Effects of Uterine Removal on the Growth of the Fetus in the Remaining Uterine Horn of Mice

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Declaration and Statement

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Herbert Sipahutar

29 May 1996

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Chapter 1
Introduction

1.1 General Review

1.1.1 Reproductive Biology of the Mouse

a. Female Reproductive System

The female reproductive system of the mouse is composed of paired ovaries, oviducts uterii, cervices and a single vagina and clitoris with clitoris gland (Fekete, 1941; Hummel *et al.*, 1966; Rugh, 1967).

The ovaries:

The ovaries are small, pink and paired spherical bodies located at the posterolateral poles of the kidneys functioning to produce mature female sex cells, the ova, and sex hormones. Each ovary is attached to the dorsal body wall by the ligamentum suspensorium ovarii (mesovarium) and connected to the anterior end of the uterus by the ligamentum ovarii proprium. Each ovary is enclosed in a thin transparent elastic capsule or bursa from which ovulated ova cannot escape. The surface of the ovary is smooth in the prepubertal female but becomes nodular after sexual maturity because of the

presence of follicles and corpora lutea (CL). It is covered by a thin, transparent connective tissue membrane covered on both surfaces by mesothelium. Ovarian size is different in strain and age because of the differences in numbers of follicles and CL.

The oviduct

This is a tubular, long (1.8 cm), narrow, coiled structure, extending from the periovarian space to the uterine horn and functioning as a transport tunnel for the ova or spermatozoon. Anatomically, this tunnel can be distinguished in three different portions, i.e. ampulla, isthmus and intramural portion (Hunumel *et al.*., 1966). The ampulla begins with ciliated and fimbriated infundibulum near the ovarium bursa. The ampulla appears to be an expandable sac in which the ova may accumulate before fertilisation and it is not highly ciliated (Rugh, 1967). The isthmus is a long, narrow, tightly and coiled tube lined with simple, low columnar non-ciliated epithelium which exhibits rhythmic peristaltic contraction during transportation of the ova. The last, intramural portion, joins the uterine horn to the oviduct within the uterine wall.

The uterus:

The uterus consists of two tubular lateral horns (cornua) forming a Y-shaped structure with a single median body (corpus). Each horn is attached to the dorsal body wall by the heavy broad ligaments (mesometria) through which blood and lymph vessels and nerves course at regular intervals. The body of the uterus consists of a cranial and a caudal or cervix portion.

The vagina and clitoris:

The vagina is a short extension of the corpus uteri and cervix with an external opening anterior to the anus on the ventral body surface.

b. Reproduction of the Mouse

Mice are polyestrous and spontaneous ovulators. Under normal diurnal light cycles they ovulate and exhibit oestrus, including mating behaviour, every 4 or 5 days. Periodicity of oestrous is a direct result of ovarian cyclic changes and this cycle is a reflection of the alteration in hypothalamus activity and changes in gonadotropin secretion. The key role in cyclic reproductive activity is played by the hypothalamus.

Apart from the litter size and strain differences, the length of gestation is affected by the reproductive state of the animal. In the non-suckling mouse the gestation period lasts 19 or 20 days, but in the mated suckling female this period is regularly lengthened for a significant period of time (Snell, 1941; Gruneberg, 1952; Rugh, 1967). Prolongation of gestation in suckling females mated at the post-partum oestrous seems affected by parity. The multiparae female has an additional 6 - 16 days of gestation beyond 19, whereas the primipara female has 8 - 13 days (Gruneberg, 1952).

The female mouse is sexually mature at about 30 days of age. Sexual maturity is characterised by the opening of the vagina, cornification of the vagina, mating capability (the first mating), rate of pregnancy after the first mating (Bronson *et al.,* 1966) and a maximal growth rate (Cheek and Holt, 1963). Although the majority of females gave birth to the first litter at about 3 months of age, many of them can give birth before the age of 2 months (Gruneberg, 1952). The highest receptivity of the female to the male occurs at the period of oestrus (heat) but mating may also take place during proestrous or metestrous-1. However, the most successful mating occurs at oestrus, followed by proestrous, late oestrus and metestrous-1 with the rate of mating (characterised by the vaginal plug) of 83%, 57%, 36% and 22% respectively and almost 80 to 90% of mice become pregnant (Bronson *et al.*, 1966).

Litter size depends on the number of eggs ovulated and the rate of prenatal mortality (Snell, 1941; Gruneberg, 1952; Rugh, 1967). Both of these factors seem to be affected by the strain, parity, age, health and vigour of the mother (Snell, 1941; Gruneberg, 1952; Rugh, 1968). The normal litter size averages between 10 and 11 (range: 1 - 19) in CFI-S strain (Rugh, 1967), or 5 to 6 (range: 1 - 14) in the *dba* strain (Gruneberg, 1952) or 7.4 (range: 2 - 12) in a random bred strain (Snell, 1941). The first litter is generally the smallest and the third or fourth or fifth the largest; thereafter, litter size gradually declines, while the variability increases (Gruneberg, 1952; Rugh, 1967). Multiparous mice of 7 to 9 months of age have more implantation sites than those of virgin mice, either young or older, while ex-breeders have the smallest average number of implantation sites (Rugh, 1967). Several authors have reported that litter size is more affected by parity than maternal age; if these two variables are held constant, maternal weight also affects litter size (see Gruneberg, 1952). This difference in litter size may be explained by the pre-implantation loss since embryonic pre-implantation loss is correlated with parity and with the weight of the mother but only slightly correlated with age of the mother (Snell, 1941).

The theoretical males/females sex ratio of 1:1 of the offspring is rarely found even in a large normal population. The number of males is usually higher than females (Snell, 1941; Gruneberg, 1952; Rugh, 1967). Rugh (1967) postulated that this imbalance in favour of males may be related to the size of the Y chromosome, which is slightly smaller than the X chromosome. However, a differential prenatal mortality between the two sexes, the diet given to the mother (especially in a pure line) and seasonal conditions when the mother gave birth may also cause a disturbed sex ratio at birth (Gruneberg, 1952).

The two sexes can be easily distinguished at birth by morphological appearance. The ano-genital papilla distance is greater in males than females, and males are generally larger and heavier than females (Gruneberg, 1952; Rugh, 1967). Morphological differences between the two sexes become more clear by the age of about 8 to 10 days, when the nipples of the female appear. At a similar age, females are slenderer than males; males are aggressive and females are docile (Gruneberg, 1952; Rugh, 1967).

Rugh (1967) noted that the growth of the placenta, which in turn affects the growth of the embryo, seems to be affected by the position in the horn, since embryos implanted nearest the cervix appear to be resorbed more frequently, lighter in weight and more retarded in development with the average size of the placenta smaller than that of other positions. However, contradictory evidence has also been found by Hashima (1956) and McLaren and Michie (1960) who recorded that the smallest fetuses were located in the middle region, not in the cervix end, of the uterine horn. A similar trend was observed in guinea-pigs (Eckstein *et al.*, 1955) and rabbits (Bruce and Abdul-Karim, 1973).

1.1.2 Utero-ovarian Relationships

1.1.2.1 Anatomical Relationships

The anatomical basis by which the ovary and the uterus can communicate is facilitated by the blood vessels, lymphatics and nerve systems (Baird, 1984).

The blood vessels:

In species with a bicornuate uterus, such as mice, each horn of the uterus is supplied with blood from an artery formed by the anastomosis of the uterine and ovarian artery.

The characteristics of the vascular anatomy of the uterus and ovaries in some laboratory animals has been studied by DelCampo *et al.* (1972). They found that the uterine artery provided a major portion of the ovarian blood. Much of the uterine venous blood drains cranially into a common trunk, which drains both the uterus and ovaries in the guinea pig, rat and hamster. In the rabbit, however, the uterine artery provides only a minor portion of the ovarian blood and most of the uterine blood drains caudally. Unfortunately, little detailed work has been reported on the vascular anatomy of the mouse reproductive system, especially on the utero-ovarian point.

The lymphatic vessels:

The lymphatic vessel enters the uterus and creates a main plexus between the circular and longitudinal muscle layer in the rat or in the subserosa, muscularis and junction of the muscularis and endometrium in the rabbit and rat. The plexus then sends small vessels into the serosa, and in some species into the endometrium (Finn and Porter, 1975). In the rat, the lymphatic vessels of the uterine horns and ovary commonly fuse into dual vessels which run parallel to the ovarian blood vessels (Finn and Porter, 1975). These vessels drain into lymphatic node renalis. Caudal parts of the uterus and vagina are drained by afferent vessels of another group of lymphatic nodes. The myometrium contains an extensive plexus of lymphatic capillaries; however, the endometrium contains only a few vessels along the base of the uterine glands (Hebel and Stromberg, 1986).

The nervous system:

Both parasympathetic and sympathetic nerves terminate in the ovary, but their importance in regulating ovarian function is not known.

1.1.2.2 Functional Relationships

The functional relationships between the uterus and the ovary, whether in cyclic, pseudopregnant or pregnant animals have been studied extensively in some species. Total or partial uterine removal, ovariectomy, or the cutting or blocking of the physical relationship between the uterus and ovary were the usual techniques used. The results of these studies have been reviewed by several authors (Bland and Donovan, 1966; Anderson, 1973; 1977; Hilliard, 1973; Finn and Porter, 1975; Niswender and Nett, 1988).

How the uterine horn regulates ovarian function, and vice versa, has attracted attention for many years. It is well known that hysterectomy extends the duration of luteal function (Perry and Rowland, 1961; Melampy *et al.*, 1964; Butcher *et al.*, 1969; Malven *et al.*, 1967). Both unilateral hysterectomy and unilateral hysterectomy combined with contralateral ovariectomy extends the luteal life span of the rat (Butcher *et al.*, 1969; Anderson, 1973). Further, it has been found that the quantity of tissue removed at hysterectomy is important in determining the final duration of luteal life or pseudopregnancy (Bland and Donovan, 1966; Butcher *et al.*, 1969). Butcher *et al.* (1969) also observed that the luteolytic effect of the nongravid uterus had a local component which was dependent upon the physical connection between the uterine horn and ovary.

Based on their observation on the hysterectomized rat, Melampy *et al.* (1964) and Malven *et al.* (1967) suggested that the uterus plays a role in determining the duration of luteal function indirectly by reducing the effect of LH secreted by the pituitary. This was later confirmed by Macdonald *et al.* (1970) who reported that the duration of luteal function of hypophysectomised rats treated with prolactin and LH was moderately extended by hysterectomy. However, Christian *et al.* (1968) suggested that the uterus may exert its luteolytic effect directly on the CL rather than by affecting the secretion of LH.

In the rabbit and hamster, hysterectomy prolongs the duration of pseudopregnancy, but has no effect on the periodicity of the oestrus cycle (Bland and Donovan, 1966; Anderson, 1973). Furthermore, Kelley and Brinkley (1971) found that the uterus not only influences the regression of the CL of pseudopregnant rabbits but may also influence luteal development. Hysterectomy in cyclic guinea pigs results in prolongation of the luteal phase of the cycle, and the time of the cycle at hysterectomy determines the duration of progesterone secretion. The earlier the uterus is removed, the longer the CL will be functional (Rowlands, 1961). However, in the dog, luteal regression occurs independently of a uterine luteolysin, but it may play a role in control of duration of anoestrus (Hoffmann *et al.*, 1992).

In the mouse, hysterectomy has no significant effect on subsequent cycle, on the duration of pseudopregnancy, or on other characteristics of pseudopregnancy (Dewar, 1973). Even up to day 8 of pregnancy luteal function was independent of the presence or absence of the uterus. However, removal of the placenta after day 14 of pregnancy

was followed by the cessation of luteal function (with the possible exception of the 18th day) (Dewar, 1973).

A stimulus from the uterus of non-fertile cycles in the beef heifer and its effect on the change of active CL into the corpora albicantia was found by Anderson *et al.* (1962). In the ewe, hysterectomy alters the pattern of plasma progesterone and prevents the premature regression of the CL (Southee *et al.*, 1988). In the latter case, secretory activity of the CL is reduced by the time of hysterectomy.

In humans and primates, some contradictory effects of uterine removal on the ovarian function have been found. On the one hand, some authors consider that hysterectomy can cause the ovarian function to cease and can stimulate early menopause (for review, see Bland and Donovan, 1966). Conversely, other investigators have failed to find evidence of depressed gonadal activity after hysterectomy (Neill *et al.*, 1969) and argue that the resulting abnormalities in the previous studies were due to inadvertent interference with the blood vessels or nerves of the ovary (Anderson, 1973; 1977). These authors, however, have reached the same conclusion that hysterectomy alters neither the menstrual cycle nor the onset of menopause. Quite recently, Metcalf *et al.* (1992) after studying the ovarian function on hysterectomized women over a long period of observation reported that the ovaries of woman without uterii are physiologically similar to intact women.

How the luteal function can be affected by the uterine factors was examined by several investigators (for review, see Heap and Flint, 1984). In the rat the life of the CL of pregnancy is initially extended by the secretion of pituitary luteotrophin at the time of mating and supported by the placenta after the first half of the gestation period (Heap and Flint, 1984). The life of the CL can also be extended by an antiluteolytic stimulus sent by the conspectus to inhibit the normal luteolytic action of the uterus mediated by PGF_{2a}, as occurs in sheep (Weems *et al.*, 1992). In human beings the placental secretion of a luteotrophin substance (human chorionic gonadotrophin, hCG) which also has an antiluteolytic action.

It can be summarised that, although there is considerable variation in its effects on mammals, uterine removal results in prolongation of the luteal function in cyclic and/or pseudopregnant animals. This suggests that uterine tissue plays a role in regulating luteal regression at the end of the oestrus cycle or pseudopregnancy, and thus is an

important factor in maintaining luteal activity in the absence of pregnancy. Furthermore, the regulatory function of the uterine horn upon luteal activity is time dependent. Thus the reproductive state of females on which uterine removal is performed is critical in determining whether the CL will regress normally or remain functional. In addition, in many species, regulation of luteal activity by the uterine tissue is exerted locally.

1.1.2.3 Functional Relationships During Pregnancy

To maintain a uterine environment conducive to pregnancy, progesterone must be secreted throughout gestation and the CL of pregnancy must survive and grow larger than the CL of the normal cycle (Hilliard, 1973). In mice, placental progesterone secretion is apparently of less importance for the maintenance of pregnancy than it is in many other species since gonadectomy at any time during gestation is followed by the termination of pregnancy (Hall, 1957; Bronson *et al.*, 1966).

Luteal growth, function, and regression, as well as the maintenance of pregnancy, depends upon complex relationships between ovary, pituitary, uterus and conspectus. However, there are differences in each species in terms of the relative importance of these components. The ovary (rat and mouse), both the placenta and pituitary (hamster), the uterus (guinea pig), and both the ovary and pituitary (rabbit) play a dominant role in maintaining luteal function during pregnancy (Hilliard, 1973). However, in all five species the role of the conspectus is important in sustaining the luteal function and the luteotrophic effect is exerted either by preventing the action of prostaglandin or by inhibiting its production (Hilliard, 1973).

It is well known that the role of the pituitary during pregnancy is different in different species. Although in all species hypophysectomy before implantation results in termination of pregnancy, and hypophysectomy before midpregnancy frequently leads to fetal death and resorption due to the loss of anterior lobe secretion, removal of the pituitary in the second half of gestation has a different effect in different species (Heap *et al.*, 1973; Hilliard, 1973). In the pregnant mouse, the pituitary is required for the first half of the gestation period only, and the conspectus has developed a functional relationship with the endometrium as early as day 5 of pregnancy (Hilliard, 1973). In this species and another animals such as the rat, guinea pig and sheep, pregnancy is maintained when the pituitary is removed after midpregnancy. In these four species, especially in the rat, the main components of the pituitary luteotrophic hormones during

the first half of gestation are prolactin and LH; these are then supplemented by a placental luteotrophic hormone during the second half of gestation. Prolactin and placental lactogen are needed to maintain synthesis of oestradiol, which is the determinant factor in maintaining progesterone secretion (Heap *et al.*, 1973; Macdonald, 1978; Heap and Flint, 1984).

In the rabbit, the role of the luteotrophic hormone complex, which consists of prolactin, FSH, and possibly a low level of LH is to stimulate the follicles to secrete oestrogens, which have a direct trophic influence on luteal cells, prolonging their life and promoting progesterone secretion (Heap and Flint, 1984). Since hypophysectomy is followed by abortion, it indicates that in this species luteotrophic hormone derives solely from the pituitary and the placenta does not secrete an adequate amount of progesterone to maintain pregnancy.

In human females and primates, either the pituitary or the ovaries are not essential from a relatively early stage of gestation and the main site of progesterone synthesis for the greater part of gestation is in the placenta (Heap *et al.*,1973). The maintenance of the CL of pregnancy probably depends initially on the secretion of human chorionic gonadotrophin (hCG) by the trophoblast shortly after implantation. A placental lactogen (hPL) may also be involved later in gestation (Heap and Flint, 1984).

It is clear that in all these species the pituitary has a critical role in the initial events of pregnancy but, in some species the subsequent endocrine role of the pituitary in pregnancy maintenance is transferred to the placenta.

1.1.3 Progesterone and Pregnancy

1.1.3.1 The Importance of Progesterone During Pregnancy

Progesterone is the most potent of the progestagens (Heap *et al.*, 1973; Mauvais-Jarvis, 1983; Johnson and Everitt, 1995) and is also the most important hormone in pregnancy. So far as we know, there is no species in which pregnancy can be maintained in the total absence of progesterone (Heap *et al.*, 1973; Heap and Flint, 1984) because the onset, maintenance and termination of pregnancy, especially in mammals, are regulated by changes in the progesterone to oestradiol concentrations ratio (Raziano *et al.*, 1972; Heap *et al.* 1973; Ryan, 1973). Since pseudopregnancy in mice is only 10 to 12 days in

length (Bartke, 1970; Dewar, 1973), successful pregnancy for 18 to 19 days requires some factors that prolong the functional life span of progesterone-secreting organs.

The principal functions of progesterone during pregnancy are to prepare and maintain the uterine environment conducive to the growth and development of the conceptus, and to stimulate the growth of mammary glands but, at the same time, to suppress milk secretion (Simmer, 1968; Heap *et al.*, 1973; Johnson and Everitt, 1995). The observation that immunisation of the female against progesterone before and during implantation periods in mice (Wright *et al.*, 1982) or before and after implantation in rats (Raziano *et al.*, 1972) prevents implantation or interferes with pregnancy suggests the critical role of progesterone. The key roles of progesterone in maintaining pregnancy after implantation may be exerted by one or more of the following actions: 1) inhibition of myometrial contractions, 2) prevention of immunologic rejection of the embryo, 3) suppression of endometrial PG production, and 4) maintenance of uterine growth and plasticity (Rothchild, 1983).

Several researchers have studied the progesterone profiles of the circulating blood in intact pregnant mice (McCormack and Greenwald, 1974a; Murr *et al.*, 1974; Virgo and Bellward, 1974; Pointis *et al.*, 1981). The progesterone profiles observed by these researchers are remarkably similar. In general, progesterone concentration shows a bimodal curve pattern during pregnancy. The concentration reaches the first peak on day 4 (Virgo and Bellward, 1974) or day 6 (McCormack and Greenwald, 1974a) or day 7 (Murr *et al.*, 1974) and the second peak, which is the highest during gestation period, on day 15 (Murr *et al.*, 1974) or day 16 (McCormack and Greenwald, 1974a; Virgo and Bellward, 1974) and between the two peaks, at about day 10 to 11, progesterone decreases to a significantly low level. In all these observations, the profile always begins with low levels during the first 2 days and terminates with approximately the same levels on the day of parturition.

The low levels of progesterone on days 1 and 2 correlated with the minimum weights and morphological (McCormack and Greenwald, 1974b) and functional development (Finn and Martin, 1971) of the CL. Finn and Martin (1971) proposed that either the CL may not be sufficiently developed to secrete progesterone before day 3, that some other factor may be involved in the initiation of progesterone secretion, or that there is a delayed time of up to 48 hrs between prolactin reaching the ovary and the secretion of progesterone. The increase in the progesterone concentration during the first half of

pregnancy is needed to induce and maintain decidualization (Virgo and Bellward, 1974) and the decrease in the middle of gestation may represent a decrease in steroidogenesis due to the transitory status from a pituitary to a placental source of luteotrophin (McCormack and Greenwald, 1974a). The increase in progesterone concentration, reaching a peak on day 15 or 16 of pregnancy, reflects the additive effect of placental and pituitary sources of luteotrophin (Finn and Martin, 1971; McCormack and Greenwald, 1974a; Virgo and Bellward, 1974). These researchers also agree that progesterone withdrawal at the end of pregnancy is a prerequisite for parturition.

Similar changes in progesterone concentrations during pregnancy in mice have been observed in the rat (Grota and Eik-Nes, 1967; Wiest, 1970; Morishige *et al.*, 1973; Pepe and Rothchild, 1974). Progesterone concentration changes in the rat are inversely related to the serum luteinizing hormone (LH) concentration during the period from day 11 to term (Morishige *et al.* 1973).

1.1.3.2 The Sources of Progesterone

In the non-pregnant female animal progesterone is biosynthesized in the ovary and adrenal cortex. In the pregnant female an additional and temporary source of progesterone, the placenta, is formed. Biosynthesis of progesterone in the placenta reveals some significant differences from that in the ovary and adrenal cortex, but the steroids formed are chemically identical with those produced elsewhere (Heap *et al.*, 1973).

a. The ovary

The observation that pregnancy in mice or rats is terminated by the removal of the ovaries or destruction of the CL at any stage of pregnancy unless exogenous progesterone is provided (Bronson *et al.*, 1966; Jaitly *et al.*, 1966; Csapo and Wiest, 1969; MacDonald, 1978) suggests that the ovaries are the principal source of progesterone during the entire period of gestation (Pointis *et al.*, 1981). Although the placenta synthesises progesterone during the second half of gestation, its contribution to overall maternal progesterone concentration is small compared with the ovarian contribution in the mouse (Pointis *et al., 1981*) and pig (Kensinger *et al.*, 1986). In contrast to this, the role of the extra-ovarian source of progesterone is very important in the maintenance of gestation in the human being, monkey, ewe, mare and guinea pig,

since bilateral ovariectomy after definite stages of gestation does not interfere with pregnancy in many species (see Simmer, 1968; Allen, 1975).

