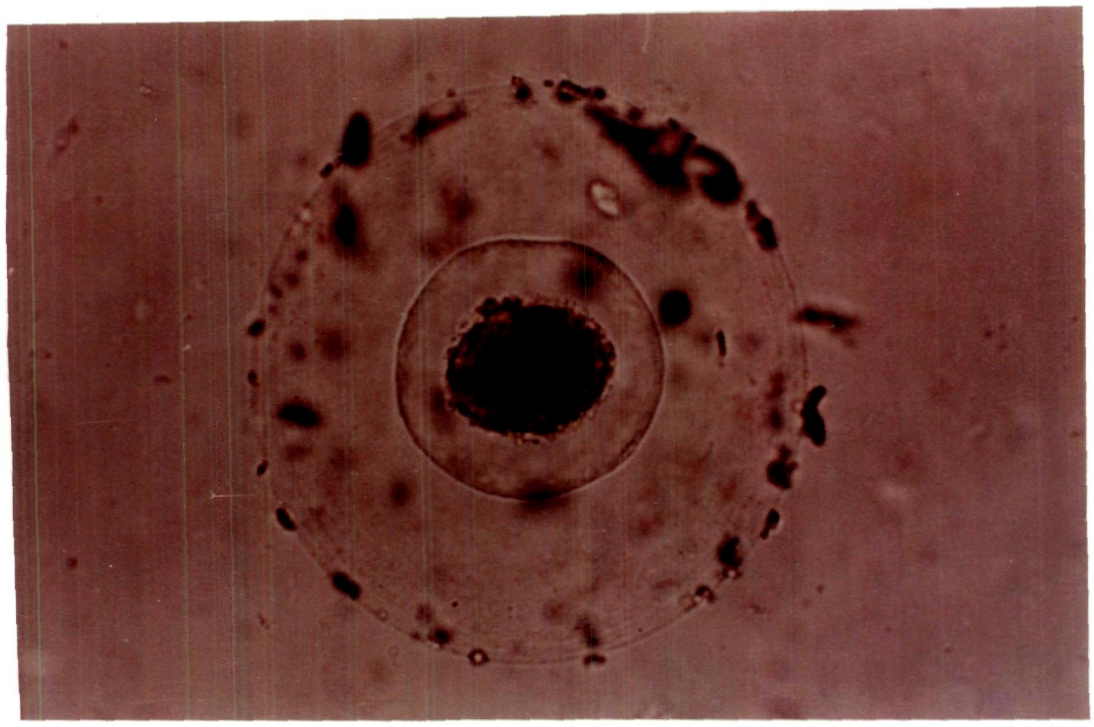


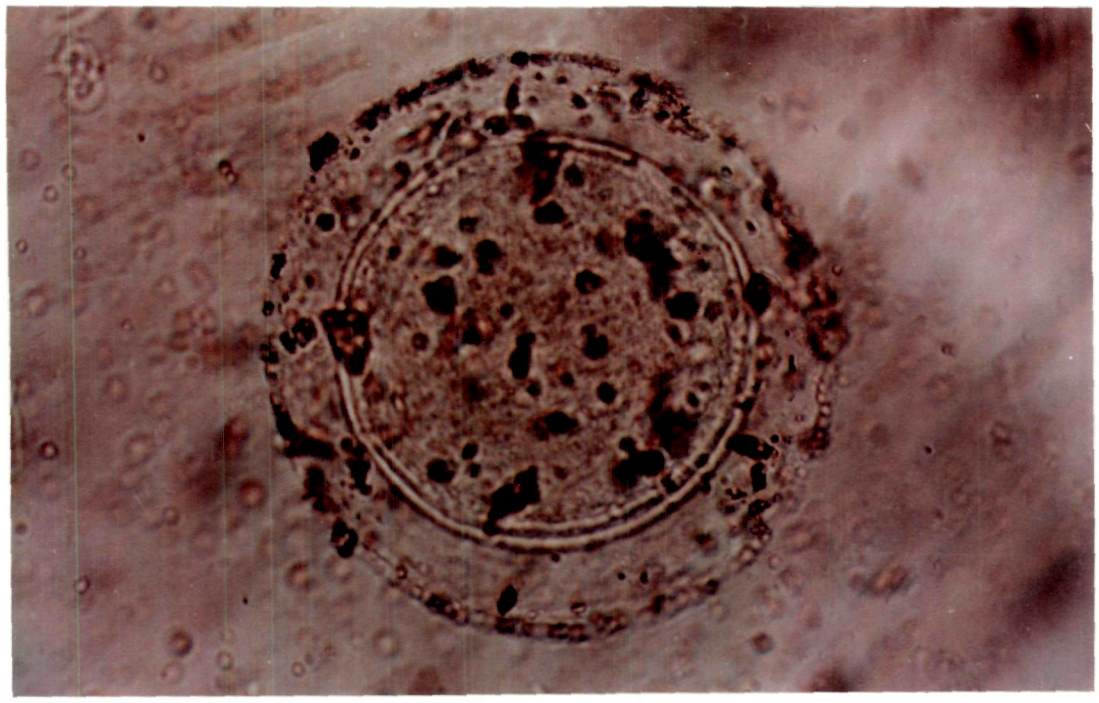
Photomicrograph of a bovine embryo, 100X



A bovine embryo showing first signs of degeneration, 200X

A degenerated bovine embryo, 200X





RESULTS OF HORMONE ASSAY

LH assay was carried out on six cows [3 cows each of unprimed group(cow number 1,2 and 3) and primed group(cow number 6,7 and 8)] plus two cows of the control group.

FSH assay was carried out on three cows of the unprimed group only (cow number 1,2 and 3) plus two cows of the control group.

Tables showing the concentration of LH and FSH in the above mentioned cows and their respective graphs showing the pattern of changes in LH and FSH concentration are given below :-

Table 4.14 : LH assay - table for Control cows no. 1 and 2.

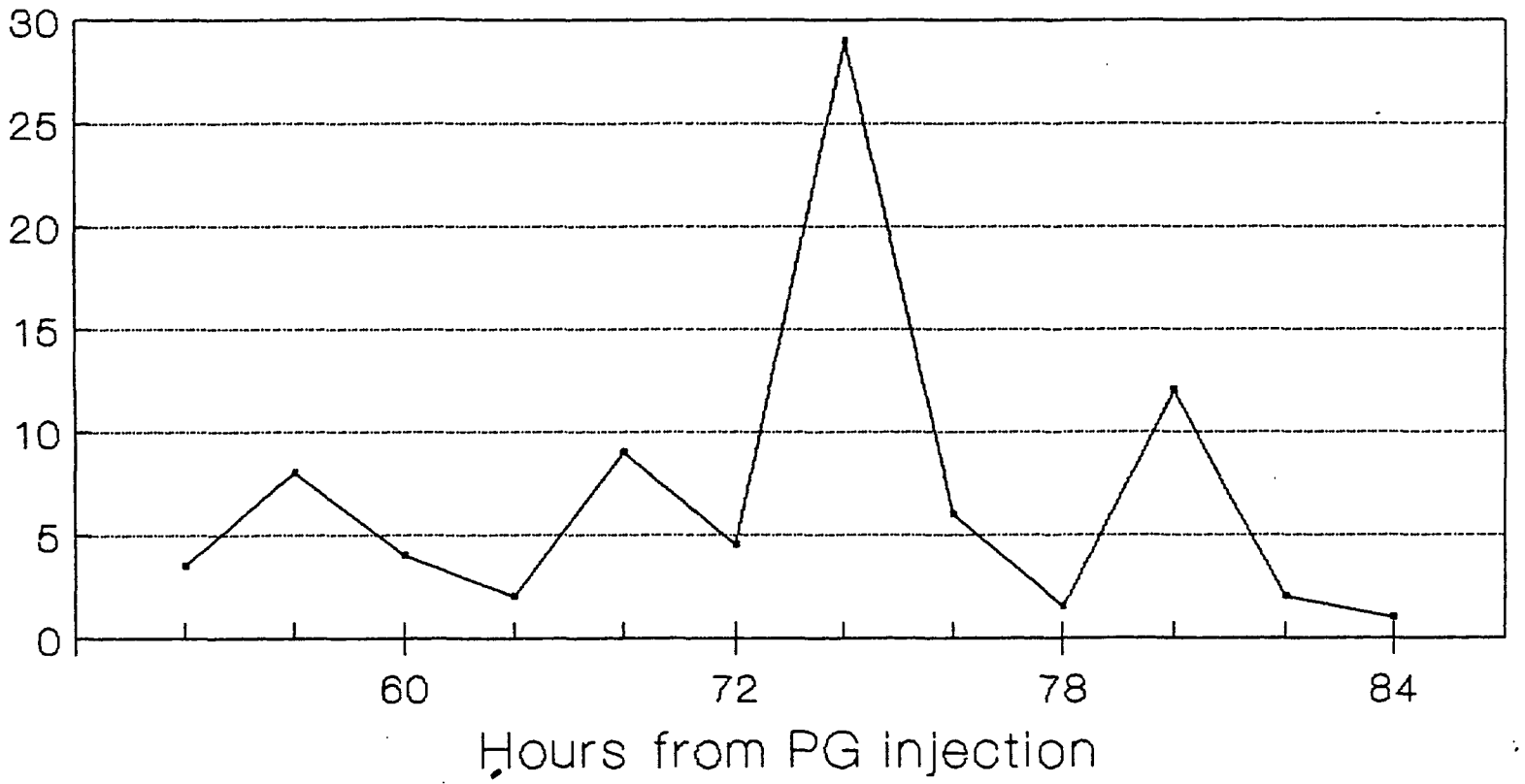
Sample No.	Hours from PG injection	Concentration (m I.U./ml)	
		Cow no. 1	Cow no. 2
1	52	3.5	1.75
2	56	8	3
3	60	4	5
4	64	2	10
5	68	9	4.5
6	72	4.5	2
7	74	29	11
8	76	6	5
9	78	1.5	1.5
10	80	12	6
11	82	2	28
12	84	1	3.5

Basal level : The lowest value or concentration of the hormone observed amongst all the samples belonging to a particular cow.

Peak level : The highest value or concentration of the hormone observed amongst all the samples belonging to a particular cow.

The control cows exhibited, on an average, a basal level of 1.25±0.25 m I.U./ml (1.0 and 1.5 m I.U./ml for control cow no. 1 and 2, respectively) of LH and a peak level of 28.5±0.5 m I.U./ml (29 and 28 m I.U./ml for control cow no. 1 and 2, respectively) of LH.

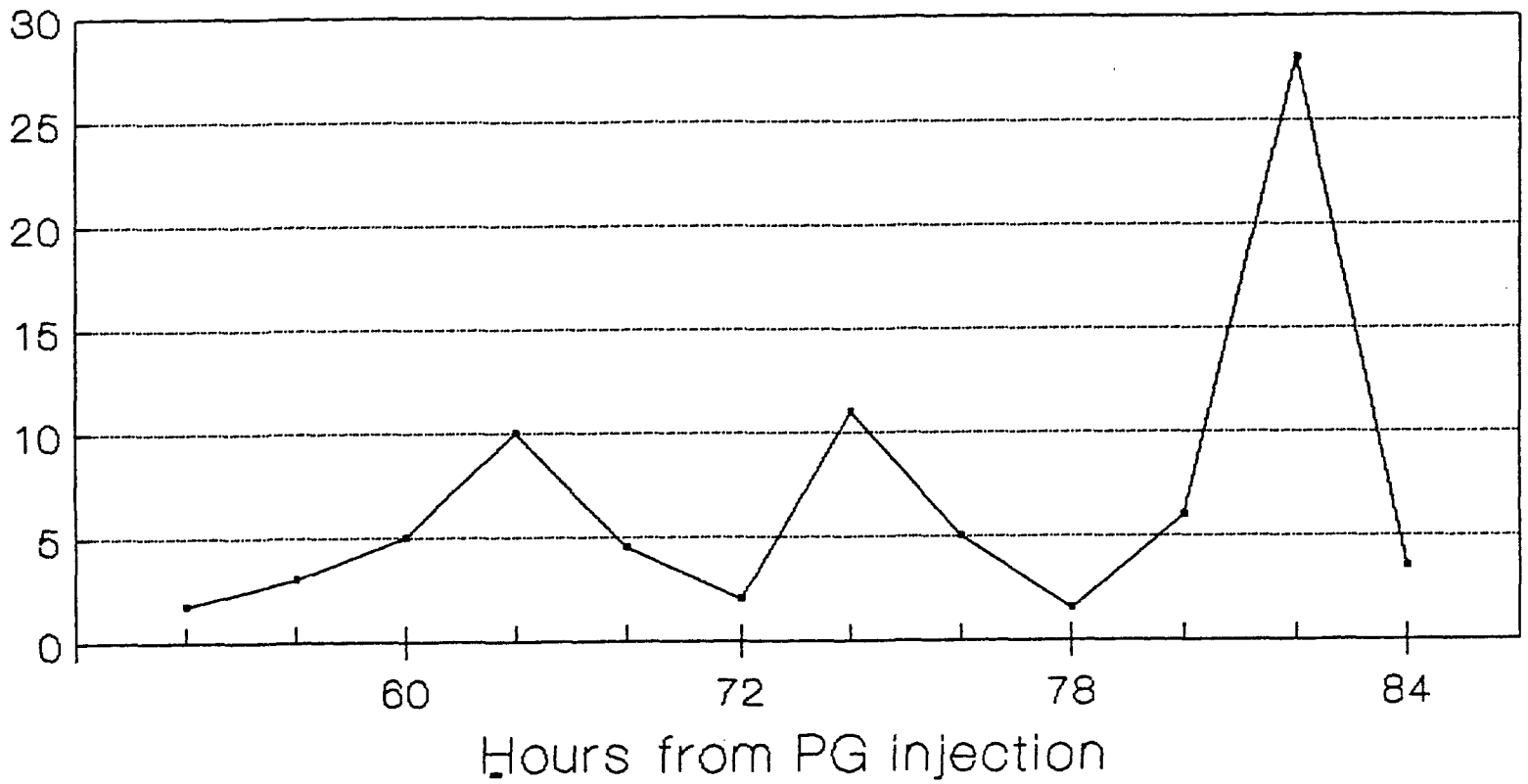
Graph depicting LH secretion-Control cow no. 1



Y axis = Concentration of LH (m I.U./ml)

Fig. No. 3.

Graph depicting LH secretion-Control cow no. 2



Y axis = Concentration of LH (m I.U./ml)

Fig. No. 4.

Table 4.15. : LH assay - table for Unprimed cows no. 1,2 and 3.

