LUTEAL FUNCTION IN ESTRUS-INDUCED PREPUBERTAL GILTS

by

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B.Sc.(Hons.) (A.B.U.), M.Sc. (Sask.)

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ABSTRACT

Agu, Gabriel Ogaba, Ph.D., The University of Manitoba, February, 1990. Luteal Function in Estrus-Induced Prepubertal Gilts. Major Professor: Mary M. Buhr.

Three in vitro experiments were conducted, using corpora lutea (CL) from prepubertal gilts (age: 120-150 days) induced to ovulate by injecting 400 IU Pregnant Mare's Serum Gonadotrophin (PMSG) and 200 IU human Chorionic Gonadotrophin (hCG) to determine in Experiment I, progesterone (P4) production by the large and small cell types isolated on days 10, 15 and 18 after induced ovulation when incubated in the presence or absence of various doses of homologous Luteinizing Hormone (LH), low density lipoprotein (LDL), high density lipoprotein (HDL), or combinations of lipoproteins (LP) and LH; Experiment II, effects of various doses of prostaglandin F2a (PGF2a) on P4 production by each cell type isolated on day 10 and 15 in the presence or absence of LP and LH and Experiment III, the effects of a stimulator (S-adenosyl-L-methionine, SAM) or inhibitors (3-deazaadenosine, 3-DZA, or S-adenosyl-L-homocysteine, SAH) of membrane phospholipid methylation on the effects of PGF2a on P4 production by each cell type from day 10. Basal P4 production decreased from days 10 to 18 for both cell types and LH had minimal effects on P4

production by both cell types. In contrast, both LDL and HDL were highly stimulatory to P4 production on all days studied. PGF2a elevated P4 production by small cells on day 10 after 24 hr incubation. SAM interacted significantly with 1 ng/ml PGF2a to decrease P4 secretion in small cells but not large cells on day 10. It was concluded that 1) both cell types appeared steroidogenically competent when supplied with a natural source of cholesterol; 2) the induced CL were minimally sensitive to PGF2a in vitro and 3) phospholipid methylation may be involved in PGF2a induction of luteolysis in small luteal cells.

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INTRODUCTION

Several reports have established a high incidence of abortion in prepubertal pigs with hormonally-induced CL that have been mated naturally or through artificial insemination. Many causes of this anomaly have been suggested which include insufficient progesterone production from the corpora lutea (CL) and pronounced susceptibility of the induced CL to the purported natural luteolysin in swine, prostaglandin F2 alpha (PGF2a). Although the length of gestation improved in pregnant pigs given supplemental steroid, pregnancy still failed in most treated gilts (Shaw et al., 1971). Furthermore, it has been suggested that the induced CL of prepubertal pigs are more sensitive to PGF2a than the spontaneous CL of the adult (Puglisi et al., 1978, 1979). However, the high levels of PGF2a often given in vivo to gilts render the interpretation of the effects of PGF2a on induced CL of prepubertal pigs inconclusive.

Several mechanisms of action have been suggested as part of the initial events in the action of PGF2a. One of these theories is that PGF2a induces the loss of LH receptors (Grinwich et al., 1976). However, the loss of LH receptors following PGF2a treatment occurs after progesterone levels have dropped (Barb et al., 1984) and the affinity of the LH receptor for LH remains unchanged after PGF2a treatment

(Grinwich et al., 1976; Barb et al., 1984). These events suggest that other mechanism(s) of action of PGF2a may be involved, especially considering reports that PGF2a-induced luteolysis is accompanied by changes in the integrity of the luteal cell membrane.

Therefore, the overall aims of this research program were 1) to establish the ability of the CL of hormonally-induced prepubertal gilts to utilize progesterone precursors (in the form of lipoproteins) or respond to LH, using the induced CL of days 10, 15 and 18 after induction 2) to investigate the sensitivity of the induced CL to PGF2a and 3) to establish if membrane phospholipid methylation is involved in the luteolytic effects of PGF2a on the induced CL of prepubertal gilts.

LITERATURE REVIEW

1.1 Maintenance of Corpora Lutea in Pigs

1.1.1 Corpora Lutea Maintenance in Sexually Mature Pigs

Adult pigs reproduce throughout the year according to an 18 to 21 day estrous cycle (Bazer et al., 1982), with a gestation length of 110-120 days (Hughes and Varley, 1980). Ovulation occurs 36 to 42 hours after the onset of estrus and CL are formed by day 4 or 5 of the cycle. Progesterone secretion increases to a maximum between days 12 and 14 (Guthrie et al., 1972) or between days 11 and 12 (Connor et al., 1976). Average plasma progesterone levels between days 8 and 13 in cyclic gilts were not significantly different from those of pregnant pigs (King and Rajamahendran, 1988) suggesting that the function of both types of CL are similar. In the absence of fertilized ova or blastocysts, luteal regression begins from mid-cycle (day 12) and progesterone reaches a nadir by day 17 to 18, culminating in another period of estrus somewhere between day 18 and day 23.

In the presence of a blastocyst, an endometrial-blastocyst interaction occurs around day 11-12 after ovulation (Bazer and Thatcher, 1977). The presence of the

embryo prevents luteal regression and the CL remain the primary source of progesterone throughout pregnancy.

1.1.2 Embryonic Mortality in Prepubertal Pigs

An important goal in swine production is to farrow more pigs per sow per year. This could be achieved, in part, by lowering the age at first farrowing (Britt, 1979). However, attempts to induce a successful, full-term pregnancy by gonadotropin treatment in 90- to 180-day-old prepubertal gilts have had limited success (Rampacek et al., 1976). In an experiment designed to evaluate the effectiveness of a combination of 400 IU of Pregnant Mare's Serum Gonadotrophin (PMSG) and 200 IU of human Chorionic Gonadotrophin (hCG) (referred to as "PG 600") for induction of estrus in gilts, Britt et al. (1989) reported that the percentage of gilts (aged 165 to 225 days) in estrus within 28 days ranged between 42 to 97 in the treated group compared with 31 to 90 for controls. Interval from treatment to estrus was shorter (3.9 to 12.1 days) in the treated group than for controls (4.6 to 17.5 days) and the percentage of gilts that returned to estrus after the first breeding (rebred) did not differ between the two groups (12.8% for control and 16.4% for treated group). Farrowing rate did not differ between the two groups (55 to 97% for the treated group and 50 to 100% for controls). Similarly, litter traits and pigs weaned per litter did not differ between the groups. The number born alive ranged from 7.1 to 9.5 for the treated group and 7.3 to 8.9

for the control group. Pigs weaned per litter in the control group exceeded by 1.3 to 1.6 pigs per litter those in treated group.

Only 3 of 18 gilts treated with gonadotropins between 95 to 130 days of age maintained pregnancy to day 23 (Dziuk and Gehlback, 1966) and embryonic survival (the number of embryos relative to CL) was low for each pig. None of these gilts went to term. Although 55 of 57 prepubertal gilts (90-120 days) ovulated in response to gonadotropin treatment, only 28% were pregnant on day 30 following insemination (Ellicott et al., 1973). Corpora lutea had partially regressed in bred prepubertal pigs by day 20 (Segal and Baker, 1973), although they appeared normal in structure and progesterone secretion. Pregnancy could be maintained to day 60 with a combination of progesterone and estrogen (Ellicott et al., 1973). However, an estrone implant failed to prevent luteolysis between days 12 to 19 of the cycle (Tolton et al., 1985). Bred prepubertal gilts that received exogenous steroid therapy 48 hours post-PMSG to maintain the CL, had high fetal mortality after day 20 of pregnancy (Shaw et al., 1971) and second generation corpora lutea induced by gonadotropin administration (days 9 and 11 of gestation) regressed on day 50 and the pig aborted. Daily hCG treatment between 11 to 17 days of gestation increased the proportion of gilts that were pregnant on day 25. Furthermore, 20 of 31 gilts induced to ovulate with gonadotropins between 155 to 163 days of age were pregnant on day 30 (Guthrie, 1977) but the number of embryos on day 30 was

significantly less than the number of fertilized ova on day 3. Rampacek et al. (1976) also reported a low pregnancy rate of between 10 to 33% by day 25 in 155 to 175-day old gilts.

Greater abnormalities were found in blastocysts from gilts at first spontaneous estrus than those from second estrus (Menino et al. 1989). Some blastomeres failed to become incorporated into the morula or blastocyst stage which resulted in fewer cells involved in embryo development. It is not known if the same situation occurs with induced pregnancy in prepubertal gilts. However, Kineman et al. (1987) reported that induced CL of prepubertal pigs responded less to gonadotropin stimulation than CL of sexually mature sows and concluded that the prepubertal CL is more sensitive to the endogenous luteolysin.

Since exogenous progesterone or gonadotropins following induced ovulation improved the percentage of prepubertal gilts that maintained pregnancy (Shaw et al., 1971; Ellicott et al., 1973; Segal and Baker, 1973; Rampacek et al., 1976), it was suggested that premature luteolysis in prepubertal gilts is due to inadequate luteotropic support. However, others suggest that the induced CL of prepubertal gilts may be overly sensitive to PGF2a (Puglisi et al., 1978; 1979) because hysterectomy following induced ovulation maintained the CL to at least day 30. Since the CL of adult, sexually-mature pigs respond poorly to gonadotropin treatment (Cook et al., 1967; Buhr, 1987) and since hysterectomy does not guarantee maintenance of CL to term in prepubertal pigs

(Puglisi et al., 1978) the argument seems to be in favour of a lack of luteotropic support.

1.2 The Role of Lipoproteins and Luteinizing Hormone In Steroidogenesis by Corpora Lutea of Domestic Mammals

1.2.1 The Two Cell Types of the Mammalian Corpora Lutea

From early studies in the sow (Corner, 1919) and ewe (Warbritton, 1934) it was recognized that the CL, endocrine organs whose primary function is the secretion of progesterone (Rothchild, 1981; Keyes et al., 1983; Niswender et al., 1985; Stormshak et al., 1987) possess two steroidogenic cell types. These cells were differentiated on the basis of size, structure, and biochemical attributes. Other reports since then have supported these findings in all domestic species studied (Niswender et al., 1985; O'Shea, 1987).

The more conspicuous but less numerous is the large cell type (Deane et al., 1966; Donaldson and Hansel, 1965; Lemon and Loir, 1977; O'Shea et al., 1979; Ursley and Leymarie, 1979; Koos and Hansel, 1981; Fitz et al., 1982). In bovine CL large luteal cells made up only 3.5% and small luteal cells 26.7% from a total cell population of $393.4 \pm 52 \times 10^3$ cells per mm^3 of luteal tissue, which is a ratio of 1:7.6, at about day 12 of the estrous cycle (O'Shea et al., 1989). Although large cells are fewer than the small cell types and account for only 4% in ovine (Rodgers et al., 1984) or 8 to 12% in

bovine (Chegini et al., 1984) total cell population in the CL, they occupy more space than any other cell type (Rodgers et al., 1984). It is not clear whether the differences in the two studies are due to species, stage of the cycle or method of determination of the cell populations. On the basis of volume, the large cell type accounts for about 30% of the ovine CL (Niswender et al., 1976) compared to 16% for the small cell type.

Electron microscopic and morphometric studies estimated that the diameter of the large cell type ranges from 30 to 50 micrometers (um) (McClellan et al., 1975), 22 to 35 um (Fitz et al., 1982) 18 to 45 um (Chegini et al., 1984) or 20 to 50 um (Fields et al., 1985) while that of the small luteal cell type ranges from 12 to 22 um(Fitz et al., 1982;) or 10 to 15 um (Glass et al., 1984; Fields et al., 1985).

1.2.2 Origin of the Two Cell Types

The large cell type is now believed to originate from granulosa cells while the small cell type derives from the theca interna in the ewe (Warbritton, 1934; Donaldson and Hansel, 1965; Gemmel et al., 1974) and sow (Lemon and Loir, 1977). Using monoclonal antibody to bovine theca and granulosa cells, Alila and Hansel (1984) reported that granulosa antibody bound mainly to large luteal cells and labeled only a few small cells at the early stage of the estrous cycle. Theca antibody bound mainly to small luteal cells but also labeled some large cells later in the cycle.

Alila and Hansel (1984) suggested that this probably represented early stages of differentiation of small cells to large cells. Recently, Farin et al. (1988) reported that in vivo treatment of ewes with either LH or hCG resulted in the conversion of small luteal cells to the large cell type. The significance of these findings is that the theca-derived cells probably have a shorter life-span than the large luteal cells. If this is the case, progesterone production during the latter part of gestation would be derived mainly from the large cell type in both the cow and the ewe.

1.2.3 The Secretory Function of the Two Cell Types

The small cells of the sow (Lemon and Loir, 1977) possess numerous lipid droplets, and a few densely-staining granules are found in large cells on day 3 of the estrous cycle. In ewes, these densely staining, membrane-bound granules were thought to contain progesterone since they were found near the periphery of the cells coincident with increased progesterone secretion (Gemmel et al., 1974; O'Shea et al., 1979; Sawyer et al., 1979; Paavola and Christensen, 1981) and they were presumably released through exocytosis into the extracellular spaces (Gemmel et al., 1974; Sawyer et al., 1979) or perhaps by simple diffusion. During the onset of luteal regression, the cytoplasmic granules were sparse in ovine CL (O'Shea et al., 1986).

Despite such speculations, the nature of the contents of these granules remains controversial. Reports have suggested

they contain oxytocin in sheep (Rodgers et al., 1983b; Theodosis et al., 1986; Wathes et al., 1983) or relaxin in rats (Anderson and Sherwood, 1984) pigs (Kendall et al., 1978) and cows (Fields et al., 1980) or progesterone binding protein in the cow (Parry et al., 1980).

Both cell types secrete progesterone in vitro but basal secretion was higher in tissue fractions containing predominantly large cells (Fitz et al., 1982; Rodgers and O'Shea, 1982; Hoyer and Niswender, 1985; Niswender et al., 1985) and on a per cell basis, large cells produce more progesterone than small luteal cells (Rodgers et al., 1983a). Small luteal cells were frequently found among the large cell types with interdigitating processes between adjacent cells in sheep CL (O'Shea et al., 1979) and both cell types produced more progesterone in vitro than each cell type perfused separately (Lemon and Mauleon, 1982). This report suggests a synergistic interaction between the cell types. However, Rodgers et al., (1985) found no evidence from progesterone production in vitro to support synergism between the two cell types in ovine CL. The problem with such comparative studies is that the same number of cells may not be duplicated in all experiments. Such discrepancy in cell population could confound the results.

1.2.4 The Role of Lipoproteins in Steroidogenesis

1.2.4.1 Primary Structure and Purification of Lipoproteins

Lipoproteins are macromolecules composed of multiple proteins called apoproteins and lipid subunits (Gwynne and Strauss, 1982). They are classified on the basis of hydrated density into about 5 classes of which the most biologically relevant appear to be low density lipoprotein (LDL) and high density lipoprotein (HDL) (Jackson et al., 1976; Osborne and Brewer, 1977) because they carry most of the cholesterol in the circulation (Chapman, 1980; Havel et al., 1980).

Lipoproteins of different species differ in molecular weight, apolipoprotein component, weight ratio of cholesterol to protein and circulating levels (Gywnne and Strauss,1982) which complicates results of heterologous lipoprotein studies. Lipoproteins are separated by sequential ultracentrifugation at an increasing density. This is achieved by adding potassium bromide to whole plasma (Chapman et al., 1979; Buhr, 1987). Porcine LDL is distributed between the region of 1.024 to 1.045 gm/ml density (Chapman and Goldstein, 1977; Janado et al., 1966) and has apolipoprotein B as its protein component (Chapman and Goldstein, 1977) while HDL has a lower limiting density of 1.070 gm/ml. and apolipoprotein A-1 is its predominant protein moiety (Knipping et al., 1975; Knipping et al., 1978). High density lipoprotein from porcine serum has essentially a single component (Cox and Tanford, 1968) and is the predominant lipoprotein in porcine

plasma (Mills and Taylaur, 1971; Knipping et al., 1978).

1.2.4.2 Lipoproteins and Progesterone Production. The report that the lipid droplets in the cell cytoplasm contain cholesterol in bovine CL (Flint and Armstrong, 1971), that the concentration of these droplets varied inversely with the rate of progesterone production (Parry et al., 1980) and that the small cell type possessed the enzyme delta 5-3B-hydroxysteroid dehydrogenase which catalyses the conversion of pregnenolone to progesterone (O'Shea et al., 1979) suggest that cholesterol is a substrate for progesterone synthesis.

Progesterone production was enhanced in vitro by perfused large cells but not small cells of the sow, in the presence of cholesterol (Lemon and Mauleon, 1982) but both cell types responded with increased progesterone production in the presence of pregnenolone in the perfusion medium. Progesterone production was enhanced in ovine luteal cells in vitro in the presence of 25-hydroxycholesterol (25-OH cholesterol) (Hoyer and Niswender, 1985) with a greater response occurring in the small cell type-enriched fraction.

Lipoproteins are required for progesterone production by porcine luteal or granulosa cells in vitro. Porcine granulosa cells in medium containing lipoprotein-depleted serum produced less progesterone than those cultured in medium with complete serum (Veldhuis et al., 1984) and canine lipoprotein augmented progesterone release by dissociated luteal cells from pregnant pigs (Grinwich et al., 1983). Since the cell

types or the lipoprotein fractions were not separated in this study, any differential response of the individual cell types to the lipoprotein components was not determined.

Human LDL and porcine LDL but not human HDL increased progesterone production by porcine granulosa cells in vitro (Veldhuis et al., 1984; Tureck and Strauss, 1982). Recently, it was reported that porcine LDL stimulated progesterone production by large cells isolated from porcine CL on day 10 of the estrous cycle but HDL either did not affect or inhibited progesterone secretion (Buhr, 1987). However, both cell types were stimulated by both LDL and HDL on day 15 although LDL was more potent than HDL. In contrast to the porcine CL, both LDL or HDL stimulated progesterone production in dissociated bovine luteal cells (Pate and Nephew, 1988).

1.2.5 The Role of Luteinizing Hormone in Steroidogenesis

Reports on the role of LH in porcine luteal function are scanty in the literature. As early as 1961, Duncan and co-workers (1961) reported that LH or hCG had minimal effects on progesterone production by porcine CL in vitro. One of the first in vivo studies which investigated the possible role of LH in luteal function in swine demonstrated that hypophysectomy shortly after the initiation of estrus permitted the development of a fully functional CL (du Mesnil du Buisson and Leglise, in: Stormshak et al., 1987). But in hysterectomized gilts, hypophysectomy induced luteal

regression in about a week while daily injections of hCG, LH or crude pituitary extracts maintained luteal function (du Mesnil du Buisson and Leglise, in: Stormshak et al., 1987).

Bovine (Cook et al., 1967; Mattioli et al., 1985) or porcine or ovine (Cook et al., 1967) LH induced progesterone production by porcine luteal slices but a greater response was elicited from incubations of ovine or bovine luteal tissue. Perfusion of both CL cell types in vitro with media containing porcine LH (Lemon and Loir, 1977) or treatment of both cell types from day 12.5 but not day 6.5 of the porcine estrous cycle with bovine LH in vitro (Hunter, 1981) increased progesterone levels in both cell types. While progesterone stimulation was higher in the small cell fraction (Lemon and Loir, 1977) the large cells were minimally stimulated by porcine LH in a 2 hr incubation of dispersed cells while the small cells were unaffected by LH treatment (Buhr, 1987).

These reports put together suggest that the function of the porcine CL may be independent of LH or that LH has a permissive role. This is in contrast to the sheep in which LH stimulated progesterone production maximally in small but not the large cells (Bourdage et al., 1984; Fitz et al., 1982; Hoyer and Niswender, 1985; Niswender et al, 1985; Rodgers and O'Shea, 1982). The small cells of the sheep have significantly more LH receptors (Fitz et al., 1982; Lemon and Mauleon, 1982) and are more responsive to N^6-2'-O-Dibutyryladenosine 3' 5'-Cyclic Monophosphate (db-cAMP) stimulation than large cells

(Rodgers et al., 1983a). This would explain the response to LH by large cell fractions in the reports cited here. Experiments in cattle (Donaldson and Hansel, 1965) and sheep (Fitz et al., 1982; Niswender et al., 1985) have suggested that LH induces the differentiation of small cells into large cells, but with no change in the total number of the steroidogenic cell population. This has not been determined in the pig.

1.3 Luteolytic Role of Prostaglandin F2a in Swine

1.3.1 The Uterine Factor

Experiments have indicated that PGF2a is luteolytic in swine (Connor et al., 1976; Moeljono et al., 1976; Rampacek et al., 1979) and the uterine endometrium has now been identified as a source of PGF2a (Puglisi et al., 1978). The progress to this conclusion was made through experiments involving hysterectomy of pigs during the early part of the cycle (Anderson et al., 1961; du Mesnil du Buisson, 1961), autotransplantation of luteal phase uterine tissue in early or mid-cycle (Spies et al., 1960) or chemical destruction of the endometrium (Anderson et al., 1961). In each case it was reported that the CL persisted in the absence of the uterus. In addition, total hysterectomy maintained CL up to at least day 30 (du Mesnil du Buisson, 1961) but when more than 1/4 of the uterus was retained the CL found on day 40 or 55 were on the opposite side of the uterine fragment. These early studies suggested that a uterine factor caused luteal

regression. Recently it was demonstrated that plasma progesterone levels in hysterectomized gilts were greater than those of pregnant or cyclic pigs between days 8 and 15 of the estrous cycle (King and Rajamahendran, 1988).

1.3.2 Chemical Nature of the Luteolysin and Levels of Uterine PGF

The chemical nature of the luteolysin in swine endometrial filtrates was assessed by Duncan et al. (1960). Raising the temperature of the filtrate to 100 C for 15 minutes or dialysis of the preparation failed to eliminate its inhibition of progesterone synthesis from CL tissues. A buffered solution of ashed preparations of endometrial filtrates from day 13 or 18 of the cycle, or filtrates extracted under acidic, basic or neutral conditions (Duncan et al., 1960) still retained their progesterone-inhibitory characteristics. These experiments indicated that the luteolysin was a stable, non-proteinaceous macromolecule.

Later, it was reported that indomethacin, a prostaglandin synthesis inhibitor (Flower, 1974), prolonged luteal life in the pregnant pig (Nara and First, 1977; Sherwood et al., 1979) and cycling gilts (Kraeling et al., 1981). Twice daily treatment with indomethacin from day 109 to 116 of pregnancy (Nara and First, 1981) or prior to infusion of PGF2a (Guthrie, 1985) delayed luteolysis and extended the gestation period. Collectively, these studies indicated that the uterine luteolytic factor was PGF2a.

1.3.3 Luteolysin at the Late Cycle

Duncan et al. (1960) reported that subtotal hysterectomy at day 7 or 15 of the cycle maintained the CL and that endometrial filtrates from days 16 and 18 but not 12 and 13 inhibited progesterone synthesis from luteal tissue in vitro. Endometrial extracts from days 12 and 13 (Duncan et al., 1961), or filtrates obtained between days 13 to 17 of the cycle (Christensen and Day, 1972) induced luteolysis while those from other stages of the estrous cycle were less effective in vivo or in vitro. Similarly, Patek and Watson (1976) observed that superfusion of CL slices with media containing PGF2a or uterine flushings from the mid or late luteal period decreased progesterone secretion. It seems then, that the uterine luteolysin appears around mid cycle or about the time of implantation in pregnant animals.

Endometrial extracts or uterine flushings from swine during the late phase of the estrous cycle decreased in vitro progesterone synthesis by luteal slices (Schomberg, 1967) and late luteal phase uterine endometrium produced more PGF2a than from pigs during early pregnancy (Patek and Watson, 1976). Prostaglandin F2a production by uterine tissue increases from days 8 to 16 of the cycle (Guthrie and Rexroad, 1980) and its levels for days 16 and 18 were higher than those of days 8, 12, or 14 in utero-ovarian plasma of non-pregnant gilts during the period of expected luteolysis (Gleeson et al., 1974; Frank et al., 1977; Moeljono et al., 1977). The highest level of PGF2a was reached in the late

luteal phase of the cycle at a time that progesterone had declined to basal levels (Guthrie and Rexroad, 1981). These studies demonstrated that the peak production of PGF2a is correlated with the time of luteal regression.

1.3.4 The Refractory Period

In the pig it has been demonstrated that the effectiveness of PGF2a as a luteolysin depends on the stage of the estrous cycle or pregnancy (Connor et al., 1976; Diehl and Day, 1974; Hallford et al., 1975). Natural luteal regression in non-pregnant gilts starts between days 14 and 18 of the estrous cycle (Cavazos et al. 1969; Moeljono et al., 1977) and plasma progesterone declines to basal levels by days 17 to 18 (Bazer et al., 1982). Other workers reported that the onset of sensitivity of porcine CL to PGF2a-induced luteolysis occurs between days 12 and 14 of the estrous cycle (Moeljono et al., 1977). Intramuscular injection of PGF2a on days 10 or 12 (Diehl and Day, 1974) or at 12 hour intervals on days 4 and 5 (Hallford et al., 1975) or infusion of PGF2a into the anterior vein of sows for 10 hours on either day 12, 14 or 15 of the estrous cycle (Krzymowski et al., 1976) did not affect CL weight or induce luteolysis. However, PGF2a administration on day 12 or 13 (Hallford et al., 1975) or its analogue (ICI 79939) given between days 12 and 15 (Guthrie and Polge, 1976) reduced the estrous cycle length. Cloprostenol injection on day 13 of the cycle caused a greater reduction in serum progesterone than PGF2a, 15-keto-

PGF2a or 15-methyl-PGF2a (Buhr et al., 1986). Injection of PGF2a to hysterectomized gilts on day 17 decreased serum progesterone within 10 hours (Moeljono et al., 1976) and intramuscular injection of PGF2a on day 12 but not day 9 (Connor et al., 1976) abbreviated the length of the estrous cycle. The discrepancy between the results of Diehl and Day (1974) and those of Hallford et al. (1975) could be due to different dosages of PGF2a or that the CL early in the luteal was in a refractory period in the experiments of Hallford et al. (1975).

Henderson and McNatty (1975) proposed that the occupancy of the luteal LH receptors by LH released during the preovulatory surge prevents PGF2a from interfering with the LH receptors. This may account for CL refractoriness to PGF2a early in the cycle. However, since maternal LH is released in pulses during the proestrous surge in the rat (Gallo, 1980) it is unlikely that LH will occupy its receptors for a prolonged period of time. Also, infusion of LH for several hours failed to inhibit PGF2a-induced luteolysis in heifers (Gonzalenz-Mencio et al., 1977) Therefore, this hypothesis may not explain early CL refractoriness to PGF2a. Gonzalenz-Mencio et al. (1977) suggested the phenomenon may be due to low populations of PGF2a receptors. This is supported by Silvia et al. (1984) who showed that the large luteal cell population which possess the receptors for PGF2a, was low in early CL of the ewe. Regardless of the cause of early CL refractoriness to PGF2a, the refractory period is nature's

intriguing means of preventing the premature destruction of the CL before attachment of the embryo occurs.

1.3.5 Resistance of Pregnant CL to PGF2a

The reports that plasma progesterone concentration remained similar to control levels for 4 hours after injection of PGF2a on day 108 of gestation (Wetteman et al., 1977), and that a continuous infusion of PGF2a was required to induce a rapid drop in plasma progesterone in pregnant pigs (Guthrie, 1985), suggest that the CL of pregnancy temporarily resist the onslaught of the luteolysin. In an in vitro study Watson and Maule Walker (1978) also suggested such a resistance: a 2 hour superfusion of CL from 18 to 22-day pregnant pigs with 1 ug/ml PGF2a was ineffective in permanently suppressing progesterone levels. Evidence suggests that the resistance of the CL of pregnancy may be due to the presence of the fetus (Bazer and Thatcher, 1977).