Ovarian progesterone can be synthesised in the follicle, interstitial and luteal tissue (Heap *et al.*, 1973). However, the main source of ovarian progesterone is the CL tissue (Elbaum *et al.*, 1975; Heap *et al.*, 1973; Mauvais-Jarvis, 1983). Elbaum *et al.* (1975) observed that total CL weight is significantly correlated with serum progesterone concentration on day 16 of pregnancy of the rat. In addition, the prominence of the progesterone-secreting interstitial tissue varies greatly in different species. For example, this tissue is very prominent in the ovaries of mice, rats and rabbits but absent in the ovary of large domestic animals such as the cow, ewe, sow, and mare (Hansel *et al.*, 1973; Heap *et al.*, 1973).

Luteal biosynthesis of progesterone is regulated by hormones or substances originating from both the pituitary gland and the uterus. For example, both gonadotrophin and prostaglandins (PGs) exert dual effects, i.e. stimulation and inhibition, on luteal progesterone biosynthesis (reviewed by Dorfman, 1973; Hansel *et al.*, 1973). In the *in vitro* system, progesterone secretion of the ovary is higher after stimulation of the LH in the cow; and progesterone secretion in the rabbit increases in the presence of PGE_2 (Dorfman, 1973; Hansel *et al.*, 1973). However, several authors reported a luteolytic effect of PGs, especially $PGF_{2\alpha}$, in the *in vivo* system (Dorfman, 1973; Hansel *et al.*, 1973). In addition, PGs did not stimulate the progesterone synthesis when added to saturating amounts of LH or hCG (Dorfman, 1973). It has also been reported that a small dose of $PGF_{2\alpha}$ injected into the ovarian bursa of a pseudopregnant hamster resulted in stimulation of luteal function (luteotrophic effect) while a single larger dose produced a depression (luteolytic) effect (Lukaszewska *et al.*, 1972). More recently, Mauvais-Jarvis (1983) observed that the stimulating effect of LH on progesterone synthesis and secretion by the CL is mediated by an increased synthesis of cAMP.

b. The placenta

The fact that placental pregnancy can be induced by removing the fetuses from the uterus of the rabbit, rat, cat and rhesus monkey (see Allen, 1975), and that the removal of either the fetuses or the placenta at delivery resulted in a decrease in progesterone concentration (Grota and Eik-Nes, 1967) suggests that the endocrine function of the placenta is important in maintenance of gestation. In the guinea pigs, in fact, placental progesterone is sufficient to support and to continue pregnancy after bilateral

ovariectomy at day 28 of pregnancy (Heap and Deanesly, 1966). In the rat, the fetal placenta is responsible for the secretion of an enzyme inhibitor, possibly a placental LTH, that regulates the activity of ovarian 20α-OH-SDH (Heap *et al.*, 1973). The latter hormone has a key position in the control of ovarian steroid biogenesis. It is also reported that the placenta not only contains enzymes for steroid synthesis but it also synthesises a small quantity of progesterone during the second half of pregnancy in the mouse (Salomon and Sherman, 1975; Pointis *et al.*, 1981; Kensinger *et al.*, 1986) or in the guinea pig (Heap and Deanesly, 1966).

In the human being the placenta is the main source of progesterone during the gestation period. Some evidence for this conclusion has been provided by Simmer (1968). Firstly, there is a positive relationship between peripheral serum concentration of progesterone and placental size. Secondly, hypophysectomy, oophorectomy, and adrenalectomy treatments fail to induce a significant change in pregnanediol levels. Finally, progesterone concentration is higher in the umbilical and uterine venous blood than in the corresponding arterial or venous blood of the mother at term. Placental progesterone of the pig, goat and rabbit, however, is never produced in sufficient quantities to maintain gestation in the absence of the ovaries (Heap and Flint, 1984).

Although placental contribution on the maternal progesterone concentration is relatively small in several species (rat: Elbaum *et al.*, 1975; pig: Kensinger *et al.*, 1986), the placenta has another means of determining maternal progesterone concentration since it can stimulate CL to grow and increases its rate of progesterone secretion by stimulating the production of intraluteal oestrogen (Kato *et al.*, 1979). A direct relationship between the number of conceptuses, the size of the CL and the serum progesterone concentration on day 15 of pregnancy has been reported by Kato *et al.* (1979).

The importance of placental endocrine function during gestation period can be interpreted from the experimental results reviewed by Heap *et al.* (1973). Hysterectomy results in termination of the CL function in the rabbit, rat, hamster, guinea pig and cat, and removal of the fetuses alone, while the placenta left *in situ* does not alter the course of pregnancy in the rabbit, rat, mouse, monkey and cat (Heap *et al.*, 1973). In addition, observations on mice revealed that plasma progesterone concentrations increase in the second half of gestation (Pointis *et al.*, 1981) where these values are higher in mice selected for large litters than for small (Michael *et al.*, 1975) or are proportionally related to litter size (Soares and Talamantes, 1983; Humphreys *et al.*, 1985). In addition, the

uteroovarian venous blood ratio of progesterone concentrations during the second half of gestation in unilaterally ovariectomized mice is increased, ranging between 0.006 (at day 13) to 0.04 ng/ml plasma (day 16 - 17) (Pointis *et al.*, 1981). Progesterone concentration in the ovarian venous blood ranges between 958 - 3096 ng/ml (with the maximum values reached at day 13 and the minimum at day 18) and in the uterine venous blood ranges between 17.7 - 114.9 ng/ml (with the maximum values reached at day 17 and the minimum at day 13). Progesterone concentrations in the peripheral plasma in the same period of gestation never exceed a value of 25 ng/ml (Pointis *et al.*, 1981). This information suggests that the placenta has an endocrine function during pregnancy and that placental endocrine function is increased when the ovarian function is decreased.

c. The adrenal

The fact that maternal adrenals of many species are capable of producing appreciable amounts of gonadal steroids including androgens, oestrogens, and progestagens has been reported by several investigators as reviewed by Heap *et al.* (1973). More recently, Macdonald and Matt (1984) measured the contribution of the adrenal on steroid (androgen and progestin) secretion in pregnant rats and suggested that the adrenal cortex contributes to the placental progesterone pool during pregnancy. They observed that adrenalectomy caused lower levels of serum progesterone on days 10, 14, 16, and 18 of pregnancy. However, whether they make a significant contribution to pregnancy maintenance or not remains in question. The adrenal secretion of progestagens and other gonadal steroids is high under conditions of stress or under stimulation of ACTH but adrenalectomy does not result in termination of pregnancy in the rat, cat, dog, and ferret (Heap *et al.*, 1973).

1.1.3.3 The Rate of Progesterone Secretion

The rate of progesterone secretion seems to be different in different stages of the reproductive cycle or of pregnancy. For example, the rate of secretion in early pregnancy in the rabbit is about 4.37 µg/min, whereas the ovaries in late pregnancy secrete about 4.57 µg/min. Stimulation of LH can double the secretion rate to 7.43 and 7.72 µg progesterone per minute for early and late pregnancy, respectively. In the cyclic rat, ovarian venous blood concentrations vary between 0.02 to 0.08 µg/ovary/min with a maximal levels reached in proestrous (Heap *et al.*, 1973). In the pregnant mare the production rate of progesterone in the ovary is about 300 pg/cell/day with a secretion

rate of about 78.7 μg/min, and in the pregnant guinea-pig the values are 419 pg/cell/day and 0.83 μg/min for production and secretion rates respectively (for details see Dorfman, 1973). In pregnant goats, the secretion rate is dependent upon the number of CL in the ovaries. For example, there is 4.2 μg/min in the ovary with single CL and 7.2 μg/min in one with three CL (Linzell and Heap, 1968). In sheep, however, this CL-dependent progesterone secretion rate can not be detected (Linzell and Heap, 1968). It is suggested that in ovary-dominated animals (the ovary as a main source of progesterone during pregnancy), but not in placenta-dominated animals, the ovarian progesterone secretion rate is closely related to the number of CL.

The maximum concentration of progesterone produced by the rat placenta is 0.02 μg/g placenta (Heap *et al.*, 1973). Placental secretion rate in sheep is about 9.7 μg/min but the placenta does not secrete progesterone in goats (Linzell and Heap, 1968). In humans, the rate varies between 0.06-0.22 μg/min (these values are summarised from several authors who measurde the rate at different stages of pregnancy with different methods of measurement) (Simmer, 1968). In general, the secretion rate of the placenta is increased with the age of gestation.

In the goat, adrenal secretion of progesterone can make a small but significant contribution during pregnancy in the range of 60 - 415 ng/min (about 1 - 10% of that from the CL) but in the sheep the adrenal secretion rate is very low, valued between 0.7 - 2.8 ng/min (Linzell and Heap, 1968).

1.1.4 Prenatal Growth

According to Cockburn (1989) fetal growth is governed by the rate of cell division in different cells and tissues, and total size reflects changes in cell number rather than changes in cell size or the quantity of extracellular materials. Some of the factors affecting the prenatal growth rate are uterine size (uterine environment), genetic potential, maternal health and nutrition, blood supply and placental function. Of these, the major determinant is the uterine environment (Brumby, 1960; McCarthy, 1965; Cockburn, 1989). Brumby (1960) noted that sex linked genes were not responsible for any marked effect on body size. However, Moore *et al.* (1970) and Aitken *et al.* (1977) found that greater control on prenatal growth was exerted by the embryonic genotype and that maternal effects were of minor importance. However, both Brumby (1960) and

Moore *et al.* (1970) agree that genetic factors have a greater effect in regulating postnatal growth than that of uterine or maternal effects.

The influence of non-genetic maternal factors on prenatal growth have been studied extensively. For example, a constraining influence of the uterine horn on the growth of fetus has been reported by Healy (1960) and McCarthy (1965). Fetal growth was negatively correlated with both the number of implants in the same horn (local effect) and the number of implants in the whole litter (systemic effect) (McCarthy, 1965). Furthermore, Healy (1960) reported that the fetal position in the horn, the quantity of the nutrient taken up from maternal blood circulating, the size of placenta, the blood pressure at which maternal blood reaches the placenta and the growth of the placenta itself all have a great effect on fetal growth.

There are two stages at which maternal factors affect the growth of offspring, i.e. 1) Prenatal maternal effects, when the fetus is entirely dependent upon its mother nutrition, and 2) Postnatal maternal effects, where the new born offspring is entirely dependent upon maternal care (maternal milk supply). The effect of both prenatal and postnatal maternal factors on total body weight tends to decrease with the age of the offspring, however, the effect of the genotype tends to increase (El Oksh *et al.*, 1967).

In his review on the effect of genetic factors on prenatal growth, Snow (1989) draws two important conclusions. Firstly, genetic constitution is the most important factor in the control of embryonic growth (although environmental factors have an impact). Secondly, for successful growth an interaction between embryonic and maternal genome is needed. The maternal genotype may act on the embryo in two ways, i.e. it may affect the development of the oocyte and the ability of the uterus to support embryonic/fetal development.

In most litter-bearing mammalian species prenatal growth depends on the litter size. Fetal weight is negatively related to litter size in guinea pigs (Eckstein and McKeown, 1955b), mice (McLaren and Michie, 1960; Healy, 1960; McLaren, 1965) and rats (Barr *et al.*, 1970). This effect of litter size is due to the limitation of nutrients available in the shared maternal blood stream. Eckstein (1955, in Healy, 1960), on the basis of observations on the rabbit, suggested a theory of competition to explain the effect of intrauterine overcrowding on fetal growth: 1) Fetal size is positively correlated with placental size, 2) Placental size is affected by the number of implants through both local

and systemic influences, 3) Fetal size is affected by the fetal number, both locally and systemically, and finally 4) There is a blood supply limitation to the uterus.

Besides the size of litter, prenatal growth is also affected by other factors such as maternal metabolism and fetal metabolism as well, and placental regulation of nutrient production and its transportation to the fetus (Healy, 1960; Jones, 1976). The effect of maternal metabolism on prenatal growth can be seen in the occurrence of fetal overgrowth in the diabetic pregnancy. Conversely, either the reduction of food intake during pregnancy or surgical alteration of uterine blood flow to the placenta or to the umbilical blood flow to the fetus, is associated with intra-uterine growth retardation (Jones, 1976).

Transportation and hormonal function of the placenta has a significant effect on prenatal growth. Because the quantity of nutrients taken up from the maternal blood is under placental regulation, the placental size and maternal blood pressure reaching the placenta are also factors which should be considered in studying the prenatal growth. The provision of nutrition from the mother to the fetus is controlled by the concentration of nutrients in the maternal circulation and the blood supply to the placenta (Jones, 1976). The reduction in placental mass, leading to decreased maternal blood flow, reduces the nutrients available for fetal growth. The composition of the nutrient supply to the fetus may also be influenced by the placental metabolism. The placenta has the capacity for amino acid metabolism and inter-conversion, substantial lactate production and the synthesis of lipids (Jones, 1976). Placental hormones may exert effects on maternal metabolism that in turn can promote fetal growth, eg. a shift in the glucose supply to the fetus or an increase in free fatty acids late in gestation.

Although genetic and environmental influences are also important factors in regulating prenatal growth, there is considerable variation between mammals (McKeown *et al.*, 1976). Birth weight in species where the reserves of the uterus are sufficient to support the full growth of the fetus to the end of pregnancy is determined by the fetal genes and is related to the size of both parents. However, in species where the capacity of the uterus is more limited, maternal influences predominate in determining the rate of fetal growth. In these species weight at birth is related to the size of the mother rather than to that of the father (McKeown *et al.*, 1976).

Endocrine and metabolic factors interact in a complex fashion in controlling fetal growth. Multiple pregnancy is associated with a reduction in placental and fetal size. Chromosomal or antigenic dissimilarity seem to play a minor role (Dawes, 1976). However, the fact that there are gender difference in prenatal growth suggests that the antigenic dissimilarity between mother and fetus plays an important role in regulation of prenatal growth (Snow, 1989).

Maternal factor and prenatal growth

Maternal influences primarily determine the rate of fetal growth only in species where the reserves of the uterus are insufficient to support the full growth of the fetus to the end of pregnancy. In species where the uterus can support the full growth of the fetus, birth weight is determined by the fetal genes and is related to the size of both parents (McKeown *et al.*, 1976).

Uterine size

In multiple pregnancy, birth weight is related to litter size: there is a tendency for growth retardation in large litters. McKeown *et al.* (1976) have proposed three explanations for growth retardation: 1) lack of space, 2) limitation of nutrients, and 3) restriction of the channel. The last explanation (restriction of channel) was made on the basis of an observations on feto-placental relationships.

Maternal health and nutrition

Some reviews have described how the maternal health and nutritional state affects prenatal growth (Widdowson, 1968; Eisen, 1976). In the earlier stages of gestation, food and accommodation in the uterus are not limiting factors. However, in the later stages the number of young sharing the uterine blood supply can have a profound effect upon size at birth. Nutrition of the mother during pregnancy and lactation has a profound effect on the growth of her progeny. The fact that litter size is inversely related to the weight of the fetus is an indication of the prenatal competition that occurs among the young for the limited supply of nutrients in the maternal circulation.

Blood supply

As mentioned previously, the nutritional state of the mother plays an important role in regulating the growth of the young *in utero*. Conversely, in mice, there is evidence that fetal weight is unaffected by the nutritional state of the mother (Healy, 1960), and there are several aspects of the prenatal growth pattern which cannot be described solely on the basis of competition for nutrition (McLaren and Michie, 1960). For example, in mice, McLaren (1965) found that fetal growth was affected by both local and systemic factors and that there was a causal dependence of fetal growth on placental size. The importance of haemodynamic factors in relation to prenatal growth has been suggested by several authors (Eckstein and McKeown, 1955b; McKeown *et al.*, 1953; Healy, 1960; McLaren and Michie, 1960; McLaren, 1965). They believe that haemodynamic factors might be responsible for the effect of position in the horn on placental growth, and hence on fetal growth, and the systemic effect of litter size on fetal and placental growth. Also, both the pressure at which maternal blood reaches the placenta and the size of placenta are important in determining the quantity of nutrients available for fetal growth. More recently, Even *et al.* (1994) examined the relationships between fetal body weight and both uterine and placental blood flow in the rat. They found that blood flow was greater at the cervical and ovarian end than in the middle region of the uterus, but they failed to find a significant difference in the pattern of fetal weight. Although variations in blood pressure down the length of the horn do not directly affect the growth of the fetus (McLaren, 1965; Bruce, 1976), it has been found that both placental and fetal size are limited by the rate of utero-placental blood flow (Clapp, 1989).

Placental function

The growth hormone releasing factor (GHRF), originally identified in the hypothalamus, has been found in the placenta of the rat, mouse (in which it is called mGHRF) and human but little is known about its function and even less is known about factors that regulate its production. Quite recently, Endo *et al.* (1994) reported that the production of mGHRF mRNA could be detected as early as day 11 of pregnancy with the maximum values on day 15-17, followed by a slight decline on day 18. Endo *et al.* (1994) suggested that the mGHRF gene expression in the placenta is regulated by intracellular cAMP concentration.

The placenta of a number of species produces polypeptides that are members of the prolactin (PRL)-growth hormone (GH) family. The best-studied members of this family

in the mouse are mouse placental lactogen (mPL)-I, mPL-II, and proliferin (PLF). The known biological activities of mPL-I and mPL-II are similar to those of PRL. The function of PLF has not been determined, although it is known to differ from that of the mPLs. Each of these three proteins is produced by giant cells at mid pregnancy. Yamaguchi *et al.* (1994) reported that the same giant trophoblast giant cells express these three proteins simultaneously at midpregnancy, so their gestational profiles in maternal blood during this period result at least partly from changes in gene expression in one population of cells and not from a differentiation of several subsets of giant cells, each expressing only one member of the gene family.

1.1.5 Growth of the Neonate

The prenatal and preweaning periods are probably the most critical in determining adult body weight and reproductive efficiency (Eisen, 1976). Prenatal and preweaning growth are affected by both internal factors (genotype of the fetus or neonate and birth weight of the neonate) and external factors, especially parental factors, litter mates and other environmental (physical) features (Hafez, 1963). Parental factors, primarily maternal factors affect the growth of neonates mainly by the mother's ability to produce and supply milk which, apart from genetic differences, is dependent on maternal age, parity, diet and many other variables (Gruneberg, 1952). Maternal behaviour including pup retrieval, licking, nursing and nest building also affecting the growth of neonates.

At the beginning of the weaning period the growth of the neonate is slow for a period of 14 to 15 days, but rapid growth is resumed after the change to solid food is completed (Gruneberg, 1952). No differences are observed in the growth rate of males and females until 4 weeks post-natal, but after this age, the growth rate of males becomes higher than females (Gruneberg, 1952).

1.1.6 Normal Histology of the Ovary and Uterus

1.1.6.1. The Ovary

Mammalian ovaries usually consist of cortical and medullary areas which are clearly identified. In the mouse, however, these areas are not easily identified (Gude *et al.*, 1982). The CL is the most prominent tissue in the ovary. This tissue is a complex, but temporary, endocrine organ of the ovary which develops from the ovulated follicle and secretes a relatively large amount of progesterone for maintenance of pregnancy. The

ovaries may contain many sets of CL. The most recent CL are easily distinguishable from the older ones by their blue colour, the latter stained more heavily with eosin.

The CL of pregnancy enlarges until about day 13 of pregnancy (mean diameter 976 mm), and this size remains constant until about day 18 when the CL starts to regress (Fekete, 1941). At days 10 - 12 of pregnancy all the CL present in the ovary (except those of pregnancy) rapidly degenerate, forming fibrous masses containing large fat globules.

The CL is formed by luteal cells, namely large luteal cells (originating from granulosa cells) and small luteal cells (originating from theca cells), and non luteal cells, namely vascular cells (endothelial cells, pericytes, macrophages, lymphocytes) and connective tissue cells (fibroblast or fibrocytes) (Gibori, 1993). On a volume basis, the CL of pregnancy of sheep consisted of large luteal cells (25 - 35%), small luteal cells (12 - 18%), (vascular elements (11%), connective tissue (22 - 29%) and fibroblasts (7 - 11%) (Niswender and Nett, 1988). Both small and large luteal cells differ not only in size but also in morphology. In the rat, for example, the small luteal cells (12 to 20 μ in diameter) are characterised by a large oval nucleus and a few lipid droplets while the large luteal cells (30 μ in diameter) have a small spherical nucleus and are filled with lipid droplets (Nelson *et al.*, 1992). In addition, large luteal cells are polyhedral in shape with a lightly staining cytoplasm while small luteal cells are spindle shape with a darkly staining cytoplasm (Niswender and Nett, 1988).

Although both small and large luteal cells respond to LH stimulation with an increase in steroidogenic output, the cell content of LH receptors is greater in the large than in the small luteal cells. The large luteal cells, with or without LH stimulation, are the principal source of steroids (Nelson *et al.*, 1992).

1.1.6.2 The Uterus

The endometrium or mucosa of the mouse uterus are composed of three main tissues, i.e. luminal epithelium, a simple columnar epithelium lining, branched tubular glands, and vascular connective tissue. The size, shape and internal organisation of the uterine endometrial cells are controlled to a large extent by the ovarian hormones. The number of glands in the endometrium varies according to the reproductive state of the animals. The myometrium consists of an inner circular and outer longitudinal layer of smooth

Uterine horn removal was performed on four different days of gestation, i.e. day 2, day 6, day 10, and day 14 in two different ways, either with its ipsilateral ovary or not. To access the effect of treatment, operated females were autopsied on several different days of gestation, i.e. day 3, day 7, day 10. and day 14; when fetal weight was measured. Additional observation was made on the uterine and placental growth after halving the size of litter (by removing one of the uterine horns).