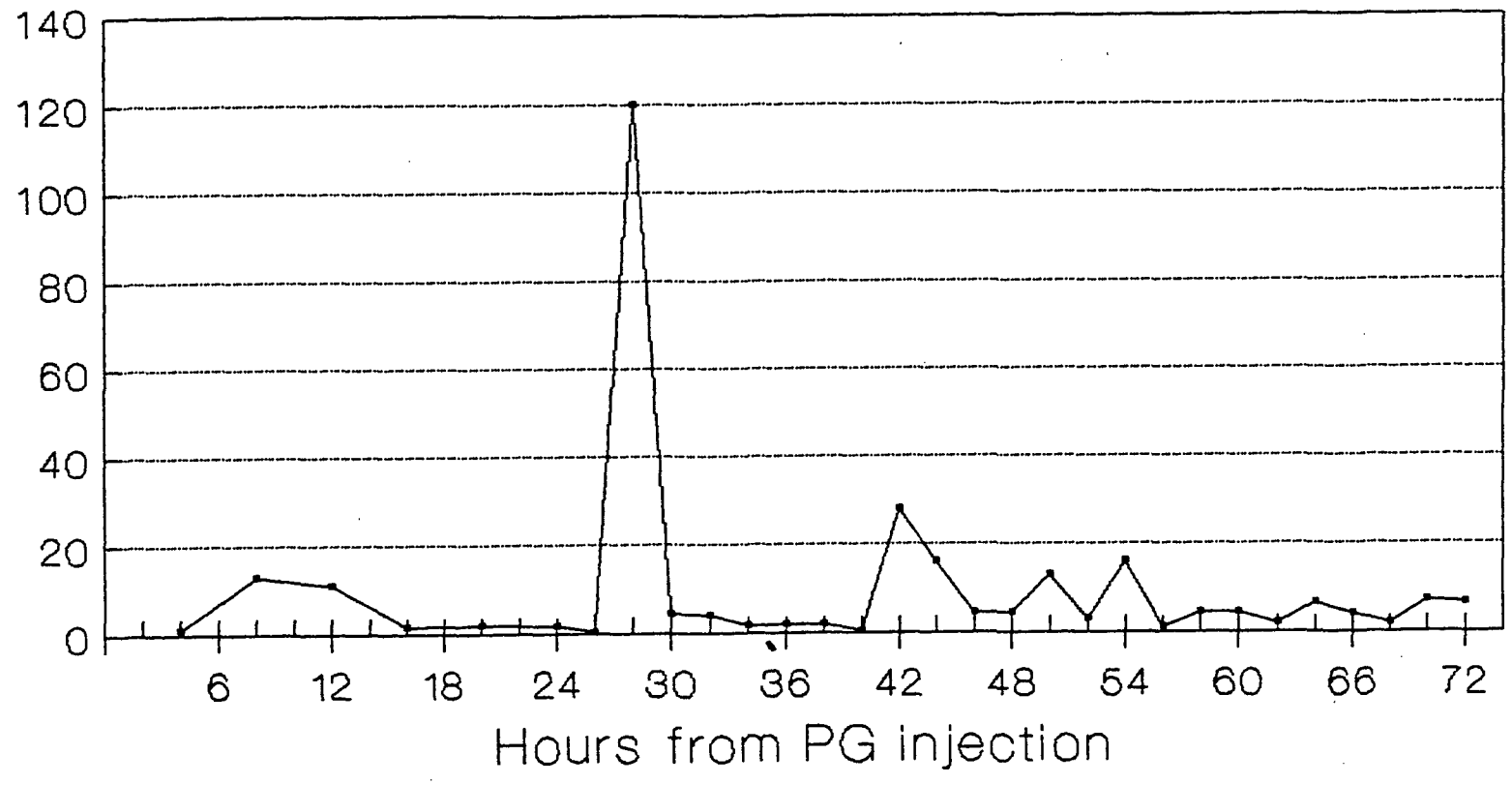
Sample No.	Hours from PG injection	Concentration (m I.U./ml)		
		Cow no. 1	Cow no. 2	Cow no. 3
1	4	6	1	0.25
2	8	2	13	4
3	12	1	11	1
4	16	8	1.5	11
5	20	5	2	0.75
6	24	4	1.75	1
7	26	3	0.5	34
8	28	5	120	1
9	30	10	4.5	0.75
10	32	2	4	2
11	34	1.5	1.75	1.5
12	36	2	2	20
13	38	2	2	0.75
14	40	1.5	0.5	2
15	42	1	28	2
16	44	2	16	4
17	46	12	4.5	3.5
18	48	1.5	4.5	28
19	50	4.5	13	6
20	52	2	3	12
21	54	5	16	0.25
22	56	5	1	2
23	58	6	4.5	4
24	60	8	4.5	5
25	62	2	2	5
26	64	2	6.5	8.5
27	66	1.75	4	3.5
28	68	5	2	2
29	70	7	7	9
30	72	2	6.5	3

Graph depicting LH secretion-Unprimed cow no. 1

Y axis = Concentration of LH (m I.U./ml)

Fig. No. 5.

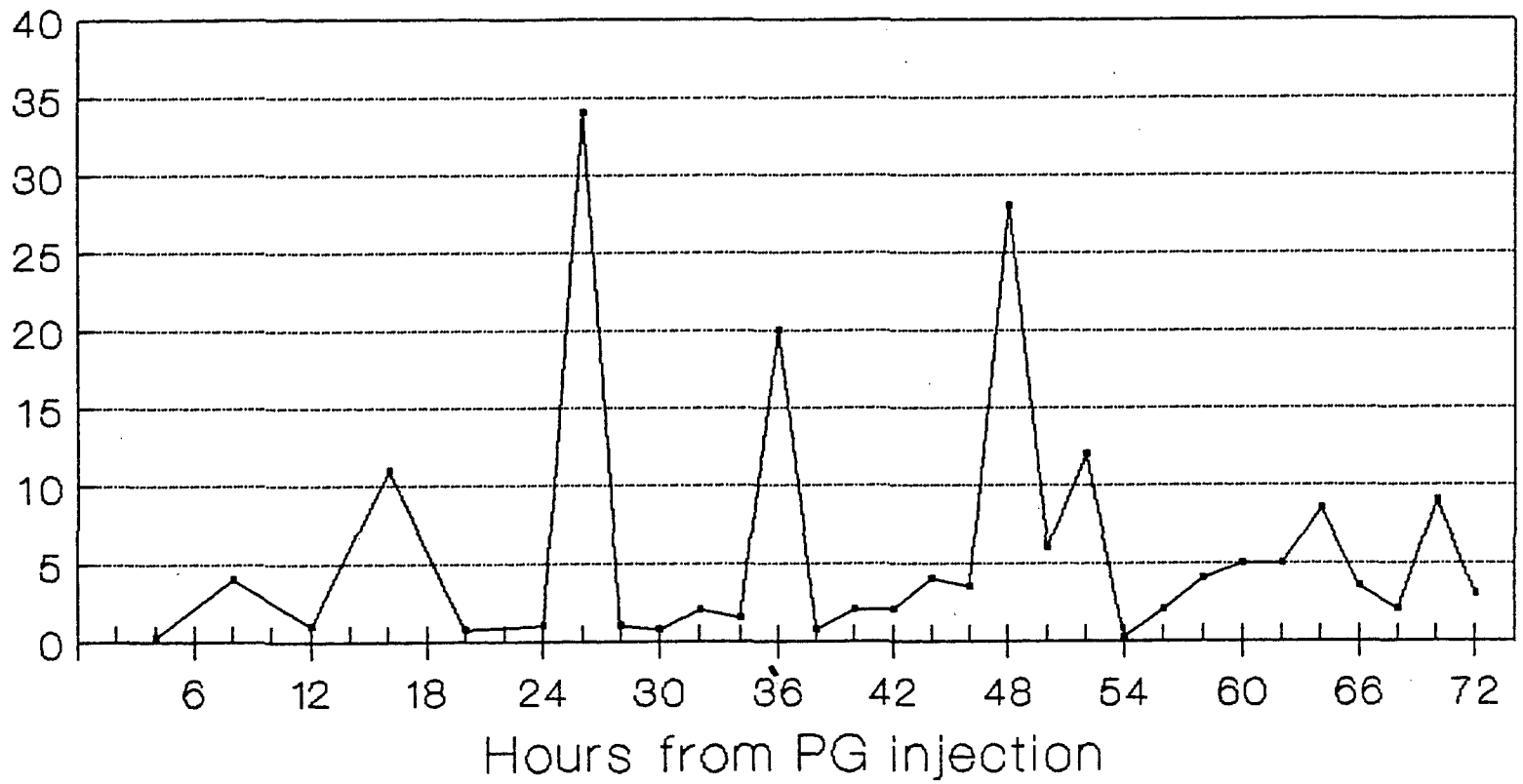
Graph depicting LH secretion-Unprimed cow no. 2



Y axis = Concentration of LH (m I.U./ml)

Fig. No. 6.

Graph depicting LH secretion-Unprimed cow no. 3



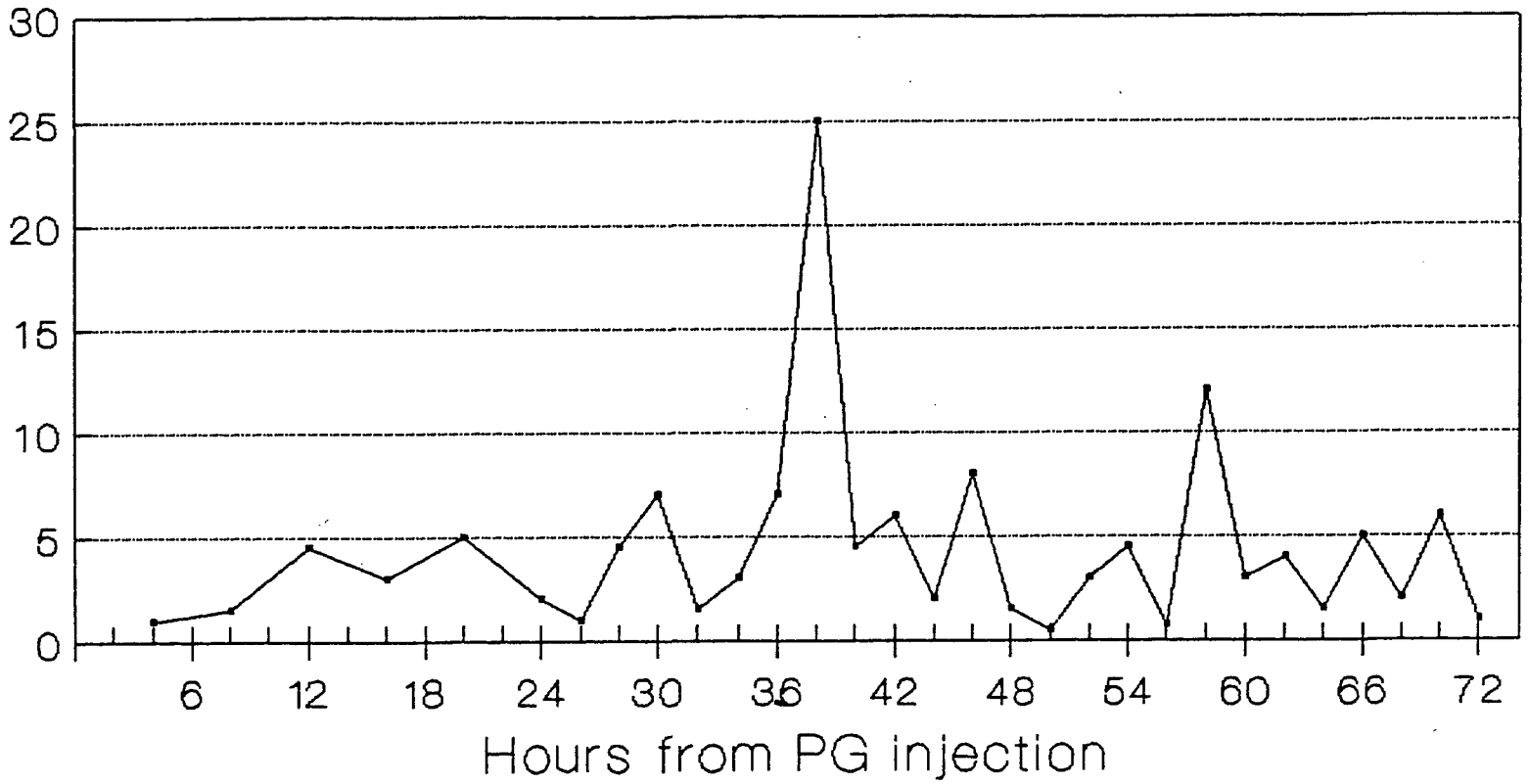
Y axis = Concentration of LH (m I.U./ml)

Fig. No. 7.

Table 4.16. : LH assay - table for Primed cows no. 6,7 and 8.

Sample No.	Hours from PG injection	Concentration (m I.U./ml)		
		Cow no. 6	Cow no. 7	Cow no. 8
1	4	1	2	1.5
2	8	1.5	1.5	3.5
3	12	4.5	5	2
4	16	3	9	2
5	20	5	0.75	6
6	24	2	6	3.5
7	26	1	3.5	8
8	28	4.5	12	6
9	30	7	4.5	2
10	32	1.5	6	4.5
11	34	3	16	12
12	36	7	3	9
13	38	25	1.5	10
14	40	4.5	11	6
15	42	6	4.5	2
16	44	2	1.75	4.5
17	46	8	5	7
18	48	1.5	7	5
19	50	0.5	110	38
20	52	3	5	8
21	54	4.5	2	2
22	56	0.75	8	1.5
23	58	12	3	4
24	60	3	1.5	3
25	62	4	4	0.5
26	64	1.5	1.75	6
27	66	5	7	4
28	68	2	4.5	2
29	70	6	2	3.5
30	72	1	1	3

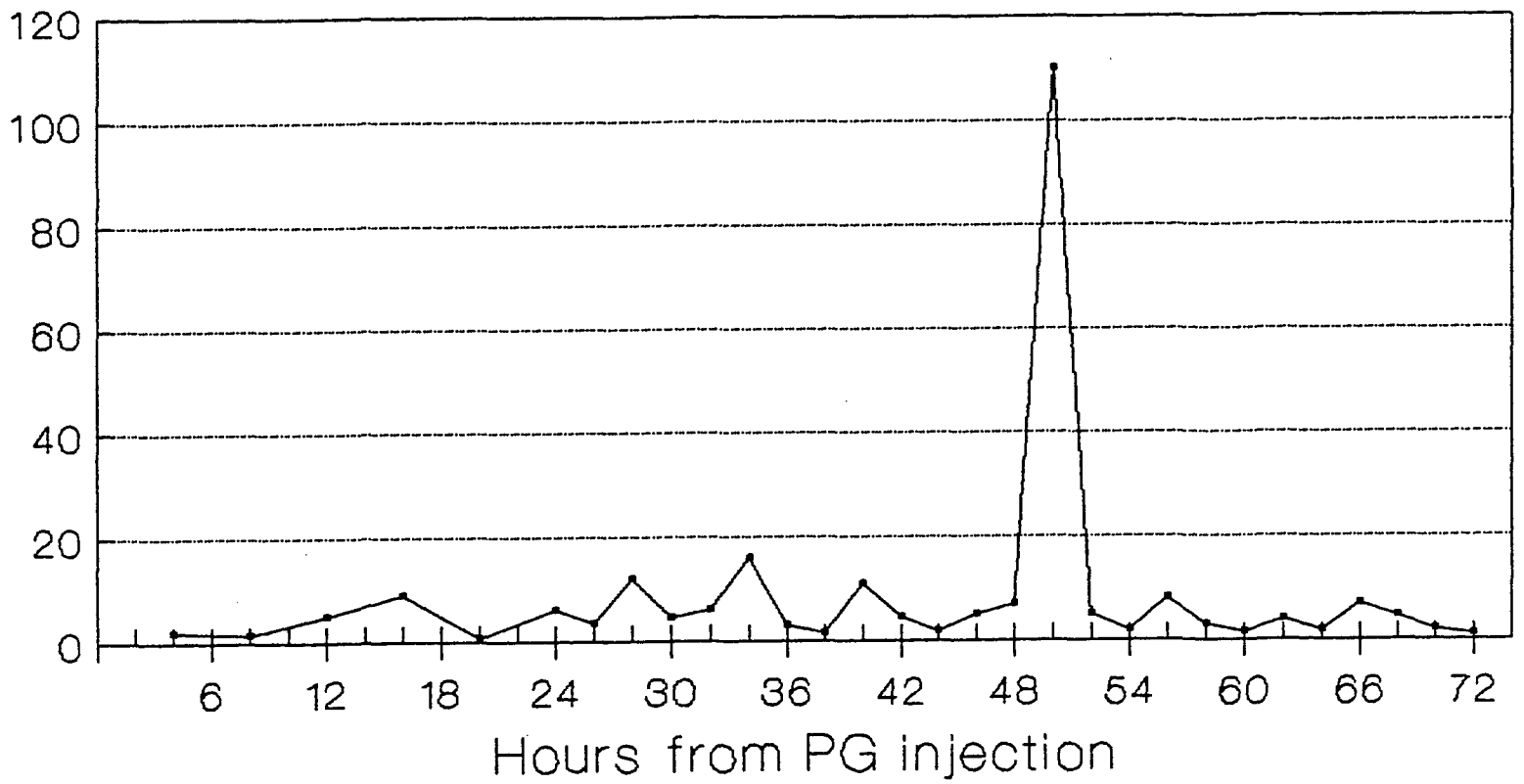
Graph depicting LH secretion-Primed cow no. 6



Y axis = Concentration of LH (m I.U./ml)

Fig. No. 8.