The theory of the maternal recognition of pregnancy in the pig proposed by Bazer and Thatcher (1977) is that premature demise of the CL of pregnancy is prevented by a change in the release of endometrial PGF2a from an endocrine (towards the uterine venous drainage) to an exocrine direction (into the uterine lumen) stimulated by fetal estrogen (Bazer and Thatcher, 1977). It was also reported that late luteal tissue of the cycle synthesized more PGF2a than CL from pregnant animals of similar stage (Watson and Patek, 1979; Guthrie and Rexroad, 1981). However, PGF2a

production in vitro by endometrium and CL of pigs during early pregnancy was not significantly different from that of the mid luteal phase (Watson and Patek, 1979).

Ball and Day (1982) presented a series of experiments indicating that temperature-resistant saline extracts from day 16 to 25 pig embryos contained a substance that directly inhibited the luteolytic effects of PGF2a. More recently, data collected from endometrial tissue in perfusion experiments indicated that the secretion rate of PGF2a was higher from the luminal side during day 12 and 14 of pregnancy and day 14 in pseudopregnant gilts (Gross et al., 1988). This in vitro result supports the theory of redirection of PGF2a secretion into the uterine lumen when embryos are present in vivo.

1.3.6 Luteotrophic Effects of PGF2a in the Pig and Other Domestic Species.

Although the luteolytic effects of PGF2a in most domestic species are now accepted based on in vivo experiments, evidence from in vitro experiments suggests either a neutral or luteotrophic effect of PGF2a. Prostaglandin F2a enhanced basal and LH-stimulated progesterone production by dispersed porcine luteal cells from the mid to late luteal period (Mattioli et al. 1985). Superfusion or incubation of bovine luteal slices (1987 Speroff and Ramwell, 1970; Hansel et al., 1973; Hoedmaker and Grunert, 1987) or incubation of dispersed luteal cells (Hixon and Hansel, 1979) with PGF2a led to

increased progesterone production. Fredriksson et al. (1986) also reported a temporary elevation of progesterone in the mare with PGF2a treatment. Conversely, incubation of CL cells from regularly cycling, non-lactating dairy cows with PGF2a had no effect on basal progesterone production (Pate and Condon, 1984).

Using cultures of bovine granulosa cells, Henderson and McNatty (1977) reported that PGF2a inhibited the progesterone production only if added at the start but not later in the culture period. Incubation of separated bovine luteal cells with PGF2a caused a dose-dependent increase in both basal (Benhaim et al., 1987; Alila et al., 1988a & b) and LH-stimulated (Alila et al., 1988a & b) progesterone secretion in small cells. Levels of PGF2a as high as 1000 ng/ml had no effects on basal progesterone production but inhibited LH-stimulated, progesterone production by the large cells (Alila et al., 1988a).

These reports clearly demonstrated that in species in which PGF2a has been reported as luteolytic, in vitro results conflict with those obtained in vivo. This contradiction may stem from the higher dose levels of PGF2a used in in vivo experiments compared with in vitro studies or be related to the mechanism of action of PGF2a. Due to its luteolytic effects in vivo in most domestic species, several theories have been proposed to account for its mechanism of action.

1.4 The Mechanism of PGF2a-Induced Luteolysis

1.4.1 Effect of PGF2a on Blood Flow to the Corpus Luteum

Pharris and Wyngarden (1969) suggested that PGF2a-induced luteolysis in the rat was due to its constriction of the ovarian vein but Behrman et al. (1971) did not find similar results in an ovarian vein cannulation experiment. Goding et al. (1972) and Novy and Cook (1973) demonstrated a small decrease in luteal blood supply in the rabbit CL using labeled microspheres and concluded that the luteolytic effect of PGF was due to a reduced blood supply to the CL. Bruce and Hillier (1974) also supported these findings although plasma progesterone reduction preceded the vascular effects of PGF2a. However, the report that CL involution (Bruce and Moore, 1976) or PGF2a induced-luteolysis (Nett et al., 1976) was accompanied by a dramatic decline in blood flow and that plasma progesterone decreased simultaneously with reduced blood flow to the ovary in the ewe during spontaneous luteal regression (Niswender et al., 1976; Ford et al., 1979) seem to support the blood flow hypothesis.

A limitation of the blood flow hypothesis is that the loss of blood to the CL may be a consequence but not the cause of CL involution. Further, the constrictive effect of PGF2a should not be limited to the CL and blood flow to other endocrine organs should be affected, especially during the peak of PGF2a production. Such hypotheses are not supported in the literature.

1.4.2 Effects of PGF2a on Luteal LH Receptors

Functional luteolysis in the rat was characterized by the loss of LH receptors (Hichens et al., 1974) and Grinwich et al. (1976) hypothesized that PGF2a-induced luteolysis involved an early direct antagonism of PGF2a on gonadotropin receptors followed by the loss of these LH receptors. The hypothesis was supported by previous studies in which in vivo administration of PGF2a to the rat (Behrman et al., 1971) or in vitro to hamster CL (Behrman et al., 1974) resulted in a loss of LH stimulation of steroidogenesis. The capacity of the CL tissue to bind hCG and the loss of LH receptors or binding sites correlated with a drop in progesterone (Hichens et al., 1974) following in vivo PGF2a treatment. Injections of PGF2a analogue in rats (Torjesen and Aakvaag, 1976) or PGF2a treatment of rat luteal cells in vitro (Thomas et al., 1978) suppressed LH-induced progesterone secretion or reduced serum progesterone and the number of ovarian LH binding sites. Also, the number of LH receptors declined during spontaneous luteal regression in the rat (Behrman et al., 1976), sheep (Evrard-Herouard et al., 1981) and the cow (Spicer et al., 1981) occurring concomittantly with a decrease in serum and luteal progesterone in the ewe (Roser and Evans, 1983) and the mare (Diekman et al., 1983).

For the loss in LH receptors to be the initial luteolytic action of PGF2a, the PGF2a-induced loss of LH receptors should occur before or concurrently with the drop in progesterone levels. In the rat, there was no change in

the binding affinity of LH receptors following PGF2a treatment in vivo (Grinwich et al., 1976) and LH receptor capacity did not decline until 8 to 10 hours after PGF2a injection in rats (Behrman et al., 1976; Torjesen et al., 1978). Progesterone concentration, however, declined within 25 minutes (Torjesen et al., 1978). Further, progesterone levels decreased prior to a measurable drop in LH receptors in the ewe (Diekman et al., 1978; Evrard-Herouard et al., 1981) and the cow (Spicer et al., 1981). Although PGF2a treatment reportedly reduced progesterone concomittant with a drop in unoccupied LH receptors in pigs (Ziecik et al., 1980), Barb et al. (1984) reported that progesterone declined prior to a drop in LH receptors and no measurable change occurred in the binding affinity of unoccupied receptors.

In addition to this controversy, reports also indicate that the inhibitory effect of PGF2a is not restricted to LH. Pretreatment of intact rat CL with PGF2a inhibited not only LH-induced adenylate cyclase and cholera toxin-induced progesterone release but also the response of luteal membranes to epinephrine and fluoride (Khan and Rosberg, 1979). In vitro PGF2a treatment also reduced isoproterenol-induced adenylate cyclase activity in bovine luteal slices (Fletcher and Niswender, 1982). These reports suggest that the effects of PGF2a on adenylate cyclase may not be specific to LH-induced adenylate cylase.

1.4.3 Effects of PGF2a on Adenylate Cyclase and Cyclic AMP

According to the second messenger theory of LH action, LH binds to its receptors on the CL plasma membrane and activates adenylate cyclase (Marsh, 1976; Stormshak et al., 1987). Adenylate cyclase converts adenosine triphosphate to cAMP, which in turn activates cAMP-dependent protein kinase(s). The protein kinase(s) phosphorylate proteins required for progesterone synthesis. This model implies that the effects of PGF2a on LH function in the CL could be due to PGF2a inhibition of any of these biochemical steps.

The loss of LH receptors following in vivo administration of PGF2a correlated with the block of LH-stimulable cAMP and progesterone production (Grinwich et al., 1975). Pate and Condon (1984) reported that PGF2a inhibited dibutyryl cAMP-stimulated steroidogenesis in bovine luteal cells. However, there was no change in phosphodiesterase activity or the sensitivity or capacity of cells to respond to cAMP stimulation either directly (Khan et al., 1979) or through hCG-stimulated adenylate cyclase activity (Torjesen and Aakvaag, 1984) when isolated rat CL were incubated with PGF2a or cloprostenol. In the presence of IBMX, a potent phosphodiesterase inhibitor, PGF2a did inhibit LH-induced cAMP (Torjesen and Aakvaag, 1984). Since a deactivation of phosphodiesterase was required to elicit the effect of PGF2a, it appears that PGF2a was not acting directly through cAMP but merely replaced the deactivated phosphodiesterase. Although PGF2a reportedly prevented LH stimulation of adenylate cyclase

in dissociated rat luteal cells (Thomas et al., 1978; Khan and Rosberg, 1979; Wakeling and Green, 1981; Dorflinger et al., 1983) or ovine luteal cells (Agudo et al., 1984; Fletcher and Niswender, 1982) at least 60 minutes was required for a measurable drop in progesterone or adenylate cyclase activity. It appears, therefore, that PGF2a may not induce luteolysis through deactivation of adenylate cyclase or cAMP.

1.4.4 Effects of PGF2a on the Rate Limiting Step in Steroidogenesis

Since the rate limiting step in all steroidogenic tissue is the conversion of cholesterol to pregnenolone (Leaver and Boyd, 1981) some workers have suggested that PGF2a may act at some level of the control system that regulates pregnenolone formation (Torjesen and Aakvaag, 1984). The report that the concentration of lipid droplets increased in luteal cells in ewes during luteal regression (Corteel, 1975; Umo, 1975; Gemmell et al., 1976; McClellan et al., 1977; Parry et al., 1980) seems to support this hypothesis. However, they did not prove that the accumulation of lipid droplets was specifically due to PGF2a. Lipid accumulates in the cell because the cell loses the ability to use cholesterol in the lipid for steroidogenesis (Umo, 1975) as its secretory functions decline with regression. This explanation suggests that the accumulation of lipid in the cell may be the result but not the cause of luteal regression.

Heath et al. (1983) reported degranulation of dispersed bovine luteal cells following PGF2a treatment in vivo that was correlated with a decline in progesterone secretion, suggesting that these granules were secretory vesicles. But the workers did not indicate if this phenomenon eventually led to luteal regression.

1.4.5 Effects of PGF2a on Luteal Cell Membranes

The effects of PGF2a on the structure of the cell membrane has gained more attention since the reports that PGF2a-induced luteolysis is accompanied by cell membrane changes (Buhr et al., 1979; Goodsaid-Zalduondo et al., 1982). Both X-ray diffraction of microsomal membrane preparations from regressing rat CL (Buhr et al., 1979) and analysis of fatty acid composition in bovine microsomal membrane of regressing CL (Goodsaid-Zalduondo et al., 1982) demonstrated that luteolysis involves changes in the phospholipid bilayer. The workers reported a more fluid phase at mid-cycle than during the period of CL regression. Gel-phase lipid detected in the microsomal membranes persisted above the normal body temperature of the rat (Buhr et al., 1979) and became undetectable at a temperature approximately 10 C higher than for membranes during peak progesterone secretion. Similarly in the bovine model (Goodsaid-Zalduondo et al., 1982), microsomal membranes from the regressing CL showed a larger mole fraction of higher melting lipids such as sphingomyelin. Kim and Yeoun (1983) demonstrated a lower activation energy

of the membrane-linked Na^+-K^+-ATPase enzyme in rat CL in vitro following incubation of luteal slices with PGF2a and concluded that a change in membrane-bound enzyme activity may be an early step in PGF2a-induced luteolysis. The significance of these phase changes is that a more rigid membrane would prevent gonadotropin receptor aggregation (Luborsky et al., 1984) or interfere with other membrane-mediated events.

1.4.5.1 Enzymatic Methylation of Membrane Phospholipids

Enzymatic methylation of phospholipids is an event in the receptor-mediated signals on the cell membrane (Hirata et al., 1978; Hirata and Axelrod, 1980). Studies with rat brain indicated the presence of a membrane-bound two-enzyme system which sequentially methylated phosphatidylethanolamine to phosphatidylcholine (Crews et al., 1979). Tritiated S-adenosyl-L-[methyl-^{3}H] methionine (SAM), a methyl donor, can be incorporated into phosphatidyl-monomethylethanolamine and phosphatidylcholine (Hirata et al., 1978). These reactions are mediated by two methyl transferases. Methyl transferase I catalyzes the formation of phosphatidylmonomethylethanolamine from phosphatidylethanolamine while methyl-transferase II catalyzes the step-wise methylation of phosphatidyl-monomethylethanolamine to phosphatidylcholine (Hirata et al., 1978). Subsequent studies indicated that methylation began on the cytoplasmic side of the membrane where phosphatidylethanolamine and methyltransferase I were located

(Hirata and Axelrod, 1980).

Milvae et al. (1983) demonstrated that substances that affect phospholipid methylation modulate LH-induced progesterone production by dispersed bovine luteal cells. Corpora lutea were obtained from heifers on day 10 of the estrous cycle and dispersed cells were incubated in the presence or absence of S-adenosyl-L-homocysteine (SAH, a methyl-transferase inhibitor), 3-deazaadenosine (3-DZA, inhibitor of methylation) and SAM. The effects of the drugs were tested with LH, epinephrine, isoproterenol, cholera toxin or cAMP. SAH alone did not alter progesterone synthesis or dbcAMP or cholera toxin-stimulated progesterone production but reduced the stimulatory effect of LH, epinephrine or isoproterenol. 3-DZA also inhibited the stimulatory effects of LH in a dose-dependent manner. SAM alone did not affect progesterone production but concommitant treatment with LH increased the stimulation of progesterone production above that which occurred with LH alone (Milvae et al., 1983). Results obtained with tritiated SAM also indicated that methyl groups were incorporated into monomethylphosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol in the presence of LH (Milvae et al., 1983). Recently, Davis (1987) demonstrated that LH also induced increases in the levels of inositol triphosphate and cAMP in dispersed bovine luteal cells during early pregnancy; these were correlated with increases in intracellular Ca^{++}.

1.4.5.2 Phosphoinositol Turnover in Cell Membranes

Several studies suggest that some ligands cause phospholipid turnover and recycling when they interact with their membrane receptors (Nishizuka, 1984a) a process that involves intracellular Ca^{++}. The sequential hydrolysis of phospholipids involves the formation of intracellular messengers: inositol 1,4,5-triphosphate which mobilizes Ca^{++} (Irvine et al., 1984) from the endoplasmic reticulum (Burgess et al., 1984) and 1,2-diacylglycerol transiently formed from inositol phospholipids in response to the extracellular signals, which increases the affinity of protein kinase C for Ca^{++} (Nishizuka, 1984b). In mammals, phospholipase C attacks the inositol phospholipids such as phosphatidylinositol-4-monophosphate and phosphatidylinositol-4,5,-bisphosphate (Shukla, 1982). This hydrolysis results in the formation of 1,2-diacylglycerol and, depending on the inositol phospholipid being degraded, myoinositol 1-monophosphate, myoinositol 1,4-bisphosphate or myoinositol 1,4,5-triphosphate are formed. A small fraction of the byproduct of this reaction, 1,2-diacylglycerol is deacylated by the actions of 1,2-diacylglycerol lipase and 2-monoglycerol lipase to generate arachidonic acid and eicosanoids (Nishizuka 1984a,b). The product 1,2-diacylglycerol increases the affinity of protein kinase C for Ca^{++}. Protein kinase C is now recognized to be involved in the transmembrane signal transmission by cells (Nishizuka, 1984b; Nishizuka, 1986)

Recently, Hoyer et al. (1988) reported that protein

kinase C has differential regulatory effects in the small and large steroidogenic cell types of the CL. They isolated large and small cell types from day 10 ovine CL and tested the effects of phorbol-12-myristate-13-acetate (TPA), a protein kinase C activator, on progesterone secretion by the two cell types. Less kinase C activity was reported in large than small cells whether stimulated by Ca^{++}, phosphatidylserine or TPA. In another report, TPA inhibited basal progesterone secretion in ovine luteal cells in vitro by 30% while PGF2a in the same system caused a 10% reduction (Conley and Ford, 1988). The synergistic role of protein kinase C and Ca^{++} mobilization has been extended to several other systems including catecholamine release from bovine adrenal medullary cells, aldosterone secretion from porcine adrenal glomerulosa cells (Kojima et al., 1983) and insulin release from rat pancreatic islets (Zawalich et al., 1983).

Studies with ligands that bind to granulosa cells (Davis and Clark, 1983; Naor et al., 1984) or luteal cells (Davis et al., 1981; Milvae et al., 1983) including PGF2a (Leung et al., 1986; Davis et al., 1987) demonstrated that these ligands also stimulate phospholipid methylation, inositol phospholipid turnover and Ca^{++} mobilization. Luteinizing hormone stimulated incorporation of $^{32}PO_4$ into phosphatidylinositol and increased steroidogenesis in bovine luteal cells (Davis et al., 1981) and LH and GnRH increased phosphatidylinositol metabolism in ovarian granulosa cells (Davis and Clark, 1983). Also, the GnRH analogue [D-Ala^6] des-Gly^{1}0-N-ethylamide

increased the specific labelling of inositol and phosphatidic acid with ^{32}P by 4.5 and 3.5-fold respectively, producing a dose and time related increase in progesterone production in cultured granulosa cells (Naor et al., 1984).

1.4.5.3 Interaction of PGF2a with Membrane Phospholipids

Prostaglandin F2a has been reported to cause mobilization of intracellular Ca^{++} (Gore and Behrman, 1984; Dorflinger et al., 1984) and increased incorporation of ^{32}P into phosphatidic acid and phosphatidylinositol in rat luteal and granulosa cells in 2, 5 and 10 minutes (Raymond et al., 1983; Minegishi and Leung, 1985). Labeling of other phospholipids was unaffected in granulosa cells. Prostaglandin F2a also increased phosphatidylinositol bisphosphate hydrolysis in rat luteal cells (Leung et al., 1986). Luteal cells labeled with myo-[2-^{3}H]inositol and incubated with PGF2a showed a rapid decrease in the level of ^{32}P found in phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate. The effect of PGF2a reached a maximum after 20 seconds (Leung et al., 1986). Although these studies suggest a rapid effect of PGF2a, progesterone levels were not monitored in these cells so it was not clear whether these changes were associated with luteolysis. Prostaglandin F2a caused a rapid reduction in labelled phosphatidylinositol bisphosphate that was associated with ^{32}P incorporation into phosphatidic acid and phosphatidylinositol after 5 minutes incubation (Davis et al., 1987). It also increased inositol triphosphate

accumulation following 5 minutes of incubation. Furthermore, incubation of bovine luteal cells with PGF2a for 60 minutes significantly increased the incorporation of $^{32}PO_4$ into total luteal phospholipids. It increased the incorporation of $^{32}PO_4$ into phosphatidic acid by 150% and into phosphatidylinositol by 50% (Davis et al., 1987). Prostaglandin F2a-induced changes in ^{32}P-labeled phospholipids were detected within 5 minutes of treatment in cells that were not labeled prior to treatment. Davis et al. (1987) also reported that incubation of luteal cells with 1 uM PGF2a for 30 minutes caused incorporation of ^{3}H into inositol phosphate, inositol bisphosphate and inositol triphosphate by 5, 7 and 10-fold respectively and the reduction in free ^{3}H-inositol reflected the increases in the labeled inositol phosphates. Other workers have earlier reported that the effect of PGF2a on inositol phospholipids in intact cells (Dorflinger et al., 1984) was dose dependent and required PGF2a binding to its receptors (Powell et al., 1975; Kimball and Lauderdale, 1975).

Prostaglandin F2a rapidly increased inositol triphosphate when 1 mM $CaCl_2$ or 1 mM EGTA were added to luteal cells in Ca^{++}-free medium, although the EGTA reduced the magnitude of the response to PGF2a by 12% (Davis et al., 1987). Within the first 15 to 30 seconds of addition of PGF2a, intracellular Ca^{++} was increased 2 to 3 times above the resting level.

Thus, prostaglandin F2a-induced luteolysis may be due to one or a combination of events both at the cell surface level

or beyond it. Since PGF2a-associated membrane changes and the decline in progesterone which often follows PGF2a administration in vivo are rapid events, it is tempting to speculate that the membrane changes could result in the rapid drop in luteal progesterone production. For this reason, the effect of PGF2a on membrane phospholipids has become a subject of intense investigation.

MATERIALS AND METHODS

2.1 Lipoprotein Preparation

The lipoprotein isolation procedure was according to the methods of Terpstra et al. (1981). Blood was collected from sows by venipuncture on day 4 of the estrous cycle (day 0 = first day of standing heat), centrifuged for 15 minutes at 2000 x g and supernatant serum was harvested. One hundred microlitres of Sudan black (Eastman Kodak Co., Rochester, N.Y.) solution (0.05 gm Sudan black in 50 ml ethylene glycol), 0.385 gm potassium bromide (Fisher Scientific, Fair Lawn N.J.) and 0.025gm of sucrose (Fisher) were added per ml of serum. Six ml of serum were placed in a polyallomer centrifuge tube (25x89 mm) and overlayed with a discontinuous KBr density gradient (0.998, 1.049, 1.10, 1.163 and 1.225) in 0.571% sodium chloride (NaCl, Fisher Scientific), and 0.01% disodium ethylene diamine tetrachloride (EDTA, Fisher Scientific). The tubes were centrifuged in a Beckman L3-50 preparative ultracentrifuge for 16 hours at 160,000 x g, 22 C. The layers of LDL and HDL were transferred with silicon-coated (Specialty Chemicals, Gainsville, USA) pasteur pipettes (Fisher Scientific) into dialysis bags (Spectrum Medical Industries, Los Angeles, Mol. Wt. cut-off: 6,000 to 8,000) and dialyzed at 4 C for 72 hours in phosphate buffered saline (PBS) containing

0.598% sodium phosphate monobasic dihydrate (NaH_2PO_4 .$2H_2O$, Fisher Scientific), 1.635% sodium phosphate dibasic heptahydrate (Na_2HPO_4 .$7H_2O$, Fisher Scientific) and 0.9% NaCl, pH 7.4. The dialyzed lipoproteins were concentrated to desired levels against 15% (w/v) of polyethylene glycol in phosphate-buffered saline for approximately 12 hours at room temperature. Each lipoprotein fraction was stored at 4 C in 50 ml plastic flasks and used within 4 weeks.

2.2 Protein Estimation in Lipoproteins

Protein concentration in the lipoprotein preparation was estimated using the Bradford method (Bradford, 1976). Protein colour reagent was prepared by adding 100ml 83% phosphoric acid to a solution of 100gm brilliant blue G250 (Sigma Chemical Co., St Louis, MO) in 50ml of absolute ethanol. This solution was made up to 1 litre with deionized distilled water on day 0. Bovine gamma globulin (Sigma) was used as the protein standard. The absorbances were read in a spectrophotometer at 595nm wavelength and the protein level was expressed as mg/ml.

2.3 Treatment of Pigs and Surgery

Prepubertal gilts, 120 to 143 days of age at treatment, were injected intramuscularly in the flank with 400 IU pregnant mare's serum gonadotrophin (PMSG, Ayerst Laboratories, Montreal, Quebec) and 200 IU human chorionic gonadotrophin (hCG, Ayerst Laboratories) in sterile water. At day 10, 15 or

18 (Experiment I), 10 or 15 (Experiment II) or day 10 only (Experiment III) following gonadotrophin treatments ovaries were surgically removed into sterile Ham F12 nutrient media (Gibco Laboratories, Grand Island, N.Y.) supplemented with 1.17 ug/ml sodium bicarbonate ($NaHCO_3$, Fisher Scientific), 100 ug/ml dihydrostreptomycin sulfate (Sigma), 40 ng/ml dihydrocortisone (Sigma), 2 ug/ml porcine insulin (Sigma) and 5 ug/ml human transferrine (Sigma).

2.4 Cell Preparation and Incubation

Cell preparations and incubations were as previously described (Buhr, 1987). All surgical instruments and glassware were sterilized by autoclaving (American, model 57CR) and solutions were sterilized by filteration through sterile 0.45 um filters. All procedures, whenever possible, were done in a sterile horizontal flow hood. Flasks containing tissue were placed on ice at 4 C between incubations and centrifugations. Corpora lutea were identified and removed from the ovaries with forcepts transferred to fresh media in a glass petri-dish and the outer connective tissue was removed with forceps. The CL were weighed in a flask containing media, and the weight and number of CL were noted. The tissue was minced with scalpels in two 250ml polycarbonate flasks containing an aliquot of media. Each aliquot throughout the proceedure was 5 ml per gm of CL tissue. Flasks were capped with foam stoppers and the tissue was incubated for 10 minutes in a Dubnoff shaking water-bath (120 rpm) at 37 C. The flasks were

placed on ice and the tissue was allowed to settle for about three minutes and the supernatant containing mainly red blood cells, was discarded. Collagenase (type V, 2.5 mg/ml, Sigma) was added to the media for all subsequent incubations and dispersion processes. A second aliquot of the media was added to the CL tissue and incubated for 30 minutes at 37 C and 120 rpm. The supernatant was aspirated with a silicon-coated pipette into 50 ml polycarbonate tubes containing lul/ml sterile aprotinin (Sigma) and kept at 4 C. Incubation of the remaining tissue was repeated for 2 x 45 minutes and an additional 30 minutes. The supernatants from each incubation were saved. At the end of the incubations any remaining tissue remaining in the flask was dispersed with a pipette and the samples were centrifuged at 500 x g for 12 minutes at 4 C. The supernatant was discarded and the pellet gently resuspended in an aliquot of media with silicon-coated pipette. Fresh media and ethylenebis-(oxyethylenenitrilo)tetraacetic acid (EGTA, 0.38×10^{-3} gm/ml, Fisher Scientific) was added to the tissue and incubated for 10 minutes as before. The suspension was filtered through 295 um nylon mesh (Nitex) into a polycarbonate tube containing aprotinin (Sigma) and centrifuged (500 x g, 12 minutes, 4 C). The cells was washed with fresh media alone in three centrifugations and resuspended with 30 ml of media. It was then layered on a discontinuous ficoll (Pharmacia, Uppsala, Sweden) gradient (1% and 3% ficoll, modified from Ursley and Leymarie, 1979). To prepare the gradient, first 500 ml of 0.5% BSA in PBS was

placed into a silicon-coated 500 ml separatory funnel and drained into another silicon-coated, 2 litre separatory funnel. The 500 ml funnel was then filled with 1% ficoll in PBS which was slowly drained into the 2 litre flask, maintaining a distinct interface between the BSA and the ficoll. This step was repeated for 3% and 5% concentrations of ficoll. The 5% ficoll was used to push the remaining gradient to the top of the funnel to enable layering of the cells. The cells were carefully layered on top of the BSA with a rubber tubing attached to a 30 ml syringe and the 5% ficoll was immediately drawn off. This pulled the cells into the gradient and hastened the separation of the cell types. After 45 minutes, the 3% ficoll layer containing the large cell-enriched fraction was filtered through a 73 um nylon mesh and the 1% layer containing the small-cell enriched fraction was filtered through a 25 um nylon mesh into two 250 ml polycarbonate flasks. The interface between the two layers was discarded. The cells were pelleted (500 x g, 12 minutes, 4 C) and washed with media in 3 centrifugations and cell viability was estimated by the trypan blue exclusion method (Buhr, 1987). Cells were counted with a hemocytometer using a light microscope and the cell concentrations were adjusted with media to 1 x 10^3 cells per ml for large cells and 10 x 10^3 cells per ml for small cells. Cell counts were based on total cell numbers and the concentration was adjusted based on the cell type of interest. One millilitre aliquots of each cell fraction was plated in flat-bottom, Falcon 24-well plates

(Corning Glassworks, Corning, New York) and incubated overnight for 14 to 16 hours (5% CO_2, 95% air) in a VIP Imperial II CO_2 incubator at 37 C and 100% humidity. This was referred to as the 'pretreatment' incubation.