To examine the hormonal effect of the treatment, plasma progesterone concentration was measured at an interval of 4 days during the gestation period and on the day of delivery. This observation was enriched by the histological examination of the ovary and uterine tissues collected on day 18 of pregnancy.

The effect of the removal of one uterine horn on the maternal responsiveness towards pups and on growth of the neonate was noted by observing maternal behaviour during the first 5 days post partum and by recording the weight of the neonate daily during the first 21 days after birth (preweaning period). Observations were made after reducing litter size by up to 4 young per litter (2 males and 2 females).

Chapter 2
General Methods

2.1 The Animals

Ten pairs of mice of the Quackenbush strain, aged between 3 to 4 months obtained from the Zoology Department, University of Tasmania, were propagated to produce a colony stock of animals for experimental purpose. The experiments were carried out between August 1994 and September 1995.

2.1.1 Breeding and Handling

Each female mouse was housed with a single fertile male. Pregnant females were then separated from the male at least one week before the expected parturition day. At the time of parturition, litters were sexed and weighed individually; female pups with an extremely low birth weight and most of the male pups were discarded at delivery. The remaining litters were then weighed three times a week during the preweaning period and separated from the parents (weaned) at 3 weeks of age. At 5 weeks of age, the sexes were caged separately into a colony stock. Pups with an abnormal increase in

body weight during the first five weeks from birth were discarded. The colony of offspring was used as the subject for these experiments.

Mice were housed in opaque plastic cages of two sizes, small (30 x 12 x 12 cm) or large (40 x 30 x 15 cm), fitted with stainless steel lids. The small cages were used for mating and experimental treatments and the large for the colony stock. The cages were washed once a week, and given a fresh 0.5 cm thick bedding of dry paper pellets. The cages were attached to the shelves of a mobile rack.

2.1.2 Mating and Judgement of Gestation

Virgin female mice from the colony aged between 2 to 3 months were used in all of the experiments (Figure 2.1). Proestrous or oestrous mice were placed overnight with males of the same age (2 females per male) at about 0500 to 0530 PM. Female mice, with or without a vaginal plug, were separated from the males on the following morning. Either the presence of the vaginal plug checked the subsequent morning or the increase in body weight several days after mating was used to judge the stage or age of pregnancy. The day of vaginal plug presence was designated as day 0 of pregnancy. For the females without a vaginal plug in the morning, but which showed an increase in body weight several days after mating, the stage or age pregnancy was determined from the day when separation was made. Before the operations pregnant females were kept in groups of three or four in small cages. After operation, however, females were kept separately in an individual cage until autopsy.

All animals were fed commercial laboratory pellets (Gibson Ltd, Hobart, Tasmania, Australia) and tap water *ad libitum*. Food and water were changed once a week at the time of cage cleaning, and at the same time the water bottles were washed. Old food or water was first discarded before being replaced with fresh food.

2.1.3 Room Environment

Breeding (animal propagation) colony stock and treated animals were kept in a temperature- and light-controlled room. Room temperature was maintained in the range of 19°C to 22°C or 20.41 ± 0.13 (mean ± SEM). Light (L) and dark (D) periods were scheduled so that there were 12L/12D hours with the light switched on at 06.00 AM and off at 06.00 PM. The only source of light was from two pairs of 35 watt fluorescent lamps regulated by an automatic timer. The humidity of the experimental

room was uncontrolled. The experimental room was kept clean and quiet. The animals were disturbed only for cage cleaning once a week, feeding and watering twice a week, mating and separating once a day, and inspecting the delivery. All observations were made in the daylight period.

Figure 2.1 An adult virgin female Quackenbush mouse (aged between 2 and 3 months) used in this study.

2.2 Experimental Design

This study was arranged in a 4 x 3 factorial design with the day of pregnancy (Day factor) and the type of operation technique applied (Operation factor) as the main factors.

The Day factor:

The day factor was the stage or age of pregnancy when the operation was performed. Mice were examined on four different days of pregnancy, i.e. day 2, day 6, day 10 and day 14.

The Operation factor:

The operation factor was set based on the type of operation applied for each animal. There were 3 levels of operation factor performed, i.e. one uterine horn was removed (unilateral uterectomy, UX), one uterine horn and its ipsilateral ovary were removed (unilateral utero-ovariectomy, UOX), and neither the uterine horn nor the ovary was removed (sham-operated control, SOP).

This experiment, therefore, consists of 4 x 3 = 12 treatment groups (Table 2.1). Pregnant mice were allocated *at random* to these 12 groups of 5 to 8 animals each.

Table 2.1 Two way table showing the factorial design used in the experiment. Each cell represents the experimental groups.

Day of operation	Operation techniques		
	UX	UOX	SOP
2	2UX	2UOX	2SOP
6	6UX	6UOX	6SOP
10	10UX	10UOX	10SOP
14	14UX	14UOX	14SOP

2.3 Surgery

All operations were performed between 10.00 to 13.00 on the designated day for each group under a light anaesthesia by intra peritoneal injection of 0.30 to 0.45 ml of a freshly prepared 1.2 % solution of *2,2,2-tribromoethanol* (*Avertin:* Fluka Chemika, French) in distilled water. This dosage was equal to 0.01 ml per gram body weight. All operations were performed under clean, but not sterile, conditions. Surgical procedures are summarised in Figure 2.2.

The UX groups:

Removal of one uterine horn was carried out through a mid-ventral incision along the abdominal wall. One uterine horn was then exposed and one ligature placed around the distal or cervical ends of the uterine horn, just at the junction of the two horns

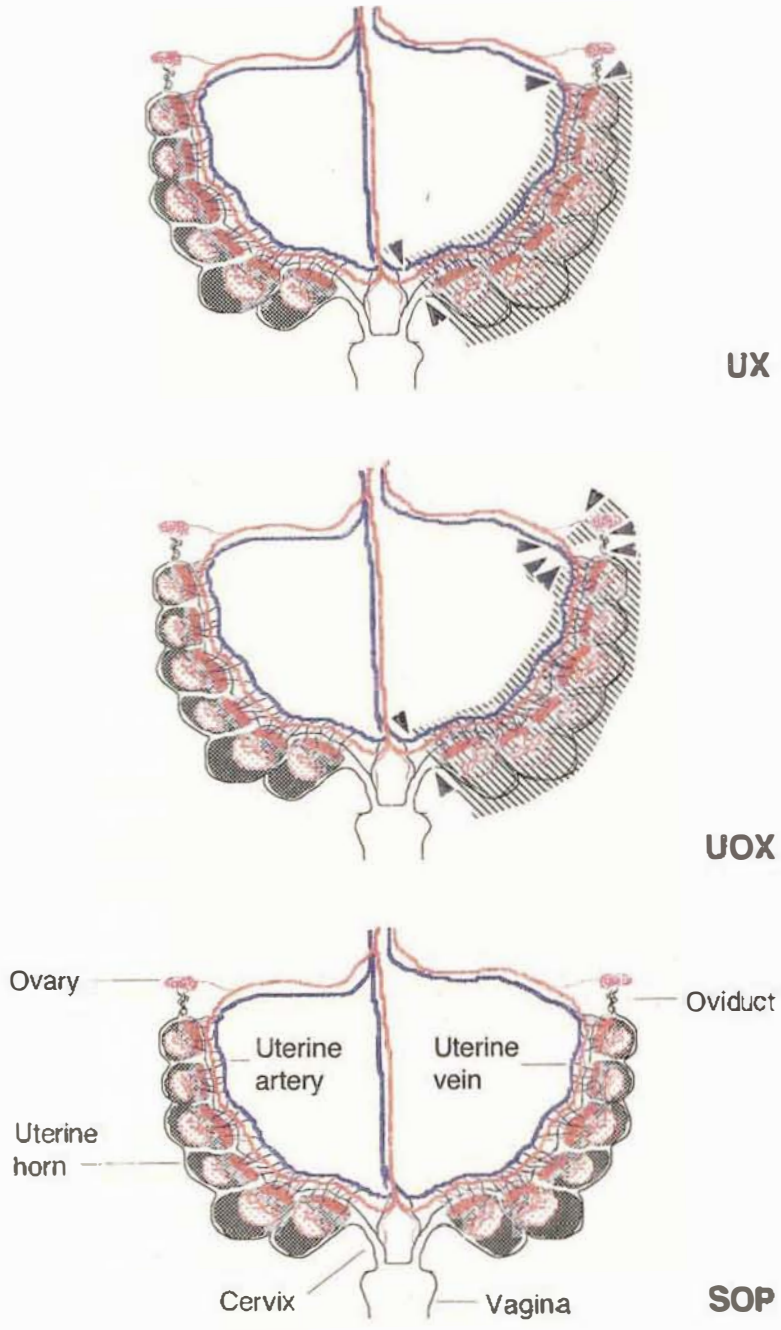

Figure 2.2 Surgical procedures carried out during experimental treatment. One uterine horn (UX group) or one uterine horn with its ipsilateral ovary (UOX group) was removed (shaded area) at several different days of gestation, i.e. Day 2, 6, 10, and Day 14. Ligatures (arrow heads), which included the uterine blood vessels, were placed around the distal or proximal of the cuts areas. In UOX group, the oviduct was left intact. Sham operated control (SOP group) consisted of laparotomy and inspection of both uterine horn, neither ligation or cuts was performed in the SOP group. (Adopted from vom Saal and Dhar, 1992).

(posterior ligature), and the other around the uterotubal junction (anterior ligature). Both the uterine tissue and the arterial and venous branches to the uterine horn were ligated tightly. Cuts were then made distal to the anterior ligature and proximal to the distal ones and the uterus and its contents then removed *en bloc* leaving the Fallopian tube (oviduct) and ovarian vasculature intact (Figure 2.2, top).

The UOX groups:

The surgical procedures for UOX animal groups followed the steps described above except that the ligation and cutting was also carried out on the distal and proximal parts of arterial and venous branches of the ipsilateral ovary. Both the uterine tissue and its ipsilateral ovary were removed. The Fallopian tube (oviduct) was left intact (Figure 2.2, middle).

The SOP groups:

A mid-ventral incision along the abdominal wall was made as previously described for the UX and UOX groups, and one uterine horn and ovary was exposed and manipulated before wound closure. However, the uterine horn and the ovary with their vasculature were neither ligated nor sectioned (Figure 2.2, bottom).

Surgery was performed on alternate sides in successive animals in an attempt to avoid possible bias between the right and left sides. Sterilised silk suture (*Ethicon:* Johnson & Johnson, Sydney, Australia) was used for all ligation and closure in the surgical procedures. Care was taken when operating pregnant females primarily on day 10 and day 14 of pregnancy to minimize physical disturbance on both gravid components and uterine vasculature. Total handling time for each operation never exceeded 5 minutes.

Post-operative care for all animals consisted of spraying antiseptic powder (*Medipulv:* Fisons, Sydney, Australia) over the incision area, and placing the animal in a supine position on a warm hot plate for recovery. The operated animals were then transferred into the experimental room only after they reached recovery status, marked by the wakening and movement of the mouse. Most animals reached recovery status in one to one and a half hours after surgery but they were only brought back into the experimental room 2 to 3 hours later. After recovery, the animals were then supplied with food and water *ad libitum.*

2.4 Autopsy

Autopsy was performed for all operated-pregnant females at the designated day for each day-based group (Table 2.2). Autopsy was carried out after a blood sample for each animal was collected (see below).

Table 2.2 The days of autopsy for each experimental group.

Group	Day of pregnancy at autopsy
2UX, 2UOX, 2SOP	3, 7, 11, 15, 18
6UX, 6UOX, 6SOP	7, 11, 15, 18
10UX, 10UOX, 10SOP	11, 15, 18
14UX, 14UOX, 14SOP	15, 18

Blood sampling

Blood samples were obtained from all treated-pregnant females on the day of autopsy. Blood samples were also collected on parturition day. Collection of the blood samples was performed under light anaesthesia as described previously (Section 2.3). Blood sample (0.8 - 1.5 ml/mice) was obtained by means of cardiac puncture with 26G needles and 2 ml heparinized disposable plastic syringes (50 IU sodium heparin per syringe), transferred into lithium heparin tubes, and then centrifuged at 2500 *g* for 10 minutes. Plasma was then harvested and stored in a freezer at -20°C until hormonal assay.

Uterine and ovarian tissue

After blood sampling, mice were killed by cervical dislocation. The remaining uterine horn (for both UX and UOX groups) or intact uterus (for SOP groups) and its ovary were removed, weighed on a Mettler balance and then placed in a petri dish containing 0.9% *(w/v)* NaCl solution for examination. Fresh examination of the uterine horn and its content from the females on day 18 of pregnancy was made directly.

Because the uterine horn and its gravid components were more difficult to distinguish and separate by morphology before day 18 of pregnancy, they were not dissected for fresh examinations. Uterine horns from day 3 to day 15 of pregnancy, therefore, were fixed directly in Bouin's solution. They were then examined under low magnification with a dissecting microscope.

2.5 Statistical Analysis

Results are expressed as means ± SEM. In most cases, data from the right and left uterine horns which originated from the SOP groups were averaged first before being analysed and presented in figures or tables. Statistical analysis (paired samples t-test) on preliminary data (data from Dr. Rose) revealed that there were no significant differences between right and left uterine horn. Two way analyses of variance (ANOVA) of general linear model using the Systat 5.2 program on a Macintosh machine were used for the overall test of effects of the day and Operation factors. One way analysis of variance was also performed to analyse the effect of the Operation factor in the specified days of operation. If the overall test was significant, a post-hoc analysis of Tukey HSD-test was used for further analysis. According to statistical theory, data in percentages are binomially distributed, the deviation from normality being greatest for small (0 - 33%) or large percentages (70 - 100%) (Zarr, 1974). Such data was transformed using *arcsine* transformation procedures before analysis. The analytical results were then presented in their original form (percentages). Regression or correlation analyses were used to examine a possible association between two parameters. Paired samples t-test was also applied when appropriate. In this study, only differences between mean at $p < 0.05$ were considered significant.

Chapter 3
Effects on Length of Gestation and Prenatal Growth

3.1 Introduction

It has been well established that the onset of parturition is timed by the fetus via secretions of adrenal cortex (fetal cortisol) which results in an increased in oestrogen (E) to progesterone (P) ratio. Increasing in E/P ratio will stimulate the uterine synthesis and release of $PGF_{2\alpha}$. Mechanical events of parturition are activated by $PGF_{2\alpha}$, the synthesis and release of the $PGF_{2\alpha}$ then enhanced by oxytocin as parturition proceeds (Thorburn, 1991; Johnson and Everitt, 1995). Thorburn (1991) proposed that the growth pattern of the fetus represents a genetically programmed 'clock' which acts by stimulating placental PGE_2 production leading to maturation of key organ systems in the fetus and finally parturition.

3.1.1 Litter Size and Length of Gestation

An inverse relationship between litter size and the length of gestation is commonly found in mammals, including the mouse. Biggers *et al.* (1963) presumed that the inverse relationship is modulated by two types of mechanism: 1) some local effect of

crowding, originating and acting in the uterus, or 2) a systemic effect, which may and may not originate in the uterus, but which must nevertheless be related in some way to the number of young carried. Further, in mice, Biggers *et al.* (1963) have described an experimental examination of this inverse relationship either by inducing a local uterine overcrowding (by unilateral ovariectomy) or by halving the number of conceptuses without interfering with the number of CL (by unilateral ligation of the Fallopian tube). They found that the controlling influences originated from the conceptuses directly, rather than the number of the CL formed in the ovary. They also showed that the effect of litter size operated systemically rather than locally. This shows that the length of gestation period is inversely related to litter size (Biggers *et al.*, 1963; Dewar, 1968), irrespective both of their distribution between uterine horns and of the number of corpora lutea (Biggers *et al.*, 1963). Since the length of gestation is unaffected by the distribution of the conceptuses between the two uterine horns, as reported by Biggers *et al.* (1963), it might be concluded that the litter size effect operates systemically rather than locally.

Similar results have previously been reported in the rabbit (Hammond, 1934), guinea-pigs and man (Widdowson, 1968) and also in the pig (Martin *et al.*, 1978). These provide evidence that the greater the number of the fetuses, no matter how they are distributed in the uterine horn, the earlier they are born and the lighter their birth weight. Of factors influencing the growth rate of the fetus, the supply of maternal blood to the placenta, the size of the placenta and the nutrition state of the mother are the important factors (Widdowson, 1968). However, it has been shown that in mice this effect was not continuously linear over the range of litter size (Dewar, 1968). In addition, Holinka *et al.* (1978) failed to detect a significant effect of litter size on the length of gestation in the 3 - 7 months-old age of mice even though this effect was very significant in the older (11 - 12 months-old age) mice. They suggested that the major factor in prolongation of pregnancy in the older mouse was the retardation in the decrease of progesterone levels at the end of gestation period.

An experimental analysis to describe the nature of the systemic effect of litter size on gestation period in mice was constructed by McLaren and Michie (1963). They showed that the total mass of fetal or placental tissue affects the length of gestation directly, irrespective of number in the uterine horns. In this case, the weight of conceptuses was inversely related to the length of gestation period. However, whether the influence regulating the onset of parturition solely exerted by the fetuses or placenta or both remains unclear. Biggers *et al.* (1963) failed to detect a mechanical effect of litter size in

regulating the length of gestation since the stretching of the uterine wall does not appear to induce parturition by increasing uterine irritability. One way in which litter size might affect the length of gestation would be if oestrogen, produced by the placenta, stimulates the uterus to become responsive to oxytocin (Biggers *et al.*, 1963).

The involvement of the placenta in regulating the length of gestation period has become obscured since Harkness *et al.* (1964) failed to detect any oestrogen in the mouse placenta. In addition, Dewar (1968) also failed to show the effect of a physiological dose of oestradiol on the length of gestation period, but with higher doses pregnancy was significantly prolonged. The answer to the question of whether the fetuses or placenta or their combination is involved in regulating the length of gestation was revealed by McLaren (1967), who proposed that the length of gestation is affected more by the fetuses mass, no matter whether it is produced by a greater number of fetuses or by an increase in their mean weight, rather than placental factors.

Kihlstrom (1972) has produced a literature review on the relation between period of gestation and body weight in some placental mammals. After examining data from about 208 species, varying in weight from 10 to 10^8 g, he found that the length of gestation (G) was related to body weight (W) by the equation $G = a.W^b$. The numerical value of parameter a varies with the type of placenta, while the exponent b is approximately the same for all terrestrial animals. Kihlstrom also found that the size and developmental stage of the newborn animals is likely to be related to the length of gestation.

3.1.2 Utero-ovarian Relationship and Length of Gestation

In the pig, like in mice, the ovary is necessary throughout pregnancy and CL is regressed at term. The disconnection of the normal anatomical relationship between the uterus and ovary (by transplanting the ovary to the uterus or the abdominal wall, or by removing one uterine horn and its contra lateral ovary) has no effect on the length of gestation of the pig (Martin *et al.*, 1978). Since the levels of both oestrogen and progesterone were also unaltered after treatment in this study, Martin *et al.* (1978) concluded that the usual connection between the uterus and the ovary is not an absolute requirement for pregnancy maintenance and parturition.

3.1.3 Objectives

This study attempted to examine the effect of removal of one uterine horn on the prenatal

growth in the remaining uterine horn of the mouse. Removal of the uterine horn was carried out in two ways, ie. by cutting one uterine horn only and by cutting the horn with its ipsilateral ovary, and on four different days of pregnancy, ie. day 2, 6, 10, and 14 of pregnancy. Before examining the effect on prenatal growth, examination on the effect of the reduction of the conceptuses into a half of normal number on length of gestation was made. The possible effect of the uterine horn removal on the development of the uterine vasculature, especially on the arteries supplying blood to the uterus and/or to the placenta, was also examined.

3.2 Methods

3.2.1 Determination of Gestation Length

Ninety-six pregnant mice were used in the investigation of gestation length. The initial weight of the animals (at the day of vaginal plug) varied from between 26.00 to 49.00 g and mean (± SD) of 35.55 ± 4. 31. The animals were caged in groups of 3 or 4 and randomly placed in 12 groups of 8 at the time of surgical operation. The environmental conditions, with respect to room temperature and lighting period are described in Chapter 2.

Pregnancies were visually timed from the finding of vaginal plug. Only the animals with vaginal plug in the morning of examination were directly included for experimental observation. The remaining animals (without a vaginal plug on the morning of examination) were separated from the males and were kept separate for at least 10 days after mating, during which period they were weighed daily. In this case, in an animal without a vaginal plug but showing a significant increase in body weight, the age of pregnancy could be referred to that of animals with a vaginal plug. The day of vaginal plug or the day of separation from male (for animals without vaginal plug) was designated as day 0 of pregnancy and the end of day 0 of pregnancy was taken as midnight after the morning on which the plug was found.

As day 17 of pregnancy approached, the mice were separated and placed into small individual cages. The animals were then checked for births three times a day during daylight period between 0600 - 0700 h (morning), 1200 - 1300 h (mid day), and 1800 - 1900 h (late afternoon).

Except for weighing, surgical procedures, cage cleaning, feeding and watering, and

birth checking, gravid females were not disturbed during the course of pregnancy.

3.2.2 Calculation of Gestation Length

Parturition was considered to have occurred after midnight and before the morning on which parturition was discovered. The timing of the gestation period was made by modification of the method used by Holinka *et al.* (1978). The accuracy of timing of gestation length was fitted to within 0.25 day or 6 hours, as summarised in Table 3.1.

Table 3.1 Calculation of length of gestation period for each time of observation

Time of observation when pups were found	Gestation length
a. morning (0600-0700 h)	Number of days from day 0
b. mid day (1200-1300 h)	Number of days from day 0 + 0.25
c. late afternoon (1800-1900 h)	Number of days from day 0 + 0.50

For example, a mother found with pups on the morning of day 19 had a 19 days gestation period, whereas one giving birth on day 19 at midday had a 19.25 day of pregnancy and one giving birth on day 19 in the late afternoon had a 19.50 day period.