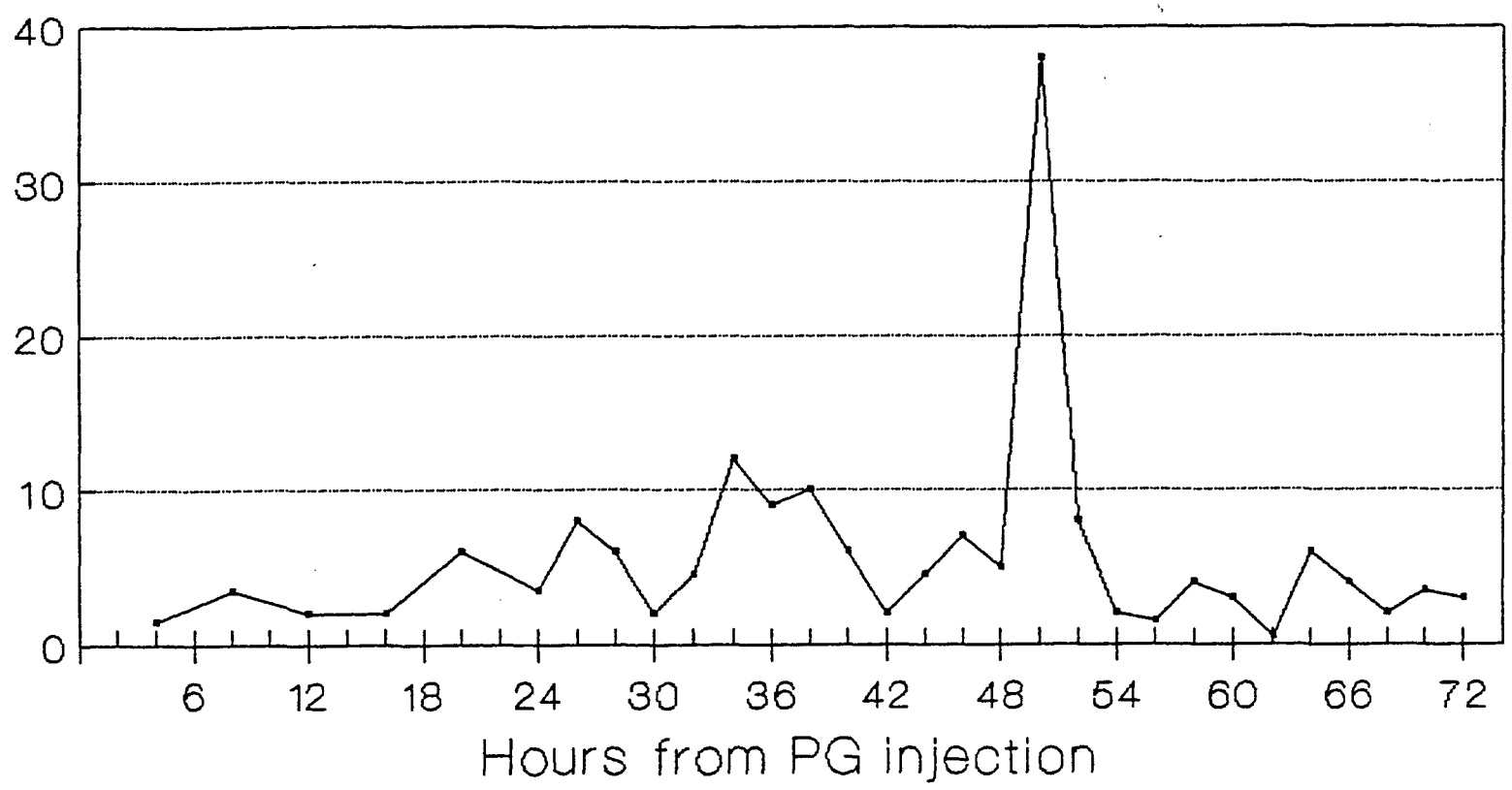
Graph depicting LH secretion-Primed cow no. 7



Y axis = Concentration of LH (m I.U./ml)

Fig. No. 9.

Graph depicting LH secretion-Primed cow no. 8



Y axis = Concentration of LH (m I.U./ml)

Fig. No. 10.

The unprimed cows exhibited, on an average, a basal level of 0.4166±0.0833 m I.U./ml (0.5, 0.5 and 0.25 m I.U./ml for cow no.1,2 and 3,respectively) of LH whereas in the primed cows this value was 0.5833±0.0833 m I.U./ml (0.5, 0.75 and 0.5 m I.U./ml for cow no. 6,7 and 8, respectively) of LH. The difference between the two groups was non-significant (P>0.05).

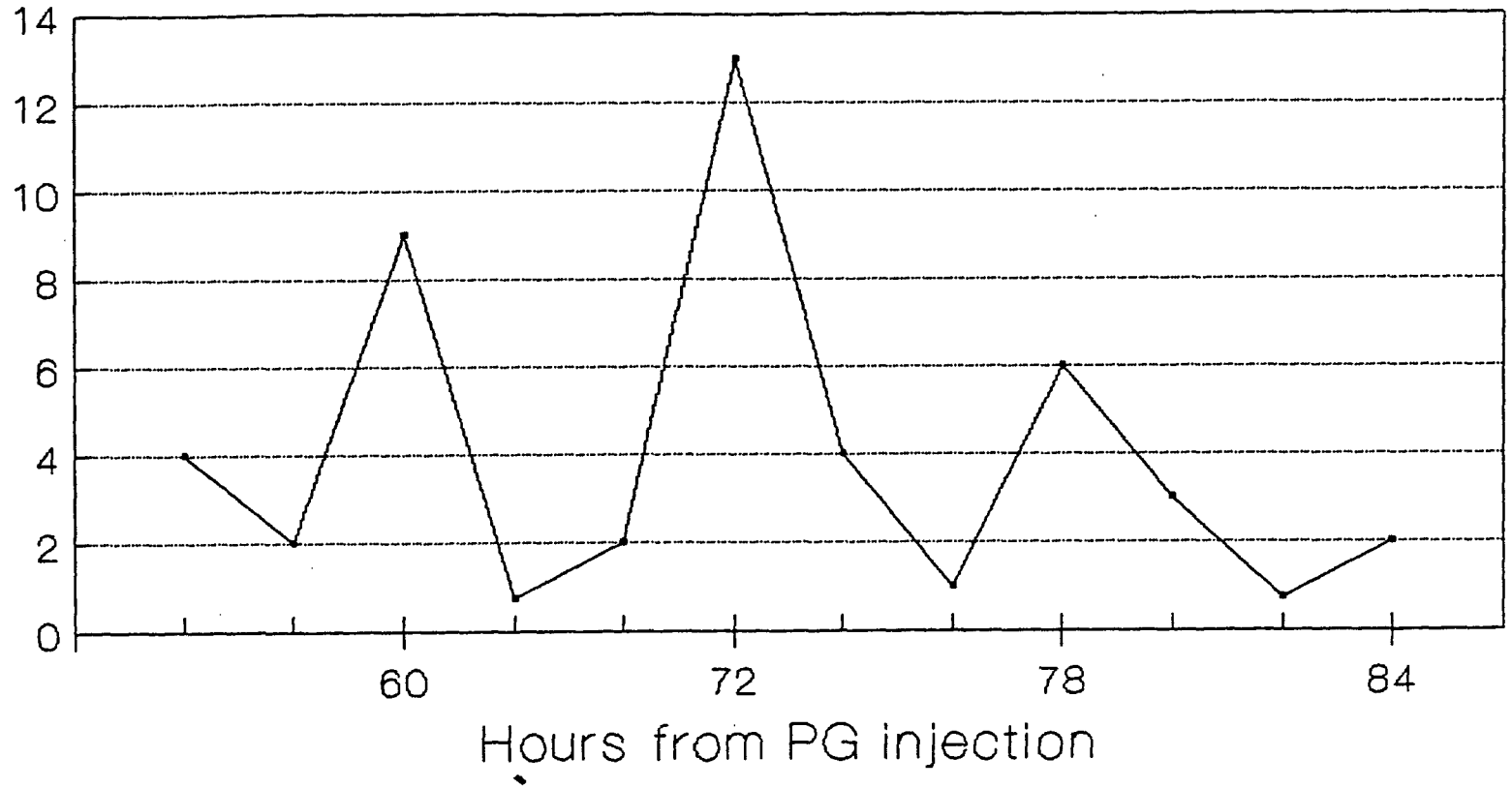
The unprimed cows exhibited, on an average, a peak level of 55.33±32.95 m I.U./ml (12, 120 and 34 m I.U./ml for cow no. 1,2 and 3, respectively) of LH whereas in the primed cows this value was 57.66±26.43 m I.U./ml (25, 110 and 38 m I.U./ml for cow no. 6, 7 and 8, respectively) of LH. The difference between the two groups was found to be non-significant (P>0.05).

Table 4.17 : FSH assay - table for Control cows no. 1 and 2.

Sample No.	Hours from PG injection	Concentration (m I.U./ml)	
		Cow no. 1	Cow no. 2
1	52	4	3
2	56	2	0.5
3	60	9	1
4	64	0.75	4
5	68	2	8
6	72	13	0.75
7	74	4	10
8	76	1	2
9	78	6	6
10	80	3	20
11	82	0.75	4
12	84	2	3

The control cows exhibited, on an average, a basal level of 0.625±0.125 m I.U./ml (0.75 and 0.5 m I.U./ml for control cow no. 1 and 2, respectively) of FSH and a peak level of 16.5±3.5 m I.U./ml (13 and 20 m I.U./ml for control cow no. 1 and 2, respectively) of FSH.

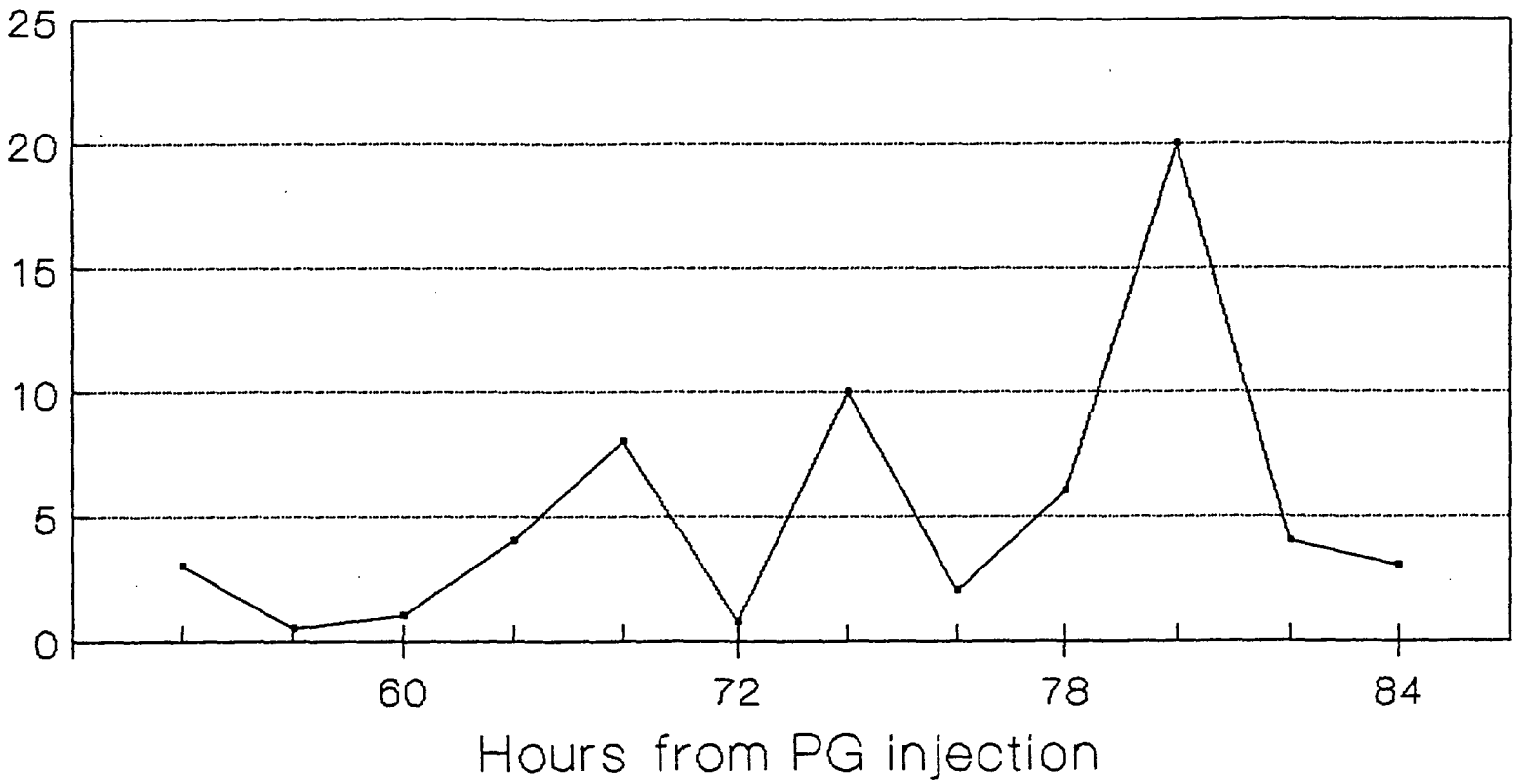
Graph depicting FSH secretion-Control cow no. 1



Y axis = Concentration of FSH(m I.U./ml)

Fig. No. 11.

Graph depicting FSH secretion-Control cow no. 2



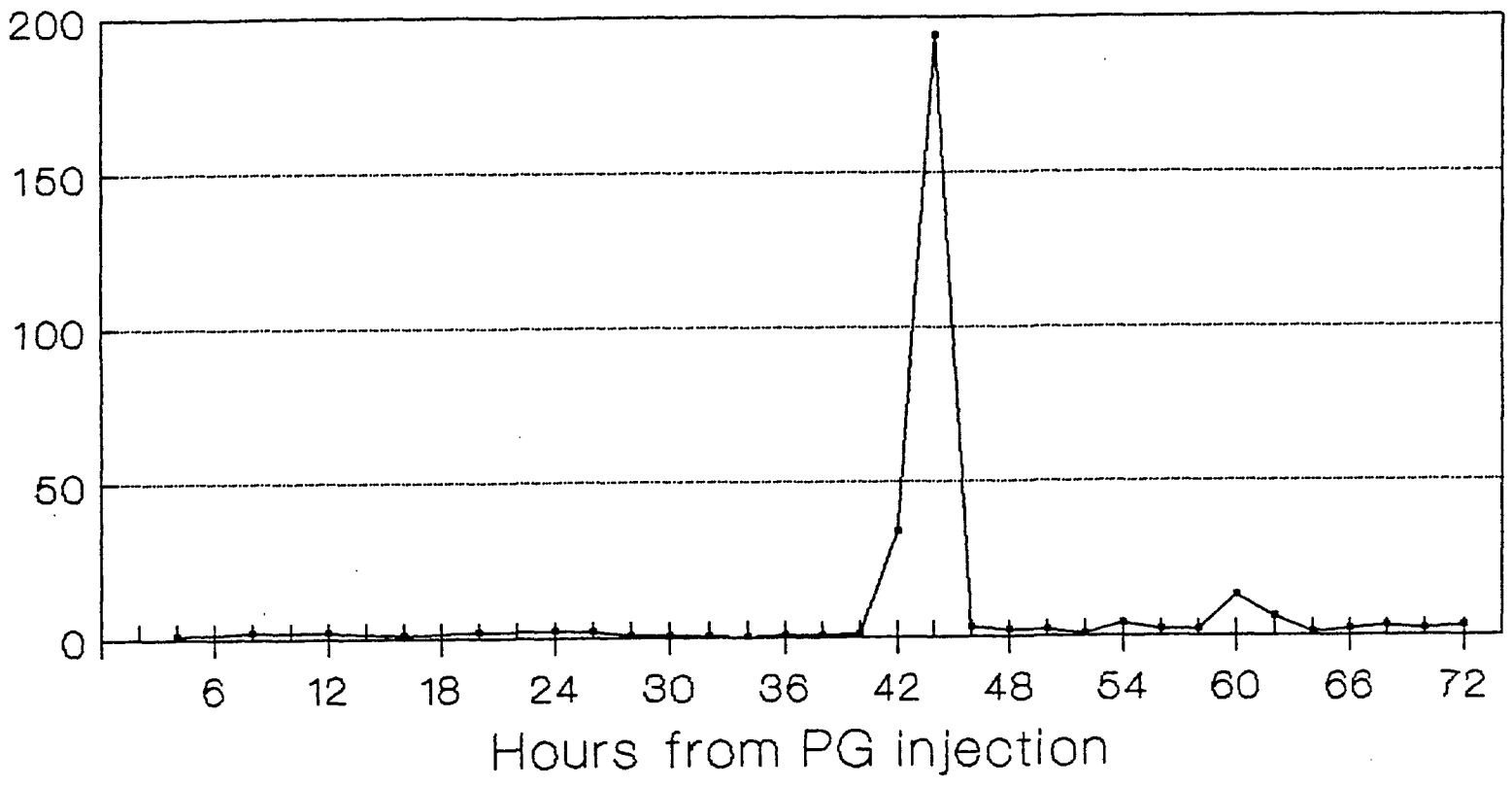
Y axis = Concentration of FSH(m I.U./ml)

Fig. No. 12.