2.5 Experimental Design

2.5.1 General Incubation Procedure

For each experiment a control in duplicate received media only. The volume of each incubate was made up to 1 ml with media following the addition of test substances and incubated for 2 hours. At this time, 500 ul was removed and replaced with 500 ul of test substances and media and incubation was continued for a further 22 hours. Samples from each incubation were placed in 1 ml plastic vials and immediately frozen and stored at -20 C until assayed for progesterone.

2.5.2 Experiment I. Experiment 1 was a randomized 3x2x3 factorial experiment with 3 days (day 10, 15 and 18), 2 cell types (large and small) and 3 treatment substances (LH, LDL and HDL). The number of pigs (n) used was 3, 4 and 4 for day 10, 15 and 18 respectively. Following pretreatment incubation media was aspirated from the wells with a pasteur pipette and replaced with 1 ml of media containing lipoproteins (LDL or HDL at 0, 10, 50 or 100ug/ml) $\pm$ porcine luteinizing hormone (USDA-pLH-B-1, USDA Reproduction Laboratories, Beltsville, MD) at 10, 50 or 100 ng/ml, or all possible combinations of each

lipoprotein and LH in duplicate.

2.5.3 **Experiment II.** This was a randomized 2x2x3 factorial experiment with 2 days (days 10, n = 4 and 15, n = 3), two cell types (large and small) and 3 treatment substances (PGF2a, LH and LDL). At the end of pretreatment incubation, media was aspirated as described for experiment I and replaced with LH (100 ng/ml) or LDL (100 ug/ml) $\pm$ PGF2a (0.001, 0.1, 1 or 100 ng/ml) or combination of LH, LDL and each level of PGF2a in duplicate. Prostaglandin F2a was prepared by a serial dilution of sterile Lutalyse (Sigma). Two hundred microlitres of sterile lutalyse containing 5 mg/ml of dinoprost (as dinoprost tromethamine, Sigma) was diluted with 9.8 ml media. Two hundred microlitres from this preparation was further diluted with 39.8 ml media to give 100 ng/ml. From this concentration, 200 ul was taken and diluted with 19.8 ml of media. This concentration was 1 ng/ml. One milliliter from this concentration was then diluted with 9 ml of media to give 0.1 ng/ml. Finally, 200 ul from this final concentration was diluted with 19.8 ml of media to give 1 pg/ml.

2.5.4 **Experiment III.** Six gilts were used for this study. This was a randomized 2x4 factorial experiment with 2 cell types (large and small) obtained at day 10 and 4 treatment substances (PGF2a, SAM, Sigma; SAH, Sigma; 3-DZA, Southern Research Labs. Birmingham, Alabama). The drugs SAM, SAH and 3-DZA were prepared as follows: SAH, (anhydrous mol. wt. 384.4)

was prepared at a concentration of 1 mM (0.3844 mg/ml). SAM, (anhydrous mol. wt. 399.4) was used at a concentration of 200 uM (0.08 mg/l) while 3-DZA was administered at 10 uM concentration (0.0026625 mg/l). Following pretreatment incubation, media was aspirated from the wells with a pasteur pipette and replaced with 1 ml of media containing PGF2a (1 or 100 ng/ml) $\pm$ SAM (200 uM), SAH (1 mM) or 3-DZA (10 uM) as per experiment I and II.

2.6 Progesterone Assay

Progesterone,[1, 2-^{3}H(N)] (specific activity 53.4 Ci/mMol), was purchased from New England Nuclear, Boston. Phosphate buffered saline (as described under lipoprotein preparation) containing 1 mg/ml gelatin (Sigma) was the assay buffer. Antiserum (Antiprogesterone #11) raised in sheep by repeated immunization against progesterone conjugated to bovine serum albumin (11alfa-hydroxy-4-pregnene-3, 20-dione hemisuccinate: BSA, Steraloids) was obtained from Dr. N.C. Rawlings, University of Saskatchewan, and stored frozen in 0.1ml aliquots. Progesterone was used as standard in 50, 100, 200, 400 and 800 pg/tube concentrations.

Progesterone was assayed by the method of Yuthasastrakosol et al. (1974). At room temperature, known aliquots of incubation samples were placed in labeled 12 x 75 mm culture tubes in duplicates and diluted to 500 ul/tube with assay buffer. Progesterone, [1,2-^{3}H(N)] was diluted with assay buffer to give between 10,000-12000 counts per minute (cpm/100

ul) on a LKB Rackbeta scintillation counter. One hundred microlitres of the radiolabeled progesterone was added to each assay tube, followed by 100 ul of 1:500 dilution of the antiserum except for total count and non-specific count tubes which received no antiserum. This concentration of antiserum bound between 45 and 48% of tritiated progesterone at 11,000 cpm. The volume in each assay tube was 700 ul. Assay tubes were briefly agitated on the multi-tube vortexer (Scientific Manufacturing Industries, model 2600) and incubated overnight at 4 C.

A charcoal solution was made by combining 0.375 gm of purified powdered Norit A charcoal (Matheson Coleman and Bell) per 100 ml of assay buffer. This solution was vortexed for 60 minutes at 4 C and stored at 4 C for 2 days. Working at 4 C, 500 ul of this continuously agitated solution was added to each assay tube. Tubes were briefly vortexed and incubated at 4 C for 10 minutes. Incubation was followed by centrifugation (4 C) at 1500 x g for 10 minutes using a CR3000 centrifuge (Jouan Inc, Winchester, Virginia). The supernatants were decanted into scintillation vials and 5 ml of scintillation fluid (Scintiverse II, Fisher Scientific) was added and mixed. Radioactivity was measured and progesterone level was determined in the Rackbeta counter using a RIA program.

Aliquots from a frozen pool of incubation samples containing known levels of progesterone were used to compute interassay coefficient of variation. The intraassay coefficient of variation, assessed from samples of known

concentrations used with each assay, was 7.5% and interassay coefficient of variation, calculated from the results of ten assays, was 11.02%.

2.7 Statistical Analysis

2.7.1 Pretreatment Incubation Progesterone Analysis

Least Square Analysis of progesterone data was performed using the General Linear Model Procedure (SAS). For Experiment I, analysis of variance (ANOVA) was performed to determine if differences existed between pretreatment progesterone production by large cells isolated on days 10, 15 and 18 of the estrous cycle. For this analysis, cycle day (i.e day of the estrous cycle) and pigs within cycle day were the sources of variation. ANOVA was also performed to compare the pretreatment progesterone production by small and large cells obtained on days 15 and 18 of the cycle. Because of inadequate numbers of small cells from day 10 CL, progesterone production by small cells was only analysed for day 15 and 18 CL. In the analysis of progesterone production by both cell types from day 15 and 18 CL, the sources of variation were cycle day, pigs within cycle day and cell type. Pigs within cycle day was the error term for cycle day.

2.7.2 Treatment Incubation Analysis

For each Experiment, ANOVA was performed for each cell type and cycle day to determine the overall response to treatment.

The model included treatment as well as pigs. For Experiment I, the intended full treatment applications were as shown in Table 1. The objective of the ANOVA was not only to determine treatment differences, but to establish the causes underlying treatment differences. This was done by considering the factorial nature of the treatments used. Note that in Table 1 all LH treatments appear with all lipoprotein treatments. It is possible with this factorial arrangement, to determine main effects of all levels of LH or lipoproteins and their interaction effects. However, because of insufficient number of cells to complete all treatments, this full model was not used. Instead, the analysis to establish the causes of treatment differences was performed in 3 factorial patterns (Tables 2 and 3) to obtain as much information from the data as possible. In addition to these analyses, linear, quadratic and cubic contrasts (SAS, 1985) were used to determine the response of each cell type to the various levels of lipoproteins in the absence of LH within each day of the estrous cycle.

For each experiment, cell type and day of cycle, a preliminary ANOVA was performed using the pretreatment progesterone levels as covariates with the 2 and 24 hour progesterone samples. Another ANOVA was then used to determine the response to the test substances. Linear contrasts and estimate analysis were used to assess the main effects of each test substance and their interactions. Bonferroni's inequality (SAS, 1985) test was then used to test for significant

TABLE 1. The intended full pattern of treatments for both cell types in Experiment I.

		LH (ng/ml)			
		0	10	50	100
LDL (ug/ml)	0	x	x	x	x
	10	x	x	x	x
	50	x	x	x	x
	100	x	x	x	x
HDL (ug/ml)	10	x	x	x	x
	50	x	x	x	x
	100	x	x	x	x

Note: Each 'x' represents one treatment, done in duplicate.

differences between pairs of treatment using an experimentwise error rate of 0.05%.

In all analyses, progesterone level was expressed as least square means ng progesterone/hr/ 10^3 cells. The 24 hour values were corrected mathematically to incorporate progesterone left in the media after the 2 hour incubation, using the equation: P = {M/(V1/500) + (N/(V2/1000)}/(C*24). P = total progesterone level at 24 hour incubation (ng) and M = progesterone level assessed from sample assay volume (V1) at 2 hour incubation. N and V2 are the same values following the 24 hour incubation. C = the total number of cells in each well calculated using the population of both large and small cells in each incubation well.

2.7.2.1 Description of Main Effect and Interaction Means: With reference to Table 2, the main effects for each treatment of lipoprotein was calculated as the average of the horizontal 'x' values for that treatment while the main effects for LH are the means of the vertical 'x' values for that treatment. For example: from pattern A, the main effect for control HDL is the mean of x1 and x2 and the main effect for HDL at 100 ug/ml is the mean of x3 and x4. Similarly, the main effect for control level of LH is the mean of x1 and x3 while that for LH at 50 ng/ml is the mean of x2 and x4. x1, x2, x3 and x4 are 'interaction means' of LH and HDL.

TABLE 2. The three factorial patterns of treatments used to analyse data from Experiment I.

Pattern A

	LH 0	LH 50
HDL 0	x1	x2
HDL 100	x3	x4

Pattern B

		LH 0	LH 50
LDL	0	x1	x2
	50	x3	x4
HDL	50	x5	x6

Pattern C

		LH 0	LH 50
HDL	0	x1	x2
	10	x3	x4
	50	x5	x6
	100	x7	x8

Note Each 'x' represents a treatment.

TABLE 3. Number of treatments (nTrt) applied in each cycle day and cell type and factorial patterns of LH and lipoproteins that were used in Experiment I.

Day of Estrous Cycle	Cell Type*	nTrt	Factorial Patterns of LH and Lipoproteins		
			A	B	C
10	L	9	x		
15	S	23	x	x	
15	L	14	x	x	
18	S	21	x	x	x
18	L	17	x		x

* L = Large cells
  S = Small cells

RESULTS

3.1 Ovulation Rates and Corpora Lutea Weight.

Ovulation rates were determined by calculating the number of CL for both ovaries for each animal. In experiment I on day 10, the total number of CL ranged from 6 to 42 for both ovaries. The average number of CL was 20.75, for 4 animals (n). Ovulation rate ranged from 4 to 20 CL for the left ovary and 6 to 28 for the right ovary on day 15 post injections with an average of 25.16 CL, n = 6. On day 18, the CL number ranged from 4 to 34 and 10 to 31 for the left and right ovaries respectively with an average of 35.5 CL, n = 6. The average luteal weight was 0.381 gm for day 10, 0.453 gm for day 15 and 0.449 gm for day 18 pigs.

In Experiment II, ovulation rates ranged from 11 to 51 and 20 to 40 for day 10 and 15 respectively. The average number of CL were 28.5, n = 4 for day 10, and 26.3 n = 3 for day 15. The ovulation rates ranged from 9 to 43 with an average of 16.6 CL, n = 5 for Experiment III.

3.2 Experiment I

3.2.1 Pretreatment Incubation Progesterone Analysis

3.2.1.1 Analysis of Covariance. Please refer to Appendix Tables 1 to 19 for a summary of analysis of variance (ANOVA), progesterone data and probability levels for all linear contrast analysis. Analysis of covariance indicated that the pre-treatment progesterone levels for large cells did not significantly vary with the 2-hour treatment progesterone levels ($P > 0.05$) on days 15 and 18, but the effect of the pretreatment progesterone production as covariates in the analysis was significant for large cell type on day 10 and for small cell type on day 18. However, the differences between the variabilities in the analysis (as indicated by R^2) with and without the inclusion of the pretreatment progesterone level in the ANOVA model were small in both cases. For day 10 large cells, R^2 was 0.826 in the covariance analysis and 0.815 using only treatments and pigs in the model. For day 18 small cells, the values for R^2 were 0.887 and 0.882 respectively. Therefore, the pre-treatment progesterone levels were not used as covariates in the ANOVA of either the 2 or 24 hour progesterone levels in subsequent analysis.

3.2.1.2 Analysis of Variance. The ANOVA indicated that the effects of cycle day (i.e day of the induced cycle) and pigs within cycle day were highly significant ($P = 0.0208$ and

0.0001 respectively). When the data for P4 production by large and small cells during pretreatment incubation were pooled, the effect of cycle day approached significance (P = 0.052). Further analysis found cycle day by cell type interaction highly significant (P = 0.0001), indicating that the amount of progesterone produced by the cell types depended on the day of estrous cycle. Progesterone production by the large cell type was greater on day 10 (4.278 $\pm$ 0.898) than either day 15 or 18. However, the difference between day 10 and 15 was not significant (P > 0.05, Fig. 1). Progesterone production by either cell type was significantly higher on day 15 than day 18 (Fig. 1). On each cycle day, progesterone production per cell by large cells was significantly higher (P = 0.0001) than the corresponding production by small cells. The decrease in progesterone from day 15 to 18 was more drastic (60%) for large cells than small cells (40%), which resulted in a highly significant cycle day and cell type interaction (P = 0.0001).

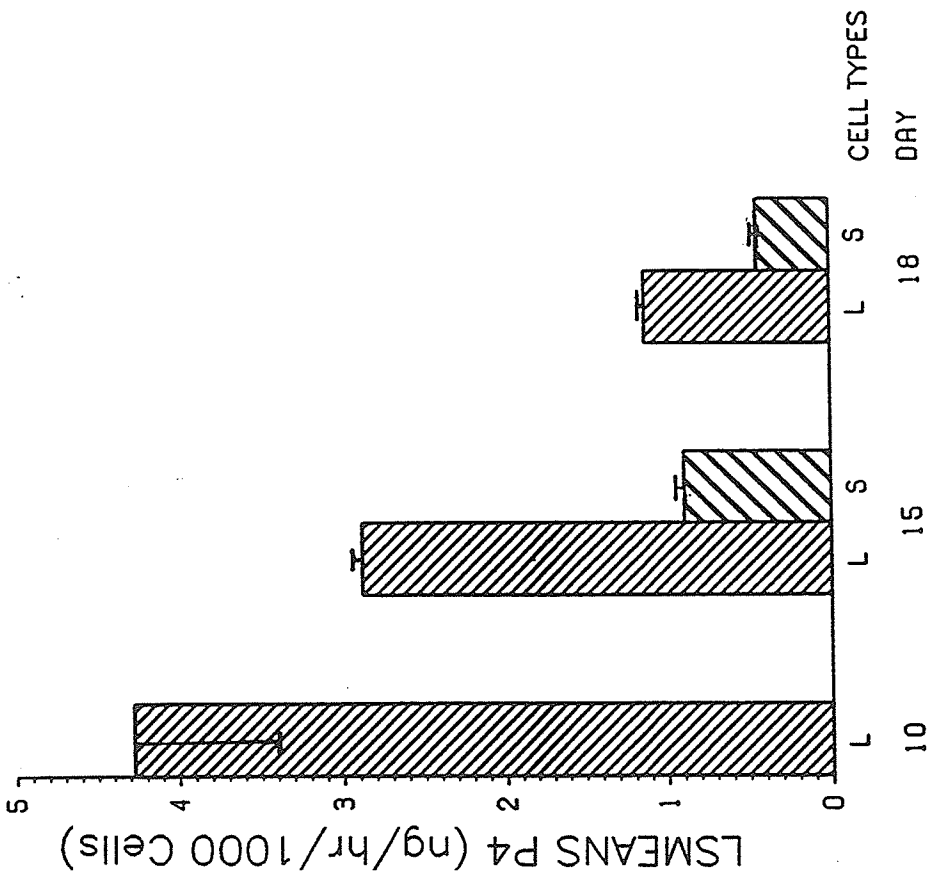
3.2.2 Treatment Analysis

3.2.2.1 Large Cells. There was a highly significant effect of individual pigs on all days investigated (P = 0.0001). In addition, exposure of day 10 large cells to HDL at 100 ug/ml for 2 hours increased progesterone in the media relative to its appropriate control (P = 0.0038, Fig. 2). In this and all subsequent figures unless otherwise stated, displayed values correspond to main effect means. Further analysis indicated

Fig. 1 Pretreatment basal progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) during the estrous cycle by large luteal cells (days 10 n = 3, 15 n = 4 and 18 n = 4) or small cells (days 15 and 18). (L = large cells; S = small cells; Day = day of estrous cycle; n = number of gilts; P4 = progesterone).

P4 PRODUCTION BY SMALL AND LARGE CELLS

DAYS 10, 15 AND 18: PRETREATMENT INCUBATION



that 100 ug/ml of HDL had no significant effect on progesterone production at 24 hours incubation of day 10 cells (Fig. 3). Both LDL and HDL at 50 ug/ml significantly increased progesterone above basal levels at 2 ($P = 0.0454$, LDL; $P = 0.0351$ HDL, Fig. 4) but not 24 (Fig. 5) hours incubation of day 15 cells. There was no significant interaction between LH and the lipoproteins. This means that addition of LH (50 ng/ml) to media alone had the same effect as addition of LH to media containing lipoproteins. HDL at 100 ug/ml significantly increased progesterone level above basal at both 2 ($P = 0.0013$, Fig. 4) and 24 ($P = 0.0253$, Fig. 5) hours on day 15.

Neither HDL nor LH had any significant effect on progesterone production by day 18 large cells at 2 hours incubation (Fig. 6) but 100 ug/ml HDL significantly increased progesterone production ($P = 0.0462$, Fig. 7) at 24 hours incubation. There was no significant interaction between LH and lipoproteins ($P > 0.05$).

In the absence of LH, the responses of large cells to HDL were linear on day 15 at 2 hour incubation ($P = 0.0081$) indicating a dose-dependent effect of HDL on progesterone production. The interaction of LDL with zero levels of LH was quadratic ($P = 0.0258$) because LDL at 10 ug/ml stimulated a greater production of progesterone than at 50 ug/ml concentration. There were no significant patterns for other days and incubation times.

Fig. 2 In vitro effects of HDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 10 large cells (3 gilts) at 2 hr incubation (P4 = progesterone; * = different from control level, $P < 0.05$).

PROGESTERONE PRODUCTION BY LARGE CELLS

DAY 10, 2 HRS

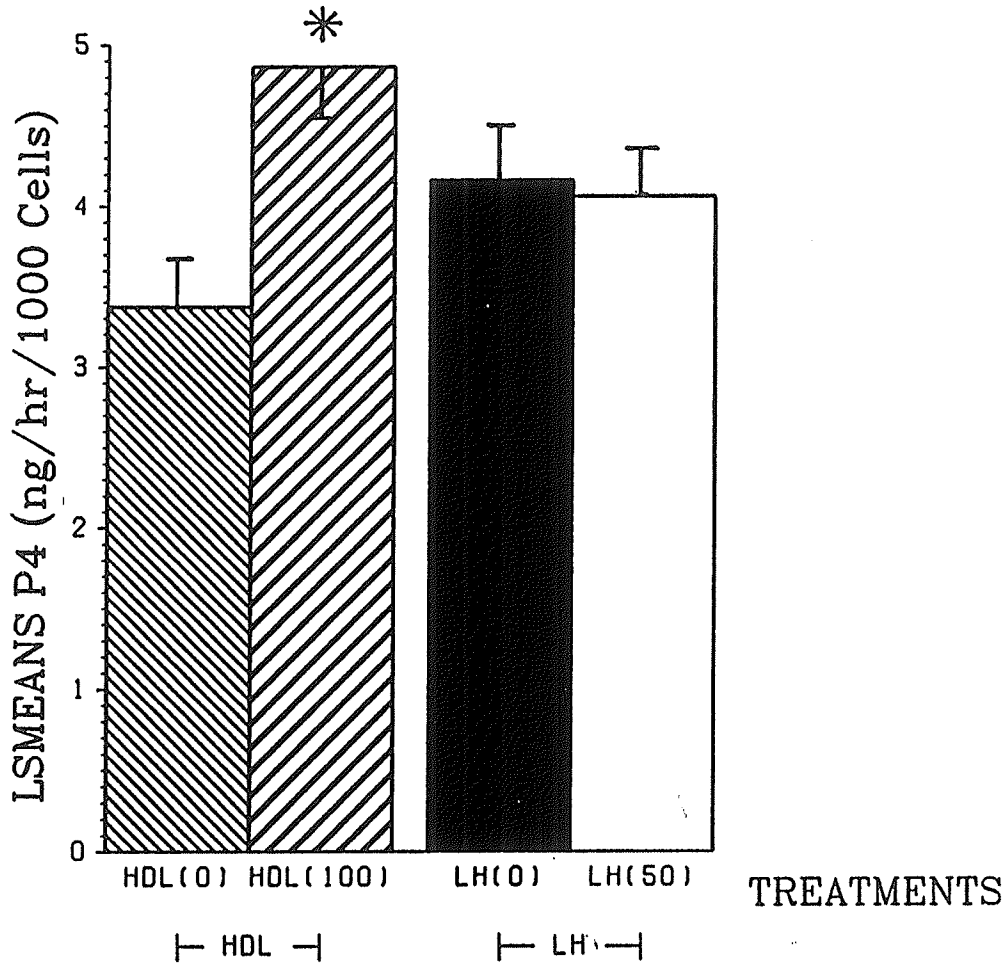


Fig. 3 In vitro effects of HDL (ug/ml) or LH (ug/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 10 large cells (3 gilts) at 24 hr incubation (P4 = progesterone).

PROGESTERONE PRODUCTION BY LARGE CELLS

DAY 10, 24 HRS

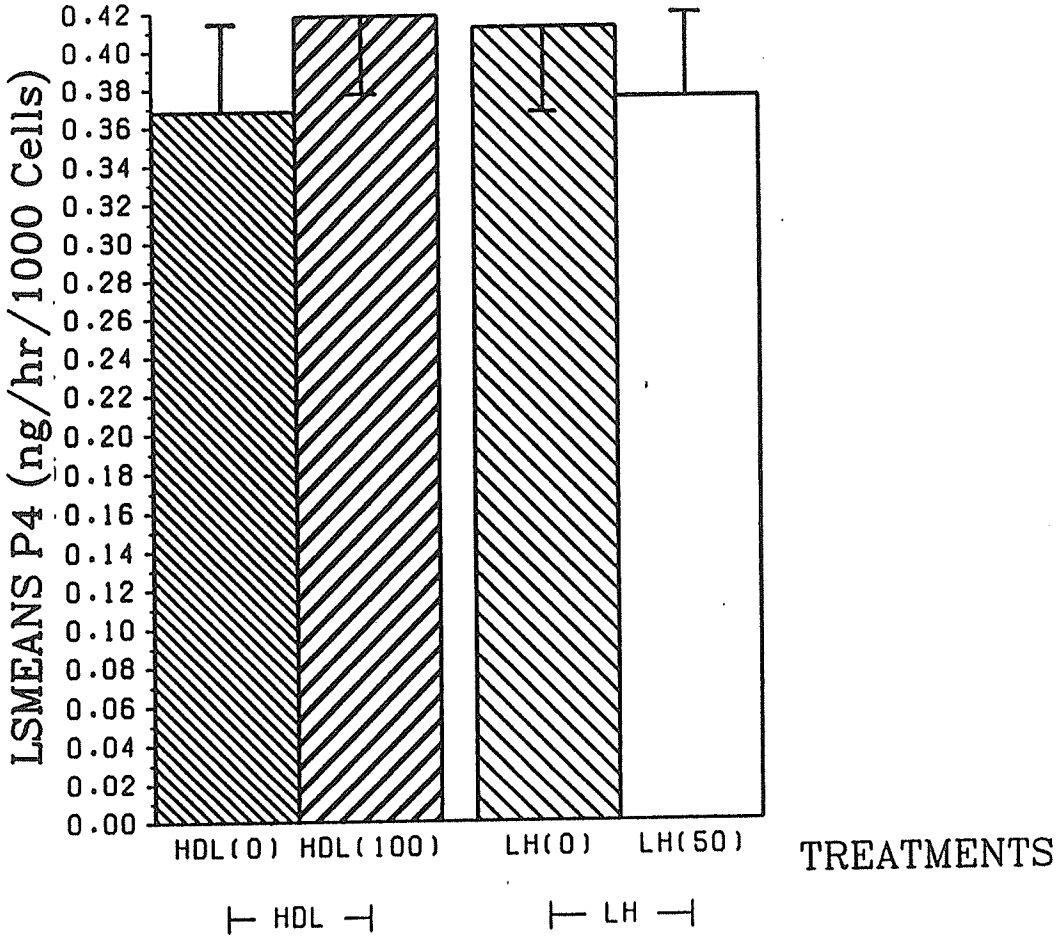


Fig. 4 In vitro effects of LDL, HDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 15 large cells (4 gilts) at 2 hr incubation (P4 = progesterone; * = different from control level, $P < 0.05$).