The mice were weighed and anesthetized after delivery for blood sampling and then killed by cervical dislocation. Young mice were sexed visually and weighed individually to the nearest 0.0001 g by placing the mouse in a tared plastic cup on a Mettler direct reading balance. The C-R length of the young mice was measured to the nearest 0.02 mm using a metal calliper. All delivered young mice were killed after measurement of their weight and length by placing them in the freezer.

Calculated length of gestation period, litter size, and mean weight and C-R length for each litter were used for statistical analysis.

3.2.3 Prenatal Growth

A total of 210 pregnant mice was used in this experiment. The mice were mated and judged for age of pregnancy as described in Chapter 2. The growth of the uterus and its gravid components during gestation period were examined, starting just one day after surgery for each group. Examination was performed on five different days of

pregnancy, ie. day 3, day 7, day 11, day 15, and day 18. Thus, the groups of pregnant mice operated on, on day 2 of pregnancy were examined five times, the first examination being day 3 of pregnancy, one day after operation. The other examinations were on day 7, day 11, day 15, and day 18 of pregnancy for the second, the third, the fourth, and the fifth examination respectively. For the group of pregnant mice operated on, on day 6 of pregnancy, uterine and ovarian examination were performed four times, i.e. on day 7, day 11, day 15 and day 18 of pregnancy. In the remaining groups, pregnant animals operated on, on day 10 and day 14 of pregnancy were examined three and two times, starting at day 11 and day 15 of pregnancy respectively as described in section 2.4 (Chapter 2). Five animals per group were examined (Table 3.2).

Table 3.2 Number of animals autopsied for each designated day of pregnancy per group. Prefix number for each group denotes the day of pregnancy when the operation was performed.

	Number of animals autopsied on each designated days of pregnancy				
Groups	3	7	11	15	18
2UX	5	5	5	5	5
2UOX	5	5	5	5	5
2SOP	5	5	5	5	5
6UX	-	5	5	5	5
6UOX	-	5	5	5	5
6SOP	-	5	5	5	5
10UX	-	-	5	5	5
10UOX	-	-	5	5	5
10SOP	-	-	5	5	5
14UX	-	-	-	5	5
14UOX	-	-	-	5	5
14SOP	-	-	-	5	5
Total	15	30	45	60	60

The diameter of the rostral end and middle portion of the uterine loop artery (henceforth these arteries abbreviated as Uo and Ut for the rostral end and middle portion of uterine loop artery respectively) and the diameter of uterine segmental arteries (in this study these arteries were referred to as placental arteries, abbreviated as Pl) at three different locations (ovarian end, middle point, and vaginal end) were measured using a metal calliper (see Figure 3.1). Measurement was performed at two different times under light anaesthesia. The first measurement was carried out at surgery and the second one at autopsy on day 18 of pregnancy. At surgery, after the ventral body wall was opened and before the uterine horn was removed, uterine horn and its gravid components were

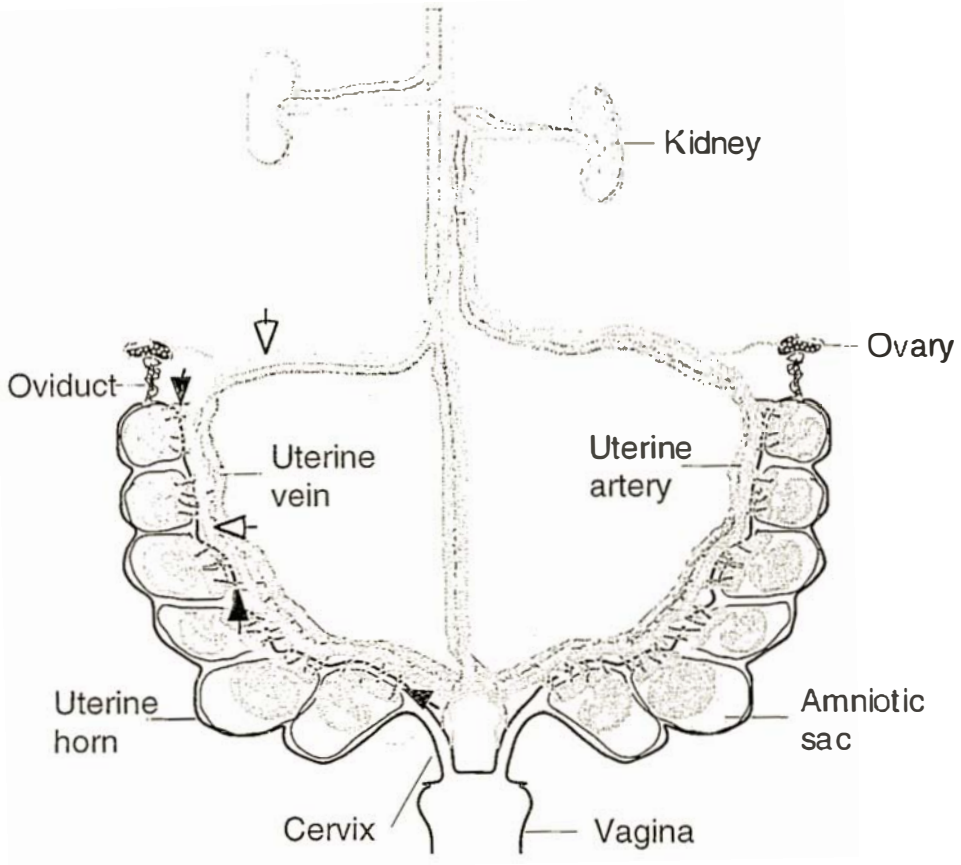

Figure 3.1 Diagram of the uterine vasculature of the mouse near term, showing the positions at which diameter of the arteries was measured (arrow heads). Measurements were made on the utero-ovarian artery (Uo) (open arrow, upper left), uterine artery loop (Ut) (open arrow, lower left), and on the uterine segmental or placental arteries (Pl) at three different locations (ovarian end, middle portion, and vaginal end) (solid arrow). For each group, artery diameter was measured two times, at the day of operation (initial diam eater) and at the day of autopsy, i.e. Day 18 of pregnancy (final diameter). (Adopted from vom Saal and Dhar, 1992).

exposed and stretched lightly. In such conditions the diameter of the uterine-related

arteries was determined easily to the nearest 0.02 mm by using a metal calliper. A similar procedure was applied when measuring the artery diameter at autopsy on day 18 of pregnancy. Measurement on day 18 of pregnancy was performed before the blood sample was collected.

After bleeding, mice were killed for the uterine examination by cervical dislocation. The remaining uterine horn (for both UX and UOX groups) or intact uterus (for the SOP group) and its ovary was removed, weighed and then placed in a petri dish containing 0.9% (*w/v*) NaCl solution until examined for gravid components (for horns obtained from day 18 of pregnancy) or until fixing in Bouin's solution (for horns obtained from days 3, 7, 11, and 15 of pregnancy).

All uterine horns and ovaries collected from the groups of animals at day 18 of pregnancy were examined on collection. After opening the uterus, conceptuses (fetuses, fetal membranes, and placentae) were removed in order from the ovarian end to the vaginal end of the uterine horn. For the SOP group, the left uterine horn was examined first before the right one. Each conceptus was opened and examined before the next conceptus was removed following Norman and Bruce (1979b). The number of live fetuses was recorded, dissected free from their membranes, separated from their umbilical cords, blotted lightly by transferring them four times on dry petri dish surfaces, sexed visually, and immediately weighed individually to the nearest 0.0001 gram on a Mettler direct reading balance. Visual determination of fetal sex was made by observing the ano-genital papilla distance, where the distance is larger in males than in females (Gruneberg, 1952; Rugh, 1967). Fetal C-R length was determined to the nearest 0.2 mm using a metal calliper. Each placenta was carefully separated from its umbilical cord and from fetal membranes, blotted lightly as for fetuses, and weighed. Care was taken to remove the attached fetal membranes from both the fetus and the placenta. The remaining uterine tissue plus embryonic membranes and umbilical cord were carefully blotted lightly by transferring them onto dry petri dish surfaces four times and weighed totally. The total amount of fetal fluid per horn was obtained by subtraction of total fetal weight, total placental weight, and total empty uterine tissue, embryonic membranes and umbilical cord from the total weight of uterine horn (McLaren *et al.*, 1976). This entire procedure, i.e. preparation, separation of uterine tissue, fetus, and placenta from the embryonic membranes, was conducted on a filter paper moistened with saline solution. This procedure was performed on the live conceptuses only.
Ovaries were examined for weight and number of corpora lutea after all the excessive fat covering the ovary was removed. The number of corpora lutea was counted visually

under dissecting microscope. Corpora lutea were counted in a petri dish three before recording as a unit of data .

Because the uterine horn and its gravid components were more difficult to distinguish and separate by morphology before day 18 of pregnancy, they were not dissected for fresh weight measurements. The uterine horns from day 3, day 7, day 11, and day 15 of pregnancy, therefore, were fixed in Bouin's solution until examined. These uterine horns and their gravid components were examined under a dissecting microscope at low magnification.

When examining the fixed uterine horns, the strong smell of Bouin's solution was first removed by washing them in slow running tap water for about 2 minutes. After opening the uterine horn, conceptuses were removed in order. Fetuses and placentae (from 11 and 15 days old) were separated from their membranes and/or its umbilical cord, blotted slightly by transferring them to filter paper as many as four times, sexed visually, weighed individually and determined for C-R length of fetus as described for fresh examination. The remaining uterine tissue plus fetal membranes and umbilical cord were weighed totally as empty uterus. The amount of fetal fluid per horn was calculated as for fresh examination of uterine horn from day 18 of pregnancy.

The uterus obtained from day 3 of pregnancy was examined for uterine weight only when day 7 uterine horn was examined for both uterine weight and conceptus weight.

Fetal weight, placental weight and fetal C-R length were averaged per horn before being used for statistical analysis. Therefore, for the SOP group, which had two uterine horns, the mean values of the right and the left horn for each parameter were averaged.

3.3 Results

3.3.1 Length of Gestation

a. Time and day of Parturition

Distribution of animals for each group on the time and day of parturition is given in Table 3.3. Most animals gave birth in the early morning, and parturition at mid-day and late-afternoon was rare. The overall time of birth were 68.8%, 9.4%, and 21.9% for early morning, mid-day, and late-afternoon respectively. This distribution pattern of time of birth was generally found in the UX and UOX, but not in SOP groups. Almost

60 - 90% of animals in UX and UOX groups delivered their pups in early morning. In sham-operated control (SOP) group, birth in the late afternoon commonly occurred. For example, five of 8 (62.5%) animals in 2SOP, 37.5% in both 6SOP and 10SOP, and 25% in 14SOP group gave birth in the late afternoon.

Table 3.3 Number (N) and distribution of animals in the observation period (morning, mid-day, or late afternoon) and on the day of parturition for each experimental group

		Number of animals delivered at observation period[1]			Number of animals delivered on day[1,2,3]			
Group	N	Morning	Mid-day	Late afternoon	18	19	20	21
2UX	8	6 (75.0)	0	2 (25.0)	2 (25.0)	6 (75.0)	0	0
2UOX	8	5 (62.5)	2 (25.0)	1 (12.5)	2 (25.0)	4 (50.0)	2 (25.0)	0
2SOP	8	3 (37.5)	0	5 (62.5)	6 (75.0)	2 (25.0)	0	0
6UX	8	5 (62.5)	0	3 (37.5)	2 (25.0)	5 (62.5)	1 (12.5)	0
6UOX	8	7 (87.5)	0	1 (12.5)	2 (25.0)	5 (62.5)	1 (12.5)	0
6SOP	8	5 (62.5)	0	3 (37.5)	7 (87.5)	1 (12.5)	0	0
10UX	8	7 (87.5)	1 (12.5)	0	0	8(100.0)	0	0
10UOX	8	7 (87.5)	1 (12.5)	0	0	6 (75.0)	2 (25.0)	0
10SOP	8	1 (12.5)	4 (50.0)	3 (37.5)	8(100.0)	0	0	0
14UX	8	7 (87.5)	0	1 (12.5)	1 (12.5)	5 (62.5)	2 (25.0)	0
14UOX	8	7 (87.5)	1 (12.5)	0	0	6 (75.0)	1 (12.5)	1 (12.5)
14SOP	8	6 (75.0)	0	2 (25.0)	6 (75.0)	2 (25.0)	0	0
Total	96	66 (68.8)	9 (9.4)	21 (21.9)	36 (37.5)	50 (52.1)	9 (9.4)	1 (1.0)

[1]number in parenthesis is percentage of occurrence of parturition,
[2]the length of gestation of the female delivered 0.25 to 0.50 day longer than related days are rounded down,
[3]day of vaginal plug = day 0 of pregnancy.

Parturition day (day of pregnancy when females gave birth) ranged between day 18 and day 21 of pregnancy (day of vaginal plug = day 0 of pregnancy) (Table 3.3). This range varied between groups. Most of the females in the SOP groups gave birth at day 18 of pregnancy, and in these groups no parturition occurred after day 19 of pregnancy. In contrast, both UX and UOX groups usually gave birth at day 19 of pregnancy with a rare occurrence on days 18, 20, and 21 of pregnancy (Table 3.3). Therefore, overall modal gestation length for all animals was 19 days. All the animals of both UX and UOX groups, no matter whether their uterine horn was removed with or without its ipsilateral ovary, showed a similar pattern to that of overall modal gestation length. In contrast to this, the animals of SOP group had a modal gestation length of 18 days, one day shorter than that of the UX and UOX groups, and a range of 18 - 19 days. Interestingly, all 10 SOP's animals were delivered at day 18 of pregnancy, and most of them gave birth at mid-day or late afternoon. There was no difference between the UX

and UOX groups in the distribution of animals on day of parturition (Table 3.3). Parturition on day 21 of pregnancy was a very rare occurrence for all groups. In this study, only one of 96 animals gave birth on day 21 of pregnancy.

b. Litter Size and Length of Gestation In general, with one exception in the 6SOP group, mean body weight of females at the day of parturition (final body weight) remained higher than on day 0 of pregnancy (day of vaginal plug) (initial body weight)(Table 3.4). Mean percentages in final and initial body weight differences varied between 10 - 30% of initial body weight (except for 6SOP group which had a negative value of -0.59%). These differences were statistically significant ($p < 0.05$) in almost all groups, except in the 6UOX, 6SOP, and 10UX groups. However, neither day factors nor Operation factors nor their combination influenced significantly the percentage of female body weight increase.

Table 3.4 also shows that the size of litter per female for all groups varied between 2 and 18. The smallest size was found in the 2UOX group and the biggest one in the 6SOP group. For all days of operation, the removal of one uterine horn, whether it was followed by the removal of its ipsilateral ovary or not, significantly reduced litter size by almost 50% compared to their sham-operated control (SOP) groups. However, removal of one ovary following the removal of one uterine horn had no significant effect on the size of litter. For each day of operation, the size of litter in the UX group was the same as in the UOX groups except at day 6 of pregnancy where litter size of the UOX group was significantly higher than that of UX group (Table 3.4). The age of pregnancy when the uterine horn was removed (day factors) and combination of the day and Operation factors had no effect on litter size. Thus, the animals operated on, on day 14 of pregnancy showed a similar pattern in their litter size to the animals operated on, on the other days of pregnancy regardless of whether operation techniques of UX or UOX or SOP were used.

For each day of operation, the gestation period in both the UX and UOX groups tended to be longer, but still not significantly, than that of the SOP groups (Table 3.4). The difference in length of gestation period was significant if the data was pooled on the Operation groups (UX, UOX and SOP groups) without considering the day factors (Figure 3.2). Mean ($\pm$ SEM) lengths of gestation period of UX group (19.03 ± 0.08) and UOX group (19.19 ± 0.11) were significantly longer than SOP group (18.39 ± 0.06) days ($p < 0.001$). This suggests that the reduction of the litter to a half of its

Table 3.4 Mean (±SEM) maternal body weight, litter size and length of gestation

Group	Maternal body weight (g)			Litter size per female	Length of gestation
	Initial (a)	Final (b)	Increase [(b) - (a)]%	(c)	(days)
2UX	36.63 ± 1.74	42.05 ± 1.49[1]	15.51 ± 3.15	8.00 ± 0.65 (6 - 12)	18.88 ± 0.16
2UOX	35.44 ± 1.21	41.36 ± 1.29[1]	17.18 ± 3.73	6.63 ± 0.96 (2 - 10)	19.13 ± 0.24
2SOP	35.44 ± 1.82	41.15 ± 1.73[1]	16.85 ± 1.34	13.63 ± 0.86 (8 - 16)[2]	18.56 ± 0.11
6UX	34.81 ± 1.39	40.86 ± 2.15[1]	18.31 ± 6.78	7.38 ± 0.46 (6 - 10)	19.06 ± 0.22
6UOX	35.88 ± 1.61	38.87 ± 2.38	10.42 ± 9.10	10.50 ± 0.50 (8 - 12)[3]	18.94 ± 0.19
6SOP	34.44 ± 0.69	34.38 ± 2.93	-0.59 ± 9.49	12.63 ± 1.03 (9 - 18)[3]	18.31 ± 0.13
10UX	37.38 ± 1.31	42.29 ± 2.39	14.25 ± 7.86	6.75 ± 0.59 (5 - 9)	19.03 ± 0.03
10UOX	33.31 ± 2.00	40.43 ± 1.33[1]	23.03 ± 4.50	7.00 ± 0.94 (3 - 11)	19.28 ± 0.16
10SOP	33.06 ± 0.99	42.72 ± 0.82[1]	29.78 ± 3.45	13.38 ± 1.28 (5 - 16)[2]	18.31 ± 0.06
14UX	37.88 ± 1.87	42.40 ± 1.21[1]	12.96 ± 3.77	6.00 ± 0.71 (3 - 9)	19.18 ± 0.19
14UOX	35.31 ± 0.95	41.95 ± 0.94[1]	19.23 ± 3.36	6.25 ± 0.59 (4 - 9)	19.41 ± 0.26
14SOP	37.00 ± 1.99	41.71 ± 1.05[1]	13.92 ± 3.36	13.88 ± 0.58 (11 - 16)[2]	18.38 ± 0.16

[1]significantly different from its initial body weight ($p < 0.05$; paired t-test); [2]significantly different from its UX and UOX groups ($p < 0.01$; Tukey HSD test); [3]significantly different from its UX group ($p < 0.01$; Tukey HSD-test); [a&b]initial and final body weight were recorded on day of plug and on day of parturition respectively; [c]number in parenthesis denotes minimum and maximum size of litter.

normal size could prolong the length of gestation almost one day longer than that of intact animals. Prolongation of the gestation period in the two groups of UX and UOX is seemingly determined solely by the reduction in litter size, and removal of one ovary following the removal of its ipsilateral uterine horn, made no contribution, because there was no difference between UX and UOX groups. Thus, whereas both the day factor and combination of day and Operation factors had no significant effect on the length of gestation, removal of one uterine horn with or without its ipsilateral ovary independently affected this parameter significantly.

The functional relationships between the length of gestation and litter size for the three groups of Operation factors are presented in Table 3.5. Length of gestation (y) was a function of litter size (x) in the UOX group only ($p < 0.05$). This functional relationship was not statistically significant in the UX ($p = 0.52$) and SOP ($p = 0.78$) groups. If the data was pooled without considering the day and the Operation factors, the functional relationships between length of gestation and litter size was highly significant ($r = 0.55$, $p < 0.001$).

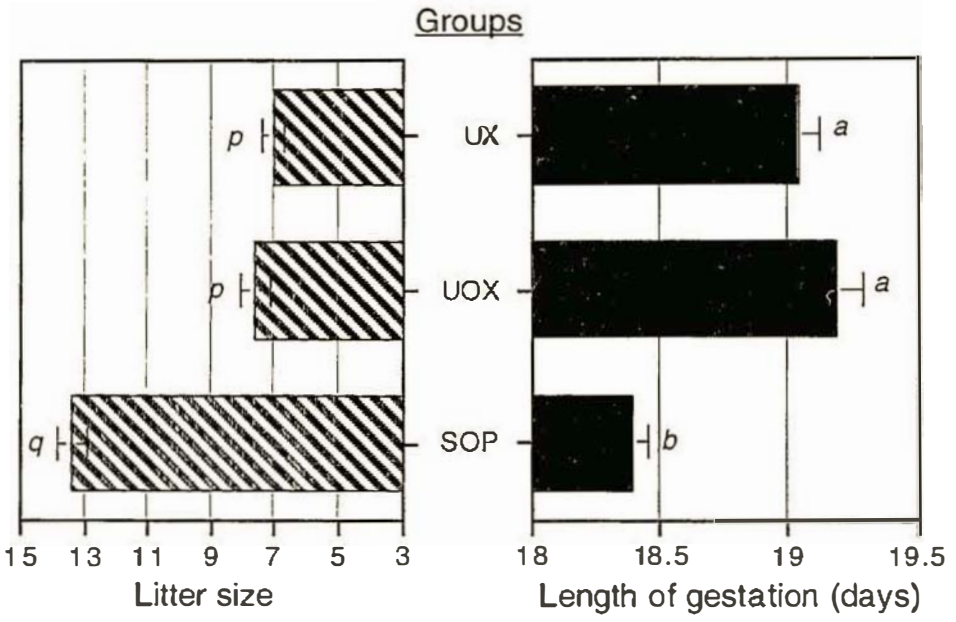

Figure 3.2 The relationship between litter size (left) and length of gestation (right). Removal of one uterine horn with or without its ipsilateral ovary (the UX or UOX group) significantly reduced the size of litter and prolonged gestation almost one day more than the sham-operated control (the SOP) group. Different letters between group are significantly different ($p < 0.001$, Tukey HSD-test, $n = 32$).

Table 3.5 Functional relationships between length of gestation (y) and size of litter (x) in the UX, UOX, and SOP groups.