Table 4.18. : FSH assay - table for Unprimed cows no. 1,2 and 3.

Sample No.	Hours from PG injection	Concentration (m I.U./ml)		
		Cow no. 1	Cow no. 2	Cow no. 3
1	4	1	2	0.5
2	8	2	2	2
3	12	2	3	1
4	16	1	3	0.75
5	20	2	2.25	1
6	24	2.25	3	200
7	26	2	2	0.75
8	28	0.75	0.1	62
9	30	0.75	2	2
10	32	0.5	2	1
11	34	0.1	2	9
12	36	0.75	2	1
13	38	0.75	2	1
14	40	1	2	1
15	42	34	2	1
16	44	194	0.75	2
17	46	3	1	2
18	48	2	0.75	8
19	50	2	0.5	2
20	52	0.75	2	2
21	54	4	2	2
22	56	2	2	2
23	58	2	1	13
24	60	13	2.25	6
25	62	6	1	4
26	64	1	0.75	2
27	66	2	1	2
28	68	3	0.5	4
29	70	2	2	3
30	72	3	1	2

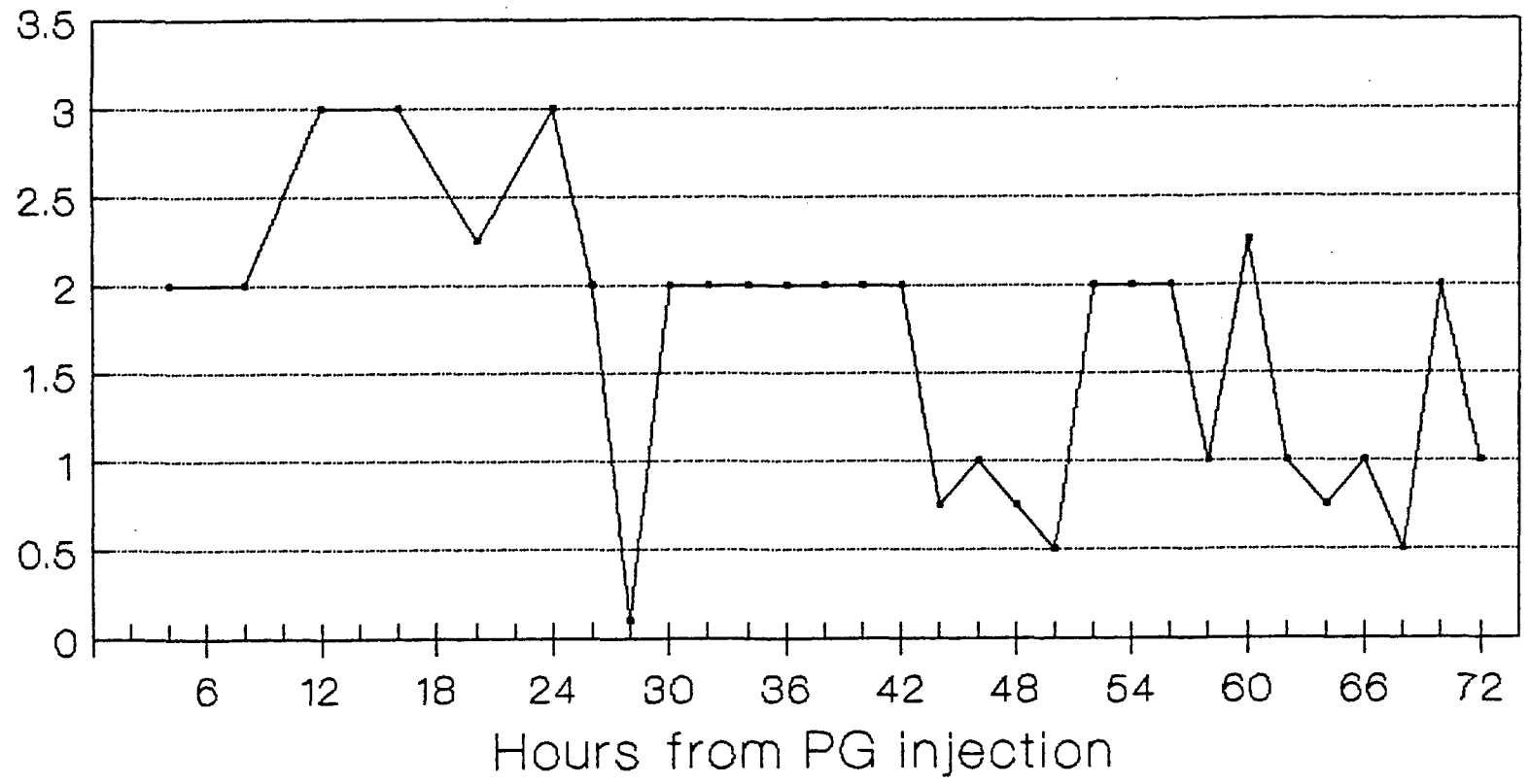
Graph depicting FSH secretion-Unprimed cow no. 1



Y axis = Concentration of FSH(m I.U./ml)

Fig. No. 13.

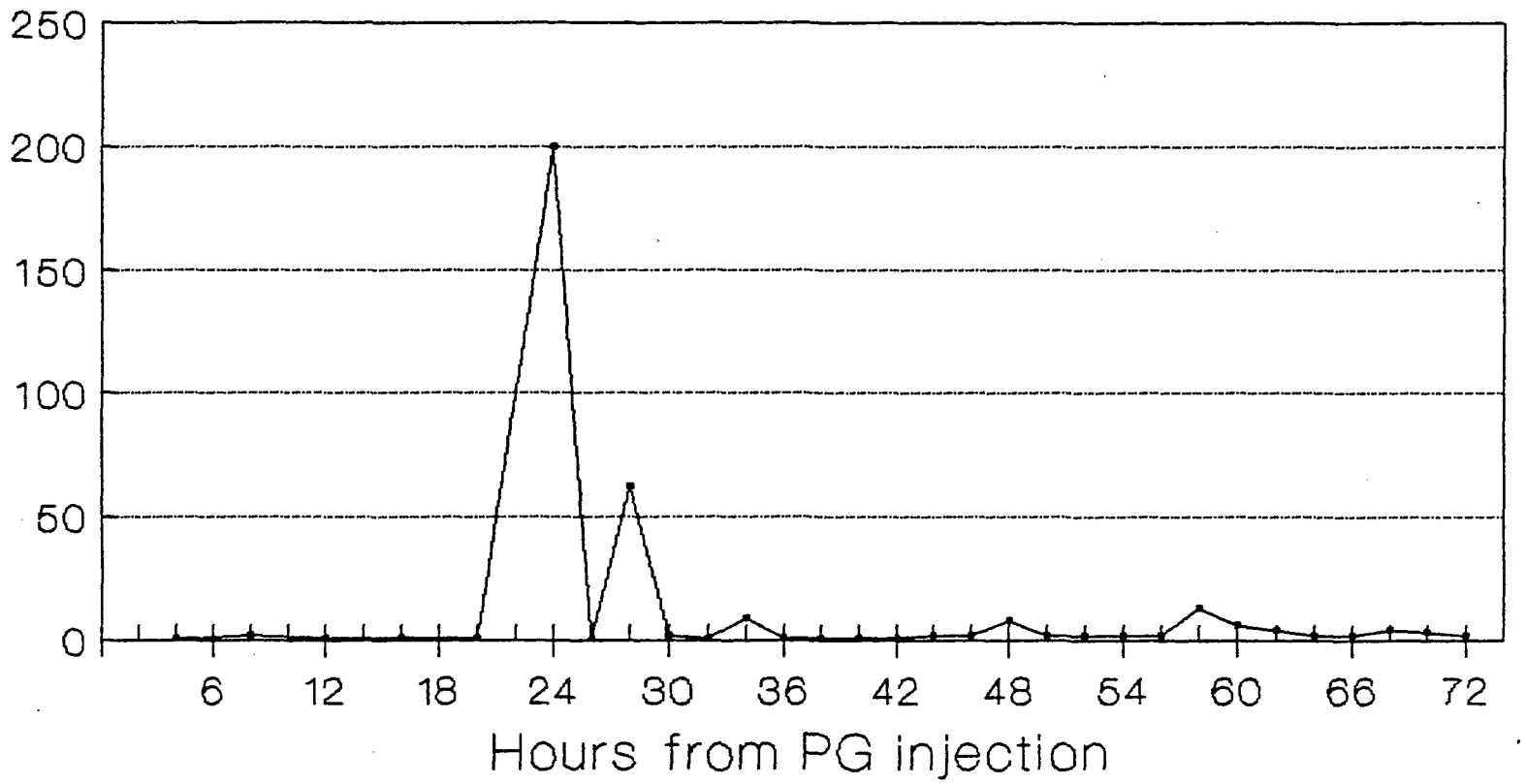
Graph depicting FSH secretion-Unprimed cow no. 2



Y axis = Concentration of FSH(m I.U./ml)

Fig. No. 14.

Graph depicting FSH secretion-Unprimed cow no. 3



Y axis = Concentration of FSH(m I.U./ml)

Fig. No. 15.

The unprimed cows exhibited, on an average, a basal level of 0.233±0.133 m I.U./ml (0.1, 0.1 and 0.5 m I.U./ml for cow no. 1, 2 and 3, respectively) of FSH and a peak level of 132.33±64.68 m I.U./ml (194, 3 and 200 m I.U./ml for cow no. 1, 2 and 3, respectively) of FSH.

The unusually low peak level of FSH (3 m I.U./ml) in the unprimed cow no. 2 could not be explained. Perhaps, the endogenous FSH level never reached a higher value.

The secretion of LH and FSH was found to be pulsatile in the superovulated and control Rathi cows. These findings are in accordance with those of Rahe *et al.* (1980), Schallenberger *et al.* (1984), Walters and Schallenberger (1984), Walters *et al.* (1984) and Yadav *et al.* (1986a).

More frequent blood sampling (every 30 minutes or every hour) might be advocated for future research programmes to determine the pulse frequency.

Table 4.19. : Table showing interval from PG injection to FSH peak (hours) and FSH peak to oestrus (hours).

Cow no.	PG injection to FSH peak (hours)	FSH peak to oestrus (hours)
Control cow no.		
1	72	0 (coinciding)
2	80	0 (coinciding)
Unprimed cow no.		
1	44	2
2	24	2
3	24	0 (coinciding)

The mean interval from PG injection to FSH peak was observed to be 76±4 hours in the control cows and 30.66±6.66 hours in the unprimed cows.

The mean interval from FSH peak to oestrus was observed to be 0 hour in the control cows i.e. the FSH peak coincided with the onset of oestrus in the control cows, while this interval was found to be 1.33±0.66 hours in the unprimed cows. In one of the unprimed cows (cow no. 3), the FSH peak was found to coincide with the onset of oestrus.

Table 4.20. : Table showing interval from PG injection to LH peak (hours) and oestrus to LH peak (hours).

Cow no.	PG injection to LH peak (hours)	Oestrus to LH peak (hours)
Control cow no.		
1	74	2
2	82	2
Unprimed cow no.		
1	46	0 (coinciding)
2	28	2
3	26	2
Primed cow no.		
6	38	2
7	50	2
8	50	2

The LH peak occurred at a mean interval of 78±4 hours from PG injection in the control group, 33.33±6.35 hours in the unprimed cows and 46±4 hours in the primed cows, the difference between the unprimed and the primed cows being non-significant (P>0.05).

The results are in accordance with the following scientists who reported this interval to be 82 hours in controls (Yadav et al., 1986a); 37 hours (Yadav et al., 1986a), 38.6±6.4 hours (Kweon et al., 1987) in PMSG treated cows; 46.8±5.9 hours (Kweon et al., 1987) in FSH treated cows.

The mean interval from oestrus to LH peak was observed to be 2 hours in control cows, 1.33±0.66 hours in the unprimed cows and 2 hours in the primed cows, the difference between the unprimed and the primed cows being non-significant ($P>0.05$). Only in one of the unprimed cows (cow no. 1) the LH peak was found to be coinciding with the onset of oestrus.

The results are in accordance with the following scientists who reported this interval to be 2 hours (Yadav et al., 1986b; Yadav, 1988), average 2 hours (Sprague et al., 1971) and 1.96±0.54 hours (Bevers and Dieleman, 1987) in superovulated cows.

CONCLUSION

The growth of scientific understanding is limited by the methods available to enhance it, can be limited by failure to use the best method to promote it and may not only be limited but inhibited by the improper application of even the most appropriate method.

In the present investigation it was seen that priming had no significant beneficial effect on the superovulatory response. Perhaps, a change in the dose (larger dose) or a change in the day of priming may exert a significantly positive effect on superovulation.

Three of the ten superovulated cows revealed anovulatory follicular cysts. It was suggested by Sreenan (1978) that these follicles persisted due to their failure to bind sufficient quantity of LH during pre-ovulatory surge. Perhaps an exogenous source of LH, in the form of GnRH or HCG injection at the time of A.I., could improve ovulation rate and hence increase the superovulatory response. GnRH resulted in a significant increase in the total number of embryos recovered and a tendency for increased number of fertilized ova and transferable embryos (Savage and Mapletoft, 1984).

Though the number of embryos recovered was small but even this rate of superovulation and embryo recovery lends credibility to this technique in its ultimate objective of rapidly increasing the number of offsprings from superior animals. It is now open for speculation that whether the eggs

which were unaccounted for had been expelled from the genital tract after premature entry into the uterus or whether they were "tube-locked" i.e. retained in the oviducts.

Because of the continued stimulation of ovaries caused by the longer half-life of PMSG, most of the cows revealed large sized ovaries at the time of flushing. Might be that the fimbriae were incapable of investing the ovary leading to a failure of recovery of the ova by the infundibulum (Hafez et al., 1963; Becker and Pinheiro, 1986; Bono et al., 1991). Perhaps, treatment with a smaller dose of PMSG and incorporation of anti-PMSG in the superovulatory regimen may alleviate this problem. PMSG antiserum was found to result in higher ovulations, a decreased number of unovulated follicles, a better fertilization rate and a shorter exhibition of external symptoms of heat with less inseminations to be performed, when used alongwith PMSG for superovulation in cows (Dhondt et al., 1978). Adverse effect on embryo quality due to ovarian hyperstimulation by PMSG after ovulation can be effectively controlled with an anti-PMSG serum injected at oestrus in cows (Saumande et al., 1984). Neutralization of PMSG at 60 hours after the PG injection prevented the rise of oestradiol concentration and improved the fertilization and embryo recovery rate. Better results can be expected by incorporating GnRH and/or PMSG antiserum in the PMSG incorporating superovulation protocols. In the end it is worthwhile to suggest that superovulation trials may be conducted on more number of animals.

SUMMARY

5. SUMMARY

The present investigation was undertaken to study (1) the superovulatory response and non-surgical embryo recovery in Rathi cows, superovulated with PMSG, with and without priming, (2) the endocrine profile (in terms of LH and FSH) in superovulated and control cows. In all 12 animals were undertaken for the study, 2 were kept in the control group and the rest 10 were allocated to two different treatment groups.

Oestrus was synchronized prior to superovulation in all animals. No significant difference ($P>0.05$) was observed in mean interval from PG injection to oestrus induction between the unprimed and the primed cows (60±7.589 hours and 69.6±7.960 hours, respectively).

The superovulatory oestrus occurred between 24 to 48 hours in all animals in the two different treatment groups, with no significant difference between the unprimed and the primed cows ($P>0.05$).

The post-superovulatory return to oestrus occurred at a mean interval of 16.8±0.663 days in the unprimed cows as compared to 19.2±1.655 days in the primed cows, the difference being non-significant ($P>0.05$).

The mean ovulation rate was 9.25±0.854 and 12.0±1.732 in the unprimed and the primed cows, respectively. Although the ovulation rate was higher for the primed group, the difference observed was non-significant ($P>0.05$).

The ANOVA for ovarian length, width and thickness revealed a highly significant ($P<0.01$) effect of the stage of the cycle and no effect of treatment and site of the ovary.

So in all, no significant effect of priming was observed on the superovulatory response.

Non-surgical embryo recovery was carried out on 7th and 8th days after A.I.. A total of 11 embryos were recovered from the donor cows, out of which only 7 were viable while the other 4 were degenerated. Three of the 10 superovulated cows exhibited anovulatory follicular cysts.