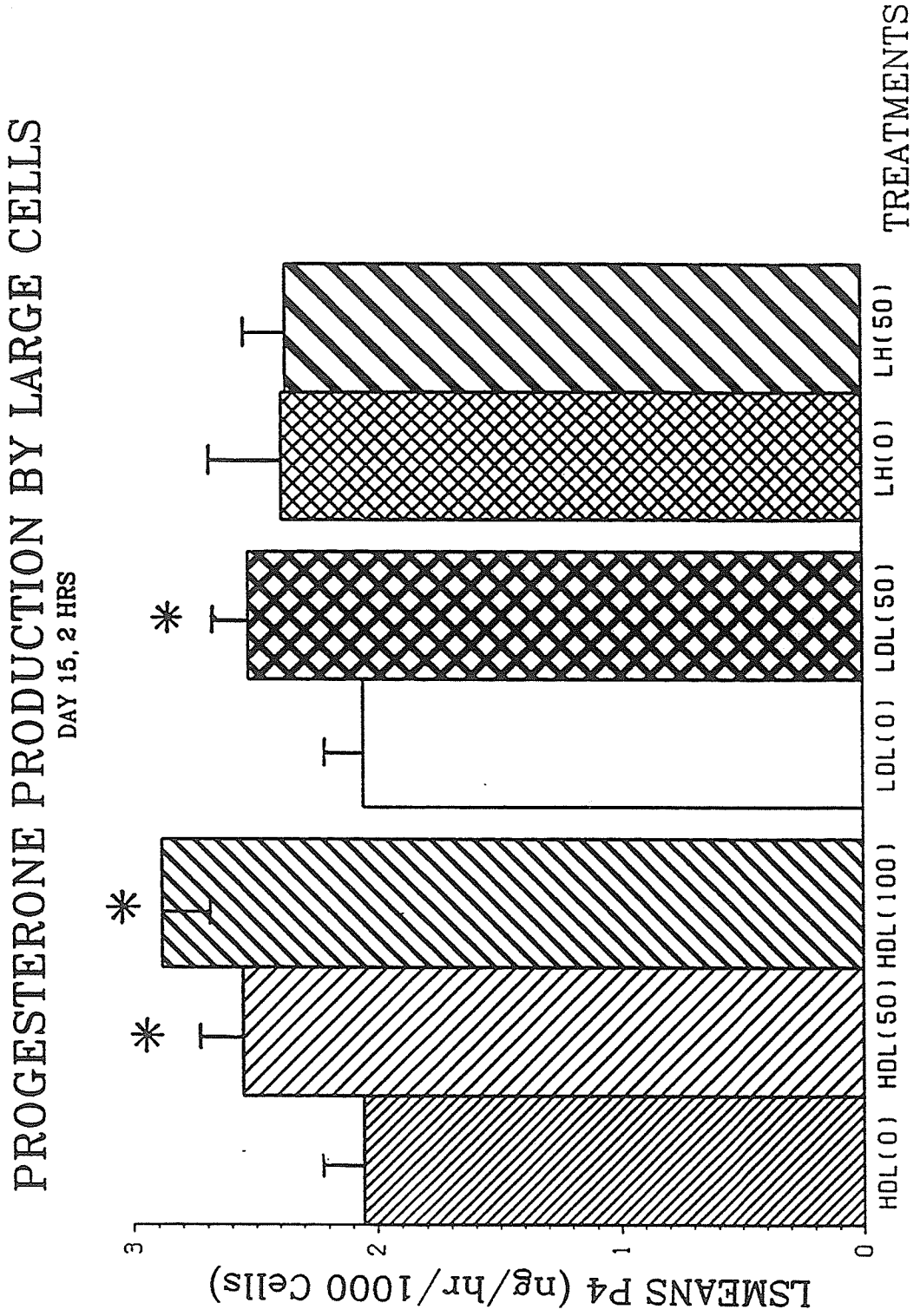

PROGESTERONE PRODUCTION BY LARGE CELLS
DAY 15, 2 HRS
LSMEANS P4 (ng/hr/1000 Cells)
0
1
2
3
*
*
*
HDL(0)
HDL(50)
HDL(100)
LOL(0)
LOL(50)
LH(0)
LH(50)
TREATMENTS
HDL
LOL
LH

Fig. 5 In vitro effects of LDL, HDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 15 large cells (4 gilts) at 24 hr incubation (P4 = progesterone; * = different from control level, $P < 0.05$).

PROGESTERONE PRODUCTION BY LARGE CELLS

DAY 15, 24 HRS

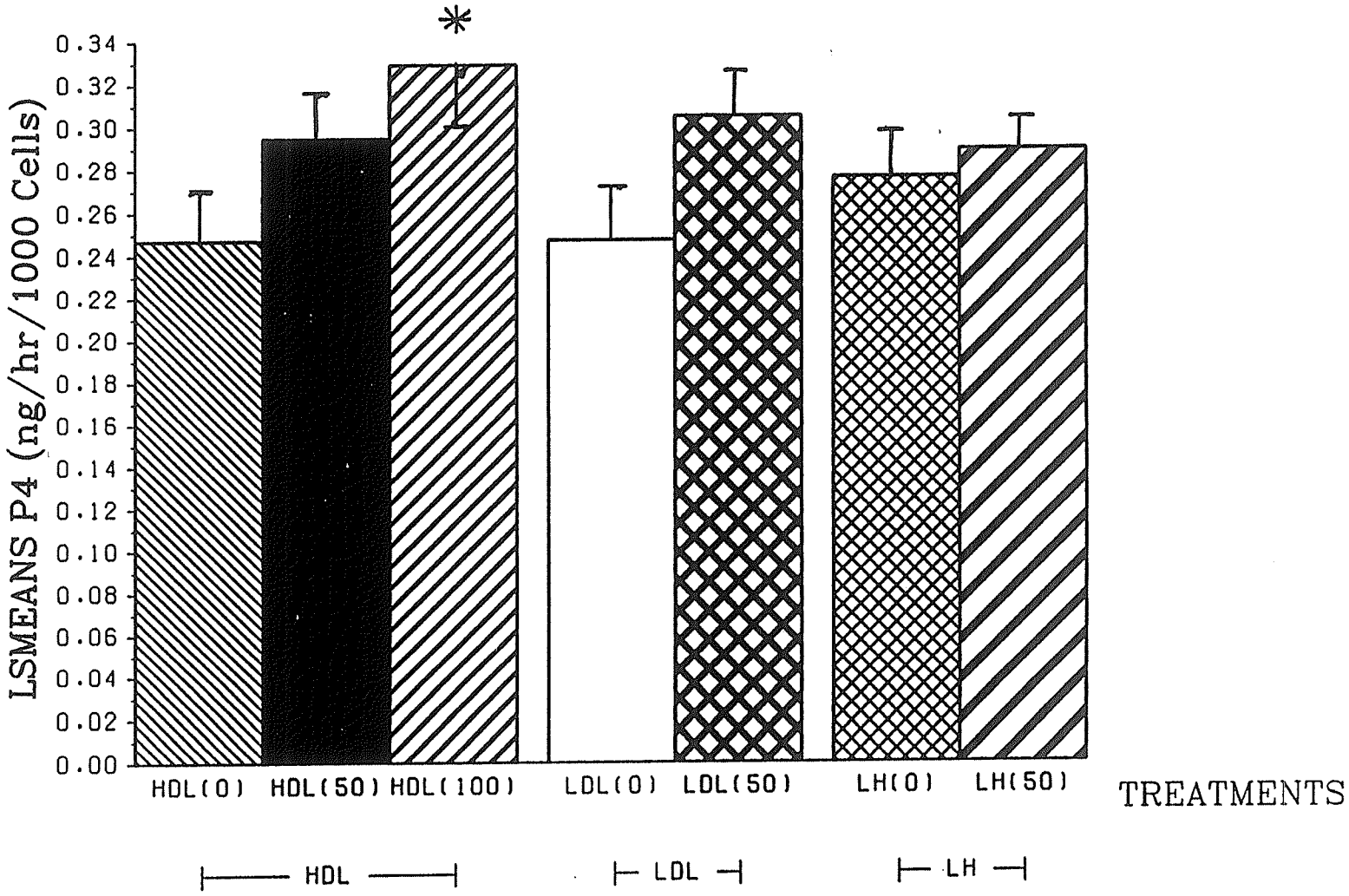


Fig. 6 In vitro effects of HDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 18 large cells (4 gilts) at 2 hr incubation (P4 = progesterone).

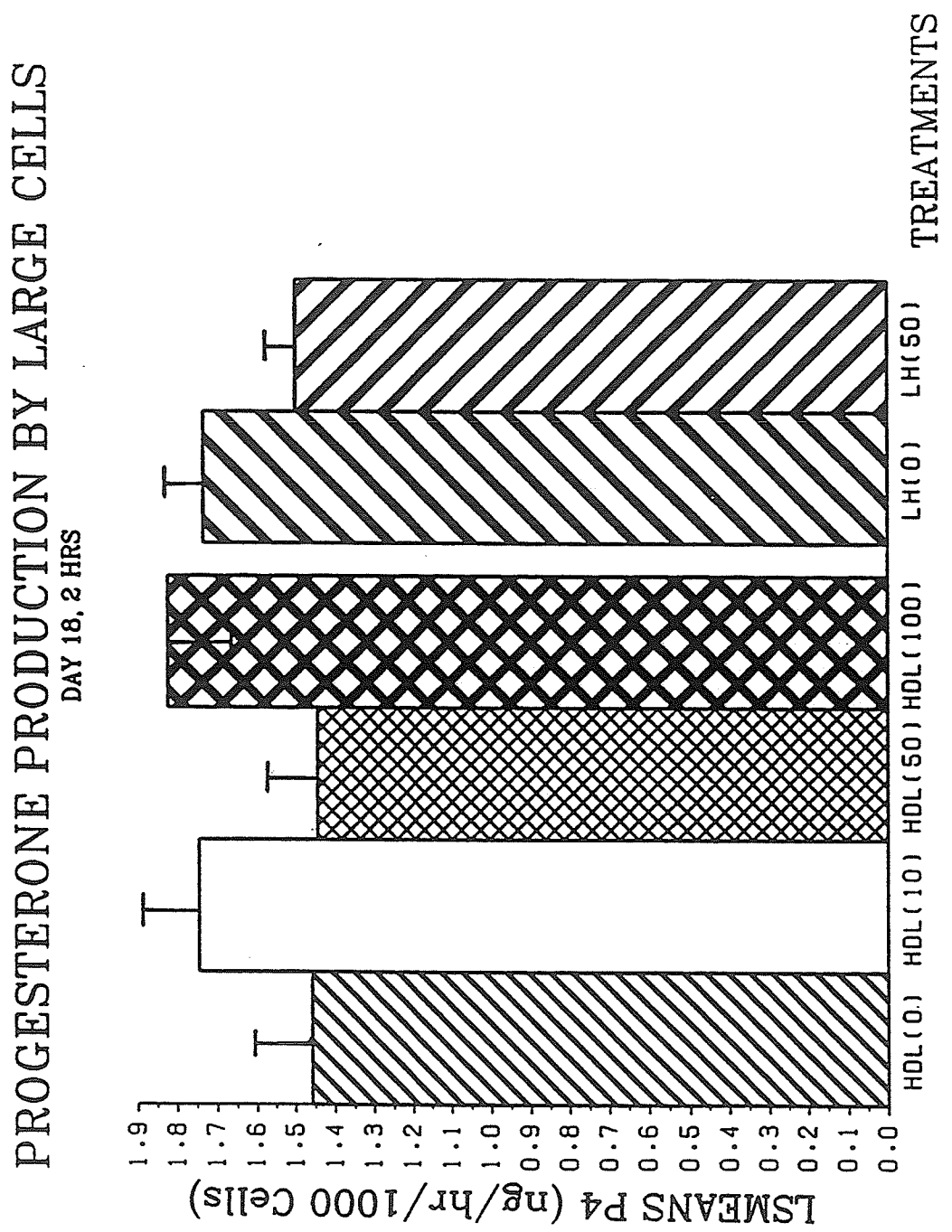
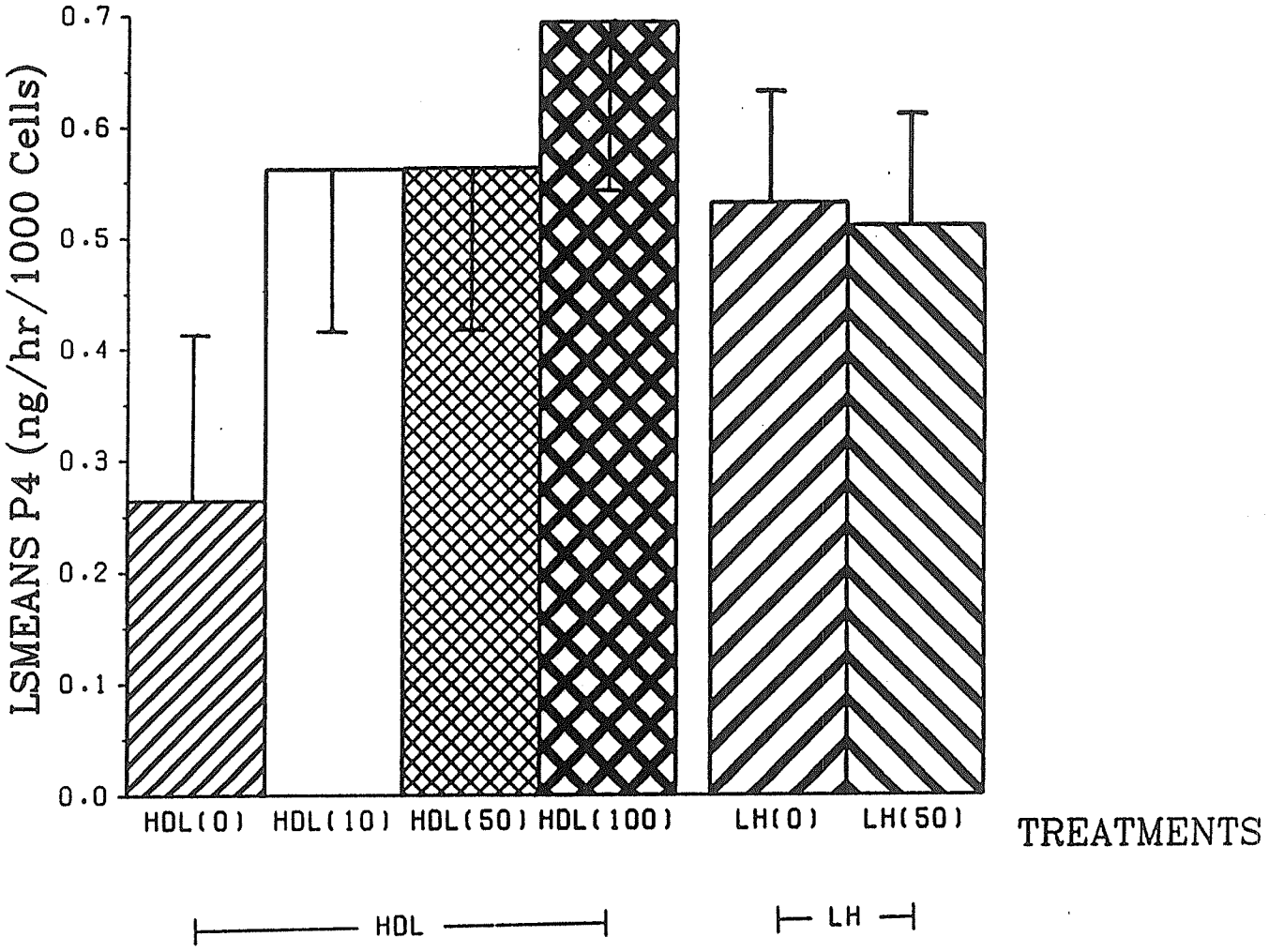

PROGESTERONE PRODUCTION BY LARGE CELLS
DAY 18, 2 HRS
LSMEANS P4 (ng/hr/1000 cells)
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
HDL(0)
HDL(10)
HDL(50)
HDL(100)
LH(0)
LH(50)
TREATMENTS
HDL
LH

Fig. 7 In vitro effects of LDL, HDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 18 large cells (4 gilts) at 24 hr incubation (P4 = progesterone).

PROGESTERONE PRODUCTION BY LARGE CELLS

DAY 18, 24 HRS



3.2.2.2 **Small Cells.** The small cell yield from day 10 CL was too low to permit the same study and analysis of these cells as was done for large cells. At 2 hour incubation of day 15 cells, LDL at 50 ug/ml significantly (P = 0.0053) augmented progesterone production above basal level and the stimulatory effect of HDL at the same concentration approached significance (P = 0.0558, Fig. 8). HDL at 100 ug/ml had no significant effect. At 24 hour incubation, neither 50 ng/ml LH nor any dose of LDL or HDL significantly affected progesterone production (Fig. 9). The interaction between LH and both levels of lipoproteins was not significant (P > 0.05).

For day 18 cells, LDL at 50 ng/ml significantly increased progesterone production at 2 (P = 0.0250, Fig. 10) but not 24 (Fig. 11) hours. At 2 hour incubation, HDL at 100 ug/ml significantly stimulated progesterone production on day 18 (P = 0.0042, Fig. 10) while 10, 50 and 100 ug/ml HDL significantly elevated progesterone at 24 hours (P = 0.0013, 0.0053 and 0.0120 respectively, Fig. 11). There was no interaction between LH and any of the levels of HDL or LDL at either incubation times.

Small cells had a linear response to increasing doses of LDL in the absence of LH on day 18 at 2 hours (Interaction means, P = 0.0282). However, at 24 hours the response was quadratic (P = 0.0007). This means that progesterone production was lower at 0 and 50 ug/ml than at 10 ug/ml LDL. There were no such patterns for other days of the cycle and incubation times.

Fig. 8 In vitro effects of LDL, HDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 15 small cells (4 gilts) at 2 hr incubation (P4 = progesterone; * = different from control level, $P < 0.05$).

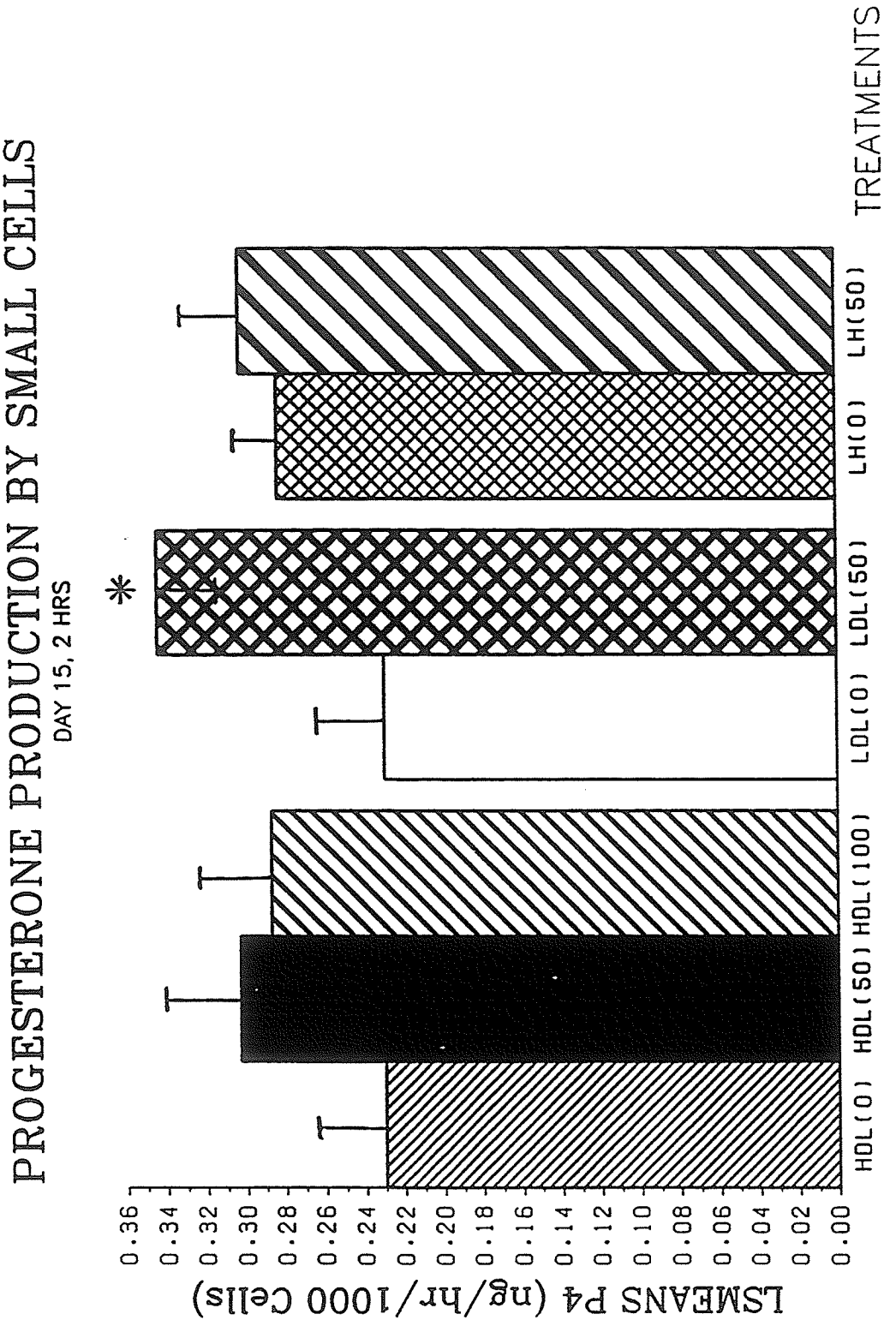

PROGESTERONE PRODUCTION BY SMALL CELLS
DAY 15, 2 HRS
LSMEANS P4 (ng/hr/1000 cells)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
0.22
0.24
0.26
0.28
0.30
0.32
0.34
0.36
*
HDL(0)
HDL(50)
HDL(100)
LDL(0)
LDL(50)
LH(0)
LH(50)
TREATMENTS
HDL
LDL
LH

Fig. 9 In vitro effects of LDL, HDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 15 small cells (4 gilts) at 24 hr incubation (P4 = progesterone).

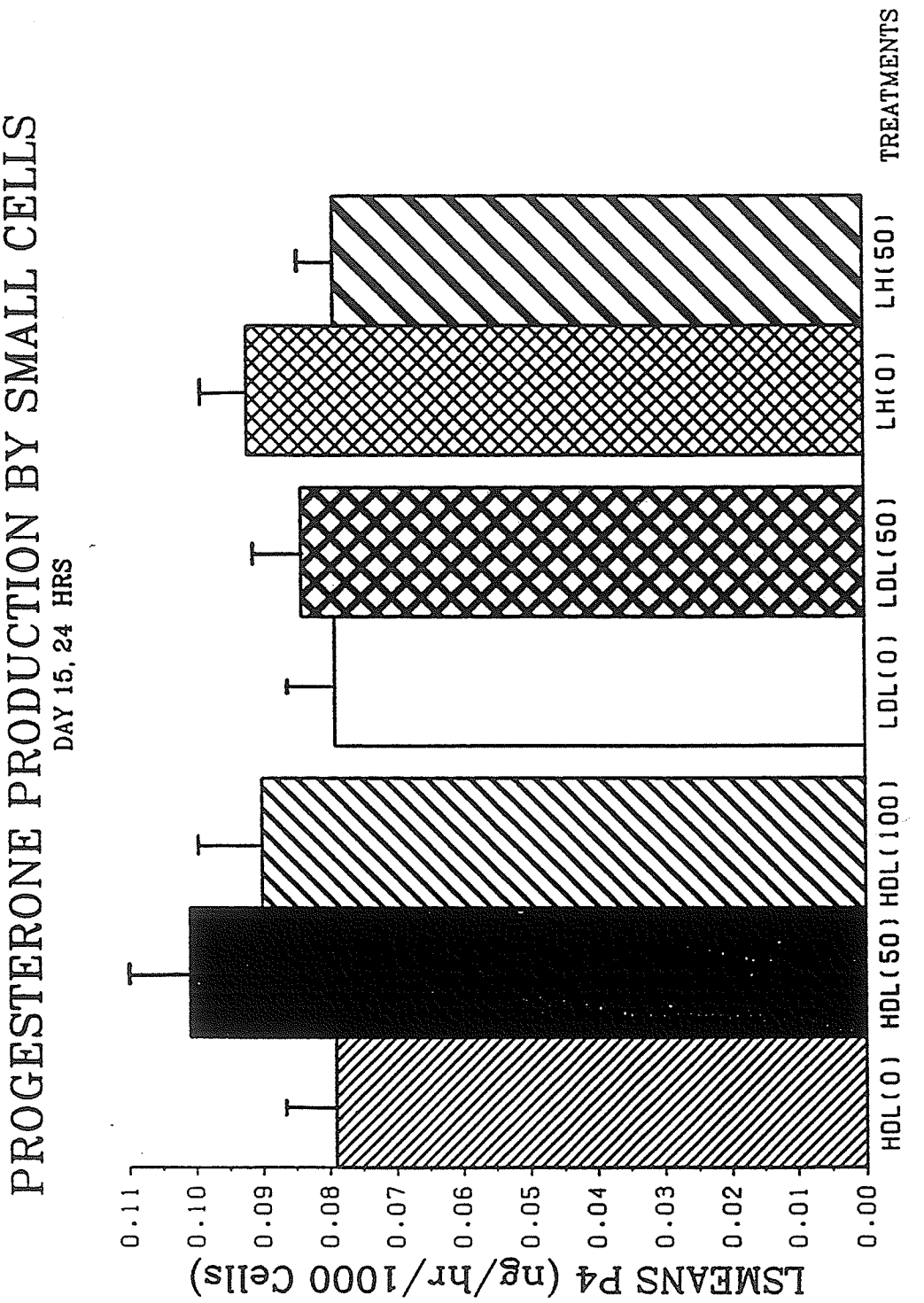

PROGESTERONE PRODUCTION BY SMALL CELLS
DAY 15, 24 HRS
LSMEANS P4 (ng/hr/1000 cells)
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
HDL(0)
HDL(50)
HDL(100)
LDL(0)
LDL(50)
LH(0)
LH(50)
TREATMENTS
HDL
LDL
LH

Fig. 10 In vitro effects of LDL, HDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 18 small cells (4 gilts) at 2 hr incubation (P4 = progesterone; * = different from control level, $P < 0.05$).