Group	Regression equation	r	p
UX	y = 19.25 - 0.03x	0.12	0.52
UOX	y = 19.86 - 0.09x	0.39	0.03
SOP	y = 18.48 - 0.01x	0.05	0.78
Overall	y = 19.69 - 0.09x	0.55	< 0.001

3.3.2 Prenatal Growth

a. Uterine weight

The growth of the remaining uterine horn in pregnant animals after removal of its counterpart horn are given in Table 3.6. During the first half of the gestation period uterine growth, as indicated by the weight, occurred at a slow rate. After that period, during the second half of gestation, the growth of uterine horn occurred very rapidly.

From day 11 to day 15 of pregnancy, the growth rate increased dramatically; the weight of the whole uterine horn for day 11 of pregnancy was below one gram while the value for day 15 of pregnancy varied between 4 to 5 grams or more. A more dramatic increase in growth of the uterine horn occurred from day 15 to day 18 of pregnancy. The figure for day 18 of pregnancy ranged between 10 to 18 grams (see Table 3.6, by day and Operation). The same tendency was observed for empty uterine tissue weight (after their gravid components, except the embryonic membranes, were removed). However, the percentage of uterine tissues relative to the whole uterine weight decreased with the age of pregnancy. There were no differences between group of day and Operation in the uterine horn weight (either in whole, empty uterine tissue or percentage of empty uterine tissue) during the gestation period examined. Therefore, it is likely that both day factor and Operation factor were worked separately and their influence on weight of the remaining uterine horn were performed independently (see the middle (By day) and the bottom (By Operation) parts of Table 3.6).

The influence of the day factor (day of pregnancy when one uterine horn was removed) on the weight of the remaining uterine horn was not as expected. Although differences between group of day factors in uterine weight were detectable, these differences did not appear in a regular way. Therefore, the expectation that females operated on earlier days of pregnancy should have a heavier uterine weight than those operated on, on the later days was not observed in this study.

Operation factors showed a more reasonable effect (see the lower part of Table 3.6, By Operation) on uterine growth. In general, uterine weight (either whole, empty, or % empty) of the operated females (either the UX or UOX group) was heavier than that of unoperated females (SOP group). This difference was statistically significant on some days of pregnancy autopsied, especially at day 15 and day 18 of pregnancy. However, once again, the removal of one ovary following the removal of its ipsilateral uterine horn had no effect on the growth of the remaining uterine horn.

Table 3 6 The growth of the uterine horn in pregnant animals after removal of one of its uterine horns on different days of gestation (prefix number in By Day and Operation groups denotes the day of operation) Data were collected on several different days of gestation (Day 3, 7, 11, 15, and 18 of gestation) starting one day after operation, and were groupped for statistical analysis either by combination of Day and Operation factors (By Day and Operation), or by Day factor only (By Day), or by Operation factor only (By Operation)

Groups[4]	Mean (± SEM) uterine weight per horn (g) on Day of pregnancy[123]														
	3			7			11			15			18		
	Whole	Empty	% Empty	Whole	Empty	% Empty	Whole	Empty	% Empty	Whole	Empty	% Empty	Whole	Empty	% Empty
By Day and Operation:															
2UX	0 037 ± 0 002		-	0 182 ± 0 032	0 059 ± 0 009	33 140 ± 2 258	0 943 ± 0 168	0 267 ± 0 033	29 794 ± 2 564	4 580 ± 0 528	0 457 ± 0 073	10 383 ± 2 087	14 096 ± 0 273	1 271 ± 0 077	8 989 ± 0
2UOX	0 040 ± 0 003	-	-	0 240 ± 0 018	0 075 ± 0 007	30 976 ± 2 184	0 810 ± 0 145	0 306 ± 0 042	28 183 ± 0 042	5 551 ± 0 536	0 556 ± 0 055	10 304 ± 1 258	13 863 ± 0 965	1 125 ± 0 056	8 229 ± 0 517
2SOP	0 030 ± 0 007			0 183 ± 0 007	0 046 ± 0 002	25 282 ± 1 114	0 915 ± 0 130	0 192 ± 0 050	20 966 ± 4 603	5 375 ± 0 520	0 462 ± 0 034	8 171 ± 0 469	10 419 ± 0 835	0 884 ± 0 048	8 647 ± 0 705
6UX	-		-	0 198 ± 0 015	0 070 ± 0 007	35 334 ± 2 437	0 900 ± 0 137	0 230 ± 0 028	26 244 ± 1 841	5 081 ± 0 765	0 704 ± 0 067	14 629 ± 1 547	14 977 ± 1 395	1 319 ± 0 125	8 836 ± 0 360
6UOX	-	-	-	0 209 ± 0 030	0 075 ± 0 009	36 398 ± 2 012	0 951 ± 0 170	0 267 ± 0 042	28 888 ± 1 647	5 252 ± 0 397	0 804 ± 0 072	15 290 ± 0 551	18 699 ± 1 173	1 569 ± 0 125	8 411 ± 0 536
6SOP	-		-	0 194 ± 0 041	0 061 ± 0 011	32 761 ± 4 396	0 797 ± 0 098	0 235 ± 0 023	30 187 ± 1 692	5 111 ± 0 514	0 460 ± 0 033	9 327 ± 0 887	12 000 ± 1 108	0 904 ± 0 077	7 603 ± 0 364
10UX	-		-	-	-	-	0 964 ± 0 063	0 322 ± 0 019	33 563 ± 1 305	4.157 ± 0 292	0 521 ± 0 053	12 510 ± 0 847	15 106 ± 1 675	1 205 ± 0 113	8 131 ± 0 529
10UOX			-	-	-	-	0 587 ± 0 029	0 193 ± 0 009	33 011 ± 1 252	4 046 ± 0 502	0 473 ± 0 046	11 906 ± 0 931	15 366 ± 0 989	1 198 ± 0 063	7 846 ± 0 315
10SOP			-	-	-	-	0 912 ± 0 071	0 269 ± 0 018	29 768 ± 1 534	4 482 ± 0 614	0 470 ± 0 063	11 199 ± 1 822	12 118 ± 1 837	1 063 ± 0 122	9 035 ± 0 633
14UX	-	-	-	-	-	-	-	-	-	4 694 ± 0 705	0 488 ± 0 061	10 602 ± 0 440	11 638 ± 0 482	0992 ± 0 081	8 511 ± 0 596
14UOX	-		-	-	-	-	-	-	-	5 191 ± 0 631	0 724 ± 0.163	13 387 ± 1 229	12 864 ± 1 084	1 120 ± 0 047	8 839 ± 0 393
14SOP	-		-	-	-	-	-	-	-	4 429 ± 0 256	0 455 ± 0 012	10 373 ± 0 532	11 656 ± 0 868	0 964 ± 0 032	8 532 ± 0 626
By Day:[5]															
Day 2	0 036 ± 0 003	-	-	0 202 ± 0 014	0 060 ± 0 005 a	29 799 ± 1 358 a	0 980 ± 0 082	0.255 ± 0 026	26 314 ± 1 941 a	5 289 ± 0 313	0 491 ± 0.032 ab	9 620 ± 0 813 a	12 792 ± 0 603 ab	1 093 ± 0 054 ab	8 622 ± 0 307
Day 6	-	-	-	0 200 ± 0 016	0 069 ± 0.005 b	34 831 ± 1 719 b	0 883 ± 0 076	0.244 ± 0 018	28 440 ± 1 023ab	5 148 ± 0 310	0 657 ± 0 050 b	13 082 ± 0 917 b	15 255 ± 0 985 a	1 264 ± 0 094 a	8 284 ± 0 266
Day 10	-	-	-	-	-	-	0 821 ± 0 054	0.262 ± 0 017	32 114 ± 0 858 b	4 229 ± 0 266	0 488 ± 0 030 a	11 871 ± 0 698ab	14 197 ± 0 915 ab	1 155 ± 0 058 ab	8 337 ± 0 303
Day-14			-	-	-	-	-	-	-	4.774 ± 0 314	0 556 ± 0 063 ab	11 454 ± 0 569ab	12 053 ± 0 479 b	1 022 ± 0 036 b	8 567 ± 0 298
By Operation:[5]															
UX	0 037 ± 0 002		-	0 190 ± 0 017	0 064 ± 0 006 ab	34 237 ± 1 608	0 936 ± 0 070	0 273 ± 0 018	29 867 ± 1 322	4 628 ± 0 286	0 542 ± 0.037 ab	12 031 ± 0 747 a	13 954 ± 0 607 a	1 197 ± 0 055 a	8 617 ± 0 231
UOX	0 040 ± 0 003		-	0 225 ± 0 017	0 075 ± 0 005 a	33 687 ± 1.666	0 873 ± 0 089	0 255 ± 0 022	30.027 ± 0 896	5 010 ± 0 274	0 639 ± 0 054 b	12 722 ± 0 635 a	15 198 ± 0 701 a	1 253 ± 0 036 a	8 331 ± 0 222
SOP	0 030 ± 0 007	-	-	0 188 ± 0 020	0 054 ± 0 006 b	29 022 ± 2 475	0 875 ± 0 057	0 232 ± 0 020	26 974 ± 1 951	4 939 ± 0 258	0 462 ± 0 018 a	9 767 ± 0 557 b	11 548 ± 0.585 b	0 951 ± 0 039 b	8 409 ± 0 299

[1]Whole = uterine horn and all its gravid components, empty = uterine tissues including embryonic membranes, % Empty = Empty/Whole x 100%,

[2]Uterine horn collected at Days 3, 7, 11, and 15 of pregnancy were fixed in Bouin's solution before examination;

[3]Hyphenated cells on the table = data not available (autopsy was started just one day after operation only), there were no gravid components which could be separated from 3 days-old uterine horns;

[4]Before analysis, the weight of the two uterine horns in SOP groups was averaged;

[5]Mean with different letter within the same column are significantly different ($p < 0.05$; Tukey HSD-test)

b. Uterine Artery Diameters

Figure 3.3 displays the mean diameter of arteries nourishing the uterine horn, measured initially at operation days for each group and finally at day 18 of pregnancy. In this case, removal of one uterine horn had a positive effect on the development of the arteries supplying the blood to the remaining uterine horn since their diameter in operated animals (UX and UOX groups) was higher than in sham-operated animals (SOP group). However, this effect was time-dependent since only animals operated on, on day 2 and day 6 of pregnancy showed significant differences in any parameters between UX or UOX group and SOP group (see Figure 3.3B and C). For example, the mean (± SEM) diameter of initial placental arteries (Pl) in the UX animals operated on day 2 of pregnancy was 0.20 ± 0.03 mm (Figure 3.3A) and it reached a diameter of 1.05 ± 0.06 mm on day 18 of pregnancy (Figure 3.3B), an increase of about 500% from the initial diameters (Figure 3.3C). The latest two values in the UX group were significantly higher ($p < 0.05$) than the SOP group's values, ie. 0.71 ± 0.05 mm and 200% respectively. A significant difference in this parameter was also found in animals operated on day 6 of pregnancy. However, the removal of one uterine horn after day 6 of gestation had no significant effect on the development of the arteries nourishing the remaining uterine horn or placentae. The increase in percentage of the arteries' diameter decreased with the stage of pregnancy when removal of one uterine horn was performed. This phenomenon was observable in all groups of animals used and in all arteries measured in this study (Figure 3.3C).

According to statistical analyses, the combination of the Operation factor and the day factor had no effect on the artery diameter. These two factors, however, affected the development of uterine artery diameter independently of each other. If the data collected on day 18 of pregnancy was grouped by Operation factor, it was found that the Uo diameters in the SOP group were significantly smaller than those of both the UX ($p < 0.01$) and the UOX ($p < 0.05$) groups. The Ut and Pl diameters also showed similar differences between the SOP group and the UX or UOX groups with the levels of significance of $p < 0.001$ (UX) and $p < 0.01$ (UOX) for Ut, and $p < 0.001$ (both UX and UOX group) for Pl.

From the data obtained from normal pregnant animals on the days of operation, as presented in Figure 3.3A, it can be concluded that the most critical stage of pregnancy for the arteries development was different in different arteries. A very rapid development of the Pl arteries, as indicated by their values in percentage of increase between two consecutive days of gestation when measurement was performed (data not shown), was

reached in the first half of the gestation period, especially between day 2 and day 6 of pregnancy. After that period the development rate of arteries decreased gradually until day 14 of pregnancy. In contrast to this, the Ut and Uo showed constant development during the gestation period examined. An even more rapid development in the Ut diameter was found in the second half of gestation period, especially between day 10 and day 14 of pregnancy.

Figure 3.3 also shows that Pl arteries underwent the most dramatic development during the gestation period compared to both of the Ut and Uo arteries. For example, at day 2 of pregnancy, when animals were operated on, the mean (SEM) diameter of Pl were 0.20 ± 0.03, 0.26 ± 0.03, and 0.26 ± 0.03 mm for UX, UOX and SOP groups respectively (Figure 3.3A). These values increased dramatically during the gestation period, reaching a value of 1.05 ± 0.06, 1.04 ± 0.04, and 0.71 ± 0.05 mm respectively for UX, UOX, and SOP groups on day 18 of pregnancy (Figure 3.3B). Thus, there was an increase in Pl diameter of about 500%, 300%, and 200% of that of initial diameter for UX, UOX, and SOP groups respectively (Figure 3.3C). At the same period, the percentage increase in Ut diameters only reached a value of about 200%, 200%, and 180%; and Uo diameters reached a value of 150%, 170%, and 140% for UX, UOX, and SOP groups respectively.

c. Gravid Components

Gravid components of the uterus, ie. uterine tissue, fetal fluid, placentae and fetuses, were examined carefully at several days of gestation. The weight of empty uterine tissue was found after all of the fetal fluid, placenta, and fetuses were removed. Placenta and fetuses were easily removed from the uterus or from their membranes at day 18 of pregnancy but not at earlier days of pregnancy. Uteri from females autopsied before day 18 of pregnancy were fixed in fixative solution, Bouin's solution, before examination. In such a condition, the components of pregnant uterus could be removed and separated as easily as at day 18 of pregnancy. Total placental weight and total fetal weight were counted by totalled individual weight of the placenta and fetus for each uterine horn. Total weight of fetal fluid was found by subtracting the total weight of placenta and fetuses from weight of the intact uterine horn. The percentage of each gravid component, relative to the total weight of the uterine horn, was then determined. For SOP groups which had two uterine horns, the gravid components of each horn were counted separately then, the two values were averaged before being analysed.

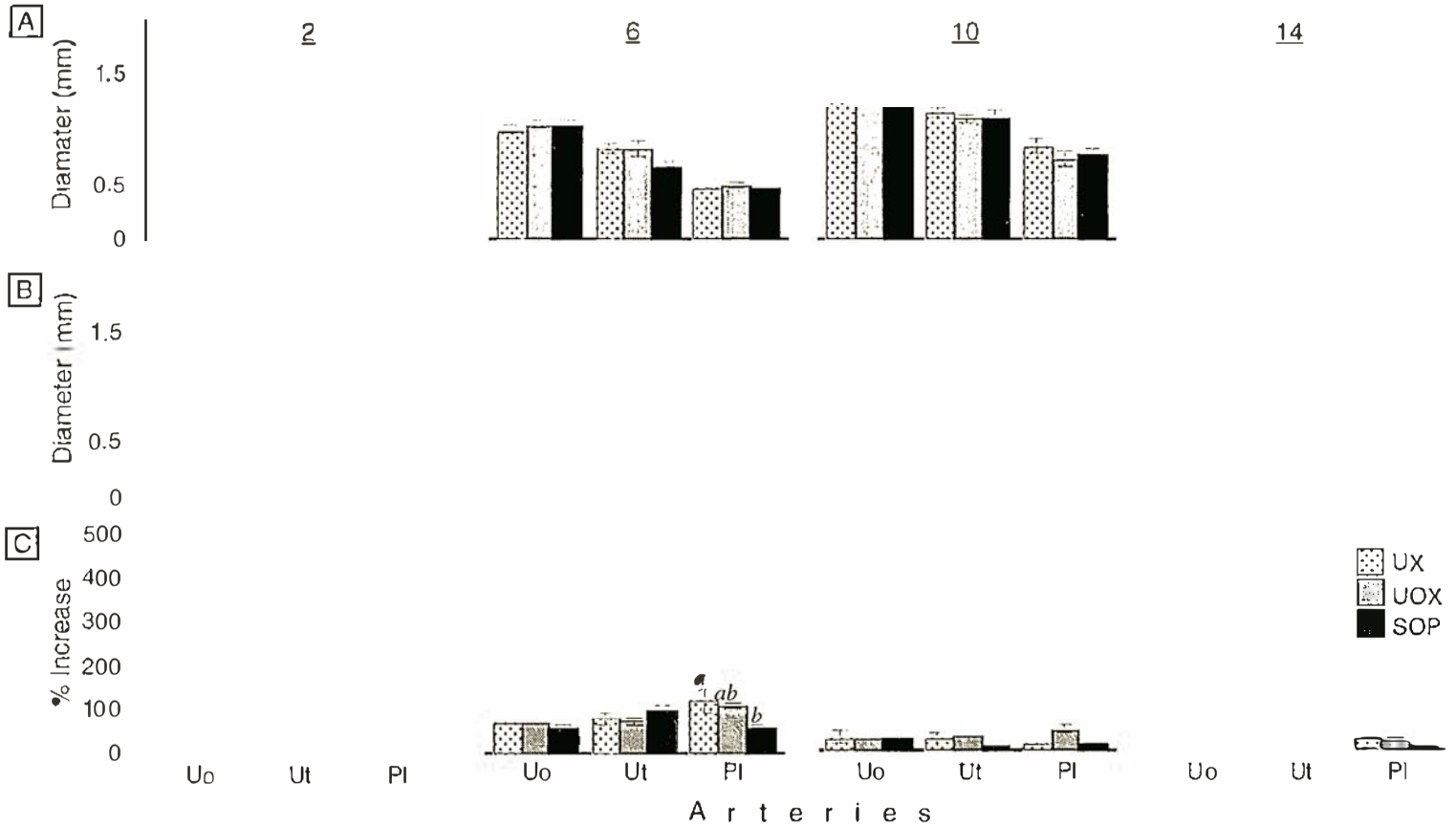

Figure 3.3 Post-operative development of Arterial diameter in the remaining uterine horn of pregnant animals after removal of one uterine horn only (⬚) or of one uterine horn and its ipsilateral ovary (▦) or of sham operation (■). Initial diameters (A) were measured at several different days of gestation (number on top), i.e. when animals were operated. Final diameters (B) were measured at Day 18 of pregnancy when animals were autopsied. Percent increase in arteries diameter (C) for each group were calculated by using the formula, (B - A) : (A) x 100%. There were three different parts of the arteries were measured, i.e. the utero-ovarian artery (Uo) at the position just before it junctioned into the ovarian artery and uterine loop artery, uterine artery (Ut) exactly at the middle part of the uterine loop, and the placental artery (Pl) (for detail see section 3.2 of this chapter). Values are mean (± SEM), different letters within the same parameter are significantly different (Tukey HSD-test; $p < 0.05$).

A two way analysis of variance revealed that the combination effect of the day and Operation factors on the uterine gravid component parameters was not significant. Data was then combined in groups of both Operation and day factors separately. Figures 3.4 and 3.5 were constructed to represent the weight proportion of each gravid component in groups of both Operation and day factors respectively.

Operation factors significantly affected the weight of the remaining uterine horn if the pregnancy of treated females was allowed to continue until day 18. At day 18 of pregnancy, mean weight of the remaining uterine horn in the UX and UOX group were significantly higher than that of SOP groups ($p < 0.05$) (Figure 3.4, Table 3.6). However, the removal of one ovary, ipsilateral to the removed uterine horn, had no effect on the growth of the remaining uterine horn. There was no difference between the uterine weight of the UX and UOX group (Figure 3.4, Table 3.6). There were no differences between operated and sham-operated control animals in the whole uterine weight if autopsy was performed earlier than day 18 of pregnancy.

At day 3 of pregnancy, the mean total weight of the uterine horn of operated animals was 0.04 g, slightly higher than sham-operated (SOP) control animals (0.03 g). At day 7 of pregnancy, the weight of the uterine horn reached a value of between 5 to 6 times its original weight at day 3. At this time, the horn consisted of uterine tissues, decidual tissue, and uterine fluid. This fluid comprised the largest proportion (about 40% of uterine weight) of the uterine weight in the SOP group but only 23% and 27% in UX and UOX groups respectively. The largest proportion of uterine content in the later two groups was decidual tissue, i.e. 42% and 39% for the UX and UOX respectively. However, there was no difference between groups in the proportion of gravid components (Figure 3.4).

At day 11 of pregnancy, the uterus consisted of uterine tissue, fetal fluid, placentae and fetuses in the proportion of 30 : 26 : 25 : 19 (in the UX group), or 30 : 23 : 28 : 19 (in the UOX group). or 27 : 32 : 23 : 18 (in the SOP group). For all groups the highest percentage of the total fetal fluid was reached at this stage of pregnancy. There was no significant difference between groups either in these proportions of the gravid components or in the total weight of the uterine horn. Total weights of the uterine horn were 0.94, 0.87, and 0.88 g for the UX, UOX and SOP group respectively (Figure 3.4).