The control cows exhibited, on an average, basal and peak values of 1.25±0.25 and 28.5±0.5 m I.U./ml of LH, respectively.

The unprimed cows exhibited, on an average, basal and peak values of 0.4166±0.0833 and 55.33±32.95 m I.U./ml of LH, respectively, whereas these values in the primed group were 0.5833±0.0833 and 57.66±26.43 m I.U./ml of LH, respectively. The difference between the two groups was non-significant ($P>0.05$).

The control cows exhibited, on an average, basal and peak values of 0.625±0.125 and 16.5±3.5 m I.U./ml of FSH, respectively.

The unprimed cows exhibited, on an average, basal and peak values of 0.233±0.133 and 132.33±64.68 m I.U./ml of FSH, respectively.

The secretion of LH and FSH was found to be pulsatile in the superovulated and control Rathi cows.

Although there are numerous sources of variation, but for superovulating the Rathi cows, slightly lower dose of PMSG (compared to that used in the present investigation i.e. 2000 to 2500 I.U) is recommended. A larger priming dose or a change in the day of priming may be tried. Preparations like GnRH or HCG and PMSG antiserum may also be incorporated in the superovulation protocols.

BIBLIOGRAPHY

6. BIBLIOGRAPHY

Afanasev, I.N. (1988). Superovulation and conception results in high-yielding donor cows. **Anim. Breed. Abstr.** 56(3) : 1326 Abstr. No.

Agarwal, S. K.; Shankar, U. and Yadav, M. C. (1992). Effect of day of initiation of gonadotropin treatment on superovulation response, recovery rate and quality of embryos in cattle. **Indian J. Dairy Sci.** 45 : 294-297.

Alcivar, A. A.; Maurer, R. R. and Anderson, L. L. (1984). Superovulatory response in FSH or Pergonal-treated heifers. **Theriogenology** 22 : 635-642.

Alfuraiji, M.M.; Broadbent, P.J.; Hutchinson, J.S.M.; Dolman, D.F. and Atkinson, T. (1990). Superovulation using different doses of PMSG and Monoclonal Anti-PMSG in cattle. **Theriogenology** 33(1) : 186.

Almeida, A. P. (1987). Superovulatory responses in dairy cows treated with PMSG. **Theriogenology** 27 : 205.

Armas, R. De; Kanitz, W.; Kanitz, E.; Blowdow, G. and Rommel, P. (1988). Concentrations of plasma progesterone, oestradiol-17B and LH in heifers superovulated with CAP, PMSG and Gn-RH in relation to the response to superovulation. **Anim. Breed. Abstr.** 56(11) : 6784(abstr. no.)

Armstrong, D. T. (1981). Prostaglandins and follicular functions. **J. Reprod. Fert.** 62 : 283-291.

Armstrong, D. T. (1993). Recent advances in superovulation of cattle. **Theriogenology** 39 : 7-24.

Aslanov. A. F. (1989). The recovery and freezing of cattle embryos. **Anim. Breed. Abstr.** 57(7) : 4813(abstr. no.).

Barnes, M. A.; Martinez Castellano, A.; Kazmer, G. W.; Wade, R. J. and Halman, R. D. (1982). Effects of exogenous FSH on oestrus, ovulation and endogenous hormone release in dairy cows. **Theriogenology**18 : 311-323.

Becker, W. A. P.; Moraes, G. V.; Pinheiro, L. E. L.; Rodrigues, C. F. M. and Carvalho, C. (1990). Quantitative and qualitative evaluation of embryo transfer in cattle **Anim. Breed. Abstr.** 58(11) : 7216(abstr. no.).

Becker, W. A. P. and Pinheiro, L. E. L. (1986). Ovarian response to superovulation in Nelore cows. **Theriogenology** 25(6) : 785-793.

Bernard, C.; Valet, J. P.; Beland, R. and Lambert, R. D. (1983). Prediction of bovine ovulation by a rapid radio-immunoassay for plasma LH. **J. Reprod. Fert.** 68 : 425-430.

Betteridge, K. J. (1977). Superovulation. **In : Betteridge, K. J. (ed.) Embryo transfer in farm animals. A review of techniques and applications. Canada Department of Agriculture. Monograph 16**, 1977, pp. 1-9.

Betteridge, K. J. (1980). Introduction to Embryo transfer to farm animals. **In : Current therapy in theriogenology. D. A. Morrow, W. S. Saunders (eds)**, pp. 69-88, Philadelphia.

Betteridge, K. J.; Sugden, E. A.; Eaglesome, M. D.; Randall, G. C. B. and Mitchell, D. (1982). Plasma progesterone levels in superovulated cattle in relation to hatched embryo sizes, ovulation rates and premature regression of corpora lutea. **Anim. Reprod. Sci.** 4 : 201-212.

Bevers, M. M. and Dieleman, S. J. (1987). Superovulation of cows with PMSG : variation in plasma concentrations of progesterone, oestradiol, LH, cortisol, prolactin and PMSG and in number of preovulatory follicles. **Anim. Reprod. Sci.** 15 : 37-52.

Bhattacharya, B. N.; Sinha, A. K.; Sinha, S. N. and Singh, B. (1989). Ovarian response after repeated superovulation in crossbred cows. **Indian J. Anim. Reprod.** 10(1) : 51-56.

Boland, M. P.; Gordon, I.; Mc Govern, H. and Lynn, G. (1988). Superovulatory responses of cows and heifers in summer in Saudi Arabia. **Theriogenology** 29(1) : 227 (abstr.).

Boland, M. P.; Goulding, D. and Roche, J. F. (1991). Alternative gonadotropins for superovulation in cattle **Theriogenology** 35(1) : 5-17.

Boland, M.P.; Mac Donell, H.F.; Ahmed, T.S. and Reid, J.F.S. (1986). Use of fenprostalene in superovulated beef heifers. **Vet. Rec.** 119 : 241-242.

Bolzoni, P.A.; Gregory, R.M.; Brass, K.E. and Mattos, R.C. (1991). Serum progesterone concentration in cows superovulated by means of PMSG and FSH prior to embryo transfer. **Anim. Breed. Abstr.** 59(12) : 8227(abstr. no.).

Bono, G.; Gabai, G.; Silvestrelli, L. and Antonella Comin (1991). Superovulatory and endocrinological responses of Simmental cows treated either with PMSG or hMG or in combination. **Theriogenology** 35(6) : 1179-1189.

Booth, W.D.; Newcomb, R.; Strange, H.; Rowson, L.E.A. and Sacher, H.B. (1975). Plasma oestrogen and progesterone in relation to superovulation and egg recovery in the cow **Vet. Rec.** 97 : 366-369.

Bouters, R.; Moyaert, I.; Corijn, M. and Vandeplassche, M. (1983). The use of a PMSG anti-serum in superovulated cattle : endocrinological changes and effects on timing of ovulation. **Zuchthygiene** 18 : 172-177.

Brand, A.; Trounson, A.O.; Aarts, M.H.; Drost, M. and Zaayer, D. (1978). Superovulation and non-surgical embryo recovery in the lactating dairy cow. **Anim. Prod.** 26 : 55-60.

Breuel, K.F.; Baker, R.D.; Butcher, R.L.; Townsend, E.C.; Inskeep, E.K.; Dailey, R.A. and Lerner, S.P. (1991). Effects of breed, age of donor and dosage of FSH on the superovulatory response of beef cows. **Theriogenology** 36(2) : 241-255.

Britt, J.H. and Holt, L.C. (1988). Endocrinological screening of embryo donors and embryo transfer recipients : A review of research with cattle. **Theriogenology** 29(1) : 189-202.

Bruijn, M.De and Booman, P. (1991). The use of bovine polyclonal and monoclonal antibodies and of murine monoclonal antibodies against PMSG for passive immunization in cattle. A comparative study and some preliminary results. **Anim. Breed. Abstr.** 59(3) : 1725(abstr. no.).

Budevich, I.I.; Pleshkevich, I.S.; Kononenko, V.L. and Zhuk, N.F. (1990). Induction of multiple ovulation in Russian Black Pied cows. **Anim. Breed. Abstr.**. 58(4) : 2005 (abstr. no.).

Callesen, H.; Bak, A.; Greve, T.; Avery, B.; Gotfredsen, P.; Holm, P.; Hyttel, P.; Pedersen, J.O.; Schmidt, M.; Smith, S. and Svanborg, N. (1989). Use of PMSG antisera in superovulated dairy heifers. **Theriogenology** 31(1):179 pp.

Callesen, H.; Greve, T. and Hyttel, P. (1986). Preovulatory endocrinology and oocyte maturation in superovulated cattle. **Theriogenology** 25 : 71-86.

Callesen, H.; Greve, T. and Hyttel, P. (1987). Premature ovulations in super ovulated cattle. **Theriogenology** 28 : 155-166.

Callesen, H.; Greve, T.; Hyttel, P.; Bak, A.; Gotfredsen, P. and Holm, P. (1990). Preovulatory plasma oestradiol-17 beta concentrations and ovulation rates in PMSG/Anti-PMSG treated heifers. **Theriogenology** 34(2) : 251-258.

Caral, J.; Solano, R.; Armas, R.De and Bernal, A. (1989a). Effect of different methods of inducing oestrus on superovulation in Holstein heifers. **Anim. Breed. Abstr.** 57(8) : 5503(abstr. no.).

Caral, J.; Solano, R.; Bernal, A. and Armas, R.De (1989b). Two methods of superovulation for Holstein cows in a commercial herd. **Anim. Breed. Abstr.** 57(8) : 5502(abstr. no.).

Choudary, J.B.; Gier, J.T. and Marion, G.B. (1968). Cyclic changes in bovine vesicular follicles. **J. Anim. Sci.** 27 : 468þþ.

Chung, K.M.; Kim, J.K. and Im, K.S. (1989). Ovarian response, embryo recovery and their viability after superovulation in pubertal, post-pubertal heifers and cows. **Anim. Breed. Abstr.** 57(10) : 6603(abstr. no.).

Chupin, D.; Combarnous, Y. and Procureur, R. (1984). Antagonistic effect of LH on FSH induced superovulation in cattle. **Theriogenology** 21 : 229 (abstr.).

Cooper, M.J. (1974). Control of oestrous cycles of heifers with a synthetic prostaglandin analogue. **Vet. Rec.** 95 : 200-203.

Cowen, P. and Sosnik, U. (1987). Effect of superovulation on lactating Holsteins. **Theriogenology** 28 : 783-788.

Critser, J.K.; Rowe, R.F.; Campo, M.R. Del and Ginther, O.J. (1980). Embryo transfer in cattle : Factors affecting superovulatory response, number of transferable embryos, and length of post-treatment oestrous cycles. **Theriogenology** 13(6) : 397-406.

Delgado, A.R.P.; Elsden, R.P. and Seidel, G.E., Jr. (1989). Effects of GnRH on superovulated cattle. **Theriogenology** 31(2) : 317-321.

Dempsey, C.W. (1973). Follicular growth rate and ovulation after various experimental procedures in the guinea pig. **Amer. J. Physiol.** 120 : 126 pp.

Dhondt, D.; Bouters, R.; Spincemaille, J.; Coryn, M. and Vandeplassche, M. (1978). The control of superovulation in the bovine with a PMSG-antiserum. **Theriogenology** 9 : 529-534.

Dieleman, S.J. and Bevers, M.M. (1987). Effects of monoclonal antibody against PMSG administered shortly after the preovulatory LH surge on time and number of ovulations in PMSG/PG-treated cows. **J. Reprod. Fert.** 81 : 533-542.

Dieleman, S.J.; Bevers, M.M. and Gielen, J.Th. (1987). Increase of the number of ovulations in PMSG/PG-treated cows by administration of monoclonal anti-PMSG shortly after the endogenous LH peak. **Theriogenology** 27(1) : 222(abstr.).

Dieleman, S.J.; Bevers, J.H.; Van Tol, H.T.M. and Willemse, A.H. (1986). Peripheral plasma concentrations of oestradiol, progesterone, cortisol, LH and prolactin during the oestrous cycle of the cow, with emphasis on the peri-oestrous period. **Anim. Reprod. Sci.** 10 : 275-292.

Dieleman, S.J.; Bevers, M.M.; Vos, P.L.A.M. and Loos, de F.A.M. (1993). PMSG/anti-PMSG in cattle : A simple and efficient superovulatory treatment. **Theriogenology** 39 : 25-41.

Dieleman, S.J.; Bevers, M.M.; Wurth, Y.A.; Gielen, J.Th. and Willemse, A.H. (1989). Improved embryo yield and condition of donor ovaries in cows after PMSG superovulation with monoclonal anti-PMSG administered shortly after the preovulatory LH peak. **Theriogenology** 31(2) : 473-487.

Dinar, M.A.; Diskin, M.G.; Mc Donagh, T. and Sreenan, J.M. (1987). Oestrus and ovulatory responses in repeatedly superovulated cows **Theriogenology** 27 : 201.

Dobson, H. (1978). Plasma gonadotropins and oestradion during oestrus in the cow. **J. Reprod. Fert.** 52 : 51-53.

Donaldson, L.E. (1984). The day of the oestrous cycle that FSH is treated and superovulation in cattle. **Theriogenology** 2 : 97-99.

Donaldson, L. E. (1985). LH and FSH profiles of superovulation and embryo production in the cow. **Theriogenology** 23 : 441-447.

Donaldson, L. E. and Hansel, W. (1968). Cystic corpora lutea and normal and cystic graafian follicles in the cow. **Australian Vet. J.** 44 : 304-308.

Donaldson, L. E. and Ward, D. N. (1985). Superovulation in cattle : dose-response to FSH-P with and without LH contamination. **Theriogenology** 23 : 189(abstr.).

Donaldson, L. E. and Ward, D.N. (1986). Effects of LH on embryo production in superovulated cows. **Vet. Rec.** 119 : 625-626.

Downey, B. R. (1980). Regulation of oestrous cycle in domestic animals. **Can. Vet. J.** 21 : 301-306.

Driancourt, M. A. (1991). Follicular dynamics in sheep and cattle. **Anim. Breed. Abstr.** 59(8) : 5315(abstr. no.).

Dufour, J.; Whitmore, H. L.; Ginther, O. J. and Casida, L. E. (1972). Identification of the ovulating follicle by its size on different days of the oestrous cycle in heifers. **J. Anim. Sci.** 34(1) : 85-87.

Eckery, D. C.; Wiltbank, J. N. and Powell, K. (1990). Superovulating beef cattle with purified ovine FSH. **Anim. Breed. Abstr.** 58(11) : 7228(abstr. no.).

Elecko, J.; Choma, J.; Maraccek, I.; Hajurka, J. and Lazar, L. (1988). Changes of surface follicles and weight of ovaries in cows tested for the response to superovulation using PMSG and cloprostenol. **Anim. Breed. Abstr.** 56(7) : 4928(abstr. no.).

Elsden, R. P.; Hasler, J. F. and Seidel, G. E., Jr. (1976). Non-surgical recovery of bovine eggs. **Theriogenology** 6 : 523-532.

Elsden, R. P. and Lawson, R. A. S. (1974). Use of prostaglandins for control of ovulation and egg transfer in cattle. **Aust. Soc. Reprod. Biol. Prostaglandin Symp. Feb. 16, Session 1.**

Elsden, R. P.; Lewis, S.; Comming, I. A. and Lawson, R. A. S. (1974). **J. Reprod. Fert.** 36 : 455.

Elsden, R. P.; Nelson, L. D. and Seidel, G. E., Jr. (1978). Superovulating cows with follicle stimulating hormone and pregnant mare serum gonadotropin. **Theriogenology** 9 : 17-26.

Erickson, B. H. (1966). Development and senescence of the postnatal bovine ovary. **J. Anim. Sci.** 25 : 800.

Ezoe, K.; Goto, K.; Kajisa, O.; Nakanishi, Y.; Ogawa, K.; Yanagita, K.; Tasaki, M.; Inohae, S.; Tateyama, S.; Ohta, H.; Yamada, I. and Hasegawa, Y. (1991). Relationships between plasma concentration of gonadotropins, ovarian steroids and inhibin and embryo production in superovulated Japanese Black cows. **Anim. Breed. Abstr.** 59(1) : 188(abstr. no.).

Foote, R. H.; Allen, S. E. and Henderson, B. (1989). Buserelin in a superovulatory regimen for Holstein cows : II. Yield and quality of embryos in commercial herds. **Theriogenology** 31(2) : 385-392.