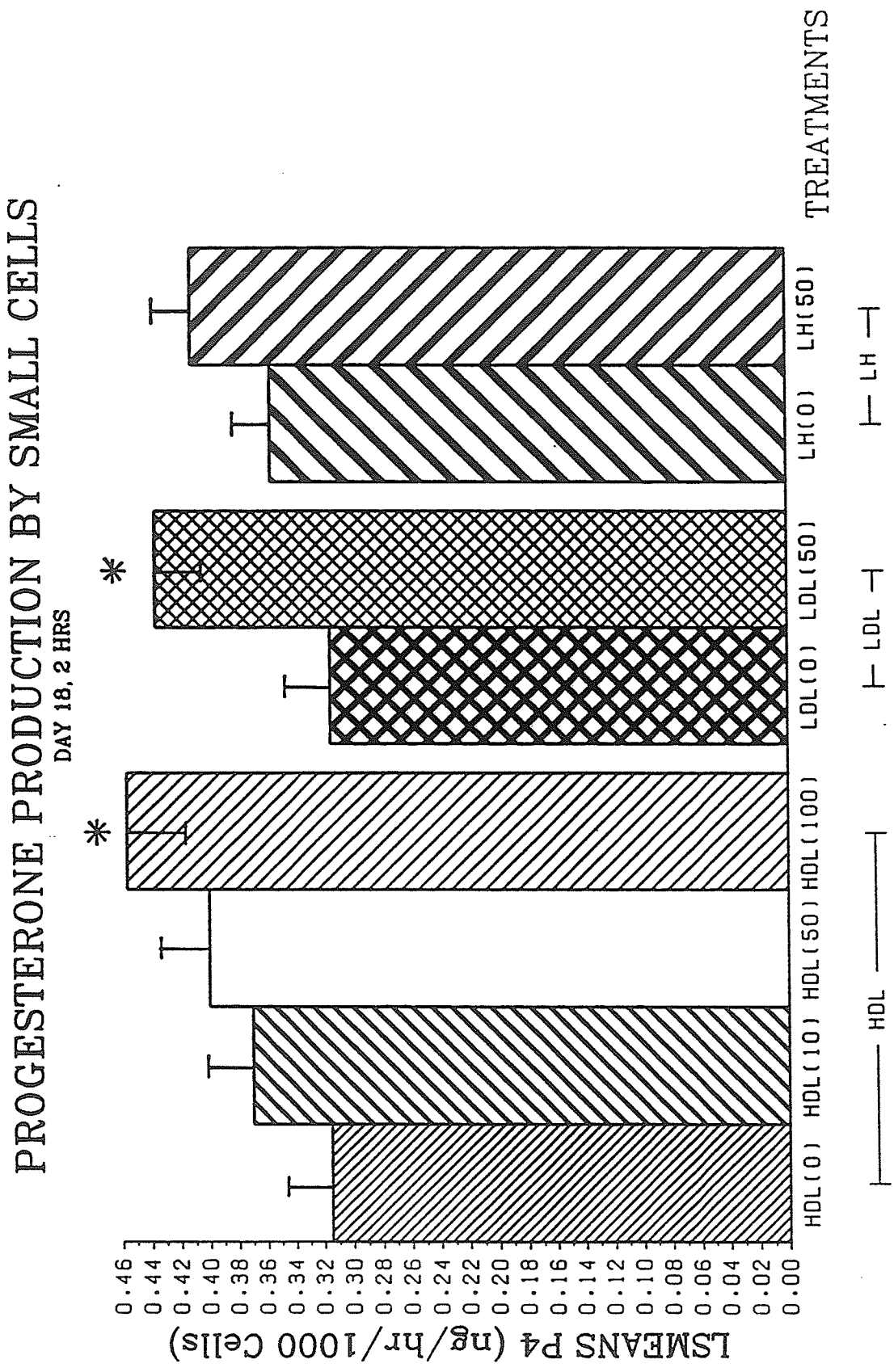
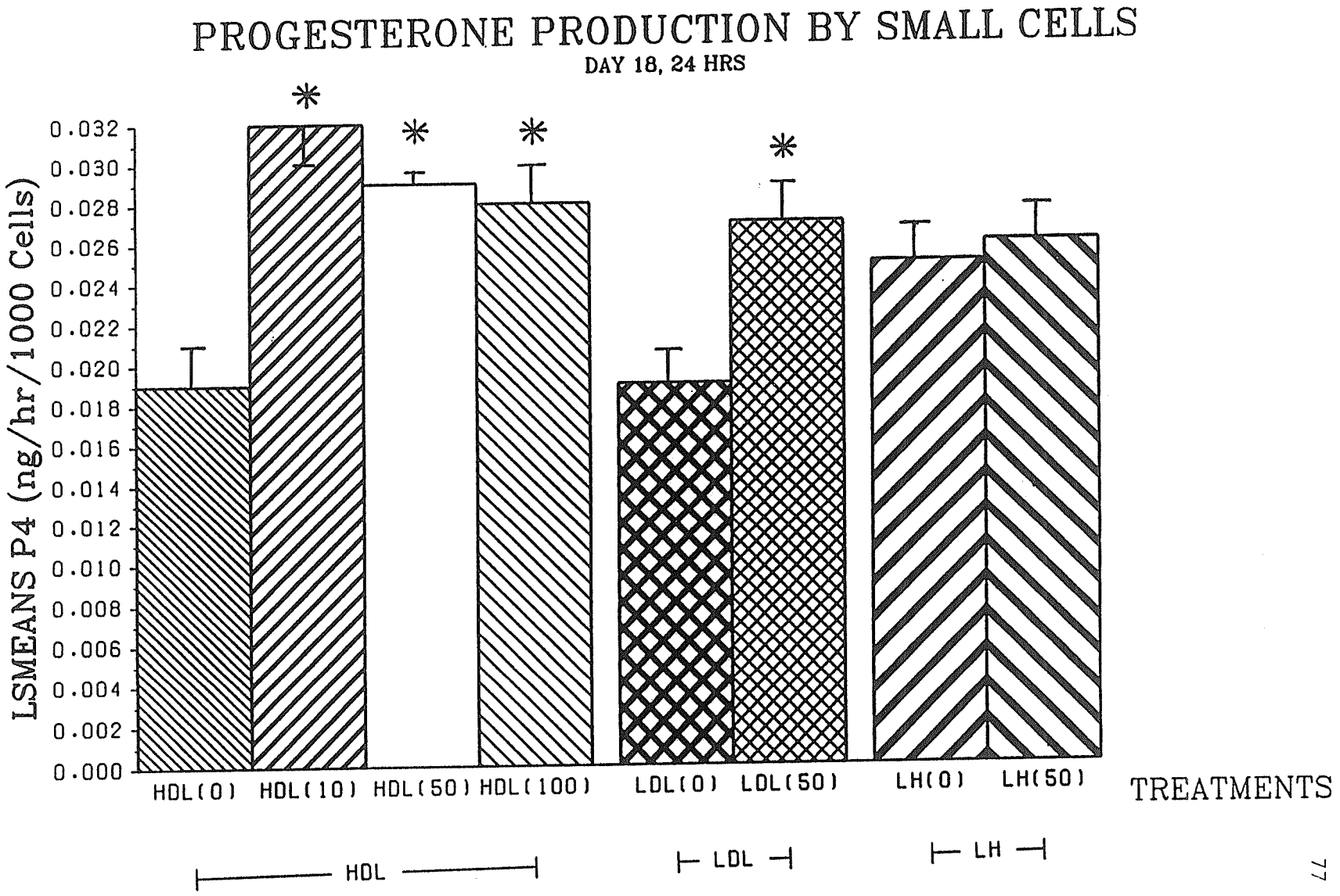

PROGESTERONE PRODUCTION BY SMALL CELLS
DAY 18, 2 HRS
LSMEANS P4 (ng/hr/1000 cells)
0.46
0.44
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
*
*
HDL(0)
HDL(10)
HDL(50)
HDL(100)
LDL(0)
LDL(50)
LH(0)
LH(50)
TREATMENTS
HDL
LDL
LH

Fig. 11 In vitro effects of LDL, HDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 18 small cells (4 gilts) at 24 hr incubation (P4 = progesterone; * = different from control level, $P < 0.05$).


PROGESTERONE PRODUCTION BY SMALL CELLS
DAY 18, 24 HRS
LSMEANS P4 (ng/hr/1000 Cells)
0.032
0.030
0.028
0.026
0.024
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
HDL(0)
HDL(10)
HDL(50)
HDL(100)
LDL(0)
LDL(50)
LH(0)
LH(50)
TREATMENTS
HDL
LDL
LH

3.3 Experiment II

3.3.1 Day 10

3.3.1.1 **Large Cells.** Analysis of variance and linear contrasts following 2 (Fig. 12) or 24 (Fig. 13) hour incubations indicated that the main effects of LH or the 4 levels of PGF2a (0.001, 0.1, 1 and 100 ng/ml) were not significant ($P > 0.05$). However, LDL (100 ug/ml) significantly elevated progesterone concentration compared to the unstimulated level at 2 hours incubation ($P = 0.0001$, Fig. 12) and 24 hours ($P = 0.0001$, Fig. 13). Progesterone production by a combination of LDL and LH was significantly different from control production. There was no significant interaction between LDL or LH and any of the four levels of PGF2a indicating PGF2a did not significantly affect cellular response to LDL or LH.

3.3.1.2 **Small Cells.** The results of the analysis of variance and linear contrasts indicated that LDL (100 ug/ml) significantly elevated progesterone ($P = 0.0001$) above basal, unstimulated production at 2 (Fig. 14) and 24 hours (Fig. 15).

Luteinizing hormone (100 ng/ml) also induced a significant increase in progesterone production at 24 (Fig. 15) but not 2 (Fig. 14) hour incubation. Progesterone release due to treatment with LDL or LH was not significantly affected by combining either LDL or LH with any of the levels of PGF2a.

Fig. 12 Effects of PGF2a, LDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 10 large cells (4 gilts) at 2 hr incubations (P4 progesterone; TRTS = "Treatments"; * = different from control level, $P < 0.05$).

PROGESTERONE PRODUCTION BY LARGE CELLS

DAY 10, 2 HRS

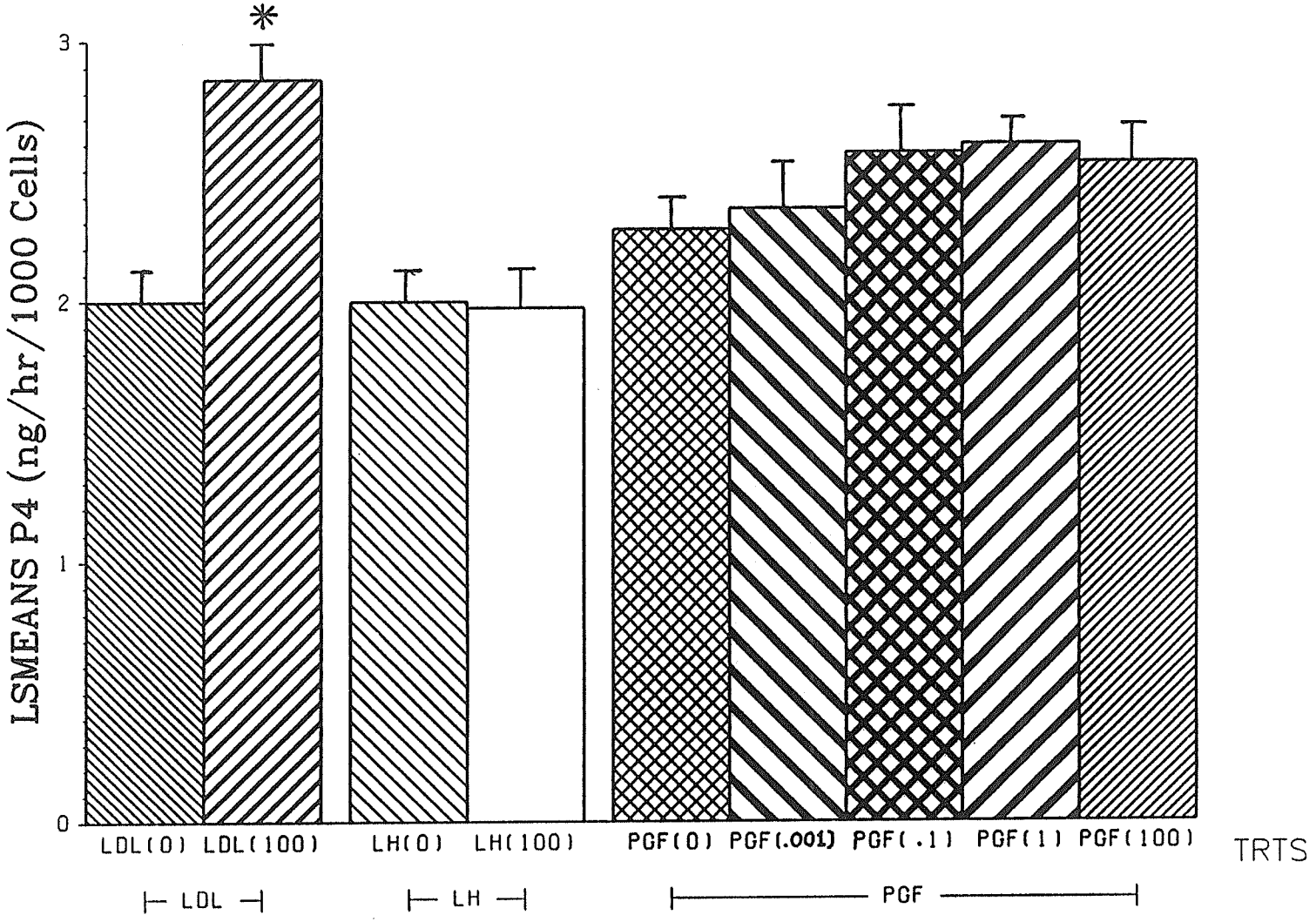


Fig. 13 Effects of PGF2a, LDL (ug/ml) or LH (ng/ml) on progesterone production (LSmeans ng/hr/10^3 cells $\pm$ SEM) by day 10 large cells (4 gilts) at 24 hr incubations (P4 = progesterone; TRTS = "Treatments"; * = different from control level, $P < 0.05$).

PROGESTERONE PRODUCTION BY LARGE CELLS

DAY 10, 24 HRS

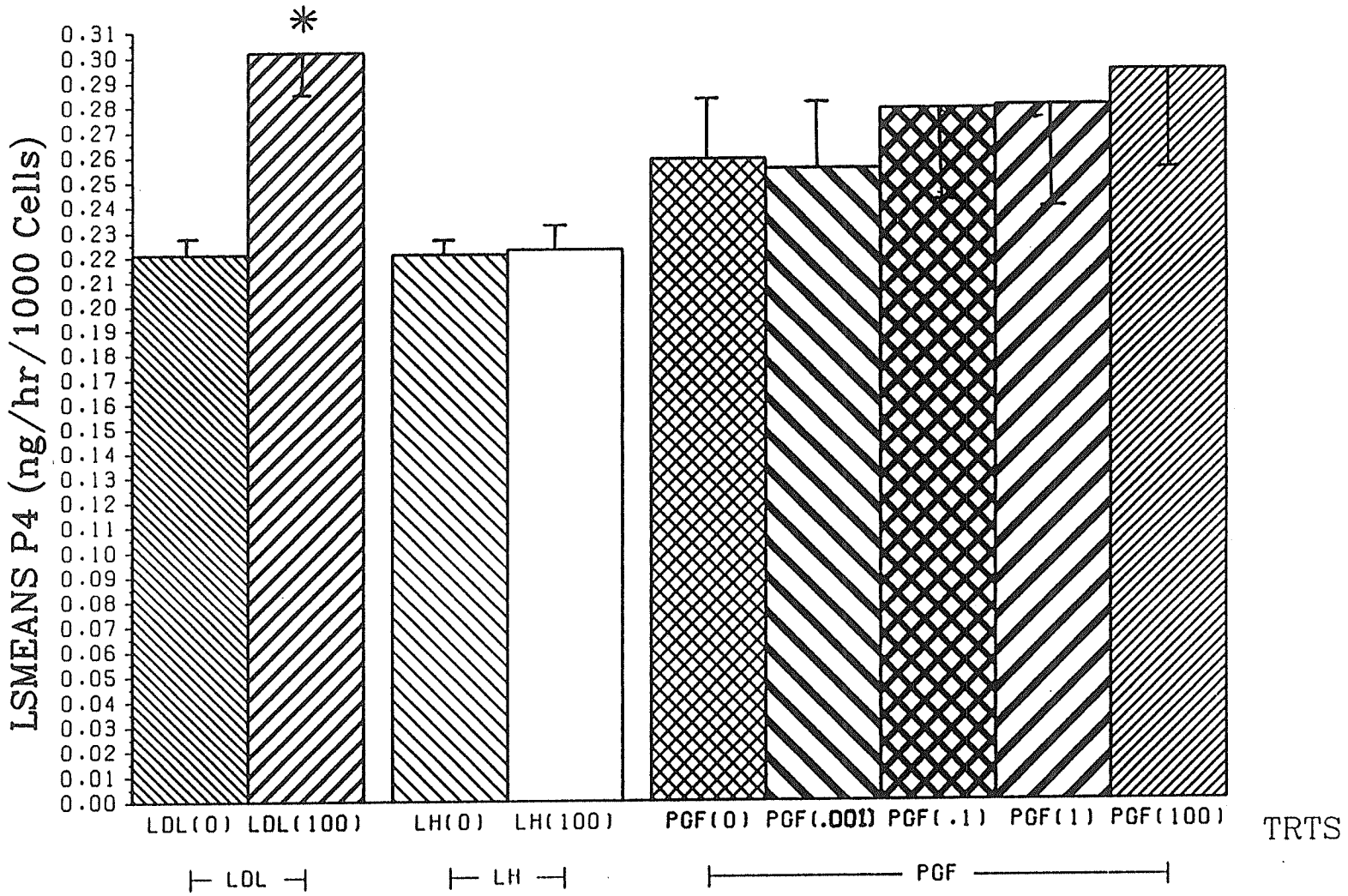


Fig. 14 Effects of PGF2a, LDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 10 small cells (4 gilts) at 2 hr incubations (P4 = progesterone; TRTS = "Treatments"; * = different from control level, $P < 0.05$).

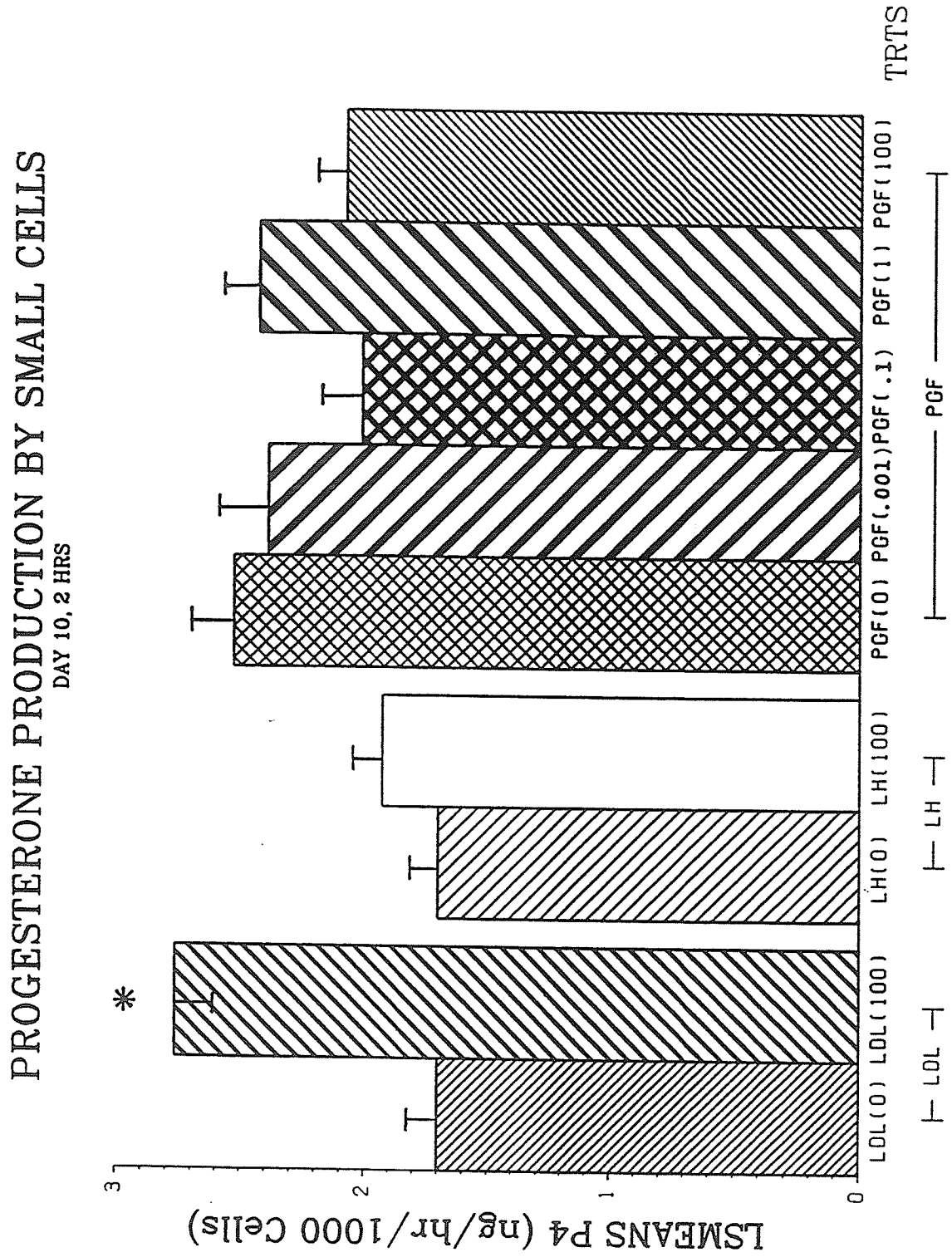
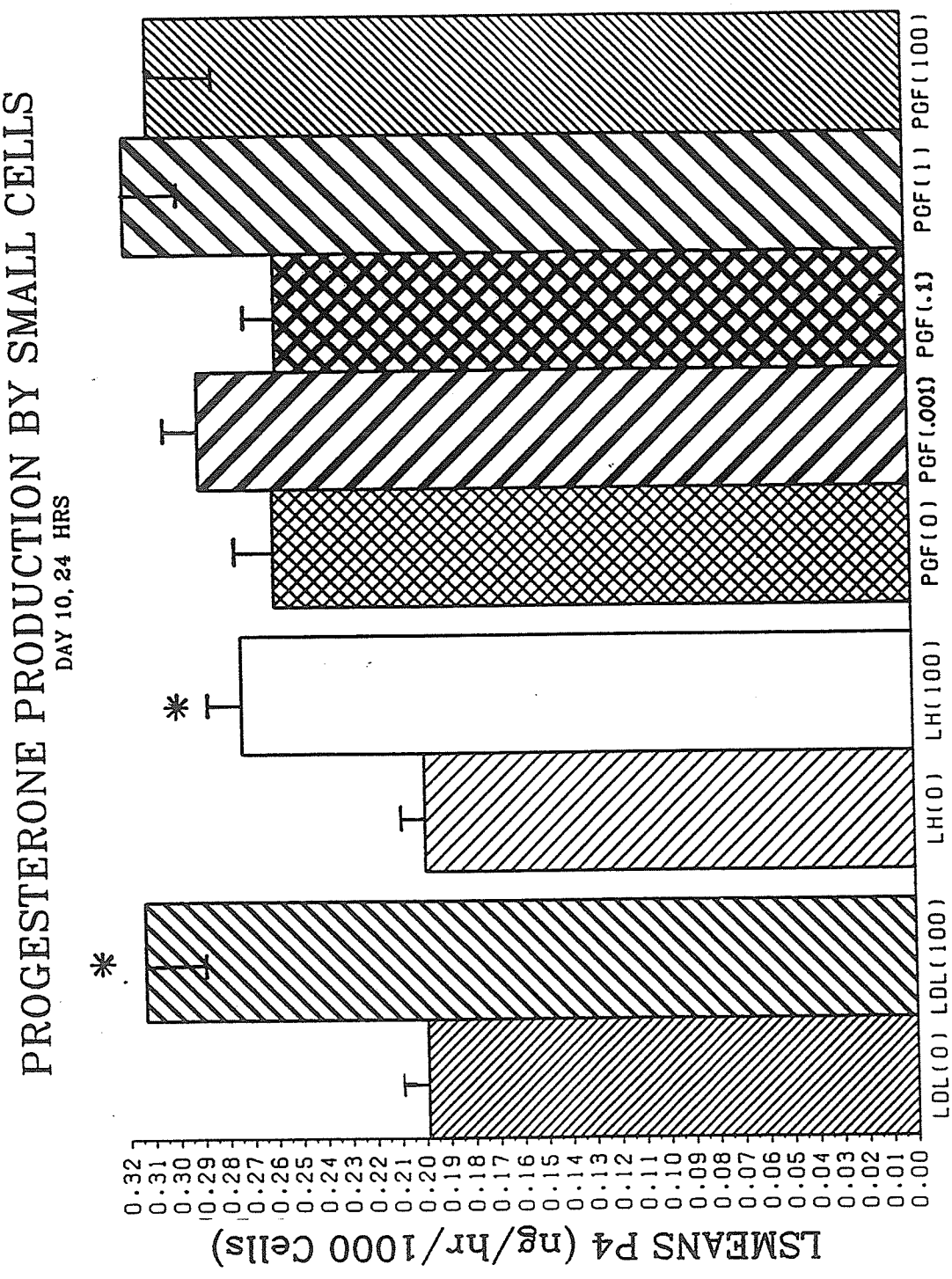

PROGESTERONE PRODUCTION BY SMALL CELLS
DAY 10, 2 HRS
LSMEANS P4 (ng/hr/1000 cells)
3
2
1
0
*
LDL(0) LDL(100) LH(0) LH(100) PGF(0) PGF(.001)PGF(.1) PGF(1) PGF(100)
TRTS
LDL
LH
PGF

Fig. 15 Effects of PGF2a, LDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 10 small cells (4 gilts) at 24 hr incubations (P4 = progesterone; TRTS = "Treatments"; * = different from control level, $P < 0.05$).


PROGESTERONE PRODUCTION BY SMALL CELLS
DAY 10, 24 HRS
LSMEANS P4 (ng/hr/1000 cells)
0.32
0.31
0.30
0.29
0.28
0.27
0.26
0.25
0.24
0.23
0.22
0.21
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
LDL(0) LDL(100) LH(0) LH(100) PGF(0) PGF(.001) PGF(.1) PGF(1) PGF(100)
TRTS
LDL
LH
PGF

3.3.2 Day 15

3.3.2.1 Large Cells. Low density lipoprotein at 100 ug/ml significantly increased progesterone release into the incubation medium at 2 hours (Fig. 16) and at 24 hours (Fig. 17). The main effects of LH at both incubation times were not significant. No significant interactions between LDL or LH in combination with any level of PGF2a was detected and there was no significant effect of PGF2a.

3.3.2.2 Small Cells. Low density lipoprotein at 100 ug/ml significantly augmented progesterone release ($P = 0.0001$) into the incubation media after 2 hours (Fig. 18) and after 24 hour incubation (Fig. 19).

In contrast, the main effects of LH at both incubation times were not significant. Progesterone production due to treatment with either LDL (100 ug/ml) or LH (100 ng/ml) were not significantly altered when either of them was simultaneously used with any of the levels of PGF2a ($P > 0.05$).

3.4 Experiment III

3.4.1 Large Cells. The model for ANOVA was treatments and pigs only. Neither PGF2a nor the membrane effectors SAM, DZA or SAH appeared to have any significant effects ($P > 0.05$) on progesterone production although PGF2a at 1 or 100 ng/ml

Fig. 16 Effects of PGF2a, LDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 15 large cells (3 gilts) at 2 hr incubations (P4 = progesterone; TRTS = "Treatments"; * = different from control level, $P < 0.05$).

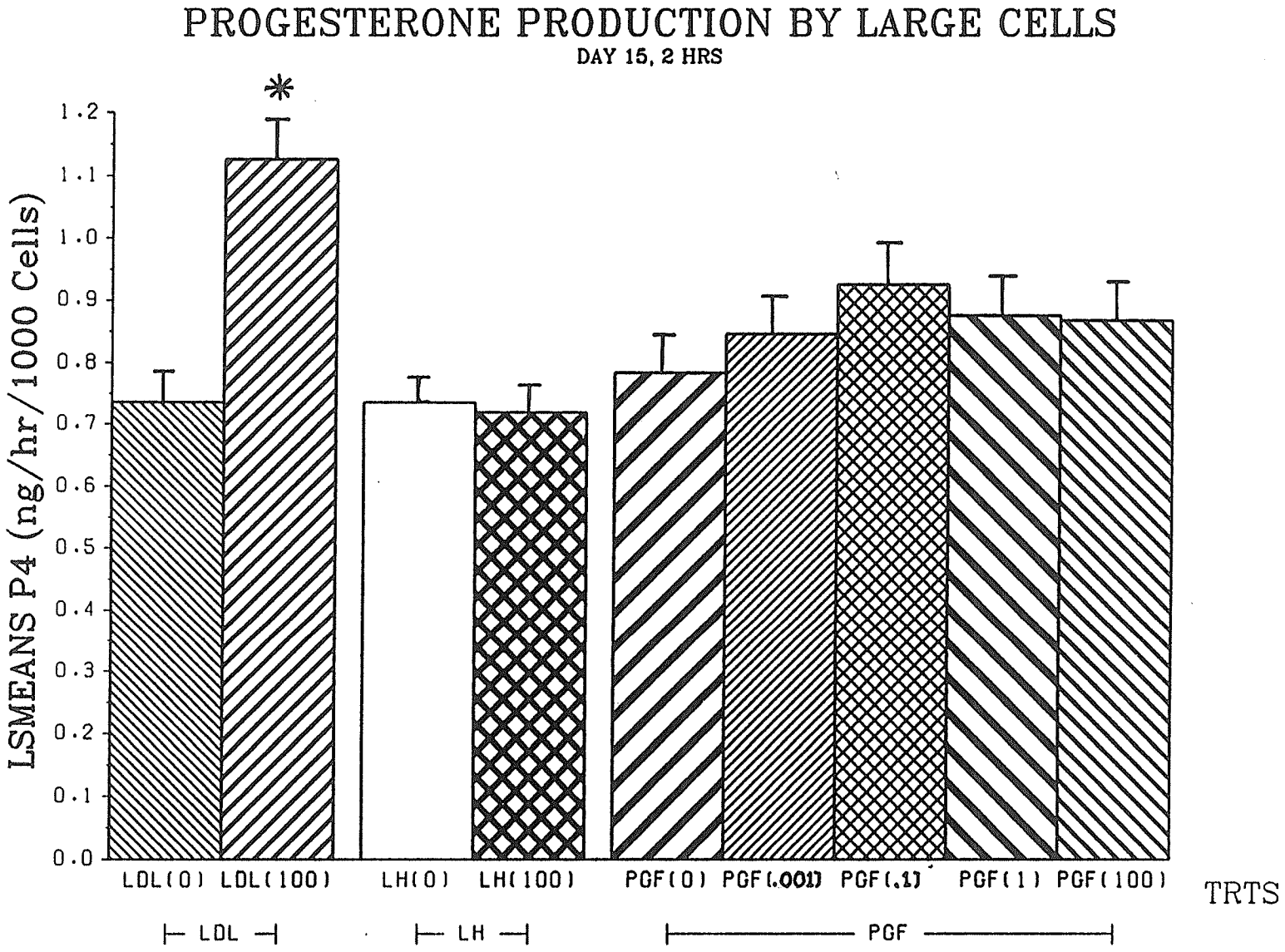

PROGESTERONE PRODUCTION BY LARGE CELLS
DAY 15, 2 HRS
LSMEANS P4 (ng/hr/1000 Cells)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
LDL(0)
LDL(100)
LH(0)
LH(100)
PGF(0)
PGF(.001)
PGF(.1)
PGF(1)
PGF(100)
TRTS
LDL
LH
PGF

Fig. 17 Effects of PGF2a, LDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 $\pm$ SEM) by day 15 large cells (3 gilts) at 24 hr incubations (P4 = progesterone; TRTS = "Treatments"; * = different from control level, $P < 0.05$).