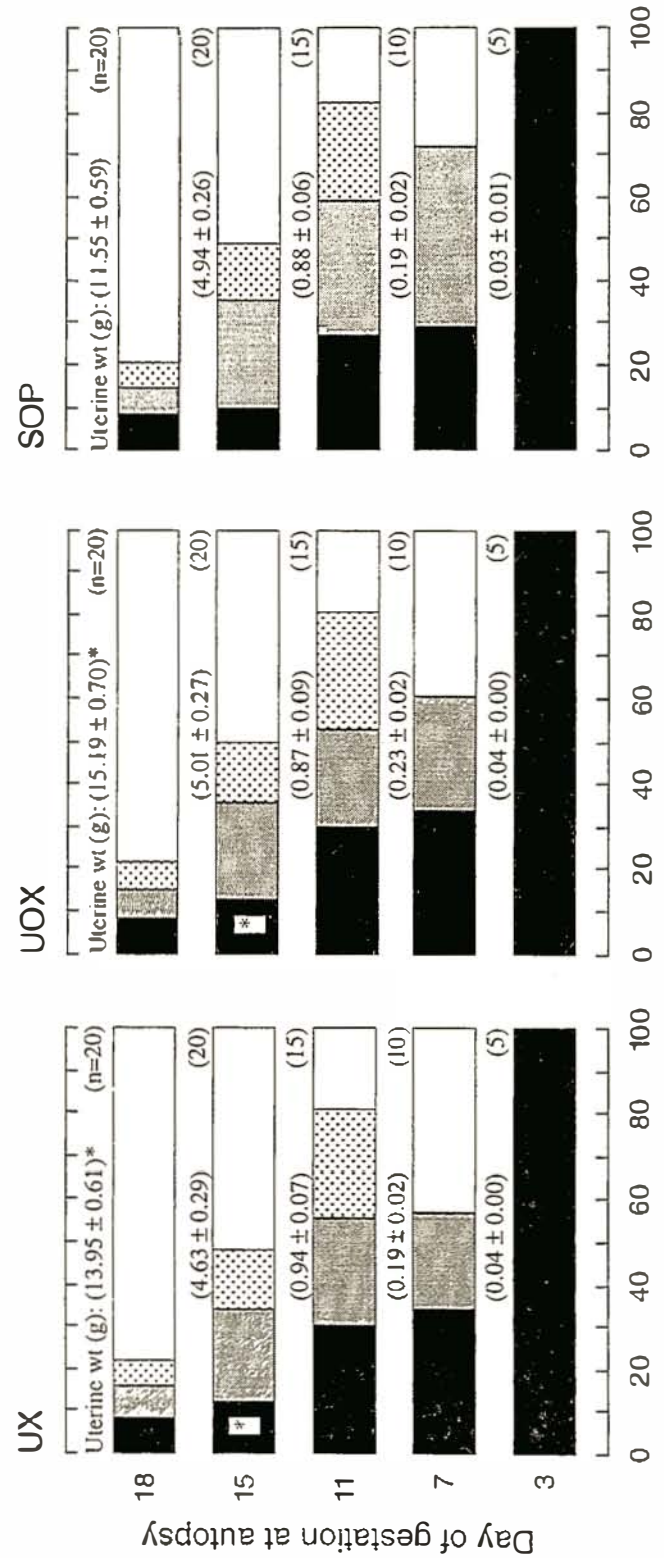

Figure 3. 4 Uterine weight (mean ± SEM) and mean per cent total weight of gravid components per uterine horn at autopsy (■ = empty uterine tissue, ▣ = fetal fluid, ⊡ = placenta, □ = fetus). At Day 7 of autopsy, (□) and (⊡) represents percent total weight of decidual tissue and percent total weight of uterine fluid respectively. At Day 3 of autopsy, (■) represents percent total uterine horn. Data was grouped into ●peration groups (UX, UOX, and SOP groups) without considering the Day factors. The percentage values for each component were relative to the total weight of the uterine horn. * significantly different from its SOP group ($p < 0.05$; Tukey HSD-test).

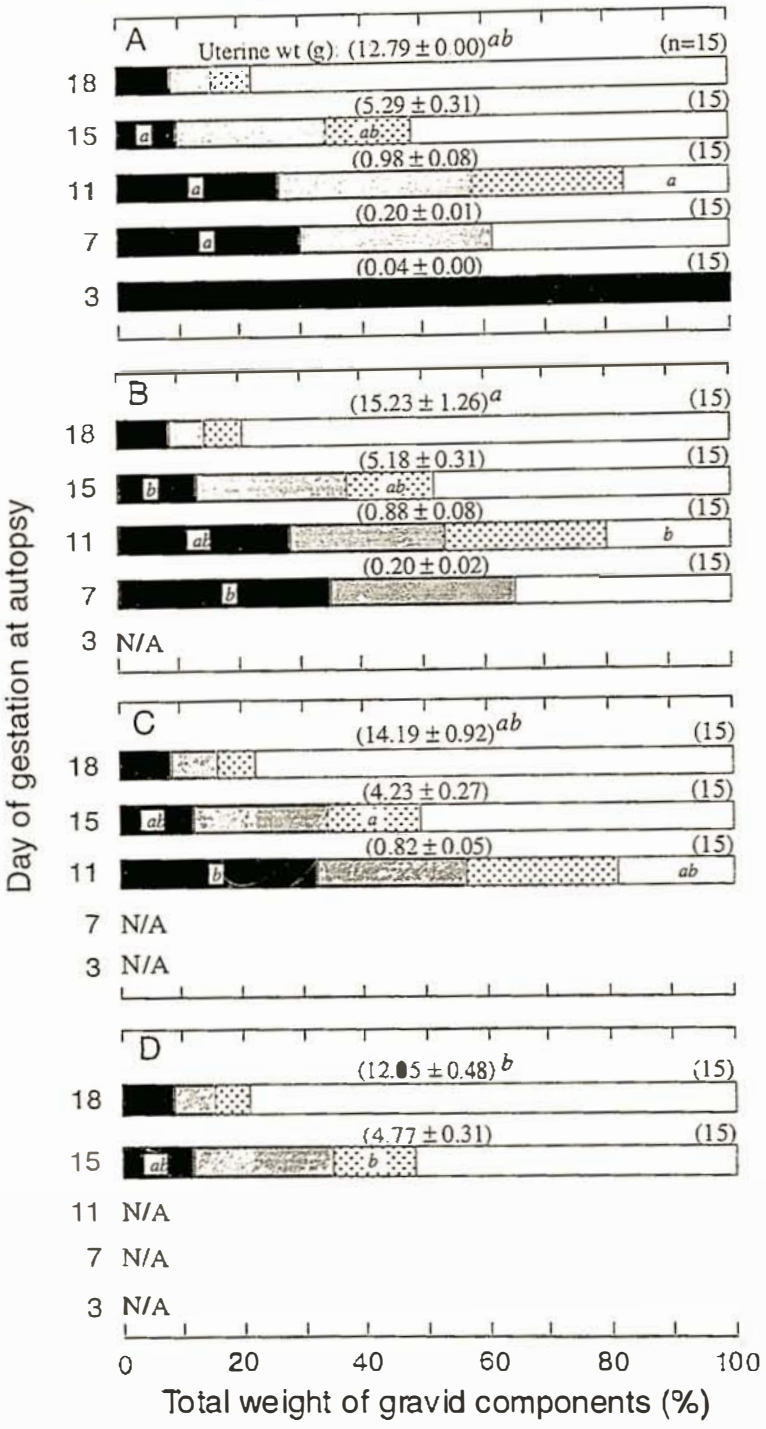

Figure 3.5 Uterine weight (mean ± SEM) and mean percent total weight of gravid components per uterine horn at autopsy (■ = empty uterine tissue, ▨ = fetal fluid, ⊡ = placenta, ▭ = fetus). At Day 7 of autopsy, (▭) and (▨) represent mean percent total weight of decidual tissue and percent total weight of uterine fluid respectively. At Day 3 of autopsy, (■) represents mean percent total weight of uterine horn. Data was grouped into the Day of factor groups (A, Day 2; B, Day 6; C, Day 10; D, Day 14) without considering the Operation factor. The percentage values for each component were relative to the total weight of the uterine horn. For each uterine horn and its gravid component, different letters between the same day of autopsy denote significant differences ($p < 0.05$; Tukey HSD-test). N/A, no data available.

By day 15 of pregnancy the percentage of total weight of the fetuses had increased dramatically from 19% on day 11 to 50% or more, while the percentage of total weight of the uterine tissue, fetal fluid and placenta had decreased moderately (Figure 3.4). There was a significant difference ($p < 0.05$) in percentage of total uterine tissue weight between operated (UX and UOX) and the sham-operated control (SOP) groups.

At day 18 of pregnancy, mean total weights (±SEM, g) of uterine horn of the UX (13.95 ± 0.61) and UOX (15.19 ± 0.70) groups were significantly ($p < 0.05$) higher than those of SOP group (11.55 ± 0.59), but there was no difference between UX and UOX groups. In all UX, UOX, and SOP groups, almost 80% of the uterine component consisted of fetal contribution while the contribution of all of the empty uterine tissue, fetal fluid, and placenta were only about 20% (Figure 3.4). Although total fetal weight and total placental weights of both UX group (10.90 ± 0.51 and 0.85 ± 0.04) and UOX group (11.96 ± 0.57 and 0.92 ± 0.05) were significantly higher than those of SOP group (9.15 ± 0.46 and 0.70 ± 0.04) (data not shown), their percentages were not significantly different. The proportion of gravid components in the remaining uterine horn, therefore, was not altered by the removal of one uterine horn.

A similar pattern of the gravid components' growth was also obtained if the data was grouped on the basis of day factor without considering the Operation factor (Figure 3.5). As can be seen in Figure 3.5, at day 18 of autopsy, total weight of the uterine horn of females operated on, on day 14 was significantly lower than those operated on day 6 but not different from those of day 2 and day 10. However, there were no significant effect of the day factor on their gravid components. Day 15, day 11, day 7, and day 3 of autopsy, showed no significant differences between day groups in total weight of uterine horn. However, in some cases gravid components showed a significant differences between groups. The differences between groups were frequently found in uterine tissue (empty), the placenta, and the fetus. In contrast, the difference in fetal fluid content was never found to be significant between groups (Figure 3.5).

d. Fetal and Placental Mass

The two-way analysis of variance revealed that there was no combination effect of the day and Operation factors on the mass of both fetuses and placentae in the remaining uterine horn. The intra-uterine growth pattern of the fetus and placenta during the gestation period examined, as indicated by their weight, are presented in Figure 3.6. Figure 6 was divided into four separate groups according to the day factor, and the day of pregnancy when the operation was performed.

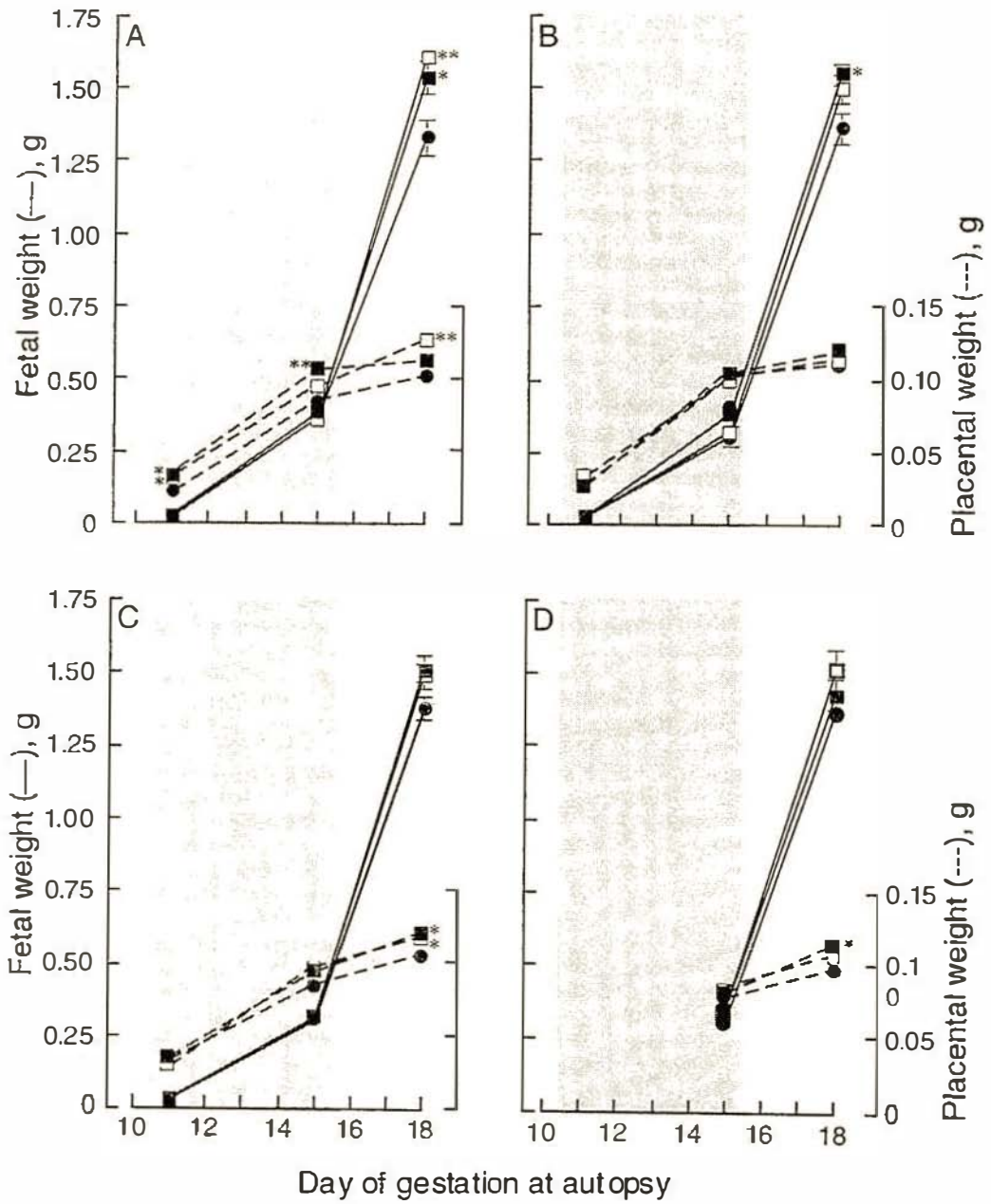

Figure 3.6 The growth of fetus (solid line) and placenta (dashed line) in the remaining uterine horn of the UX (■), UOX (□), and SOP (●) groups operated on, on day 2 (A), day 6 (B), day 10 (C), and day 14 (D). Animals were autopsied on day 11, day 15, and day 18 of pregnancy. Hatched areas indicate the data obtained from fixed preparation. Data was grouped and analysed by considering the Operation factors for each Day group. Values are mean (± SEM); *$p<0.05$ and **$p<0.01$ significantly different from its SOP group ($n = 5$; Tukey HSD-test).

Until day 15 of pregnancy the growth of fetuses was not affected by the removal of one uterine horn since fetal weight of the UX or UOX groups was not different from its sham-operated control (SOP group) whether the mother was operated on, on day 2 of pregnancy (Figure 3.5A), or on day 6 (Figure 3.6B), or on day 10 (Figure 3.6C) or on day 14 (Figure 3.6D). After day 15 of pregnancy, fetal weight of either the UX and UOX groups or the UX group alone, was higher than that of SOP group. These differences, however, are only significant in groups of animals operated on day 2 and day 6 of pregnancy (Figure 3.6A and 3.6B). In contrast, significant differences in placental weight between groups was detected from day 11 to day 18 of pregnancy in the animals operated on day 2 of pregnancy. The significant difference between the UX or UOX and SOP group can also be detected at day 18 of pregnancy in the animals operated on, on day 10 and day 14 of pregnancy (Figure 3.6C and 3.6D).

In general, Figure 3.6 also shows that between day 11 and 15 of pregnancy, both fetuses and placentae of all day groups and also of all Operation groups were grown rapidly. The fetal growth line of the UX, UOX and SOP groups was similar to each other, their lines were almost coincident with one another. After day 15 of pregnancy, the fetus showed a marked increase, while the placenta showed a decrease or a plateau in their growth rate. It seems that, the growth of the fetus continued until the day of parturition even though the placenta had reached its maximum size at the end of the first half of gestation period.

A significant effect of the removal of one uterine horn on fetal and placental growth could be detected after day 11 of pregnancy. If the data is pooled by Operation factor without considering day factor (Figure 3.7), it can be seen that both fetal growth and placental growth of both the UX and UOX groups was significantly higher than that of SOP groups with the exception that fetal weight of the UOX group on day 15 of pregnancy were not significantly different from the SOP group. At any day of autopsy, however, there were no significant differences between the UX and UOX group in fetal and placental growth. Again, Figure 3.7 shows that maximum fetal growth occurred between day 15 and day 18 of pregnancy, preceded by the maximum rate in placental growth which occurred between day 11 and day 15 of pregnancy.

Placental mass, but not fetal mass, was significantly influenced by the day factor. However, the effect of the day factor on fetal and placental mass was time-dependent. This factor only effectively induced intra-uterine mass when examined in the earlier days of pregnancy (Figure 3.8). Placental weight of females operated on, on day 2 and

6 of pregnancy was significantly higher than that of females operated on, on day 14 of pregnancy. The placental weight of females operated on day 10 of pregnancy tended to be lower, but not significant statistically, than that of females operated on both day 2 and day 6 of pregnancy, but higher than that of females operated on day 14 of pregnancy (Figure 3.8A). The trend that the earlier the removal of one uterine horn was performed the more rapid intra-uterine growth in the remaining uterine horn will occur was also observed in relation to the fetal growth. At any day of autopsy, fetal growth from females operated at day 2 of pregnancy was higher than that from females operated at day 6, day 10, and day 14 of pregnancy. Thus fetuses from females operated at day 2 of pregnancy had a higher body weight than females operated on at later days of pregnancy, while fetuses from females operated on day 14 of pregnancy had the lowest body weight at any time of autopsy. Figure 3.8 displays another fact that an optimal growth of placenta is an important prerequisite for the growth of fetuses.

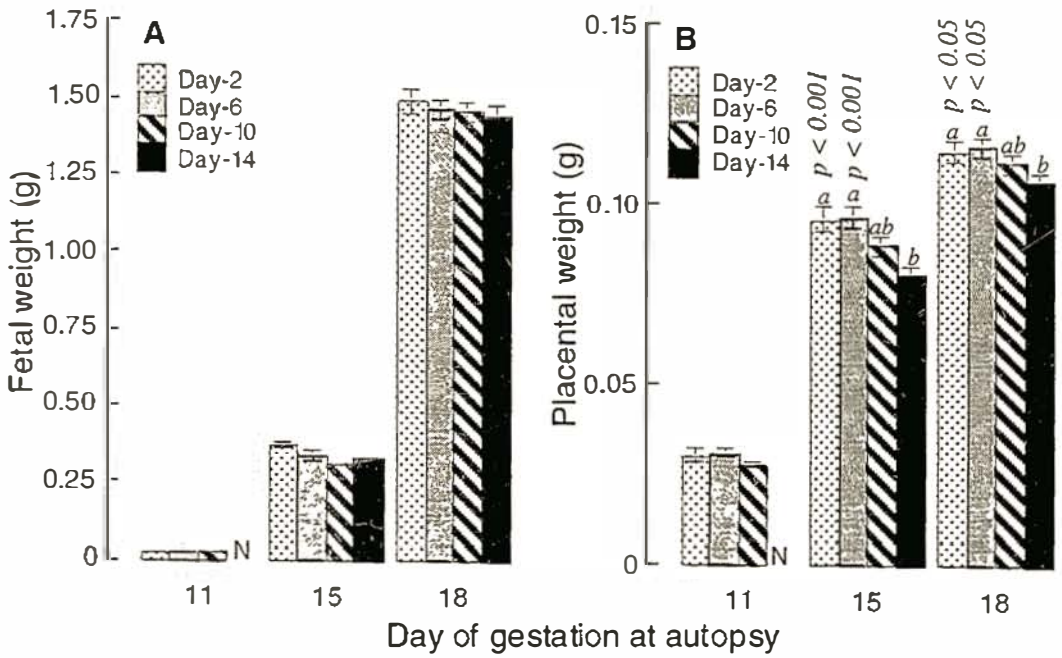

Figure 3.8 The growth of fetus (A) and placenta (B) in the remaining uterine horn of females operated at Day 2, Day 6, Day 10, and Day 14 of pregnancy. Animals were autopsied at Day 11, Day 15, and Day18 of pregnancy; uterine horn obtained from Day 11 and Day 15 of were fixed in Bouin's solution before examination. Data was grouped by the Day factors without considering the Operation factors. Data was not available form females operated on Day 14 and autopsied on Day 11 of pregnancy (N). Values are mean (± SEM), different letters within the same day of autopsy indicate significant difference(the p values are presented at the top of each bar; n = 15; Tukey HSD-test).

Therefore, it can be summarised that, apart from the time dependent, the effect of removal of one uterine horn seems to operate as follows. Removal of one uterine horn initialyy induces maximum growth of the placenta by facilitating more favourable conditions for growth, and these conditions finally prepare the optimum condition for the growth of fetuses in the remaining uterine horn.

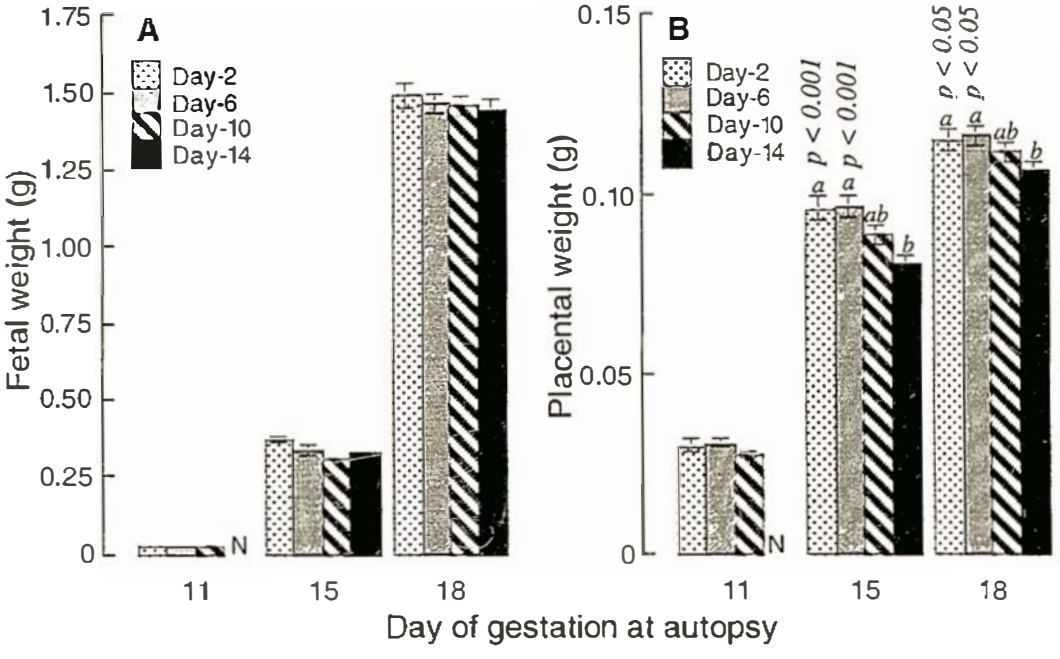

Figure 3.8 The growth of fetus (A) and placenta (B) in the remaining uterine horn of females operated at Day 2, Day 6, Day 10, and Day 14 of pregnancy. Animals were autopsied at Day 11, Day 15, and Day18 of pregnancy; uterine horn obtained from Day 11 and Day 15 of were fixed in Bouin's solution before examination. Data was grouped by the Day factors without considering the Operation factors. Data was not available form females operated on Day 14 and autopsied on Day 11 of pregnancy (N). Values are mean (± SEM), different letters within the same day of autopsy indicate significant difference(the p values are presented at the top of each bar; n = 15; Tukey HSD-test).