Foote, R. H. and Ellington, J. E. (1988). Is the superovulated oocyte normal ? **Theriogenology** 29 : 111-123.

Fortune, J. E.; Sirois, J. and Quirk, S.M. (1988). The growth and differentiation of ovarian follicles during the bovine oestrous cycle. **Theriogenology** 29(1) : 95-109.

Fortune, J. E.; Sirois, J.; Turzillo, A. M. and Lavoir, M. (1991). Follicle selection in domestic ruminants. **Anim. Breed. Abstr.** 59(8) : 5318(abstr. no.).

Fuente, J. De La; Cocero, M.; Lopez Sebastian, M. and Barragan, C. (1989). Effect of different exogenous gonadotropins (FSH and PMSG) on the quantity and quality of embryos produced by cows. **Anim. Breed. Abstr.** 57(7) : 4830(abstr. no.).

Gallo, G. F.; Algire, J. E.; Srikanda Kumar, A. and Downey, B. R. (1992). Effects of PGF-2 alpha analogue on the ovulatory response of superovulated heifers. **Anim. Reprod. Sci.** 27 : 83-90.

Garcia, G. J. K.; Elsden, R. P. and Seidel, G. E., Jr. (1983). Efficacy of PGF-2 alpha for reducing the return to oestrus interval in superovulated cattle. **Theriogenology** 19 : 129.

Garcia-Winder, M.; Lewis, P. E.; Bryner, R. W.; Baker, R. D.; Inskeep, E. K. and Butcher, R. L. (1989). Effect of age and norgestomet on endocrine parameters and production of embryos in superovulated beef cows. **Anim. Breed. Abstr.** 57(3) : 1567(abstr. no.).

Gielen, J. Th.; Roerink, G. H.; Atoon, R. E.; Vonk Noordegraaf, C. A.; Pazman, J.; Hoeijmakers, M. J. H.; Steeg, R. H. M.; Coert, A.; Aguer, D. and Nell, T. (1990). Use of PMSG plus Neutra-PMSG in dairy cows treated repeatedly for superovulation. **Theriogenology** 33(1) : 229.

Goncalves, P. B. D.; Gregory, R. M. and Rodrigues, J. L. (1987). The efficiency of two non-surgical techniques for bovine embryo recovery on days 6 and 7 of oestrous cycle. **Theriogenology** 28(1) : 25-32.

Gonzalez-Mencio, F.; Manns, J. and Murphy, B. D. (1978). FSH and LH activity of PMSG from mares at different stages of gestation. **Anim. Reprod. Sci.** 1 : 137-144.

Gonzalez-Padilla, E.; Wiltbank, J. N. and Niswender, G. D. (1975). Puberty in beef heifers. 1 The interrelationship between pituitary, hypothalamic and ovarian hormones. **J. Anim. Sci.** 40(6) : 1091-1104.

Goto, K.; Ohkutsu, S.; Nakanishi, Y.; Ogawa, K.; Tasaki, M.; Ohta, H.; Inohae, S.; Tateyama, S.; Kawabata, T.; Ishii, S.; Miyamoto, A.; Furusawa, T.; Umezu, M. and Masaki, J. (1988). Endocrine profiles and embryo quality in superovulated Japanese Black cattle. **Theriogenology** 29(3) : 615-626.

Goulding, D.; Williams, D. F.; Duffy, P.; Boland, M. P. and Roche, J. F. (1990). Superovulation in heifers given FSH initiated either at day 2 or day 10 of the oestrous cycle. **Theriogenology** 34 : 767-778.

Goulding, D.; Williams, D. H.; Roche, J. F. and Boland, M. P. (1991). Superovulation in heifers using either PMSG or FSH during the mid luteal stage of the oestrous cycle. **Theriogenology** 36(6) : 949-958.

Grasso, F.; Guibault, L. A.; Roy, G. L. and Lussier, J. G. (1989). Ultrasonographic determination of ovarian follicular development in superovulated heifers pretreated with FSH-P at the beginning of the oestrous cycle. **Theriogenology** 31 : 1209-1220.

Gray, B. W.; Cartee, R. E.; Stringfellow, D. A.; Riddell, M.G.; Riddell, K. P. and Wright, J. C. (1992). The effects of FSH-priming and dominant follicular regression on the superovulatory response of cattle. **Theriogenology** 37 : 631-639.

Greve, T.; Bak, A. and Schmidt, M. (1988). Superovulation of dairy cattle : Effect of PMSG anti-serum treatment. **Theriogenology** 29(1) : 252.

Greve, T.; Callesen, H. and Hyttel, P. (1984a). Characterization of plasma LH profiles in superovulated dairy cows. **Theriogenology** 21 : 237(abstr.).

Greve, T.; Callesen, H. and Hyttel, P. (1984b). Plasma progesterone profile and embryo quality in superovulated dairy heifers. **Theriogenology** 21 : 238(abstr.).

Griffin, J. L. and Randel, R. D. (1978). Reproductive studies of Brahman cattle. II. LH patterns in ovariectomized Brahman and Hereford cows before and after injection of GnRH. **Theriogenology** 9(5) : 437-446.

Guay, P. and Bedoya, M. (1981). A study of the equivalence between rectal palpation, laparoscopy, laparotomy and ovarian dissection for evaluation of the ovarian response of PMSG-superovulated cows. **Can. Vet. J.** 22 : 343(abstr.).

Guilbault, L. A.; Grasso, F.; Lussier, J. G.; Rouilier, P. and Matton, P. (1991a). Decreased superovulatory responses in heifers superovulated in the presence of a dominant follicle. **J. Reprod. Fert.** 91(1) : 81-89.

Guilbault, L. A.; Grasso, F.; Roy, G. L. and Lussier, J. G. (1991b). Effect of ovarian condition on the ovulation rate and embryo production of superovulated heifers. **Anim. Breed. Abstr.** 59(10) : 6658(abstr. no.).

Guilbault, L. A.; Lussier, J. G.; Grasso, F. and Roy, G. L. (1989). Concentrations of progesterone and FSH in superovulated heifers pre-treated with FSH-P at the beginning of the oestrous cycle. **Theriogenology** 31(1) : 200.

Hafez, E. S. E.; Sugie, T. and Gordon, I. (1963). Superovulation and related phenomena in the beef cow. (1) Superovulatory responses following PMS and HCG injections. **J. Reprod. Fert.** 5 : 359-379.

Halbert, G.W.; Leslie, K.E.; Walton, J.S. and Betteridge, K.J. (1989). Evaluation of return to oestrus in superovulated dairy heifers following prostaglandin treatment. **Theriogenology** 31(1) : 201 (abstr.).

Halley, S. M.; Rhodes, R. C.; Mc Kellar, L. D. and Randel, R. D. (1979). Successful superovulation, non-surgical collection and transfer from Brahman cows. **Theriogenology** 12(2) : 97-108.

Hansel, W. and Convey, E. M. (1983). Physiology of the oestrous cycle. **J. Anim. Sci.** 57(Suppl. 2) : 404-424.

Hasler, J. F.; Mc Cauley, A. D.; Schermerhorn, E. C. and Foote, R. H. (1983). Superovulation responses of Holstein cows. **Theriogenology** 19 : 83-99.

Hawk, H. W. (1988). Gamete transport in the superovulated cow. **Theriogenology** 29(1) : 125-142.

Hay, J. H.; Phelps, D. A.; Hanks, D. R. and Foote, W. D. (1990). Sequential uterine horn versus simultaneous total uterine flush to recover bovine embryos non-surgically. **Theriogenology** 33(2) : 563-567.

Helmer, S. D. and Britt, J. H. (1987). Hormone secretion and characteristics of oestrous cycles after treatment of heifers with human chorionic gonadotropin or prostaglandin F2 alpha during corpus luteum formation. **J. Anim. Sci.** 64 : 782-789.

Heranshaw, H.; Mattner, P. E.; Nancarrow, C. D. and Restall, B. J. (1974). The effect of the mode of administration of PGF-2 alpha on the synchronization of oestrus in cattle. **J. Reprod. Fert.** 36 : 486-487.

Horiuchi, T.; Shinkawa, K. and Yoshimoto, T. (1991). Non-surgical collection of 8- to 32- cell bovine embryos and *in vitro* development to the blastocyst stage. **Anim. Breed. Abstr.** 59(5) : 3218(abstr. no.).

Huhtinen, M.; Rainio, V.; Aalto, J.; Bredbacka, P. and Maki-Tanila, A. (1992). Increased ovarian responses in the absence of a dominant follicle in superovulated cows. **Theriogenology** 37 : 457-463.

Humphrey, W. D.; Murphy, B. D.; Rieger, D.; Mapletoft, R. J.; Manns, J. and Fretz, P. D. (1979). Effect of FSH/LH ratio of PMSG of ovulatory responses. **Theriogenology** 11 : 101(abstr.).

Hunter, R. H. F. (1988). Low incidence of fertilization in superovulated cows : A physiological explanation. **Vet. Rec.** 123 : 443.

Hutter, J. C. and Cabodevila, J. A. (1990). Use of FSH for superovulation of donor cows. **Anim. Breed. Abstr.** 58(5) : 2659(abstr. no.).

Hutter, J. C.; Cabodevila, J. A. and West, M. (1990). Effect of type of administration and dose of cloprostenol on the production of embryos in superovulated cows. **Anim. Breed. Abstr.** 58(5) : 2658(abstr. no.).

Hyttel, P.; Callesen, H. and Greve, T. (1986). Ultrastructural features of preovulatory oocyte maturation in superovulated cattle. **J. Reprod. Fert.** 76 : 645-656.

Hyttel, P.; Callesen, H.; Greve, T. and Schmidt, M. (1991). Oocyte maturation and sperm transport in superovulated cattle. **Theriogenology** 35(1) : 91-108.

Inskeep, E. K. (1973). Potential uses of prostaglandins in control of reproductive cycles of domestic animals. **J. Anim. Sci.** 36 : 1149-1157.

Ireland, J. J.; Coulson, P. B. and Murphree, R. L. (1979). Follicular development during four stages of the oestrous cycle of beef cattle. **J. Anim. Sci.** 49(5) : 1261-1269.

Ireland, J. J. and Roche, J. F. (1983). Development of non-ovulatory antral follicles in heifers : changes in steroids in follicular fluid and receptors for gonadotropins. **Endocrinology** 112 : 150-156.

Ireland, J. J. and Roche, J. F. (1987). Hypotheses regarding development of dominant follicles during a bovine oestrous cycle. **In : Roche and O'Callaghan, (eds). Follicular growth and ovulation rate in farm animals. Martinus Nijhoff Publishers, Dordrecht**, 1987, pp. 1-18.

Jensen, M. A.; Greve, T.; Mades, A. and Edquist, L. E. (1982). Endocrine profiles and embryo quality in the PMSG-PGF2 alpha treated cow. **Theriogenology** 18 : 33-44.

Jones, A. L.; Staples, T. R. and Page, R. D. (1986). Enhanced return to oestrus in superovulated heifers using fenprostalene. **Theriogenology** 25 : 161.

Jordt, T. and Lorenzini, E. (1989). Superovulation, collection and transfer of embryos and demi-embryos from Boran (Bos indicus) cows and heifers. **Anim. Breed. Abstr.** 57(1) : 179(abstr. no.).

Kadu, M. S.; Fasihuddin, M.; Shrivastava, O. P.; Chede, S. A.; Pawashe, C. H. and Totey, S. M. (1989). Preliminary trials on superovulation, non-surgical embryo recovery and embryo transfer in cows. **Indian J. Anim. Reprod.** 10(2) : 101-104.

Kaneko, H.; Terada, T.; Goto, K.; Nakashima, T.; Ogata, K.; Kondoh, M.; Taya, K. and Sasamoto, S. (1989). Suppression of the preovulatory surge of FSH in superovulating cattle pre-treated with porcine FSH. **Anim. Breed. Abstr.** 57(11) : 7199(abstr. no.).

Kaneko, H.; Terada, T.; Taya, K.; Watanabe, G.; Sasamoto, S.; Hasegawa, Y. and Igarashi, M. (1991a). Ovarian follicular dynamics and concentrations of oestradiol-17 beta, progesterone, LH and FSH during the periovulatory phasa of the oestrous cycle in the cow. **Anim. Breed. Abstr.** 59(12) : 8250(abstr. no.).

Kaneko, H.; Terada, T.; Watanabe, G.; Taya, K. and Sasamoto, S. (1991b). Change in peripheral levels of immunoreactive inhibin and gonadotropins in cattle induced to superovulate by porcine FSH. **Anim. Breed. Abstr.** 59(1) : 205(abstr. no.).

Kanitz, E.; Kanitz, W.; Delzer, W.; Schmidt, H. E.; Kitzig, M. and Schneider, F. (1990). Use of LH analysis to determine the optimal time of insemination after superovulation. **Anim. Breed. Abstr.** 58(11) : 7235(abstr. no.).

Kardymowicz, O. (1972). A method of vital staining for determining the viability of fertilized sheep ova stored in vitro. In : **Proc. 7th International Congress on Anim. Reprod. and A. I., Munich**, 1 : 503-506.

Khilkevich, S. and Ovchinnikov, A. (1988). Superovulation of donor cows in relation to their hormonal status prior to treatment. **Anim. Breed. Abstr.** 56(8) : 4943(abstr. no.).

Kim, J. G., Chung, K. M.; Im, K. S. and Lee, Y. B. (1988a). Effects of superovulation of immature and mature heifers on ovarian response, embryo production and pregnancy. **Anim. Breed. Abstr.** 56(11) : 6806(abstr. no.).

Kim, H. N.; Rorie, R. W.; Youngs, C. R.; White, K. L. and Godke, R. A. (1987). The use of anti-PMSG with PMSG for superovulating beef cattle. **Theriogenology** 27 : 243(abstr.).

Kim, H. N.; Roussel, J. D.; Pool, S. H. and Godke, R. A. (1988b). The effect of a commercially-available purified FSH and bovine anti-PMSG serum on the superovulation of dairy heifers. **Theriogenology** 29(1) : 267(abstr.).

Ko, J. C. H.; Kastelic, J. P.; Campo, M. R. Del; Ginther, O. J. (1991). Effects of a dominant follicle on ovarian follicular dynamics during the oestrous cycle in heifers. **J. Reprod. Fert.** 91(2) : 511-519.

Kojima, T.; Soma, T. and Oguri, N. (1990). The effects of simplification of donor preparation, several superovulatory treatments by FSH-P and the single insemination regime on embryo production in Japanese beef cattle. **Anim. Breed. Abstr.** 58(8) : 5092(abstr. no.).

Kuchukhidze, M. N. (1988). Superovulation of high yielding cows by FSH treatment. **Anim. Breed. Abstr.** 56(5) : 2604(abstr. no.).

Kummer, V.; Zraly, Z.; Holcak, Z.; Schlegelova, J. and Hruska, K. (1980). Superovulation in cattle : Effect of goat anti-PMSG serum. **Theriogenology** 14 : 383-390.

Kuttner, K. (1978). Superovulation in cows with PMSG. **Anim. Breed. Abstr.** 48 : 104.

Kweon, O. K.; Kanagawa, H.; Takahashi, Y.; Miyamato, A.; Mask, J.; Umerzu, M.; Kagbu, S.; Twazumi, Y. and Aoyogi, V. (1987). Plasma endocrine profile and total cholesterol levels in superovulated cows. **Theriogenology** 27 : 841-857.

Landsverk, K.; Refsdal, A. O. and Vatn, T. (1991). Results of non-surgical flushing of donor cows repeated after 24 hours. **Theriogenology** 35(1) : 229.

Laster, D. B. (1972). Disappearance and uptake of I^{125} FSH in rat, rabbit, ewe and cow. **J. Reprod. Fert.** 30 : 407-415.

Lauderdale, J. W. (1972). Effects of PGF-2 alpha on pregnancy and oestrous cycles of cattle. **J. Anim. Sci.** 35 : 246(abstr.).

Lauria, A.; Genazzani, A. R.; Oliva, O.; Inaudi, R.; Cremonesi, F.; Monittola, C. and Aureli, G. (1982). Clinical and endocrinologocal investigations on superovulation induced in heifers by hMG. **J. Reprod. Fert.** 66 : 219-225.

Leaver, J. D.; Glencross, R. G. and Pope, G. S. (1975). Fertility of Friesian heifers after luteolysis with a prostaglandin analogue (ICI-80,996). **Vet. Rec.** 96 : 383-384.