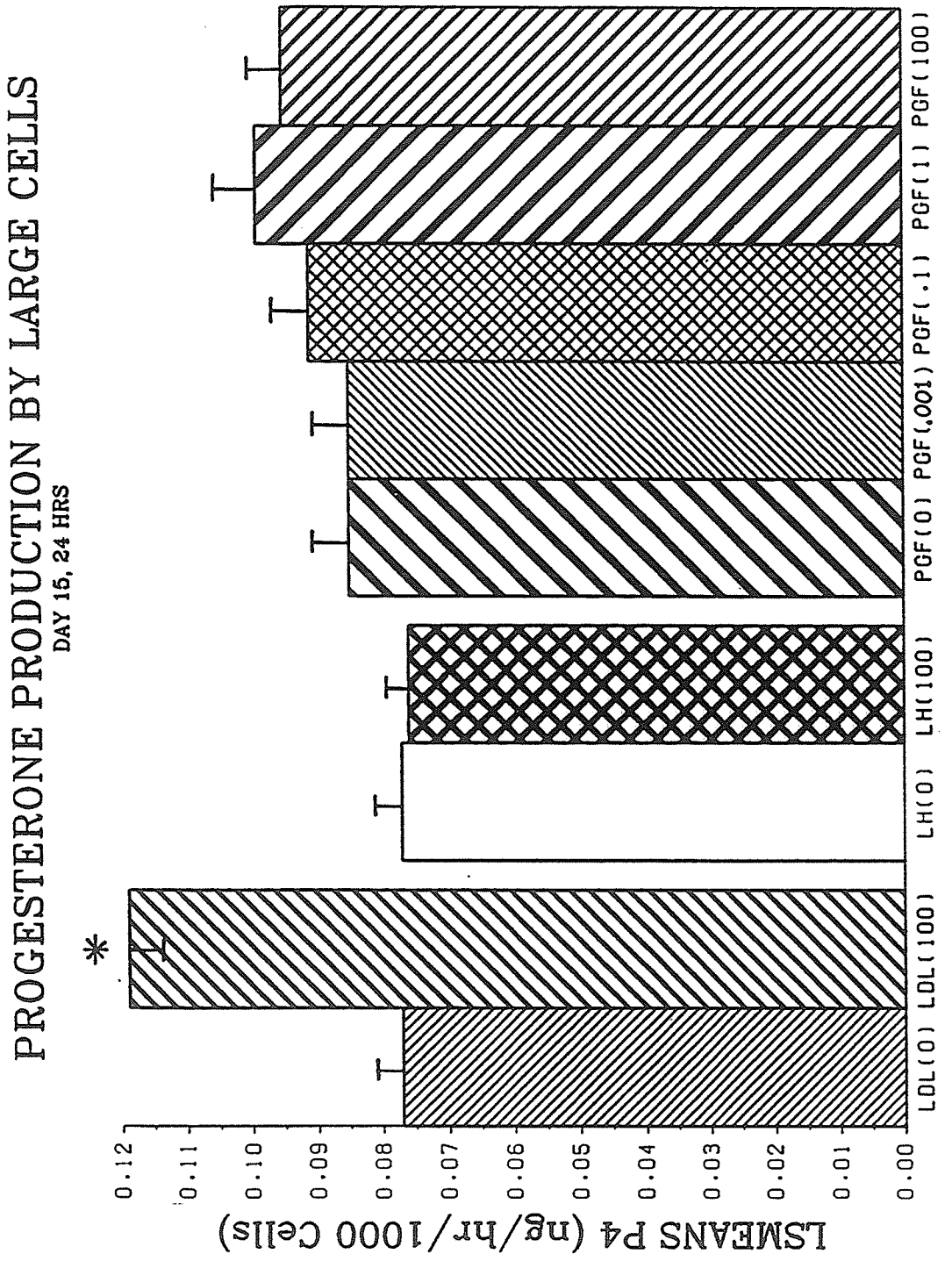

PROGESTERONE PRODUCTION BY LARGE CELLS
DAY 15, 24 HRS
LSMEANS P4 (ng/hr/1000 cells)
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
*
LDL(0) LDL(100) LH(0) LH(100) PGF(0) PGF(.001) PGF(.1) PGF(1) PGF(100)
LDL
LH
PGF
TRTS

Fig. 18 Effects of PGF2a, LDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 15 small cells (3 gilts) at 2 hr incubations (P4 = progesterone; TRTS = "Treatments"; * = different from control level, $P < 0.05$).

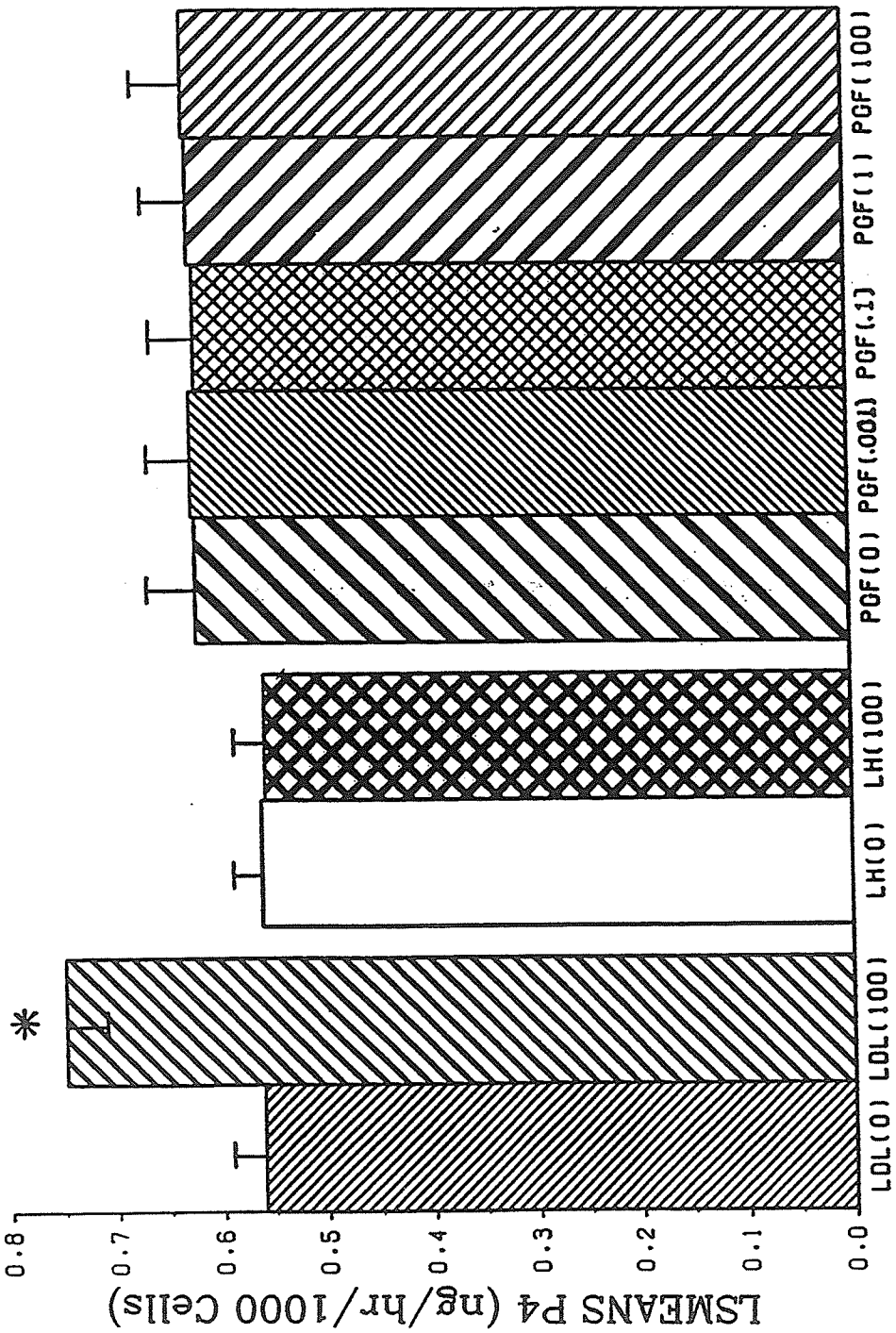
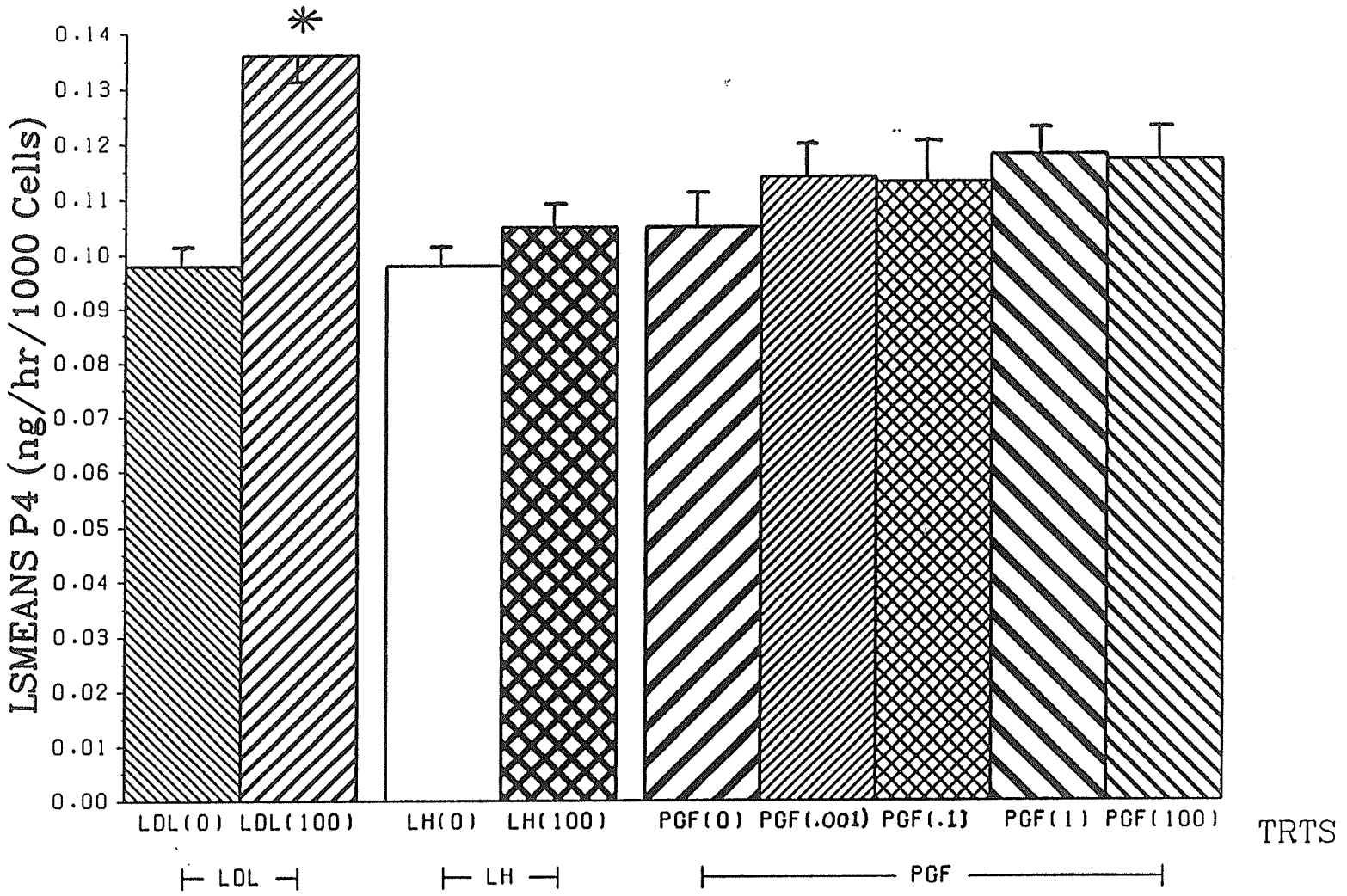

PROGESTERONE PRODUCTION BY SMALL CELLS
DAY 16, 2 HRS
LSMEANS P4 (ng/hr/1000 cells)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
*
LOL(0) LOL(100) LH(0) LH(100) POF(0) PGF(.001) PGF(.1) PGF(1) PGF(100)
LOL
LH
PGF
TRTS

Fig. 19 Effects of PGF2a, LDL (ug/ml) or LH (ng/ml) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 15 small cells (3 gilts) at 24 hr incubations (P4 = progesterone; TRTS = "Treatments"; * = different from control level, $P < 0.05$).

PROGESTERONE PRODUCTION BY SMALL CELLS

DAY 15, 24 HRS



slightly augmented progesterone production (P = 0.0642 and 0.0632 respectively) after 2-hour incubation (Fig. 20). There was no interaction between the membrane effectors and the various levels of PGF2a. Similarly, there were no statistically significant responses after the 24-hour incubation (P = 0.7574, Fig. 21).

3.4.2 Small Cells. Following a 2-hour incubation (Fig. 22) PGF2a at 1 ng/ml appeared to stimulate progesterone production (P = 0.0220) and PGF2a at 1 ng/ml interacted with SAM (P = 0.0519) to decrease progesterone release following 2 hour incubation (Fig. 23).

The stimulatory effect of PGF2a at 1 ng/ml was abolished after 24 hours (Fig. 24) although PGF2a at 1 ng/ml still interacted significantly (P = 0.0178) with SAM to decrease progesterone production (Fig. 25). Interaction between 3-DZA and PGF2a at 1 ng/ml approached significance after 24 hour incubation (P = 0.0652, Fig. 25).

Fig. 20 Effects of in vitro treatment with PGF2a (ng/ml), or the Membrane Effectors (MEF) SAM (200uM), SAH(1mM) or 3-DZA(10uM) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 10 large cells (6 gilts) at 2 hr incubation P4 = progesterone).

PROGESTERONE PRODUCTION BY LARGE CELLS

DAY 10, 2 HRS

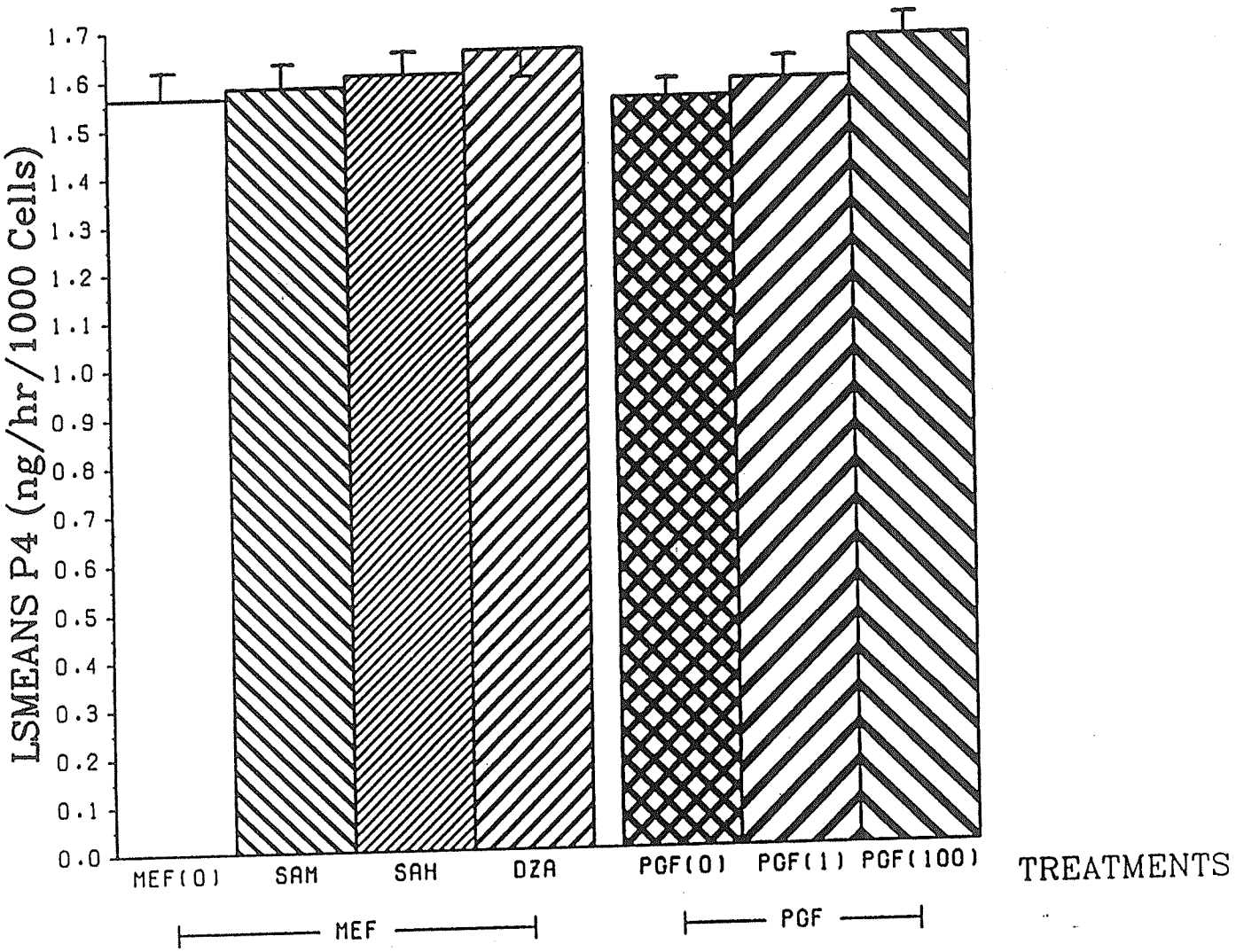


Fig. 21 Effects of in vitro treatment with PGF2a(ng/ml), or the the Membrane Effectors (MEF) SAM(200uM), SAH(1mM) or 3-DZA(10uM) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 10 large cells (6 gilts) at 24 hr incubation (P4 = progesterone).

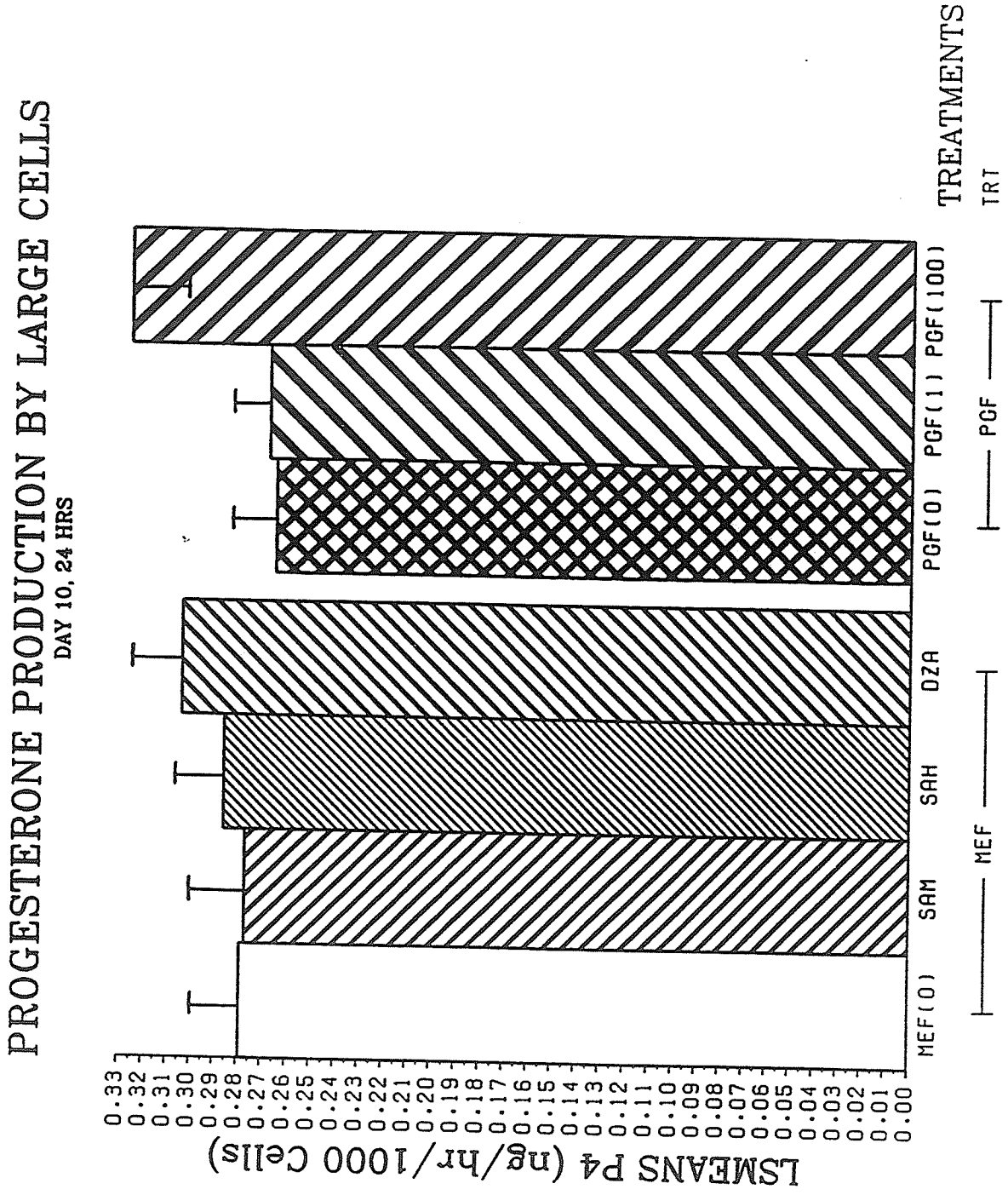

PROGESTERONE PRODUCTION BY LARGE CELLS
DAY 10, 24 HRS
LSMEANS P4 (ng/hr/1000 Cells)
0.33
0.32
0.31
0.30
0.29
0.28
0.27
0.26
0.25
0.24
0.23
0.22
0.21
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
MEF(0)
SAM
SAH
DZA
PGF(0)
PGF(1)
PGF(100)
TREATMENTS
MEF
PGF
TRT

Fig. 22 Effects of in vitro treatment with PGF2a(ng/ml), or the Membrane Effectors (MEF) SAM(200uM), SAH(1mM) or 3-DZA(10uM) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 10 small cells (6 gilts) at 2 hr incubation (P4 = progesterone; * = different from control level,
$P < 0.05$).

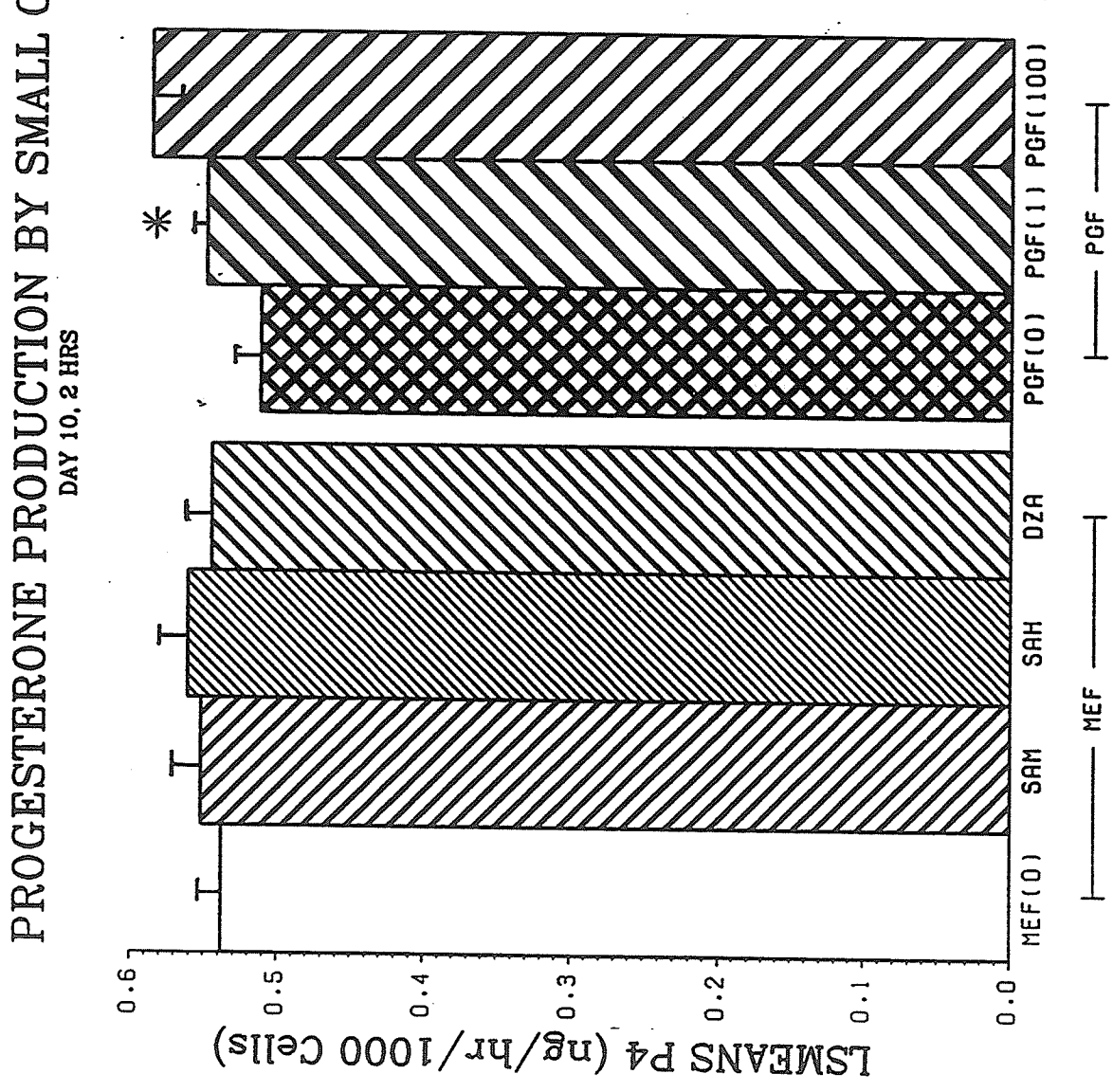

PROGESTERONE PRODUCTION BY SMALL CELLS
DAY 10, 2 HRS
LSMEANS P4 (ng/hr/1000 cells)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
*
MEF(0)
SAM
SAH
DZA
PGF(0)
PGF(1)
PGF(100)
MEF
PGF
TREATMENTS

Fig. 23 Interaction between 1 ng/ml PGF2a and 200 uM SAM at 2 hours (P = 0.0519, 6 gilts). (_____ SAM+PGF2a; _____ PGF2a only).