A positive correlation between fetal weight and placental weight was found in this study (Table 3.7 and Table 3.8). In Table 3.7 (data by group of Operation factor) can be seen that at day 11 of pregnancy this positive correlation was very strong in UOX ($r = 0.772$, $p < 0.001$) and in SOP ($r = 0.807$, $p < 0.001$) but weak in UX group ($r = 0.365$, $p = 0.181$). This correlation was became weaker; in fact, some groups showed a tendency towards a negative correlation as gestation periods were advanced (UOX group at day 15 and SOP group at day 18, $r = -0.163$ and $r = -0.091$ respectively).

Table 3.7 Correlation coefficients between fetal and placental measurements at three different days of autopsy (Day 11, Day 15, and Day 18) for the UX, UOX and SOP groups. Data was analysed without considering the Day factor.

	Day-11[1]			Day-15[1]			Day-18		
	UX	UOX	SOP	UX	UOX	SOP	UX	UOX	SOP
Mean fetal weight v. placental weight	+0.365 ns	+0.772***	+0.807***	+0.451*	-0.163 ns	+0.456*	+0.212 ns	+0.495*	-0.091 ns
Mean fetal:placental weight ratio v. fetal weight	+0.648**	+0.074 ns	+0.501 ns	+0.406 ns	+0.724***	+0.815***	+0.586**	+0.389 ns	+0.711***
Mean fetal weight v. litter size	-0.183 ns	+0.318 ns	+0.063 ns	-0.152 ns	+0.050 ns	+0.014 ns	-0.207 ns	-0.560*	+0.013 ns
Mean fetal:placental weight ratio v. placental weight	-0.456 ns	-0.569*	-0.019 ns	-0.621**	-0.788***	-0.107 ns	-0.665**	-0.606**	-0.763***
Mean placental weight v. litter size	-0.410 ns	+0.106 ns	-0.035 ns	-0.253 ns	+0.006 ns	-0.530*	-0.116 ns	-0.326 ns	+0.113 ns
Mean fetal:placental weight ratio v. litter size	+0.162 ns	+0.259 ns	+0.193 ns	+0.076 ns	+0.025 ns	+0.311 ns	-0.024 ns	-0.145 ns	-0.049 ns

*p < 0.05, **p < 0.01, ***p < 0.001, ns, not significant (p > 0.05); [1]data obtained from fixed horns (the horns were fixed in Bouin's solution before examination)

Table 3.8 Correlation coefficients between fetal and placental measurements at three different days of autopsy (Day 11, Day 15, and Day 18) for animals operated on, on Day 2 (D-2), Day 6 (D-6), Day 10 (D-10), and Day 14 (D-14) of pregnancy. Data was analysed without considering the Operation factor.

	Day-11[1]				Day-15[1]				Day-18			
	D-2	D-6	D-10	D-14	D-2	D-6	D-10	D-14	D-2	D-6	D-10	D-14
Mean fetal weight v. placental weight	+0.670**	+0.555*	+0.607*	N/A	+0.233 ns	+0.576 ns	+0.075 ns	+0.408 ns	+0.532*	+0.531*	+0.219 ns	+0.585*
Mean fetal:placental weight ratio v. fetal weight	+0.514*	+0.349 ns	+0.525*	N/A	+0.554*	+0.795***	+0.473 ns	+0.395 ns	+0.520*	+0.408 ns	+0.519*	+0.408 ns
Mean fetal weight v. litter size	-0.066 ns	+0.073 ns	+0.351 ns	N/A	-0.394 ns	-0.107 ns	-0.211 ns	-0.156 ns	+0.248 ns	-0.306 ns	+0.155 ns	-0.349 ns
Mean fetal:placental wt ratio v. placental weight	-0.281 ns	-0.547*	-0.328 ns	N/A	-0.670**	-0.003 ns	-0.821***	-0.670**	-0.443 ns	-0.554*	-0.697**	-0.494 ns
Mean placental weight v. litter size	-0.157 ns	-0.087 ns	+0.006 ns	N/A	-0.724**	-0.241 ns	-0.431 ns	-0.073 ns	+0.124 ns	-0.275 ns	+0.210 ns	-0.394 ns
Mean fetal:placental weight ratio v. litter size	+0.099ns	+0.156 ns	+0.549*	N/A	+0.301 ns	-0.067 ns	+0.271 ns	-0.113 ns	+0.129 ns	-0.014 ns	-0.023 ns	+0.059 ns

*p < 0.05, **p < 0.01, ***p < 0.001, ns, not significant (p > 0.05); [1]data obtained from fixed horns (the horns were fixed in Bouin's solution before examination); N/A, data not available.

The decrease in correlation coefficients can also be detected when the data is analysed based on group of day factors (Table 3.8). A significant positive correlation between mean fetal weight and mean placental weight was detected at day 11 of pregnancy in D-2, D-6, and D-10 groups. This association was not significant at day 15 of pregnancy before reaching a significant positive correlative association at day 18 of pregnancy again (Table 3.8).

Table 3.7 and Table 3.8 also show that the association between mean fetal placental weight ratio and mean fetal weight was positive for all groups of day of operation and also for all days of autopsy (Table 3.8). On the contrary, there was a negative association between mean fetal:placental weight ratio and mean placental weight for all groups of day of operation and also for all groups of day of autopsy. Neither mean fetal weight, nor mean placental weight, nor mean fetal:placental weight ratio showed a significant association with litter size. Significant negative correlation between mean placental weight and litter size at day 15 of autopsy in SOP group ($r = -0.530$, $p < 0.05$) (Table 3.7), or at day 15 in D-2 group ($r = -0.724$, $p < 0.01$) (Table 3.7), or between mean fetal weight and litter size at day 18 in UOX group ($r = -0.560$, $p < 0.05$) (Table 3.7), or significant positive correlation between mean fetal:placental weight ratio and litter size at day 11 in D-10 group ($r = 0.549$, $p < 0.05$) (Table 3.8) apparently was only an exclusive occurrence.

Table 3.9 Mean (± SEM) fetal-placental weight ratio for both by day and by Operation groups autopsied at three different days of pregnancy (day 11, day 15, and day 18).

	day of pregnancy at autopsy[1]		
Groups	11	15	18
By day:			
day 2	0.707 ± 0.030	3.861 ± 0.139ab	13.049 ± 0.347
day 6	0.765 ± 0.027	3.441 ± 0.166 b	12.661 ± 0.300
day 10	0.769 ± 0.017	3.482 ± 0.130 b	13.075 ± 0.327
day 14	N/A	4.088 ± 0.114 a	13.510 ± 0.279
By Operation:			
UX	0.776 ± 0.034 a	3.702 ± 0.126	12.922 ± 0.276
UOX	0.694 ± 0.018 b	3.586 ± 0.123	13.158 ± 0.236
SOP	0.782 ± 0.172 a	3.867 ± 0.145	13.143 ± 0.316

[1]Different letters within the same column are significantly different ($p < 0.05$; Tukey HSD-test); N/A, no data available.

e. Fetal:Placental Weight Ratio and Placental Weight Relationship

The efficiency of placenta after removal of one uterine horn was estimated indirectly by calculating the fetal:placental weight ratio for each conceptus (Dwyer *et al.*, 1992). Mean value per horn was then calculated and used in statistical analysis. For SOP groups, mean values of placental efficiency of the two horns were averaged before being analysed.

The ability of placenta to support fetal tissue increased dramatically as pregnancy proceeded (Table 3.9). One gram placenta can afford only less than one gram fetal tissue at day 11 of pregnancy. This ability rose up to about 4/1 (g fetal tissue/ g placental tissue) at day 15 of pregnancy and then increased again and reached a maximum level of about 13/1 at day 18 of pregnancy. Two way analysis of variance revealed that the combination of day and Operation factors had no significant effect on fetal:placental weight ratio. On certain days of autopsy, however, the day and Operation factors worked separately and significantly affecting the fetal:placental weight ratio (Table 3.9). For example, fetal:placental weight ratio of animals operated on, on day 14 of pregnancy and autopsied at day 15 of pregnancy was significantly higher than for those operated on, on day 6 and day 10 ($p < 0.05$). A small, but significant ($p < 0.05$), difference was also detected between UOX and UX and SOP groups, but there was no significant difference between UX and SOP group.

To see whether the day factor or the Operation factor influence the placental efficiency at the same day of autopsy or not, the relationship between fetal:placental weight ratio and placental weight was analysed separately in groups of Day or Operation factor. The results for each day of autopsy are presented in Figure 3.9 and Figure 3.10 (see also Table 3.7 and Table 3.8). At the same day of autopsy, fetal:placental weight ratio of most groups were negatively correlated with placental weight. This suggests that the ability of placenta to support the fetal tissue is decreased as the weight of placenta increased. These relationships were significant in the UX group at day 15 ($r = -0.621$, $p < 0.01$) and day 18 ($r = -0.665$, $p < 0.01$) but not on day 11 of pregnancy. In the UOX group this relationship was significant at all days of pregnancy examined while in the SOP group only significant at day 18 of pregnancy (Figure 3.9, Table 3.7). The negative correlation between fetal:placental weight ratio and placental weight of the animals operated on day 2 of pregnancy was only significant at day 10 of pregnancy while the animals operated on day 6 showed a significant relationship at day 11 and day 18 of pregnancy. The animals operated on day 10 showed a significant relationship at day 15 and day 18 of pregnancy.

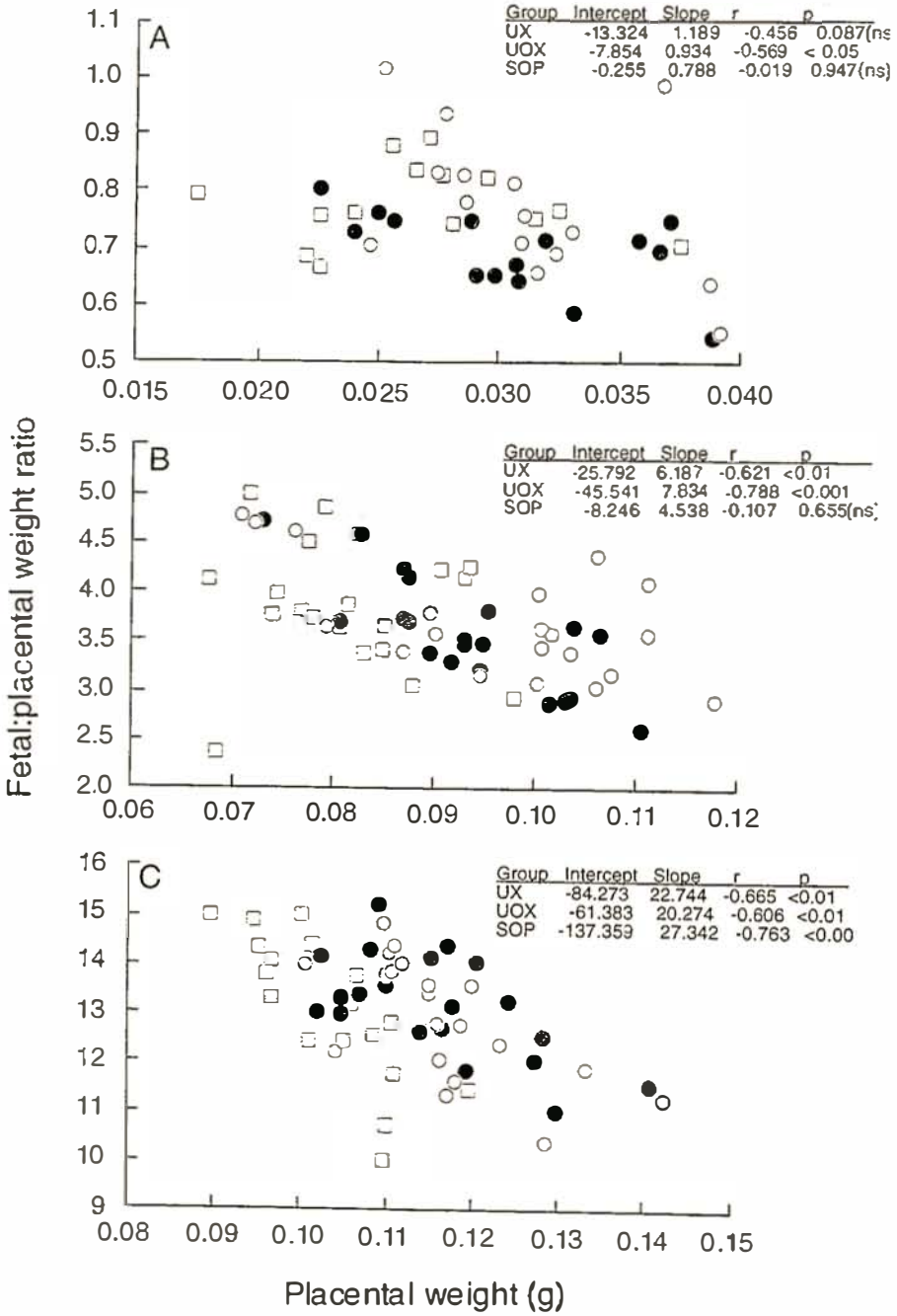

Figure 3.9 The relationship between fetal:placental weight ratio and placental weight at Day 11 (A), Day 15 (B), and Day 18 (C) of pregnancy after removal of one uterine horn at four different days of pregnancy (Day 2, Day 6, Day 10, and Day 18) for three different types of operation (○= UX, ●= UOX, and □= SOP). The values of intercept, slope, correlation coefficient (r), and the degree of significance (p) for each group are shown at the upper right of each diagram.

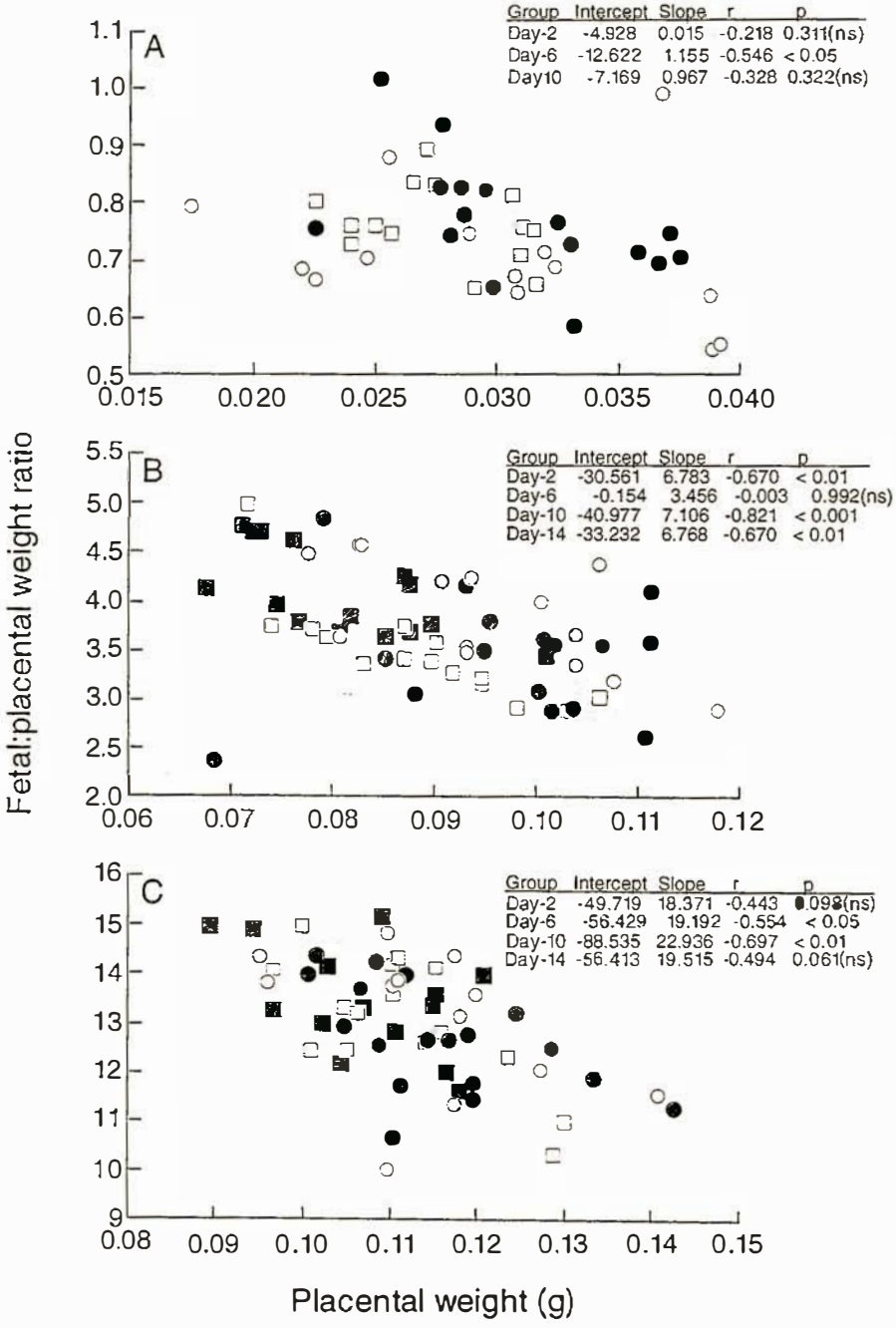

Figure 3.10 The relationship between fetal:placental weight ratio and placental weight at Day 11 (A), Day 15 (B), and Day 18 (C) of pregnancy for females operated on Day 2 (○), Day 6 (●), Day 10 (■), and Day 14 (▣). The values of intercept, slope, correlation coefficient (r), and the degree of significance (p) for each group are shown at the upper right of each diagram.

3.4 Discussion

a. Effect on the Length of Gestation

The length of gestation is controlled by a complex process including external factors, such as photoperiod, and internal factors, such as litter size. Litter size influences gestation length in normally polytocous animals such as mice; small litters are associated with a longer gestation period (Dewar, 1968). For example, Biggers *et al.* (1963), by unilaterally ovariectomizing or by unilaterally ligating the Fallopian tube before mating in mice, showed that the length of gestation was influenced by the total number of conceptuses in the female. The fact that length of gestation is negatively related to the size of litter (Biggers *et al.*, 1963; Dewar, 1968) led us to infer that removal of one uterine horn at several different days of gestation would halve the size of litter and this condition would lead to prolongation of the gestation period.

In the present study removal of one uterine horn on several different days of gestation significantly reduced the size of litter. Operated female mice, whether or not their ipsilateral ovary was removed, had a smaller litter size at birth compared with sham-operated control group (Table 3.4). Although, in mice, the remaining ovary can undergo compensatory growth (regarding the number of ova ovulated) after unilateral ovariectomy (Biggers *et al.*, 1963; McLaren, 1963), in this study, as expected, removal of ipsilateral ovary following removal of uterine horn in the UOX group, had no effect on the size of litter compared to the UX group. Surgery procedures in this study were performed after copulation. The earliest operation was performed on day 2 of pregnancy and the latest one was on day 14 of pregnancy. This procedure was the opposite to the procedure used by Biggers *et al.* (1963) who examined the effect of litter size on gestation period in mice by using females from which one ovary had been surgically removed before sexual maturity. The later procedure would allow the remaining ovary to adjust the number of ova to be ovulated.

In the present study, the UX and UOX groups where their litter size was reduced up to a half of normal size had a mean length of gestation period almost one day longer than that of the SOP group with one day difference in modal length of gestation (modal gestation length was 19 days for the UX and UOX groups and 18 days for SOP group) (Figure 3.2). This litter size-related gestation length is consistent with the previous studies reported by Biggers *et al.* (1963), McLaren and Michie (1963), and Dewar (1968). However, in mice, Dewar (1968) has failed to detect a continuously linear inverse relationship over the range of litter sizes, but this relationship was mostly confirmed in the litters below the modal size.

Because the length of gestation of pregnant mice in this study is not different in the UX and UOX group, this shows that the controlling influence of the litter size on the length of gestation period originated from the fetuses themselves or of their association with placental tissue, and not from the number of corpora lutea of pregnancy formed in the ovary. This is fully consistent with a study on the pig (Omtvedt *et al.*, 1965; Martin *et al.*, 1977) and with a study on mice (Biggers *et al.*, 1963), who found that litter size, but not the number of corpora lutea in the ovary, related inversely with the length of gestation. Biggers *et al.* (1963) suggested that the effect of litter size on the length of gestation was solely due to the total number of fetuses, and not on their distribution, in the uterine horn. In other words, the effect of litter size on the length of gestation operates systemically rather than locally.