Lee, C. N.; Maurice, E.; Ax, R. L.; Pennington, J. A.; Hoffman, W. F. and Brown, M. D. (1983). Efficacy of GnRH administered at the time of artificial insemination of heifers and post-partum and repeat-breeder dairy cows. **Am. J. Vet. Res.** 44 : 2160-2163.

Liehr, R. A.; Marion, G. B. and Olson, H. H. (1972). Effects of prostaglandins on cattle oestrous cycles. **J. Anim. Sci.** 35 : 247(abstr.).

Lindner, G. M. and Wright, R. W., Jr. (1983). Bovine embryo morphology and evaluation. **Theriogenology** 20 : 407-416.

Lindsell, C. E.; Murphy, B. D. and Mapletoft, R. J. (1986). Superovulation and endocrine responses in heifers treated with FSH-P at different stages of oestrous cycle. **Theriogenology** 26 : 209-219.

Lindsell, C. E.; Pawlyshyn, V.; Bielanski, A. and Mapletoft, R. J. (1985). Superovulation of heifers with FSH-p beginning on four different days of the cycle. **Theriogenology** 23 : 203.

Lopez-Barbella, S. R.; Warnick, A. C.; Wise, T. H. and Fields, M. J. (1979). Endocrine response of the cow to PMSG and subsequent multiple regression by PGF-2 alpha. **J. Anim. Sci.** 48 : 1135-1142.

Louis, T. M.; Hafs, H. D. and Morrow, D. A. (1972). Oestrus and ovulation after uterine PGF-2 alpha in cows. **J. Anim. Sci.** 35 : 247-248(abstr.).

Lubbadeh, W. F.; Graves, C. N. and Spahr, S. L. (1980). Effect of repeated superovulation on ovulatory response of dairy cattle. **J. Anim. Sci.** 50 : 124-127.

Lussier, J. G. and Carruthers, T. D. (1989). Endocrine and superovulatory responses in heifers pre-treated with FSH or bovine follicular fluid. **Theriogenology** 31(4) : 779-794.

Manickam, R.; Mohan, M.; Pillai, K. G. S. and Krishnamurti, C. R. (1991). Observations on non-surgical recovery and transfer of embryos in crossbred cows. **Anim. Breed. Abstr.** 59(5) : 3234(abstr. no.).

Mapletoft, R. J. (1980). Embryo Transfer for the practitioner. **Proc. 13th Annu. Meet. Am. Assoc. Bovine Pract. Toronto, Canada** pp. 154-162.

Mapletoft, R. J. (1984). Embryo Transfer Technology for the enhancemant of animal reproduction. **Biotechnology** 2 : 149-159.

Mapletoft, R. J.; Wu, M. and Murphy, B. D. (1990). Superovulation of beef cattle with Follitropin. **Anim. Breed. Abstr.** 58(2) : 606(abstr. no.).

Marion, G. B. and Gier, H. T. (1971). Ovarian and uterine embryogenesis and morphology of the non-pregnant female mammal. **J. Anim. Sci.** 32(Suppl. I) : 24-47.

Marion, G. B.; Gier, H. T. and Choudary, J. B. (1968). Micromorphology of the bovine ovarian follicular system. **J. Anim. Sci.** 27 : 451-465.

Matton, P.; Adelakoun, V.; Couture, Y. and Dufour, J. J. (1981). Growth and replacement of the bovine ovarian follicles during the oestrous cycle. **J. Anim. Sci.** 52(4) : 813-820.

Mc Gowan, M. R.; Braithwaite, M.; Jochle, W. and Mapletoft, R. J. (1985). Superovulation of beef heifers with pergonal (hMG) : a dose-response trial. **Theriogenology** 24 : 173-184.

Mehmood, A.; Anwar, M.; Ullah, N.; Baig, S. M. and Wright, R. W., Jr. (1991). Pattern of sex steroids secretion and their relationship with embryo yield in Jersey cows superovulated with PMSG. **Theriogenology** 35(3) : 513-520.

Mishra, A. K.; Joshi, B. V. and Nair, H. K. (1992). Preliminary trials of Multiple Ovulation and Embryo Transfer (MOET) in cows under field conditions. **Indian J. Anim. Reprod.**. 13(1) : 16-17.

Monniaux, D.; Chupin, D. and Saumande, J. (1983). Superovulatory responses of cattle. **Theriogenology** 19 : 55-81.

Monniaux, D.; Mariana, J. C. and Gibson, W. R. (1984). Action of PMSG on follicular populations in the heifer. **J. Reprod. Fert.** 70 : 243-253.

Monty, D. E., Jr. and Racowsky, C. (1988). In vitro evaluation of early embryo viability and development in summer heat-stressed, superovulated dairy cows. **Anim. Breed. Abstr.** 56(2) : 717(abstr. no.).

Moor, R. M.; Kruip, Th. A. M. and Green, D. (1984). Intra-ovarian control of folliculogenesis : Limites to superovulation ? **Theriogenology** 21 : 103-116.

Moyaert, I.; Bouters, R.; Schonherr, O. T.; Wilderbek, A. T. M.; Coert, A.; Coryn, M. and Vandeplassche, M. (1985). The control of superovulation in the bovine with a monoclonal PMSG antibody. **Theriogenology** 23 : 210(abstr.).

Murphy, B. D.; Mapletoft, R. J.; Manns, J. and Humphrey, W. D. (1984). Variability in gonadotropin preparations as a factor in the superovulatory response. **Theriogenology** 21 : 117-125.

Nawito, M.; Schallenberger, E. and Schams, D. (1977). Release of lutropin (LH) and follitropin (FSH) in cattle after administration of a new gonadoliberin (GnRH) analogue in comparison with the gonadoliberin decapeptide. **Theriogenology** 7 : 277-284.

Newcomb, R. and Rowson, L. E. A. (1975). Conception rate after uterine transfer of cow eggs in relation to synchronization of oestrus and age of eggs. **J. Reprod. Fert.** 43 : 539.

Newcomb, R.; Rowson, L. E. A. and Trounson, A. O. (1976). The entry of superovulated eggs into the uterus. **In : Egg Transfer in cattle. Rowson, L. E. A. (ed), Commission of the European Communities, Luxembourg-EUR 5491**, 1976, pp. 1-15.

O'Farrell, K. and Hartigan, P. J. (1990). Superovulation and non-surgical egg recovery from normal and repeat breeding dairy cows. **Anim. Breed. Abstr.** 58(7) : 4261(abstr. no.).

Oyedipe, E. O.; Pathiraja, N.; Voh, A. A., Jr. and Buvanendran, V. (1988). Use of plasma progesterone profiles for the assessment of reproductive functions in indigenous Nigerian Zebu cattle. **Theriogenology** 30(3) : 629-641.

Palasz, A.; Garcia, A.; Gonzales, A. and Mapletoft, R. J. (1989). Luteinizing Hormone release in the cow : The effect of dose of two commercial GnRH preparations. **Theriogenology** 31(1) : 237(abstr.).

Papkoff, H. (1977). Relationship of PMSG to the pituitary gonadotropins. **In : Control of reproduction in the cow. Ed. Sreenan, J. EEC Galway**, pp. 73-86.

Pawshe, C. H.; Kadu, M. S.; Fasihuddin, M. and Totey, S. M. (1992). Superovulation with FSH-P and PMSG hormones in crossbred cows and heifers. **Indian J. Anim. Reprod.** 13(1) : 18-20.

Peters, A. R. (1985). Hormonal control of the bovine oestrous cycle. I. The natural cycle. **Br. Vet. J.** 111 : 564-575.

Peters, A. R.; Pimentel, M. G. and Lamming, G. E. (1985). Hormone responses to exogenous GnRH pulses in post-partum dairy cows. **J. Reprod. Fert.** 75 : 557-565.

✓ Petr. J.; Mika, J. and Filek, F. (1990). The effect of PMSG-priming on subsequent superovulatory response in dairy cows. **Theriogenology** 33(5) : 1151-1155.

Phatak, A. P.; Whitmore, H. L. and Brown, M. D. (1986). Effect of GnRH on conception rate in repeat-breeder dairy cows. **Theriogenology** 26 : 605-609.

Pierson, R. A. and Ginther, O. J. (1988). Follicular populations during the oestrous cycle in heifers. III. Time of selection of the ovulatory follicle. **Anim. Reprod. Sci.** 16 : 81-95.

Piper, P. J.; Vane, J. R. and Wyllie, J. H. (1970). Inactivation of prostaglandins by the lung. **Nature** (Lond.), 225 : 600-604.

Plasse, D.; Warnick, A. L. and Koger, M. (1968). Reproductive behaviour of Bos indicus females in a sub-tropical environment. I. Puberty and ovulation frequency in Brahman and Brahman x British females. **J. Anim. Sci.** 27 : 94-100.

Posadas, E.; Valencia, J.; Zarco, L. and Avila, J. (1991). Evaluation of the incorporation of GnRH into a superovulatory regimen for Zebu cattle. **Theriogenology** 35(4) : 761-767.

Radhakrishnan, B. and Raman, S. R. P. (1987). Studies on induced oestrus in cattle following cloprostenol and serum gonadotropin treatment. **Indian Vet. J.** 64 : 44-47.

Rahe, C. H.; Owens, R. E.; Fleeger, J. L.; Newton, H. J. and Harms, P. G. (1980). Pattern of plasma luteinizing hormone in the cyclic cow : Dependence upon the period of the cycle. **Endocrinology** : 107 : 498-503.

Rajakoski, E. (1960). The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical and left-right variations. **Acta. Endocrinol.** 34(Suppl. 52) : 7-68.

Rajamahendran, R.; Canseco, R. S.; Denbow, C. J.; Gwazdauskas, F. C. and Vinson, W. E. (1987). Effect of low dose of FSH given at the beginning of the oestrous cycle and subsequent superovulatory response in Holstein cows. **Theriogenology** 28(1) : 59-65.

Rajamahendran, R.; Lague, P. C. and Baker, R. D. (1976). Plasma progesterone levels in cycling and gonadotropin-prostaglandin treated heifers. **Can. J. Anim. Sci.** 56 : 37-42.

Ramakrishna, O. and Ramachandraiah, S. V. (1989). Superovulation with FSH in crossbred cows. **Indian J. Anim. Sci.** 59(1) : 102-104.

Randhawa, G.S. (1980). Oestrus manifestation and ovarian activity in buffalo heifers. **Thesis submitted to the Punjab Agricultural University, Ludhiana.**

Refsdal, A. O.; Vatn, T.; Landsverk, K. and Kommisrud, E. (1989). Embryo Transfer in cattle under Norwegian conditions. **Anim. Breed. Abstr.** 57(2) : 900(abstr. no.).

Reichenbach, H. D. (1991). Treatment of cattle embryos in early stages of development. **Anim. Breed. Abstr.** 59(11) : 7534(abstr. no.).

Renard, J. P.; Philippon, A. and Menezo, Y. (1980). In vitro uptake of glucose by bovine blastocysts. **J. Reprod. Fert.** 58 : 161-164.

Rieger, D.; Desaulnier, D. and Goff, A. K. (1988). Ovulatory response and embryo yield in superovulated Holstein heifers given a priming dose of FSH-P at day 2 of the oestrous cycle. **Theriogenology** 30(4) : 695-700.

Riha, J. (1989). Superovulation effect of PMSG and follicotropin on donors of embryos. **Anim. Breed. Abstr.** 57(10) : 6647(abstr. no.).

Roche, J. F. (1974). Synchronization of oestrus and fertility following artificial insemination in heifers given PGF-2 alpha. **J. Reprod. Fert.** 37 : 135-138.

Roche, J. F. and Boland, M. P. (1991). Turnover of dominant follicles in cattle of different reproductive states. **Theriogenology** 35(1) : 81-91.

Rodrigues, J. L. and Gregory, R. M. (1986). Superovulation response in cows following administration of FSH-P and prostaglandin. **Theriogenology** 25 : 190.

Rodrigues, C. F. M.; Silva, J. C. B. Da; Carvalho, C. De and Parlange, A. (1990). Superovulation in Zebu cows. **Anim. Breed. Abstr.** 58(10) : 6617(abstr. no.).

Rommel, P. and Rehbock, F. (1991). Comparative investigations on the superovulation of high yielding cows. **Anim. Breed. Abstr.** 59(2) : 958(abstr. no.).

Roussel, J. D.; Beatty, J. F. and Koonce, K. (1988). GnRH therapy in functional infertility of dairy cattle. **Theriogenology** 30(6) : 1115-1119.

Rowson, L. E. A.; Moor, R. M. and Lawson, R. A. S. (1969). Fertility following egg transfer in the cow : Effect of method, medium and synchronization of oestrus. **J. Reprod. Fert.** 1 : 517-523.

Rowson, L. E. A.; Tervit, H. R. and Brand, A. (1972). The use of prostaglandins for the synchronization of oestrus in cattle. **J. Reprod. Fert.** 29 : 145(abstr.).

Sacher, B.; Nieman, H.; Schilling, E. and Smidt, D. (1987). Effect of repeated superovulation on embryo recovery and fertility. **Anim. Breed. Abstr.** 55 : 6148(abstr. no.).

Sarvaiya, N. P.; Chauhan, F. S. and Mehta, V. M. (1992). Comparative efficacy of different gonadotropin preparations on superovulation and embryo recovery in Jersey x Kankrej crossbred cows. **Indian J. Anim. Reprod.** 13(2) : 120-122.

Saumande, J. (1980). Concentrations of LH, oestradiol-17 beta and progesterone in the plasma of heifers treated to induce superovulation. **J. Endocrinol.** 84 : 425-437.

Saumande, J. and Batra, S. K. (1985). Superovulation in the cow : Comparison of oestradiol-17 beta and progesterone patterns in milk and plasma of cows induced to superovulate; relationships with ovarian responses. **J. Endocrinol.** 107 : 259-264.

Saumande, J. and Chupin, D. (1986). Induction of superovulation in cyclic heifers. The inhibitory effect of large doses of PMSG. **Theriogenology** 25(2) : 233-247.

Saumande, J. and Chupin, D. (1987). The search for a reference method to test the effectiveness of anti-PMSG in superovulatory treatment in cattle. **Theriogenology** 27 : 274(abstr.).

Saumande, J.; Procureur, R. and Chupin, D. (1984). Effect of injection time of anti-PMSG antiserum on ovulation rate and quality of embryos in superovulated cows. **Theriogenology** 21 : 727-731.

Saumande, J.; Tamboura, D. and Chupin, D. (1985). Changes in milk and plasma concentration of progesterone in cows after treatment to induce superovulation and their relationships with number of ovulation and of embryos collected. **Theriogenology** 23 : 719-731.

Savage, N. C.; Howell, W. and Mapletoft, R. J. (1987). Superovulation in the cow using oestradiol-17 beta or GnRH in conjunction with FSH-P. **Theriogenology** 27 : 383-394.

Savage, N. C. and Mapletoft, R. J. (1984). Superovulation in the cow utilizing oestradiol-17 beta or GnRH in an FSH-P regimen. **Theriogenology** 21 : 259(abstr.).

Savio, J. D.; Bongers, H.; Drost, M.; Lucy, M. C. and Thatcher, W. W. (1991). Follicular dynamics and superovulatory response in Holstein cows treated with FSH-P in different endocrine states. **Theriogenology** 35(5) : 915-919.

Savio, J. D.; Keenan, L.; Boland, M. P. and Roche, J. F. (1988). Pattern of growth of dominant follicles during the oestrous cycle of heifers. **J. Reprod. Fert.** 83 : 663-671.

Schallenberger, E.; Schams, D.; Bullermann, B. and Walters, D. L. (1984). Pulsatile secretion of gonadotropins, ovarian steroids and ovarian oxytocin during prostaglandin-induced regression of the corpus luteum in the cow. **J. Reprod. Fert.** 71 : 493-501.

Schams, D. and Karg, H. (1982). Hormonal responses following treatment with different prostaglandin analogues. **Theriogenology** 17 : 499-506.

Schams, D.; Menzer, Ch.; Schallenberger, E.; Hoffman, B. and Hahn, R. (1978). Some studies on PMSG and endocrine responses after application for superovulation in cattle. In : **Sreenan, J. H. (ed), Control of Reproduction in the cow. Martinus Nijhoff, The Hague**, pp. 123-143.

Schilling, E.; Niemann, H.; Cheng, S. P. and Duepke, H. H. (1979a). DAPI-a further fluorescence test for diagnosing the viability of early cow and rabbit embryos. **Zuchthygiene** 14 : 170-172.