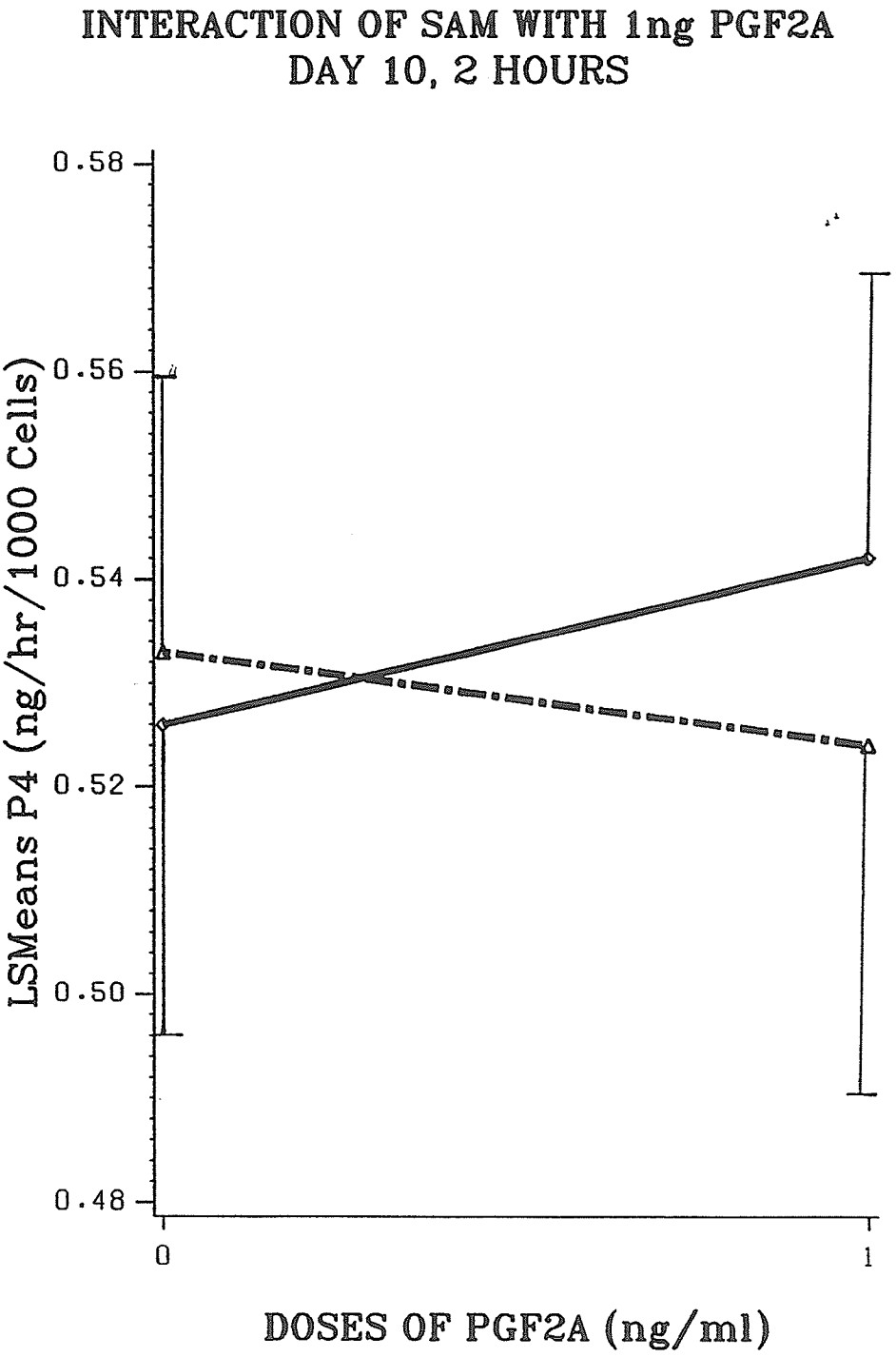

INTERACTION OF SAM WITH 1ng PGF2A
DAY 10, 2 HOURS
0.58
0.56
0.54
0.52
0.50
0.48
LSMeans P4 (ng/hr/1000 Cells)
0
1
DOSES OF PGF2A (ng/ml)

Fig. 24 Effects of in vitro treatment with PGF2a(ng/ml), or the Membrane Effectors (MEF) SAM(200uM), SAH(1mM) or 3-DZA(10uM) on progesterone production (LSMeans ng/hr/10^3 cells $\pm$ SEM) by day 10 small cells (6 gilts) at 24 hr incubation P4 = progesterone).

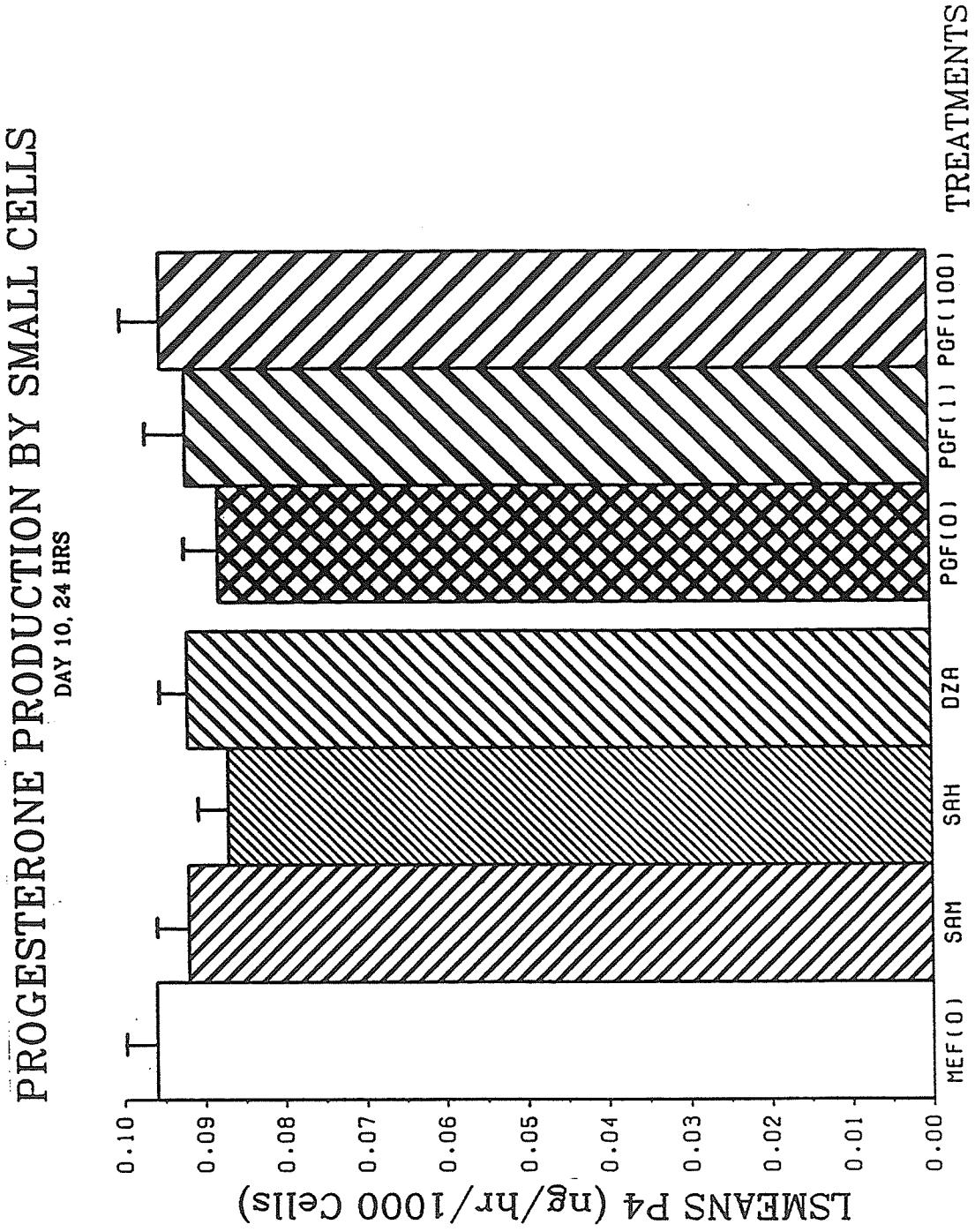
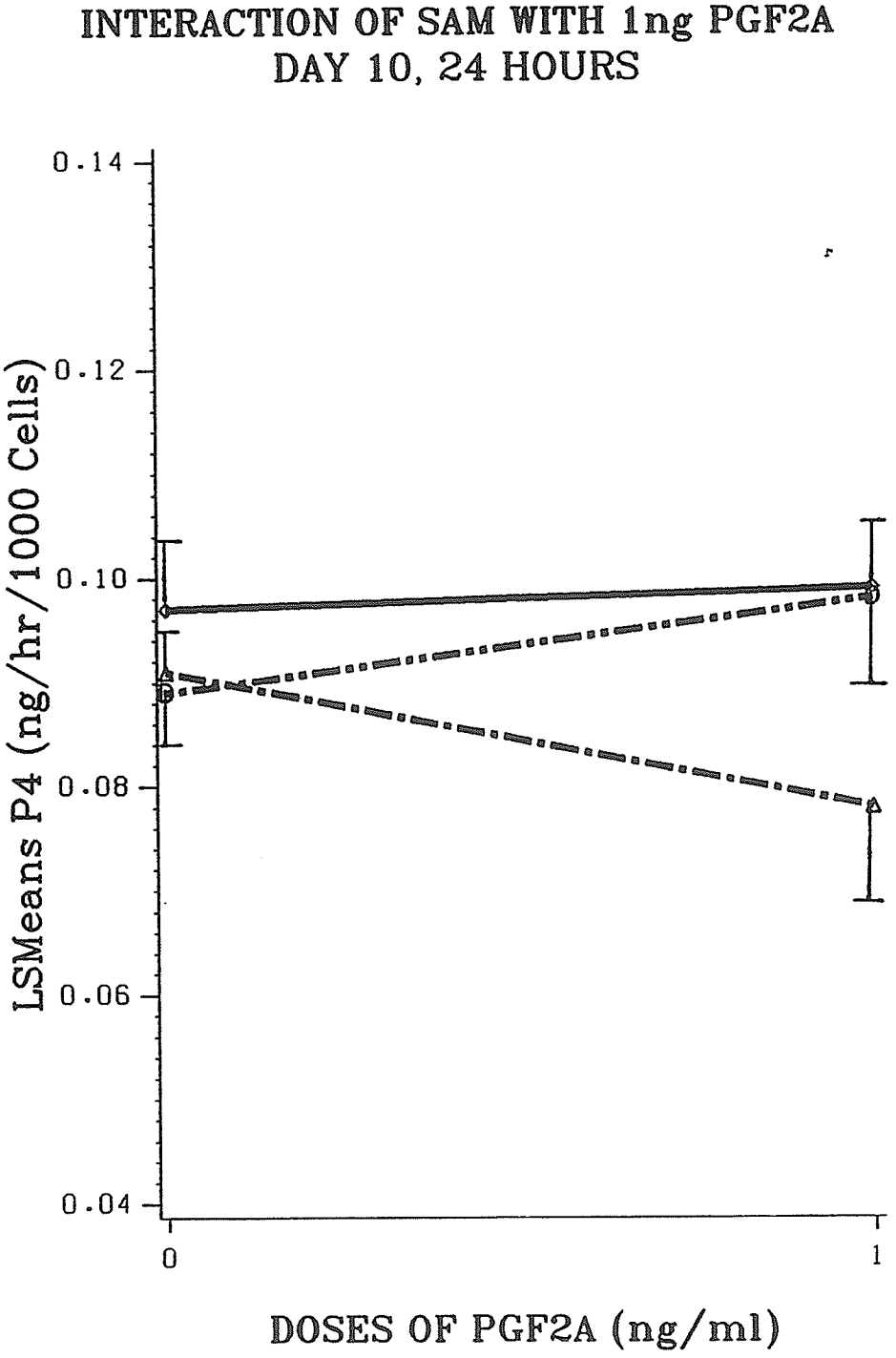

PROGESTERONE PRODUCTION BY SMALL CELLS
DAY 10, 24 HRS
LSMEANS P4 (ng/hr/1000 cells)
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
MEF(0)
SAM
SAH
DZA
PGF(0)
PGF(1) PGF(100)
TREATMENTS
MEF
PGF

Fig. 25 Interaction between 1 ng/ml PGF2a and 200 uM SAM at 24 hours incubation of small cells (6 gilts). (— - — - — SAM+PGF2a, P = 0.0178; — — — — DZA+PGF2a, P = 0.0652; ——————— PGF2a only).


INTERACTION OF SAM WITH 1ng PGF2A
DAY 10, 24 HOURS
0.14
0.12
0.10
0.08
0.06
0.04
LSMeans P4 (ng/hr/1000 Cells)
0
1
DOSES OF PGF2A (ng/ml)

DISCUSSION

4.1 Experiment I

The absence of corpora albicantia at surgery indicated that these pigs were prepubertal (Britt et al., 1989). Injection of PMSG followed immediately by hCG induced successful ovulation and CL development in these prepubertal pigs as reported by others (Shaw et al., 1971; Ellicott et al., 1973; Guthrie, 1977; Kineman et al., 1987).

Separation of the cell types of the induced CL in this experiment allowed comparison of the steroidogenic functions of the two cell types and comparisons with results from the sow (Buhr, 1987) and other domestic species. The large cell type produced more progesterone in the unstimulated state than the small cell type, a result similar to that reported for mature porcine (Buhr, 1987; Lemon and Loir, 1977), ovine (Fitz et al. 1982; Rodgers and O'Shea, 1982; Niswender et al., 1985) and bovine (Ursley and Leymarie, 1979) luteal cells in culture. However, the basal levels of progesterone obtained for each cell type from days 10, 15 and 18 in the present experiment are higher than those reported for the two cell types in a similar culture experiment from sows (Buhr, 1987). Since a similar assay system was used in the present experiment, this may be due to differences in progesterone

production by prepubertal and mature pigs. The days of the cycle studied in the prepubertal pigs differ from those of natural estrous cycle in Buhr's experiment. This may be the reason for the differences in progesterone levels. Kineman et al. (1987) reported levels as high as 249 $\pm$ 9 and 41 $\pm$ 3 ng/ml on day 10 and 15 respectively of the induced cycle following 2 hour incubations. It is not possible to directly compare these progesterone levels with those reported here since their cells were not separated into large and small subpopulations. They used a cell concentration of about 2.5 x 10^4 cells/100 ul, which is higher than that used in the present experiment.

Progesterone production by these luteal cells varied over the induced cycle. Basal progesterone production by the large cell type was higher on day 10 than either day 15 or 18. Basal production by the small cell type was not assessed for day 10 because of low cell yield but progesterone production by these cells was higher on day 15 than 18. A similar drop in progesterone was reported for both cell types of the sow from day 10 to 18 (Buhr, 1987). Rodgers et al. (1988) also reported a 70% drop in basal progesterone production by ovine luteal cells in culture from early to the mid luteal phase. These previous studies support the results obtained in this experiment. In addition, it was reported that plasma progesterone concentration rose steadily in naturally cycling gilts following ovulation, peaked between day 12 and 13 and then declined on day 14 of the estrous cycle (Connor et al.,

1976). The observed decline in basal progesterone levels from days 10 to 18 in vitro for both cell types in the present experiment suggests that the steroidogenic machinery of the induced CL of prepubertal pigs gradually ceases to function in a manner similar to CL from a mature pig.

Progesterone production fell dramatically from 2 to 24 hours, as reported over this time period for luteal cells from the sow (Buhr, 1987). Previous workers have suggested that this decline may be due to depleted substrates by 24 hours of incubation (Buhr, 1987) or product inhibition of further progesterone production (Caffrey et al., 1979; Sawyer et al., 1979). In addition to these hypotheses, the decline in progesterone could also be due to cell attachment to the culture plates. This would expose only a portion of the cell surface to the medium and thus reduce the level of progesterone released into the medium by reducing substrate intake. Furthermore, the drop in progesterone could also be due to a temporary refractoriness in the function of the steroidogenic system of the cell even in the presence of adequate substrates, or cell mortality at 24 hours. However, viability tests using trypan blue at 24 hours suggested that a 60% of these cells were still viable.

The induced luteal cells of prepubertal pigs utilized both LDL and HDL in vitro as reported for dispersed bovine cells (Pate and Nephew, 1988) and unseparated porcine luteal cells treated with whole canine lipoprotein (Grinwich et al., 1983). Porcine granulosa cells utilized LDL but not HDL

(Veldhuis, 1984) and Buhr (1987) reported that HDL was without effect or that it depressed progesterone produced by porcine large cells in culture. Based on these reports it appears that the induced CL differs from the spontaneous CL in the ability to utilize both types of lipoprotein for steroidogenesis.

Although all the levels of lipoprotein concentration were not applied to each cell type for each day of the induced estrous cycle, the response of the large cells to lipoprotein decreased with increasing day of the cycle. High density lipoprotein at 50 ug/ml elevated progesterone production significantly on day 15 but 100 ug/ml was required to raise progesterone level significantly on day 18 after a prolonged incubation of 24 hours. Low density lipoprotein was highly stimulatory to small cells on both days 15 and 18. The reduced response of large cells to lipoprotein on day 18 may account for the decrease in plasma progesterone seen towards the end of the cycle in adult pigs (Connor et al., 1976; King and Rajamahendran, 1988) because it has been reported that the large cells account for a greater proportion of progesterone levels from porcine (Buhr, 1987) and bovine (Fitz et al., 1982; Niswender et al., 1985) luteal cells. According to these reports, the steroidogenic function of the induced CL is similar to that of adult pigs.

The only response by either cell type to LH was increased progesterone production by small cells on day 18 at 2 hour incubation. It was reported earlier that LH at the dosages

employed in this study had minimal stimulatory effect on progesterone production by sow luteal cells (Buhr, 1987). In contrast, bovine LH stimulated progesterone levels from porcine luteal cells (Hunter, 1981; Mattioli et al., 1985) and Kineman et al. (1987) reported a dose-dependent effect of LH on progesterone levels by unseparated luteal cells from prepubertal pigs on day 10 and 14 of the induced cycle. The reduced response to LH could be due to several factors. It could be that the level of LH used in the present study was suboptimal. A profile of plasma LH levels in induced prepubertal pigs throughout the estrous cycle has not been determined. Such an experiment could show the relative levels of LH and its pattern of release, and might indicate the role of LH in the function of the induced CL. The previous experiments with LH (Hunter, 1981; Mattioli et al., 1985) utilized bovine LH at 0.1 uM concentration. These factors may have influenced their results. Furthermore, it is not known whether an episodic rather than a continuous presence of LH is required to induce progesterone production in pigs in vivo. In culture, LH is present throughout the incubation period. It was reported that plasma LH levels fluctuated in gilts during a 6-hourly sampling period between day 9 and 13 of the estrous cycle (Connor et al., 1976). Therefore, it could be that episodic exposure and not the absolute level of LH, is the important factor during the luteal phase.

Hunter (1981) suggested that the CL of the pig may function independently of LH during the early part of the

cycle or pregnancy. If this is the case with the induced CL one would assume that only progesterone precursors are required to sustain steroidogenesis by the induced CL of the prepubertal pig, through the estrous cycle and perhaps, through much of pregnancy. The present experiment supports this hypothesis. However, Spies et al. (1967) reported that unpurified anti-ovine LH antiserum caused atrophy of corpora lutea and complete loss of embryos in pregnant gilts. Surprisingly, the same antiserum had no effect on cycling gilts. This suggests that LH may be a luteotrophin in the pregnant gilt.

In conclusion, the cell types of the induced CL of the prepubertal pig appear normal when compared to the sow and other domestic species in progesterone production when provided with adequate levels of cholesterol in the form of either LDL or HDL. The abnormally high embryonic mortality reported in CL-induced, pregnant prepubertal pigs (Dzuik and Gehlback, 1966; Ellicott et al., 1973; Rampacek et al., 1976; Shaw et al., 1971) may be due to insufficient levels of substrates for steroidogenesis in these young pigs. Menino et al. (1989) reported that pigs bred at first spontaneous estrus had higher fetal abnormalities such as the failure of some blastomeres to incorporate into the morula or blastocyst. Although this phenomenon has not been reported for the induced pregnancy of prepubertal pigs, it suggests other potential causes of embryonic mortality in pigs. The present experiments clearly demonstrate that the induced CL

is capable of progesterone production similar to adult sows.

4.2 Experiment II

The current study examined cells from day 10 and 15 an attempt to bracket the onset of susceptibility to PGF2a and to evaluate the sensitivity of the cells to the presumptive luteolysin. Prostaglandin F2a at the doses employed did not significantly reduce progesterone production by either of the cell types from either day of the cycle.

In the sow in vivo, the CL can be induced to regress by exogenous PGF2a or an analogue from about day 12 onward (Moeljono et al., 1977; Hallford et al., 1975; Buhr et al., 1986; Guthrie and Polge, 1976; Connor et al., 1976), although failure to respond is common (Buhr et al., 1986; Krzymowski et al. 1976).

The failure of PGF2a to suppress progesterone in cells from either age of CL may be due to several reasons. Since the induced CL is functional on days 10 and 15 evidenced from the progesterone levels and lipoprotein utilization, it could be that PGF2a could not overcome the highly stimulatory effect of LDL on progesterone production by both cell fractions on both day 10 and 15. Perhaps a higher dose of PGF2a could have suppressed progesterone production, if the range of PGF2a doses used did not encompass the physiological concentration for these animals. But if the induced CL of the prepubertal pig is as highly susceptible to PGF2a as others have hypothesized (Puglisi et al., 1978,

1979; Kineman et al., 1987) one of the range of doses of PGF2a used here should have been sufficient to suppress progesterone production. Perhaps, a mediator of the luteolytic effect of PGF2a in vivo is absent in the culture environment. PGF2a reportedly had little effect on basal progesterone production by bovine large luteal cells (Alila et al., 1988a).

The purported in vivo luteolytic role of PGF2a has not always been evident in vitro. Mattioli et al. (1985) reported that PGF2a enhanced progesterone production by porcine luteal cells from late corpora lutea. In the present experiment, PGF2a tended to elevate progesterone production by small cells on day 10 after 24 hour incubation. In the cow in which the luteolytic effect of PGF2a is generally accepted, PGF2a was luteotrophic in vitro in a short-term incubation of luteal tissue slices (Hansel et al., 1973; Hoedmaker and Grunert, 1987; Speroff and Ramwell, 1970) or dispersed luteal cells (Alila et al. 1988a & b; Benhaim et al., 1987; Hixon and Hansel, 1979). Prostaglandin F2a also failed to suppress early progesterone production in the cow CL in vitro (Pate and Condon, 1984) and Fredriksson et al. (1986) reported a temporary elevation of progesterone in the mare with PGF2a treatment.

Given such quixotic effects of PGF2a in the pig and other species, it is presumptive to suggest the inability of PGF2a to reduce progesterone production in this system is due to refractoriness of the induced CL to PGF2a. The results

instead, parallel reports in other domestic species in which the in vivo luteolytic effect of PGF2a was not duplicated in vitro, either due to the culture milieu or reduced effectiveness of PGF2a in vitro. The results of this study do argue against the hypothesis that the induced CL of prepubertal pigs are highly susceptible to a direct luteolytic action of PGF2a.

4.3 Experiment III

This study was based on previous findings that PGF2a-induced luteolysis is associated with a change in the membrane fluidity from a viscous to a less fluid phase in rat (Buhr et al., 1979) and bovine (Goodsaid-Zalduondo et al., 1982) CL. It was demonstrated that methylation and translocation of membrane phospholipids reduced the viscosity of erythrocyte membranes (Hirata and Axelrod, 1978) and enhanced LH-induced steroidogenesis in dispersed, mixed bovine luteal cells (Milvae et al., 1983). Several theories have been proposed to explain the mechanism of action of PGF2a. These include reduced ovarian blood flow due to the vasoconstrictive effects of PGF2a (Pharris and Wyngarden, 1969; Nett et al. 1976), loss of LH receptors and binding sites (Grinwich et al., 1976; Hichens et al., 1974; Behrman et al., 1976; Thomas et al., 1978), inhibition of cAMP (Pate and Condon, 1984), and disruption of LH-stimulated adenylate cyclase (Thomas et al. 1978; Wakeling and Green, 1981; Dorflinger et

al., 1983). Since PGF2a-induced decline in plasma progesterone concentration is rapid (Barb et al., 1984; Grinwich et al., 1976; Torjesen and Aakvaag, 1978) the long interval between the drop in progesterone and a measurable decline in LH receptor number (Diekman et al., 1978) indicates that the loss of LH receptors is not likely the initial step in the mechanism of action of PGF2a.

Phospholipid methylation in bovine luteal cells occurred within 2 hours (Milvae et al., 1983) and the membrane changes associated with PGF2a-induced luteolysis (Buhr et al., 1979;) occurred by 24 hours after injection of PGF2a in vivo. These authors suggest that one of the initial events in PGF2a-mediated luteolysis is the change in the phospholipid composition of the luteal cell membrane.

Experiment III tested the hypothesis that agents that facilitate such methylation reactions would enhance the luteolytic effect of PGF2a and conversely, substances that inhibit phospholipid methylation would block the effects of PGF2a on progesterone secretion. Day 10 was selected based on hypothesis that the induced CL of prepubertal pigs is overly sensitive to PGF2a-induced luteolysis (Puglisi et al., 1978, 1979). Their progesterone production should decline in the presence of PGF2a at a time when adult CL are known to be refractory (Connor et al., 1976; Diehl and Day, 1974).

Prostaglandin F2a at 1 ng/ml in the presence of SAM reduced progesterone production by day 10 small cells at 2 and 24 hours. SAM alone had no significant effect on

progesterone levels and 1 ng/ml of PGF2a had either no significant effect on progesterone levels (Experiment II) or increased progesterone production (experiment III) progesterone production. It therefore appears that the membrane effects of SAM amplified the luteolytic action of PGF2a on progesterone production. Neither 3-DZA or SAH, (both inhibitors of methylation) had any effect by themselves nor was there an interaction between PGF2a and these agents. However, 3-DZA plus PGF2a at 1 ng/ml tended to increase progesterone production following 24 hour incubation of small cells further suggesting that phospholipid methylation may be involved in the effects of PGF2a. Milvae et al. (1983) reported that SAM enhanced LH-induced progesterone production in bovine luteal cells but by itself SAM had no effect on progesterone production. There were no significant effects of PGF2a with SAM in the large cell fraction which was surprising since ovine large luteal cells contain most of the receptors for PGF2a on day 10 of the cycle (Fitz et al., 1982). However, Alila et al. (1988a,b) reported that PGF2a also significantly affected progesterone production by small luteal cells of bovine CL. Based on this report and the results of the present study, luteolysis in the induced CL of prepubertal pigs may be initiated in the small luteal cells. Co-incubation of both cell types may be necessary to elicit the full effect of PGF2a.