Schilling, E.; Smidt, D.; Sacher, B.; Petac, D. and El Kaschab, S. (1979b). Diagnosis of the viability of early bovine embryos by fluorescence microscopy. **Ann. Biol. Anim. Bioch. Biophys.** 19 : 1625-1629.

Schmitz, M. E. (1987). Effect of gonadotropic hormones on responses in superovulated cows. **Anim. Breed. Abstr.** 55(6) : 3531(abstr. no.).

Schneider, H. J., Jr.; Castleberry, R. S. and Griffin, J. L. (1980). Commercial aspects of bovine embryo transfer. **Theriogenology** 13 : 73-85.

Schwartz, F. L. and Shelby, D. R. (1969). Induced multiple ovulation in the bovine. **J. Anim. Sci.** 29(1) : 198.

Seidel, G. E., Jr. (1981). Superovulation and embryo transfer in cattle. **Science** 211 : 351-358.

Seidel, G. E., Jr.; Elsden, R. P.; Nelson, L. D. and Bowen, R. A. (1968). Superovulation of cattle with PMSG and FSH. In : **Sreenan, J. H.(ed), Control of Reproduction in the cow. Martinus Nijhoff, The Hague/Boston/London, 1968**, pp. 159-168.

Sergeev, N. I.; Lebedev, V. I.; Ibragimov, Yu and Almardanov, A. (1991). The use of pituitary preparations in embryo transfer. **Anim. Breed. Abstr.** 59(9) : 6021(abstr. no.).

Shea, B. F. (1981). Evaluating the bovine embryo. **Theriogenology** 15 : 21-42.

Shea, B. F.; Hines, D. J.; Lightfoot, D. E.; Ollis, G. W. and Olson, S. M. (1976). The transfer bovine embryos. In : **Egg Transfer in cattle, L. E. A. Rowson (ed), Commission of the European Communities, Luxembourg, 1976**, pp. 145-152.

Shea, B. F.; Janzen, R. E. and Mc Dermand, D. P. (1984). Seasonal variation in response to stimulatory and related embryo transfer procedures in Albertta over nine years period. **Theriogenology** 21 : 186-195.

Shelton, J. N. (1973). PGF-2 alpha for synchronization of oestrus in beef cattle. **Aust. Vet. J.** 49 : 442-444.

Silvas, E.; Moldovan, H. and Suhareanu, M. (1981). Embryo transfer in cattle using the surgical and non-surgical methods. **Anim. Breed. Abstr.** 50 : 5549.

Sirois, J. and Fortune, J. E. (1988). Ovarian follicular dynamics during the oestrous cycle monitored by real time ultrasonography. **Biol. Reprod.** 39 : 308-317.

Slimane, N. and Quali, F. (1991). Embryo transfer in cattle in Tunisia. **Anim. Breed. Abstr.** 59(5) : 3260(abstr. no.).

Smith, J. F. (1974). Oestrus synchronization in cattle. **J. Reprod. Fert.** 36 : 483-484.

Smith, J. F. (1976). Use of a synthetic prostaglandin analogue for synchronization of oestrus in heifers. **N. Z. Vet. J.** 24(5) : 71-73.

Smith, L. E.; Sitton, G. D., Jr. and Vincet, C. K. (1973). Limited injection of FSH for multiple birth in beef cattle. **J. Anim. Sci.** 37 : 523-527.

Snedecor, G. W. and Cocharan, W. G. (1967). **Statistical methods. 6th ed. Oxford and IBH Publishing Co.**, New Delhi, India.

Sprague, E. A.; Hopwood, M. L.; Niswender, G. D. and Wiltbank, J. N. (1971). Progesterone and LH levels in peripheral blood of cycling beef cows. **J. Anim. Sci.** 33(1) : 99-103.

Sreenan, J.M. (1975). Successful non-surgical transfer of fertilized cow eggs. **Vet. Rec.** 96 : 490-491.

Sreenan, J. M. (1978). Non-surgical embryo transfer in the cow. **Theriogenology** 9 : 69.

Sreenan, J.M. and Gosling, J. P. (1977). The effect of cycle stage and plasma progesterone level on the induction of multiple ovulation in heifers. **J. Reprod. Fert.** 50 : 367-369.

Stabenfeldt, G. H.; Edqvist, L. E.; Kindahl, H.; Gustafsson, S. and Bane, A. (1978). Practical implication of recent physilogic findings for reproductive efficiency in cows, mare, sows and ewes. **J. Am. Vet. Med. Assoc.** 172 : 667-675.

Stevenson, J. S.; Schmidt, N. K. and Call, E. P. (1984). GnRH and conception of Holsteins. **J. Dairy Sci.** 67 : 141-145.

Stubbings, R. B.; Walton, J. S.; Armstrong, D. T. and Basrur, P. K. (1990). Recovery of bovine oocytes from small vesicular follicles for *in vitro* maturation and fertilization. **Anim. Breed. Abstr.** 58(8) : 5125(abstr. no.).

Subramaniam, A.; Devarajan, K. P. and Mohanan, M. (1991a). 'On farm' embryo transfer in crossbred cows under Indian field conditions. **Anim. Breed. Abstr.** 59(7) : 4738(abstr. no.).

Subramaniam, A.; Devarajan, K. P. and Mohanan, M. (1991b). Repeat Flushing and Embryo Recovery (RFER) in crossbred donor cows (Bos indicus x Bos taurus). **Theriogenology** 35(1) : 277.

Swanson, L. V.; Hafs, H.D. and Morrow, D.A. (1972). Ovarian characteristics and serum LH, prolactin, progesterone and glucocorticoid from first oestrus to breeding size in Holstein heifers. **J. Anim. Sci.** 34 : 284.

Tamboura, D.; Chupin, D. and Saumande, J. (1985). Superovulation in cows : A relationship between progesterone secretion before ovulation and the quality of embryos. **Anim. Reprod. Sci.** 8 : 327-334.

Taylor, C. and Rajamahendran, R. (1991). Follicular dynamics, corpus luteum growth and regression in lactating dairy cattle. **Anim. Breed. Abstr.** 59(11) : 7542(abstr. no.).

Tervit, H. R.; Rowson, L. E. A. and Brand, A. (1973). Synchronization of oestrus in cattle using a prostaglandin F2 alpha analogue (ICI-79939). **J. Reprod. Fert.** 34 : 179-181.

Thomas, C. K.; Xavier, F.; Leena, K. and Madhavan, E. (1991). Superovulation and embryo collection from culled cows. **Anim. Breed. Abstr.** 59(9) : 6028(abstr. no.).

Totey, S. M.; Singh, Gurpreet; Anand, R.; Singh, Gurcharan and Talwar, G. P. (1988a). Embryo Transfer in Indian cattle. **Theriogenology** 29(1) : 319(abstr.).

Totey, S. M.; Singh, Gurpreet; Talwar, G. P. and Singh, Gurcharan (1988b). Preliminary trials on superovulation, non-surgical embryo recovery and embryo transfer in cows. **Anim. Breed. Abstr.** 56(7) : 4387(abstr. no.).

Totey, S. M.; Singh, G.; Taneja, M.; Singh, Gurcharan and Chillar, R. S. (1992). Effect of repeated superovulation and flushing on embryo recovery in crossbred cows. **Indian J. Anim. Reprod.** 13 : 117-120.

Touati, K.; Beckers, J. F. and Ectors, F. (1991). Hormonal control of folliculogenesis in the bovine : Better superovulatory responses after pure FSH administration preceding classical treatment. **Theriogenology** 35 : 285(abstr.).

Trounson, A. O.; Rowson, L. E. A. and Willadsen, S. M. (1978). Non-surgical transfer of bovine embryos. **Vet. Rec.** 102 : 74-75.

Unal, F. E. (1990). Superovulation produced by various hormone combinations in cattle. **Anim. Breed. Abstr.** 58(8) : 5129(abstr. no.).

Voss, H. J.; Allen, S. E.; Aquadro, P. and Foote, R. H. (1986). Incorporation of a GnRH analogue into a superovulatory regimen for dry Holstein cows. **Theriogenology** 25(1) : 210.

Voss, H. J.; Allen, S. E.; Foote, R. H.; Im, P.; Kim, C. K. and Aquadro, P. (1989). Buserelin in a superovulatory regimen for Holstein cows. I. Pituitary and ovarian hormone response in an experimental herd. **Theriogenology** 31(2) : 371-384.

Walters, D. L. and Schallenberger, E. (1984). Pulsatile secretion of gonadotropins, ovarian steroids and ovarian oxytocin during the periovulatory phase of the oestrous cycle in the cow. **J. Reprod. Fert.** 71 : 503-512.

Walters, D.L.; Schams, D. and Schallenberger, E. (1984). Pulsatile secretion of gonadotropins, ovarian steroids and ovarian oxytocin during the luteal phase of the oestrous cycle in the cow. **J. Reprod. Fert.** 71 : 479-491.

Walton, J. S. and Stubbings, R. D. (1986). Factors affecting the yield of viable embryos by superovulated Holstein-Friesian cows. **Theriogenology** 26(2) : 167-177.

Wang, H.; Wu, M.; Patt, D.; Murphy, B. D. and Mapletoft, R. J. (1988). Superovulation in beef heifers with PMSG : Effect of dose and monoclonal antibodies to PMSG. **Theriogenology** 29(1) : 323(abstr.).

Wang, H.; Wu, M.; Xu, K.; Hagele, W. C. and Mapletoft, R. J. (1987). Control of superovulation in the cow with a PMSG antiserum. **Theriogenology** 27(1) : 291(abstr.).

Ware, C. B.; Northey, D. L.; Boland, M. P. and First, N. L. (1988). Early cycle FSH-p priming as a prelude to superovulatory gonadotropin administration in ewes and heifers. **Anim. Reprod. Sci.** 16(2) : 97-105.

Wise, T. H.; Vernon, M. V. and Maurer, R. R. (1986). Oxytocin, prostaglandin E and F, oestradiol, progesterone, sodium and potassium in preovulatory bovine follicles either developed normally or stimulated by FSH. **Theriogenology** 26 : 757-778.

Wishart, D. F. and Snowball, J. B. (1973). Endoscopy in cattle : Observation of the ovary in situ. **Vet. Rec.** 92 : 139-142.

Wright, J. M. (1981). Non-surgical embryo transfer in cattle : Embryo-recipient interactions. **Theriogenology** 15(1) : 43-56.

Wubishet, A.; Graves, C. N.; Spahr, S. L.; Kesler, D. J. and Favero, R. J. (1986). Effect of GnRH treatment on superovulatory responses of dairy cows. **Theriogenology** 25 : 423-427.

Wubishet, A.; Kesler, D. J.; Graves, C. N.; Spahr, S. L. and Favero, R. J. (1991). Preovulatory LH profiles of superovulated cows and progesterone concentration at embryo recovery. **Theriogenology** 35(2) : 451-457.

Yadav, M. C. (1988). Endocrine changes and the timing of ovulation in superovulated cows. **Anim. Breed. Abstr.** 56(2) : 746(abstr. no.).

Yadav, M. C.; Leslie, K. E. and Walton, J. S. (1985). The onset and duration of ovulation in superovulated beef heifers. **Theriogenology** 23 : 237.

Yadav, M. C.; Walton, J. S. and Leslie, K. E. (1986a). Plasma concentrations of LH and progesterone during superovulation of dairy cows using FSH or PMSG. **Theriogenology** 26(4) : 523-540.

Yadav, M. C.; Walton, J. S. and Leslie, K. E. (1986b). Timing of the onset and duration of ovulation in superovulated beef heifers. **Theriogenology** 26(4) : 509-521.

Yigezu, Z.; Boland, M. P. and Gordon, I. (1989). Progesterone profiles in heifers following repeat superovulation. **Anim. Breed. Abstr.** 57(1) : 217(abstr. no.).

Zeitoun, M. M.; Yassen, A. M.; Hassan, A. A.; Fathelbab, A. Z.; Echternkamp, S. E.; Wise, T. H. and Maurer, R. R. (1991). Superovulation and embryo quality in beef cows using PMSG and a monoclonal anti-PMSG. **Theriogenology** 35(3) : 653-667.

Zeitoun, M.M.; Yassen, A. M.; Hassan, A. A.; Wise, T. H. and Maurer, R. R. (1988). Superovulation using PMSG and anti-PMSG in cattle. **Theriogenology** 29(1) : 339.

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ABSTRACT OF THE THESIS SUBMITTED BY SANJAY BHOJWANI ENTITLED "SUPEROVULATORY RESPONSE AND NON-SURGICAL EMBRYO RECOVERY IN RATHI COWS" FOR THE AWARD OF M.V.Sc. DEGREE IN THE SUBJECT OF OBSTETRICS AND GYNAECOLOGY (INCLUDING ARTIFICIAL INSEMINATION) TO THE RAJASTHAN AGRICULTURAL UNIVERSITY, BIKANER.

ABSTRACT

The present investigation was conducted to study superovulation and non-surgical embryo recovery in Rathi cows.

Ten cows were divided into 2 groups - the unprimed and the primed groups, 2 cows were kept as controls. All the cows (except controls) were superovulated with PMSG, oestrus being synchronized by using prostaglandin F-2 alpha. A.I. was performed at superovulatory oestrus and non-surgical embryo recovery was performed on 7th and 8th days after A.I..

No significant difference (P>0.05) was observed between the unprimed and the primed cows regarding mean interval from PG injection to induction of normal oestrus, superovulatory oestrus, post-superovulatory return to oestrus, mean number of follicles, mean ovulation rate and ovarian parameters (mean length, width and thickness). A total of 11 embryos were recovered from the donor cows of which 7 were viable and 4 were degenerated. Overall, no beneficial effect of priming was observed. The LH and FSH secretion was found to be pulsatile in superovulated and control cows.

:: पशु प्रसूति एवं मादा रोग विभाग, पशु चिकित्सा एवं पशु विज्ञान ::
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स्नात्तकोत्तर उपाधि हेतु "राठी गायों में बहु अण्डक्षरण एवं शल्य क्रिया रहित भ्रूण एकत्रण" नामक थिसेस का अनुक्षेपण ।

:- प्रस्तुति- संजय भोजवानी :-

:- अनुक्षेपण -:

राठी गायों में बहु अण्डक्षरण (अति अण्डोत्सर्ग) एवं शल्य क्रिया रहित भ्रूण एकत्रण पर वर्तमान शोध कार्य किया गया ।

दस गायों को दो समूहों में विभाजित किया गया, एक समूह में बिना प्राइमिंग (प्राथमिक मात्रा) के और दूसरे में प्राइमिंग समेत बहु अण्डक्षरण कराया गया । दो गायों को कन्ट्रोल के तौर पर रखा गया । सभी गायों (कन्ट्रोल के अलावा) को बहु अण्डक्षरण के लिये प्रेगनेंट मेअर सीरम (पी॰ एम॰ एस॰ जी॰) दिया गया । गायों में मदचक्र/ श्रध्तुमति (पाली) को समकृगित/प्रेरित करने के लिये प्रोस्टाग्लैंडिन (पी॰ जी॰ एफ॰ -2 अल्फा) दिया गया । श्रध्तुमति में आने पर गायों को कृत्रिम गर्भाधान से गर्भधारण करवाया गया । कृत्रिम गर्भाधान के 7 एवं 8 दिन पश्चात् भ्रूण एकत्रित किये गये ।

दोनों समूहों की गायों में प्रोस्टाग्लैंडिन इंजेक्शन के पश्चात् से लेकर श्रध्तुमति प्रेरण, बहुअण्डक्षरणीय श्रध्तुमति तथा इसके पश्चात् पुनः सामान्य श्रध्तुमति स्थापित होने तक के औसत अंतराल, औसत अण्ड स्यूनिका संख्या, औसत अण्डक्षरण दर एवं अण्डाशय की औसत लम्बाई, चौड़ाई एवं मोटाई में कोई उल्लेखनीय अंतर नहीं पाया गया । कुल ।। भ्रूण एकत्रित किये गये, जिनमें से 7 भ्रूण सामान्य पाये गये, जबकि 4 भ्रूण असामान्य पाये गये । कुल मिलाकर प्राइमिंग से बहु अण्डक्षरण में कोई उल्लेखनीय वृद्धि नहीं पायी गयी । बहु अण्डक्षरणित एवं कंट्रोल गायों में ल्युटिनाईजिंग हॉरमोन (अर्थात् एल॰ एच॰) एवं फॉलिकल स्टीम्यूलेटिंग हॉरमोन (अर्थात् एफ॰ एस॰ एच॰) न्यासर्गों का स्राव स्पुरणीय पाया गया ।

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