Prostaglandin F2a also induces the hydrolysis and incorporation of phosphates into phospholipids in rat luteal

and granulosa cell within 2 to 10 minutes (Raymond et al., 1983; Minegishi and Leung, 1985). Prostaglandin F2a-induced phospholipid hydrolysis generates inositol triphosphate (Davis et al., 1987) which stimulates Ca^{++} and protein kinase C (Nishizuka, 1984a). However, it is not known how these reactions affect progesterone production by the cell. Further investigation will adequately address the question of whether the effects of PGF2a on the cell membrane goes beyond phase changes in the phospholipids.

In conclusion, the present experiment suggests that PGF2a-induced luteolysis may involve phospholipid methylation in small luteal cells of the induced CL of prepubertal pigs. The lack of effects of PGF2a at 100 ng/ml in the present experiment may suggest that lower levels are required in synergy with the membrane effectors, particularly SAM. The results may be confounded by the age of the CL and a study with senescent CL could shed more light into the role of membrane phospholipid methylation or hydrolysis in PGF2a-induced luteolysis.

GENERAL CONCLUSIONS

The experiments reported here clearly indicate that the induced CL of prepubertal pigs are capable of increased steroid production in the presence of either LDL or HDL. The CL do not appear to be unusually sensitive to PGF2a in vitro. Given the unpredictable effects of PGF2a in the pig, cow and the mare in vitro, this conclusion may be accepted with reservations. However, if the induced CL of prepubertal pigs are as susceptible to PGF2a as was suggested following injections of relatively high doses in vivo then low levels should suppress progesterone production.

It appears from these experiments that phospholipid methylation may be one of the events in the mechanism of PGF2a induction of regression in the CL. However, the conclusions must be tempered by recognition of the inability to demonstrate any suppression of progesterone production by PGF2a alone, and the limited stages of the induced cycle examined. Further experimentation is required on CL from varying stages of the cycle to conclusively demonstrate the role of phospholipid methylation in PGF2a-induced luteolysis in the pig.

Assuming that the CL of induced pregnancy in prepubertal pigs are similar to those of the cycle in their ability synthesize progesterone from lipoproteins, the cause of

embryonic mortality in prepubertal gilts reported by other workers do not appear to be due to inherent defects in the induced CL.

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A P P E N D I X

TABLE 1. Analysis of variance for comparison of pretreatment incubation progesterone (ng/hr/10^3 cells) produced by large cells on day 10, 15 and 18 of the estrous cycle:

Source of Variation	Degrees of Freedom	Mean Square	Denominator for F	Pr > F
Cycle Day	2	381.302	Pig within Cycle Day	0.0208
Pig within Cycle Day	12	70.110	Error	0.0001
Residual	556	0.353		

TABLE 2. Analysis of variance for comparison of pretreatment incubation progesterone (ng/hr/10^3 cells) produced by small and large cells on days 15 and 18 of the estrous cycle.

Source of Variation	Degrees of Freedom	Mean Square	Denominator for F	Pr > F
Cycle Day	1	259.016	Pig within Cycle Day	0.052
Pig within Cycle Day	9	51.883	Error	0.0001
Cell Type	1	338.671	"	0.0001
Day x Cell T.	1	79.214	"	0.0001
Residual	901	0.388		

TABLE 3. Least square means (LSMeans ± SEM) of progesterone (ng/hr/10^3 cells) produced by large cells on day 10, 15 and 18 of the estrous cycle during a pretreatment incubation of 14-16 hours

Day of Estrous Cycle	P4 ± Std. Error
10	4.3 ± 0.90^a
15	3.05 ± 0.60^a
18	1.1 ± 0.56^b

Note: Means with the same superscript are not significantly different ($P < 0.05$).

TABLE 4. LSMeans (± SEM) of progesterone (ng/hr/10^3 cells) produced by small and large cells on day 15 and 18 of the estrous cycle during a pretreatment incubation of 14-16 hour.

Day of Estrous Cycle	Cell Type	Prog. Level (±SEM)
15	L	2.9 ± 0.04^a
15	S	0.9 ± 0.05^b
18	L	1.1 ± 0.04^c
18	S	0.4 ± 0.04^d

Note: Progesterone values with the same superscript are not significantly different ($P < 0.05$).
L= large cells, S= small cells.

TABLE 5: Least square means of progesterone production (ng/hr/10^3 cells) by large and small cells in response to levels of LDL or HDL on days 15 and 18 of the estrous cycle.

Day	Cell Type	Incub. Time	Lipop. Type	Dose Levels Lipop. ug/ml.	Lin.	Quad.	Cubic
15	L	24 hr	LDL	0, 10, 100	-	(a)	-
15	L	2 "	HDL	10, 50, 100	(b)	-	-
18	S	2 "	LDL	0, 10, 50	(c)	-	-
18	S	24 "	LDL	0, 10, 50	-	(d)	-
18	L	2 "	LDL	10, 50, 100	(e)	-	-
18	L	24 "	LDL	10, 50, 100	-	-	(f)

LEGEND:

(a) $P < 0.0258$
(b) $P < 0.0081$
(c) $P < 0.0282$
(d) $P < 0.0007$
(e) $P < 0.0542$
(f) $P < 0.0058$

Lin. = Linear response, Quad. = Quadratic response.

TABLE 6. Least square means (± SEM) of main effects of levels of lipoproteins and LH on progesterone production (ng/hr/10^3 cells) by day 10, 15 and 18 small and large cells in Experiment I Pattern A.

Day of Estrous Cycle	Cell Type	Inc. Time	LH(ng/ml)		HDL(ug/ml)	
			(0)	(50)	(0)	(100)
10	L	2	4.2±0.31	4.06±0.31	3.4±0.31	4.9±0.31
10	L	24	0.4±0.04	0.4±0.04	0.4±0.04	0.4±0.04
15	L	2	2.6±0.16	2.4±0.17	2.05±0.16	2.9±0.17
15	L	24	0.3±0.02	0.3±0.02	0.2±0.02	0.3±0.02
15	S	2	0.2±0.03	0.3±0.03	0.2±0.03	0.3±0.04
15	S	24	0.09±0.009	0.08±0.009	0.08±0.009	0.09±0.009
18	L	2	1.8±0.14	1.5±0.15	1.5±0.15	1.8±0.15
18	L	24	0.5±0.15	0.5±0.15	0.3±0.15	0.7±0.14
18	S	2	0.3±0.03	0.4±0.03	0.3±0.03	0.5±0.03
18	S	24	0.02±0.002	0.02±0.002	0.02±0.002	0.03±0.002

Table 7. Least square means (± SEM) of interaction between LH and lipoproteins on progesterone production (ng/hr/10^3 cells) by day 10, 15 and 18 small and large cells in Experiment I Pattern A.

Day of Estrous Cycle	Cell Type	Inc. time	Interaction Means			
			LH0xHDL0	LH50xHDL0	HDL100xLH0	HDL100xLH50
10	L	2	3.3±0.44	3.4±0.44	5.01±0.44	4.7±0.44
10	L	24	0.4±0.06	0.4±0.06	0.5±0.06	0.4±0.06
15	L	2	2.0±0.23	2.1±0.22	3.1±0.23	2.7±0.26
15	L	24	0.2±0.03	0.3±0.03	0.3±0.03	0.3±0.04
15	S	2	0.2±0.05	0.3±0.04	0.3±0.05	0.3±0.06
15	S	24	0.08±0.01	0.07±0.01	0.1±0.01	0.08±0.01
18	L	2	1.6±0.20	1.3±0.20	1.9±0.20	1.7±0.20
18	L	24	0.3±0.20	0.3±0.20	0.7±0.20	0.7±0.20
18	S	2	0.2±0.04	0.4±0.04	0.4±0.05	0.5±0.04
18	S	24	0.02±0.003	0.02±0.003	0.03±0.003	0.03±0.003

TABLE 8. Summary of the linear contrast and estimate analysis of Experiment I showing the probability levels of the effects and interactions of levels of LH (ng/ml) and lipoproteins (ug/ml) in Pattern A.

Day of Estrous Cycle	Cell Type	Inc.Time	Main Effects		Interactions
			LH(50)	HDL(100)	LH x HDL
10	L	2	0.8155	0.0038	0.6681
10	L	24	0.5691	0.4429	0.4841
15	S	2	0.9564	0.1118	0.8732
15	S	24	0.4001	0.4217	0.9292
15	L	2	0.4178	0.0013	0.3287
15	L	24	0.6753	0.0253	0.3487
18	S	2	0.0250	0.0042	0.6322
18	S	24	0.5909	0.0120	0.8614
18	L	2	0.2669	0.0850	0.9498
18	L	24	0.8730	0.0462	0.9718

TABLE 9. Least square means (± SEM) of main effects of levels of lipoproteins and LH on progesterone production (ng/hr/10^3 cells) by day 15 and 18 small and large cells in Experiment I Pattern B.

Day of Estrous Cycle	Cell Type	Inc. Time	LH (ng/ml) (0)	LH (ng/ml) (50)
15	L	2	2.4±0.13	2.4±0.13
15	L	24	0.3±0.02	0.3±0.02
15	S	2	0.3±0.03	0.3±0.03
15	S	24	0.09±0.007	0.08±0.007
18	S	2	0.4±0.03	0.4±0.03
18	S	24	0.02±0.002	0.02±0.002

			LDL (ug/ml)		HDL (ug/ml)	
			(0)	(50)	(0)	(50)
15	L	2	2.05±0.16	2.5±0.16	2.05±0.16	2.6±0.16
15	L	24	0.2±0.02	0.3±0.02	0.2±0.02	0.3±0.02
15	S	2	0.2±0.03	0.3±0.03	0.2±0.03	0.3±0.04
15	S	24	0.08±0.008	0.08±0.008	0.08±0.008	0.1±0.009
18	S	2	0.3±0.03	0.4±0.03	0.3±0.03	0.4±0.03
18	S	24	0.02±0.002	0.03±0.002	0.02±0.002	0.03±0.002

Table 10. Least square means (± SEM) of interactions of LH with LDL or HDL on progesterone production (ng/hr/10^3 cells) by day 15 and 18 small and large cells in Experiment I Pattern B.

Day of Estrous Cycle	Cell Type	Inc. Time	Interaction Means					
			LH0xLDL0	LH50xLDL0	LH0xLDL50	LH50xLDL50	LH0xHDL50	LH50xHDL50
15	L	2	2.0±0.22	2.1±0.23	2.5±0.23	2.6±0.23	2.6±0.23	2.5±0.23
15	L	24	0.2±0.03	0.3±0.03	0.3±0.04	0.3±0.05	0.3±0.03	0.3±0.03
15	S	2	0.2±0.05	0.3±0.04	0.3±0.05	0.3±0.05	0.3±0.05	0.3±0.06
15	S	24	0.08±0.01	0.07±0.01	0.09±0.01	0.08±0.01	0.1±0.01	0.1±0.01
18	S	2	0.2±0.04	0.4±0.04	0.4±0.04	0.4±0.05	0.4±0.05	0.4±0.04
18	S	24	0.02±0.003	0.02±0.003	0.03±0.003	0.03±0.003	0.03±0.003	0.03±0.003

TABLE 11. Summary of the linear contrast and estimate analysis of Experiment I showing the probability levels of the effects and interactions of levels of LH (ng/ml) and lipoproteins (ug/ml) in Pattern B.

Day of Estrous Cycle	Cell Type	Inc.Time	Main Effects			Interactions	
			LH(50)	LDL(50)	HDL(50)	LH x LDL	LH x HDL
15	L	2	0.9306	0.0454	0.0351	0.9139	0.6316
15	L	24	0.6520	0.1070	0.1622	0.7148	0.5277
15	S	2	0.9740	0.0053	0.0558	0.9191	0.6481
15	S	24	0.5075	0.6593	0.1034	0.9710	0.7624
18	S	2	0.1613	0.0133	0.0775	0.2877	0.1809
18	S	24	0.8883	0.0336	0.0053	0.9662	0.6774

TABLE 12. Least square means (± SEM) of main effects of various levels of HDL and LH on progesterone production (ng/hr/10^3 cells) by day 18 small and large cells in Experiment I Pattern C.

Day of Estrous Cycle	Cell Type	Inc. Time	LH(ng/ml) (0)	LH(ng/ml) (50)
18	L	2	1.7±0.10	1.5±0.10
18	L	24	0.5±0.10	0.5±0.10
18	S	2	0.4±0.02	0.4±0.02
18	S	24	0.03±0.002	0.03±0.002

Day of Estrous Cycle	Cell Type	Inc. Time	HDL(ug/ml) (0)	HDL(ug/ml) (10)	HDL(ug/ml) (50)	HDL(ug/ml) (100)
18	L	2	1.5±0.15	1.7±0.15	1.4±0.15	1.8±0.15
18	L	24	0.3±0.15	0.6±0.15	0.6±0.15	0.7±0.15
18	S	2	0.3±0.03	0.4±0.03	0.4±0.03	0.5±0.03
18	S	24	0.02±0.002	0.03±0.002	0.3±0.002	0.03±0.002

Table 13. Least square means (± SEM) of interactions between LH and HDL on progesterone production (ng/hr/10^3 cells) by day 18 small and large cells in Experiment I Pattern C.

Day of Estrous Cycle	Cell Type	Inc. Time	Interaction Means			
			LH0xHDL0	LH50xHDL0	LH0xHDL10	LH50xHDL10
18	L	2	1.6±0.21	1.3±0.21	1.9±0.20	1.6±0.21
18	L	24	0.3±0.21	0.2±0.21	0.5±0.21	0.6±0.21
18	S	2	0.2±0.04	0.4±0.04	0.4±0.05	0.4±0.04
18	S	24	0.02±0.003	0.02±0.003	0.03±0.003	0.03±0.004
			LH0xHDL50	LH50xHDL50	LH0xHDL100	LH50xHDL100
18	L	2	1.5±0.21	1.3±0.21	1.9±0.21	1.7±0.21
18	L	24	0.6±0.21	0.5±0.21	0.7±0.21	0.7±0.21
18	S	2	0.4±0.05	0.4±0.05	0.4±0.05	0.4±0.04
18	S	24	0.03±0.003	0.03±0.003	0.03±0.003	0.03±0.003

TABLE 14. Summary of the linear contrast and estimate analysis of Experiment I showing the probability levels of the effects and interactions of levels of LH (ng/ml) and lipoproteins (ug/ml) in Pattern C.

Day of Estrous Cycle	Cell Type	Inc. Time	Main Effects			
			LH(50)	HDL(10)	HDL(50)	HDL(100)
18	L	2	0.1183	0.1741	0.9454	0.0850
18	L	24	0.8956	0.1625	0.1608	0.0462
18	S	2	0.1243	0.2506	0.0775	0.0042
18	S	24	0.8225	0.0013	0.0053	0.0120

			Interactions		
			LHxHDL(10)	LHxHDL(50)	LHxHDL(100)
18	L	2	0.8569	0.9098	0.9498
18	L	24	0.9350	0.9863	0.9718
18	S	2	0.1468	0.1809	0.6322
18	S	24	0.4466	0.6774	0.8614

TABLE 15. Least square means (± SEM) of interactions between LH or LDL with various levels of PGF2a on progesterone production (ng/hr/10^3 cells) by day 10 and 15 small and large cells in Experiment II. (LD = LDL; PGF = PGF2a).

Day of Estrous Cycle	Cell Type	Inc. Time	Interaction Means				
			LH0xPGF0	LH0xPGF.001	LH0xPGF.1	LH0xPGF1	LH0xPGF100
10	L	2	1.9±0.27	1.8±0.27	2.0±0.27	2.0±0.27	2.2±0.27
10	L	24	0.2±0.02	0.2±0.02	0.2±0.02	0.2±0.02	0.2±0.02
10	S	2	1.8±0.26	1.7±0.26	1.5±0.26	1.6±0.26	1.9±0.26
10	S	24	0.2±0.02	0.2±0.02	0.2±0.02	0.2±0.02	0.2±0.02
15	L	2	0.7±0.11	0.7±0.11	0.8±0.11	0.7±0.11	0.7±0.11
15	L	24	0.07±0.009	0.08±0.009	0.08±0.009	0.08±0.009	0.08±0.009
15	S	2	0.6±0.06	0.6±0.06	0.5±0.06	0.6±0.06	0.6±0.06
15	S	24	0.09±0.009	0.09±0.009	0.1±0.009	0.1±0.009	0.1±0.009
			LH100xPGF0	LH100xP.001	LH100xPGF.1	LH100xPGF1	LH100xPGF100
10	L	2	2.3±0.27	1.9±0.39	2.0±0.31	2.0±0.31	1.6±0.39
10	L	24	0.2±0.02	0.2±0.03	0.2±0.03	0.2±0.03	0.2±0.03
10	S	2	1.8±0.38	2.0±0.54	2.0±0.30	2.0±0.38	1.8±0.38
10	S	24	0.2±0.04	0.3±0.05	0.2±0.04	0.2±0.04	0.3±0.04
15	L	2	0.7±0.10	0.6±0.13	0.7±0.11	0.7±0.10	0.8±0.10
15	L	24	0.07±0.009	0.07±0.01	0.07±0.009	0.08±0.009	0.08±0.009
15	S	2	0.6±0.07	0.5±0.07	0.6±0.06	0.6±0.06	0.5±0.07
15	S	24	0.1±0.01	0.1±0.01	0.1±0.009	0.1±0.009	0.1±0.01
			LD100xPGF0	LD100xPGF.001	LD100xPGF.1	LD100xPGF1	LD100xPGF100
10	L	2	2.7±0.27	2.7±0.39	2.9±0.31	3.1±0.31	2.8±0.39
10	L	24	0.2±0.02	0.3±0.03	0.3±0.03	0.3±0.03	0.3±0.03
10	S	2	3.2±0.38	2.1±0.53	2.6±0.30	3.4±0.38	2.3±0.38
10	S	24	0.3±0.04	0.2±0.05	0.3±0.03	0.4±0.05	0.4±0.05
15	L	2	1.0±0.19	1.1±0.10	1.2±0.13	1.1±0.13	1.0±0.10
15	L	24	0.1±0.01	0.1±0.009	0.1±0.01	0.1±0.01	0.1±0.009
15	S	2	0.7±0.07	0.7±0.07	0.7±0.06	0.7±0.06	0.8±0.07
15	S	24	0.1±0.01	0.1±.01	0.1±0.009	0.1±0.009	0.1±0.01

TABLE 15 Cont'd...

Day of Estrous Cycle	Cell Type	Inc. Time	Interaction Means STMxPGF0	STMxPGF.001	STMxPGF.1	STMxPGF1	STMxPGF100
10	L	2	2.2±0.39	3.0±0.39	3.3±0.39	3.1±0.39	3.4±0.39
10	L	24	0.3±0.03	0.3±0.03	0.3±0.03	0.3±0.03	0.4±0.03
10	S	2	3.2±0.53	3.7±0.53	2.0±0.53	2.6±0.53	2.2±0.53
10	S	24	0.3±0.05	0.4±0.05	0.3±0.05	0.4±0.05	0.4±0.05
15	L	2	1.0±0.13	-	-	-	-
15	L	24	0.1±0.01	-	-	-	-
15	S	2	0.6±0.07	0.8±0.09	0.9±0.09	-	-
15	S	24	0.1±0.01	0.1±0.01	0.1±0.01	-	-

STM = "LH+LDL" given as a treatment; "-" indicates there were insufficient replications for statistical analysis.

TABLE 16. Least square means (± SEM) of main effects of PGF2a, LH and LDL on progesterone production (ng/hr/10^3 cells) by day 10 and 15 small and large cells (Experiment II).

Day of Estrous Cycle	Cell Type	Inc. Time	LH(ng/ml)		LDL(ug/ml)		LH+LDL
			(0)	(100)	(0)	(100)	
10	L	2	2.0±0.12	2.0±0.14	2.0±0.12	2.8±0.14	3.0±0.17
10	L	24	0.2±0.01	0.2±0.01	0.2±0.12	0.3±0.01	0.3±0.01
10	S	2	1.7±0.1	1.9±0.16	1.7±0.11	2.8±0.16	2.8±0.23
10	S	24	0.2±0.01	0.3±0.02	0.2±0.01	0.3±0.02	0.4±0.02
15	L	2	0.7±0.05	0.7±0.05	0.7±0.05	1.1±0.07	-
15	L	24	0.08±0.004	0.08±0.004	0.08±0.004	0.1±0.005	-
15	S	2	0.6±0.03	0.6±0.03	0.6±0.03	0.7±0.03	-
15	S	24	0.1±0.004	0.1±0.005	0.1±0.004	0.1±0.005	-

			PGF2a (ng/ml)				
			(0)	(.001)	(0.1)	(1)	(100)
10	L	2	2.3±0.14	2.3±0.17	2.6±0.17	2.6±0.16	2.5±0.17
10	L	24	0.3±0.01	0.3±0.01	0.3±0.01	0.3±0.01	0.3±0.01
10	S	2	2.5±0.17	2.4±0.19	2.0±0.15	2.4±0.17	2.08±0.17
10	S	24	0.3±0.02	0.3±0.02	0.3±0.02	0.3±0.02	0.3±0.02
15	L	2	0.8±0.06	0.8±0.07	0.9±0.07	0.9±0.07	0.9±0.06
15	L	24	0.1±0.006	0.08±0.006	0.09±0.006	0.1±0.006	0.09±0.005
15	S	2	0.6±0.04	0.6±0.04	0.6±0.04	0.6±0.04	0.6±0.04
15	S	24	0.1±0.006	0.1±0.006	0.1±0.005	0.1±0.005	0.1±0.006

TABLE 17. Summary of the linear contrasts and estimate analysis of Experiment II showing the probability levels of the main effects and interactions of LH, LDL and PGF2a (PGF).

Day of Estrous Cycle	Cell Type	Inc.Time	Main Effects						
						PGF			
			LH(100)	LDL(100)	LH+LDL	(.001)	(.1)	(1)	(100)
10	L	2	0.9024	0.0001	0.0001	0.7425	0.1965	0.1542	0.6420
10	L	24	0.9201	0.0001	0.0001	0.8425	0.3382	0.3013	0.3223
10	S	2	0.3185	0.0001	0.0015	0.6654	0.0680	0.7342	0.5410
10	S	24	0.0034	0.0004	0.0001	0.3423	0.9243	0.0647	0.0643
15	L	2	0.8258	0.0001	-	0.5696	0.2008	0.3957	0.4221
15	L	24	0.9047	0.0001	-	0.9562	0.5067	0.1311	0.2957
15	S	2	0.9400	0.0001	-	0.9771	0.9674	0.9635	0.9291
15	S	24	0.2626	0.0001	-	0.3190	0.3664	0.1409	0.1673

TABLE 17. Cont'd...

Day of Estrous Cycle	Cell Type	Inc. Time	Interactions			
			LHxPGF.001	LHxPGF.1	LHxPGF1	LHxPGF100
10	L	2	0.6997	0.4628	0.6174	0.1372
10	L	24	0.2363	0.4041	0.5185	0.1736
10	S	2	0.6359	0.3860	0.5172	0.9957
10	S	24	0.3394	0.8925	0.8952	0.6668
15	L	2	0.5253	0.4937	0.8785	0.6296
15	L	24	0.9298	0.7395	0.8035	0.5615
15	S	2	0.9642	0.8110	0.8641	0.8385
15	S	24	0.7586	0.9389	0.8834	0.2732
			LDLxPGF.001	LDLxPGF.1	LDLxPGF1	LDLxPGF100
10	L	2	0.7901	0.8436	0.4907	0.8178
10	L	24	0.8346	0.3402	0.1742	0.2927
10	S	2	0.1723	0.6283	0.5521	0.1262
10	S	24	0.1760	0.9005	0.1551	0.7704
15	L	2	0.8017	0.7501	0.8422	0.7881
15	L	24	0.6431	0.7490	0.3614	0.6943
15	S	2	0.8069	0.6440	0.8405	0.6391
15	S	24	0.3975	0.8369	0.7152	0.9279
			(STM)xPGF.001	(STM)xPGF.1	(STM)xPGF1	(STM)xPGF100
10	L	2	0.1543	0.1264	0.1971	0.1596
10	L	24	0.8968	0.8456	0.7312	0.8055
10	S	2	0.4716	0.2925	0.6664	0.2158
10	S	24	0.0919	0.8309	0.1026	0.7524

Note: (STM) = (LH+LDL).

TABLE 18. Least square means (± SEM) of main effects of PGF2a, SAM, SAH and DZA on progesterone (LSMeans ng/hr/10^3 cells) by day 10 small and large cells (Experiment III).

Cell Type	Inc. Time	PGF2a (ng/ml)		
		(0)	(1)	(100)
L	2	1.5±0.05	1.6±0.04	1.7±0.04
L	24	0.3±0.02	0.3±0.02	0.3±0.02
S	2	0.5±0.02	0.5±0.02	0.6±0.02
S	24	0.1±0.004	0.09±0.004	0.09±0.004

		Membrane Effectors			
		(0)	SAM(200uM)	SAH(1mM)	DZA(10uM)
L	2	1.6±0.05	1.6±0.05	1.6±0.05	1.6±0.05
L	24	0.3±0.02	0.3±0.02	0.3±0.02	0.3±0.02
S	2	0.5±0.02	0.5±0.02	0.6±0.02	0.5±0.02
S	24	0.1±0.004	0.09±0.004	0.09±0.004	0.09±0.004

TABLE 19. Least square means (± SEM) of interactions between PGF2a, SAM, SAH and DZA on progesterone (LSMeans ng/hr/10^3 cells) by day 10 large and small cells (Experiment III).

Cell Type	Inc. Time	PGF1xSAM	PGF1xSAH	PGF1xDZA	PGF100xSAM	PGF100xSAH	PGF100xDZA
L	2	1.5±0.10	1.5±0.09	1.7±0.09	1.7±0.09	1.7±0.09	1.6±0.10
L	24	0.2±0.04	0.3±0.04	0.3±0.04	0.3±0.04	0.3±0.04	0.3±0.05
S	2	0.5±0.03	0.6±0.03	0.6±0.03	0.6±0.03	0.6±0.03	0.6±0.03
S	24	0.08±0.008	0.09±0.007	0.1±0.008	0.1±0.007	0.09±0.007	0.09±0.008

TABLE 20. Summary of the linear contrast and estimate analysis of experiment III (Day 10 of the estrous cycle) showing the probability levels of the main effects and interactions of membrane effectors and PGF2a (ng/ml).

Cell Type	Inc.Time	PGF1	PGF100	SAM (200uM)	SAH (1mM)	DZA (10uM)
L	2	0.0642	0.0632	0.2704	0.0742	0.3981
L	24	0.0875	0.7574	0.1387	0.2184	0.3654
S	2	0.0220	0.1772	0.2954	0.0704	0.3133
S	24	0.9769	0.5988	0.2083	0.2049	0.7179

		Interactions					
		PGF1xSAM	PGF1xSAH	PGF1xDZA	PGF100xSAM	PGF100xSAH	PGF100xDZA
L	2	0.5005	0.7081	0.5333	0.2159	0.6759	0.6584
L	24	0.4686	0.5475	0.2525	0.9158	0.7803	0.3503
S	2	0.0519	0.1687	0.2544	0.2799	0.8067	0.8203
S	24	0.0178	0.3436	0.0652	0.5334	0.7622	0.2142

Note: PGF=PGF2a (ng/ml).