The nature of the systemic effect of litter size on length of gestation period has been described by McLaren and Michie (1963) and McLaren (1967). By varying total fetal mass independently of fetal number, McLaren and Michie (1963) suggested that systemic effect of the litter size on the length of gestation was a reflection of the total fetal mass or total placental mass rather than the number of conceptuses in the uterine horn. The heavier young were associated with a shorter gestation period. The present study is also in agreement with the finding of McLaren and Michie (1963). If the data was pooled by the type of operation, both mean total weight of fetus and mean total weight of placenta per female in the SOP group were heavier than those of the UX and UOX groups (data not shown).

Dewar (1973), conducted an experiment which attempted to examine the influence of total hysterectomy on the duration of pseudopregnancy in albino mice; he found no effect of total hysterectomy on the duration of pseudopregnancy. In addition, loss of the placenta on the day 8 of pregnancy did not affect the duration of corpora lutea activity but it declined to a near constant level from the day 14 of pregnancy onwards. Loss of the placenta after day 14 of pregnancy was followed by almost immediate cessation of luteal function. Dewar (1973) suggested that these findings may be due primarily to reduction in pituitary luteotrophic activity where this period corresponds to a time of increased gonadotrophin content of the pituitary. There is no indication from the present work that the presence of one uterine horn exerts a local or systemic effect in hastening the functional decline of the corpora lutea after removal of one uterine horn (see Chapter 4)

The prolongation of the gestation period in small litters (as in UX and UOX group) compared with large litters (as in SOP groups) in the present study is likely not caused by delayed implantation since the day of operation was not accompanied by any effect on the length of gestation period.

The finding that both the Day factor and the combination of Day and Operation factor had no effect on length of gestation period (Table 3.4) tallies also with the previous explanation about the effect of the uterine removal. Because of litter size was systemically (total number of conceptuses in the female) and not locally (number of conceptuses in the more crowded uterine horn) affected by the length of gestation, thus the mechanical tension on the uterine walls due to the fetal growth, which increases particularly rapidly in the later stages of pregnancy is not involved in initiating parturition by increasing uterine irritability. Recently, many reports (for review see Thorburn, 1991) revealed that the onset of parturition is initiated by the hormonal events rather than by mechanical events. Parturition is initiated by the increase in the E/P ratio due to the secretions of fetal cortisol. The changes in this E/P ratio will stimulate the uterine synthesis and release of $PGF_{2\alpha}$ which results in activation of mechanical events of parturition (Thorburn, 1991; Johnson and Everitt, 1995). Because the level of corticoid may be increased as the number of fetuses in the uterine horn increase and because the corticoids from the fetus are more important than maternal corticoids in initiating the onset of parturition thus the day factor would not alter the onset of parturition of a group of animals treated with the same type of operation.

This study was designed towards defining the effect of removal of one uterine horn on pre-natal growth of the embryo in the remaining uterine horn. It is possible of course that other factors influencing the length of gestation period in mice were not considered in the experimental design. Therefore these methodological problems in the research design limit our interpretations on the effect of treatment on the length of gestation period in mice.

b. Effect on the Uterine-related Arteries Diameter

The uterine arteries diameter measurement estimated the growth of arteries in the remaining uterine horn after removal of the contralateral uterus. It was assumed that the uterine vasculature growth in the remaining horn would be favoured by removal of one uterine horn and its vasculature. Because the uterus undergoes dramatic growth and alterations in vasculature in preparation and adaptation for pregnancy (Makowski,

1977), it was expected that the uterine-related arteries diameter in both UX and UOX groups (operated animals) would be greater than that in the SOP group (sham-operated control animals). It was also expected that arteries diameter in the remaining uterine horn of the animals operated on at earlier days of pregnancy would be greater than that of animals operated on at later days of pregnancy.

In the present study the final diameter (as measured at day 18 of pregnancy) of utero-ovarian arteries (Uo) and placental arteries (Pl), but not uterine arterial diameter (Ut), of both the UX and UOX females were significantly higher than those of the sham-operated animals (SOP) females when operation was performed on day 2 of pregnancy (Figure 3.3). If animals were operated on day 6 of pregnancy, the difference between operated and sham-operated control groups in artery diameter was only significant in the Pl arteries whereas the diameter of both Uo and Ut arteries showed no significant differences between operated and sham-operated control groups. At day 18 of pregnancy Pl arteries increased in diameter up to about five-fold (for the UX group) or three-folds (for the UOX group) compared with their initial diameter at day 2 of pregnancy, whereas in the SOP group they only reached an increase of about two-folds compared with their initial diameter. Although arteries' diameter of both the UX and UOX groups tend to be higher than that of the SOP group, removal of uterine horn after day 6 of pregnancy had no significant effect on uterine vasculature in the remaining uterus.

These findings led us to summarise that the removal of one uterine horn caused vascular hypertrophy in the remaining uterine horn, and that uterine vasculature undergoes a dramatic growth during the early gestation period, particularly between day 2 and day 6 of pregnancy, and that during that period the placental arteries had the most dramatic growth. In physical terms, flow rate is inversely related to the resistance and resistance is inversely related to the radius of a pipe. Thus, if this concept applies to the haemodynamic aspect of the artery, blood flow in a big artery should be higher than that in a smaller one, and blood flow should increase as diameter of the artery increases. Because uterine-related artery diameter continues to grow during the gestation period, as shown by the increase in their diameter, this should be accompanied by an increase in the blood flow during the course of pregnancy. These findings are in agreement with those of Garris (1983; 1984) who reported the regulative role of the uterine vasculature on the growth of fetus and placenta in the uterus. The initial fetal-placental unit formation and its subsequent maintenance in the guinea pig, for example, was marked by both dramatic growth of the uterine vasculature and fluctuation in uterine blood flow

Garris, 1984). The initial placental formation was associated with an elevation in uterine blood flow (Garris, 1984; Edward and Milligan, 1987) and reduction in the uterine blood flow during early pregnancy has the greatest effect on placental growth (Garris, 1984; Clapp, 1989). In mice implantation is completed at day 6 of pregnancy (Rugh, 1968), hence a dramatic increase in uterine-related artery diameter, particularly in PI artery diameter, during early pregnancy as observed in this study it may be necessary to support the rapid increase in uterine weight at implantation. An increase in uterine blood flow around the time of implantation (Garris and Whitehead, 1981) or around the decidual cell reaction period (Garris and Dar, 1985) as reported occurred in the guinea pig supports this speculation. Clapp (1989) hypothesised that the placenta is a site of intra uterine growth regulation because the decrease in the placental perfusion or nutrient delivery induces the placental release of one or more peptides which inhibit the effect of multiple growth factors and thereby suppress the rate of fetal and placental growth.

Mice have a duplex uterus with a loop artery feeding each uterine horn. As expected by McLaren and Michie (1960), and von Saal and Dhar (1992) revealed that blood flow in the uterine artery is bidirectional in the house mouse. As a consequence of this bidirectional flow, conceptuses at the cervical and ovarian ends of each uterine horn were favoured by the greater blood flow (vom Saal and Dhar, 1992; Even *et al*., 1994). That is why the smallest fetuses are located in the middle region of a uterine horn as reported in guinea pigs (Eckstein *et al* , 1955), mice (McLaren and Michie, 1960), and in the rabbit (Bruce and Abdul-Karim, 1973)

c. Effect on Prenatal Growth

The focus of the present study was on the effect of the removal of one uterine horn on the fetal growth in the remaining uterine horn and the questions asked were, first, would the remaining uterine horn be able to provide a more favourable condition for the growth of fetuses after removal of its counterpart horn. Secondly, if so, do any day factors exert a significant effect. Thirdly, do the Operation factor and the day factor worked independently or in combination ?

The inverse relationship between fetal weight and fetal lenght in polytocous animals, such as mice (Healy *et al* , 1960, McCarthy, 1965, McLaren, 1965), is due primarily to pre-natal competition for a limited pool of nutrients in the maternal circulation (Clapp, 1989). Clapp (1989) suggested that placental and fetal growth are limited by the rate of

utero-placental blood flow and a minor reduction in flow results in growth restriction with no evidence of metabolic abnormalities. Because of the limitation of nutrients and space provided by the mother, of course, the number of competitors is an important determinant in this competition. The rate of intra-uterine growth is partly affected by the number of fetuses in the same uterine horn (local effect) and partly by the number of litter mates (systemic effect) (Eckstein and McKeown, 1955a). It was assumed that removal of one uterine horn would result in redistribution of maternal cardiac output into the remaining uterine horn. If so, according to Clapp's flow limited-prenatal growth theory, the redistribution of blood flow as a result of uterine removal, would provide more nutrients to the conceptuses in the remaining uterine horn. Thus, the degree of pre-natal competition between conceptuses for nutrients could be reduced by the removal of one uterine horn. This more favourable condition would induce fetal growth at a higher rate.

Biggers *et al.* (1963) and McLaren (1963) showed that unilateral ovariectomy alone in unmated mice resulted in ovarian hypertrophy, thus there is no difference between operated and unoperated animals in their ovulation rate. Dziuk (1968), Webel and Dziuk (1974) and Knight *et al.* (1973; 1977) reported that unilateral hysterectomy-ovariectomy before mating resulted in ovarian hypertrophy, thus the number of ova ovulated by the operated females is not different from that of unoperated females, and that operated females have similar reproductive performance to the unoperated females up to day 30 of pregnancy. With respect to the ovulation rate, these ovarian compensatory effects, after unilateral ovariectomy in mice (Biggers *et al.*, 1963; McLaren, 1963) or after unilateral hysterectomy-ovariectomy in the pig (Dziuk, 1968; Webel and Dziuk, 1974; Knight *et al.*, 1973; 1977), and uterine compensatory effects (with respect to the space available in the uterine horn) after unilateral hysterectomy-ovariectomy in pig (Dziuk, 1968; Webel and Dziuk, 1974; Knight *et al.*, 1973; 1977) could not occur in this study because all of the operation procedures were performed during the course of gestation. Although an increase in the number of Graafian follicles in the remaining ovary after removal of one ovary in the pregnant mouse has been reported by Ross and Beaumont (1974), similar to that pattern of response in the non-pregnant mouse as reported by McLaren (1963), unilateral ovariectomy before implantation resulted in embryonic development arrest and implantation decrease due to hormonal deficiency in the adjacent uterus of the rat (Nuti *et al.*, 1971).

During the course of pregnancy, total weight of the uterus is determined by the gravid components and the weight of uterine tissue itself; and the growth of the uterus depends

primarily on the number of developing embryos contained. In the present study, the uterine horn undergoes extensive growth during the course of pregnancy (Table 3.6; Figure 3.4 and 3.5). This is in close agreement with the theory that the growth of the uterus is directed to accommodate the developing embryos contained by the uterus itself, that is, the growth of the uterus is dependent on both contact effect and hormonal effect (Huggett and Hammond, 1964). The contact effect exerts itsself as soon as the attachment of the embryo to the uterine mucosa is made, while the hormonal effect begins to take effect during mid-pregnancy (Huggett and Hammond, 1964). Although the percentage of empty uterine tissue decreased as pregnancy proceeded, the real weight, in fact, increased extensively as the whole uterine weight increased (Table 3.6). According to Huggett and Hammond (1964), the enlargement of the uterus during pregnancy is partly caused by active growth particularly in the muscular tissue and partly by the dilation of the mucosal tissue due to the growing embryonic contents.

Gravid components of the uterus comprised fetuses and placentae and their membranes, fetal fluid and the uterine tissue itself. In this study total weight (%) of fetal fluids, placenta and uterine tissue per horn reached the maximum values at day 11 of pregnancy. After day 11 of pregnancy, each value decreased gradually to reached a minimum value below or about 10% of total weight of the uterine horn at day 18 of pregnancy, just about one day before the expected parturition. In the same period total weight (%) of the fetus per horn showed a marked increase from about 20% at day 11 to a most dominant value of about 80% at day 18 of pregnancy. This tendency was observed either in the operated (the UX and UOX) or in the unoperated (the SOP) groups (Figure 3.4). This tendency can also be detected if the data is analysed in groups of day factors (Figure 3.5). This is to say, therefore, that during the first half of gestation period fetal fluids had a greater rate of growth than any other gravid components, and then this rapid growth rate is taken over by the fetuses during the second half of gestation period. The total fluid content of the conceptuses is made up of yolk-sac fluid, exocoelomic fluid and amniotic fluid. Payne and Deuchar (1972) noted that the amount of extra-embryonic fluid in the rat is largely regulated by the yolk sac. A rapid increase in the amount of fetal fluid during the early gestation period has also been reported by Renfree *et al.* (1975) who studied the developmental changes in fetal fluids in four different strains of mice from day 11 to day 18 of pregnancy. Renfree *et al.* (1975) showed that the amount of fluid was related to both the day of pregnancy (the fluid reaching a peak on day 16) and fetal weight. Later, McLaren *et al.* (1976) clearly showed that fetal fluid weight was inversely related to both litter size and fetal weight. In this case the effect of fetal weight was exerted locally while litter size affected the

fluid systemically. The finding in this study, therefore, supports the idea that the changes in the fetal fluid throughout gestation may be a necessity for continuation of fetal growth and maturation without any corresponding increase in intra-uterine pressure (Huggett and Hammond, 1964; Renfree *et al.,* 1975; McLaren *et al.,* 1976). However, the peak level of fetal fluid reached on day 11 of pregnancy in the present study does not parallel that found by Renfree *et al.* (1975) who found a peak amount of fetal fluid on day 16. It is may be due primarily to differences in the approach procedure used. In this study, the amount of fetal fluid was determined indirectly from the fixed object, except for day 18 of pregnancy, while Renfree *et al.* (1975) worked with fresh preparation for each day of gestation examined. Besides that, strain differences should also be considered. In mice, a one to two days difference between strains in reaching the fetal fluid peak volume (Renfree *et al.,* 1975), and a difference between strain in total amount of fetal fluid at the same day of pregnancy (Johnson, 1971) have been documented.

As mentioned above, the main focus of this study is to examine the effect of removal of one uterine horn on the fetal and placental growth in the remaining uterine horn. It was expected that uterine transection would result in redirection of blood flow into the remaining uterine horn which, finally, prepares a more favourable environment for growth.

In the present study, all groups, except the group of animals operated on, on day 14 of pregnancy (data before day 14 of pregnancy for this group was not available), placental growth, as indicated by weight, occurs predominantly in the first half of gestation whereas fetal growth accelerates in mid-gestation and proceeds rapidly until term (Figure 3.6; 3.7; 3.8).This result indicates that in the mouse the rapid growth of the placenta precedes the period of rapid fetal growth and that placental development remain rather static during the period of rapid fetal growth. This is supported by the positive relationship between fetal and placental weight (Table 3.7 and 3.8). The placental limitations of fetal growth in some species, particularly at the end of gestation period have been reported by some authors (mice: McLaren, 1965; pig: Knight *et al.*, 1977; rat: Norman and Bruce, 1979a; 1979b; guinea pigs: Eckstein and McKeown, 1955a; 1955b; Eckstein *et al.*, 1955). Knight *et al.* (1977), who observed the conceptus development in intact and unilaterally hysterectomised-ovariectomised gilts, reported an increase in fetal death after day 35 of gestation in a crowded uterine horn. They suggest that this increase in fetal death is primarily caused by the placental insufficiency to support fetal growth after day 35 of pregnancy. Eckstein *et al.* (1955), who studied the effect of litter size on variation in placental weight in the guinea-pig, and McCarthy (1967), who

examined the effect of litter size and maternal weight on fetal and placental weight in mice suggested that the growth of the fetus is influenced by both systemic and local effect. Although placental weight was not a good index of placental function and did not wholly explain the association between mean fetal weight and litter size (Eckstein *et al.*, 1955; McKeown *et al.*, 1973) this parameter was also influenced by both systemic and local effects (Eckstein *et al.*, 1955; McCarthy, 1967).

Recently, the basic pattern of placental regulation of fetal growth has been extensively reviewed elsewhere (Owens, 1991; Wang and Chard, 1991; Fowden, 1995; Harding and Johnston, 1995; Robinson *et al* , 1995). The effect of placenta on the growth of the fetus is exerted by both metabolic and endocrine mechanisms (Robinson *et al.*, 1995), and to allow the fetal growth this mechanisms must be supported by a sufficient nutrient supply (Harding and Johnston, 1995). The placenta controls the fetal growth by its capacity to transfer nutrients from the mother to the fetus and by its capacity to utilize and modify the available nutrients (Owens, 1991). All insulin, cortisol, thyroxine, and pituitary growth hormones are involved in both fetal tissue accretion and differentiation; this action, in part, may be mediated by the insulin-like growth factors (IGFs) (Fowden, 1995).

Both fetal weight and placental weight of the SOP group was lower than that of both UX and UOX groups. Two important findings can be revealed from this result. Firstly, the effect of uterine limitation on the growth of fetal and placental weight operated by day 14 of pregnancy. The maximal uterine carrying capacity for a large litter may be exceeded after day 14 of pregnancy. This strongly supports 'the limitation theory' suggested by McKeown *et al* (1976). Secondly, fetal growth retardation detected in the SOP groups compared to the UX and UOX groups suggests the existence of the systemic effect on fetal growth in our animals as revealed by Eckstein *et al.* (1955) in the guinea-pig, McCarthy (1967) in the mouse, and by McKeown *et al.* (1976). In the mouse, there was a negative correlation between fetal weight and litter size at birth on the two days before birth (Healy *et al.*,1960, McCarthy, 1965; McLaren, 1965); reducing litter size consequently tended to increase fetal weight.

There was no combination effect of Day and Operation factor on the fetal and placental weight at any stage of pregnancy observed. This result indicated that both the Day factor and the Operation factor acted independently.

The lack of differences in both placental and fetal weight observed on day 11 of

pregnancy may be due to either the nature of the data themselves or the methodological limitation faced in this study. In fact, due to the difficulty of separating the fetus from the embryonic membrane in fresh preparation at days 11 and 15 of observation (and also observation data at day 3 and 7), they were fixed in Bouin's solution before examination. The uterine tissue and its gravid components may not be evenly dehydrated by the Bouin's solution during fixation period or may not be dried properly during the examination period.

Both the Day factor and Operation factor are independently affected the total fetal weight, total placental weight, and total placental:fetal weight ratio at day 18. Those parameters were lower in the group of animals operated on day 14 of pregnancy than in those of day 2, day 6, and day 10 of pregnancy. The difference was only significant statistically compared to day 6 of pregnancy. Between groups and types of operation, the SOP group was significantly lower than those of the UX and UOX group. There were no a significant differences between UX and UOX groups. This is a strong confirmation to the finding on the individual mean of the same parameters described on the previous page of this report. These results also suggested that the reduction of litter size up to half the normal size (SOP group) by removing one uterine horn allows both the fetus and placenta to grow optimally in the remaining uterine horn. The optimal fetal and placental growth in the remaining uterus may be provoked by the availability of nutrients in a large amount sufficient for the growth of conceptuses.

Within groups of day, but not within groups of operation, there was a positive relationship between fetal and placental weight. The common regression coefficient for group of animals operated on day 2, day 6, day 10, and day 14 of pregnancy were 1.22 ($r^2 = 0.732$, $p < 0.001$), 0.37 ($r^2 = 0.875$, $p < 0.001$), 2.06 ($r^2 = 0.797$, $p < 0.001$), and 0.77 ($r^2 = 0.838$, $p < 0.001$) respectively. A positive relationship between fetal and placental weight can be used to argue a limitation of the placental function to support fetal growth near term (Norman and Bruce, 1979a). This relationship has been reported in rabbit, rat, guinea-pig, and in many monotocous animals (Norman and Bruce, 1979a) but not usually in the mouse (McLaren, 1965).

Chapter 4
Plasma Concentrations of Progesterone and Ovarian and Uterine Histology

4.1 Introduction

4.1.1 Uterine Involvement in Progesterone Secretion by the CL

a. Luteotrophic effect of the uterus:

It is now accepted that the luteal life span during pregnancy is controlled by the uterus, the adenohypophysis, and the conceptuses. Luteotrophic activity of the pituitary during pregnancy in mice has been reported by several authors (Jaitly *et al.,* 1966; Choudary and Greenwald, 1969). However, this activity becomes insignificant after the first half of gestation since hypophysectomy after Day 10 of pregnancy has no effect on pregnancy (Choudary and Greenwald, 1969). After Day 10 of pregnancy the luteotrophic role of the pituitary is taken over by the placenta (Choudary and Greenwald, 1969; Kohmoto and Bern, 1970). In greater detail, Critser *et al.* (1980) reported that the conceptuses play a significant role in maintaining luteal life span during pregnancy. The trophic action of the conceptuses on the corpora lutea (CL) is exerted in two ways. Firstly, by day 8 of pregnancy the conceptuses inhibit the uterine luteolytic mechanism. Secondly, by day 10, the conceptuses actively produce substances with mammotrophic and luteotrophic activity (Critser *et al.,* 1980).

Although Dewar (1973) reported that the uterus did not influence the luteal life span, several investigators have reported the presence of a regulatory effect of the uterus on circulating progesterone concentrations in mice (Michael *et al.*, 1975; Barkley *et al.*, 1977; Pointis *et al.*, 1981; Soares and Talamantes, 1983; Humphreys *et al.*, 1985) and rats (Rothchild *et al.*, 1973). The capability of the uterine tissue to concentrate progesterone above peripheral blood levels throughout early and mid-pregnancy has also been reported by Wiest (1970).

The relationship between progesterone concentrations and fetal numbers has also been studied by several investigators. For example, Humphreys *et al.* (1985) reported that plasma progesterone concentration was positively related to fetal number, and that fetal number was positively correlated with CL volume. Between days 6 and 12 after mating, the plasma progesterone level of intact pregnant mice was greater than that of both hysterectomyzed or intact pseudopregnant mice, and levels in hysterectomyzed mice were higher than those of intact pseudopregnant animals (Critser *et al.*, 1982). The observation that $PGF_{2\alpha}$ concentration was lower in pregnant and higher in intact pseudopregnant mice than in hysterectomysed pseudopregnant mice (Critser *et al.*, 1982), suggests that the luteotrophic effect of the uterine tissue is exerted by regulating the $PGF_{2\alpha}$ secretion.

b. Luteolytic effect of the uterus:

Numerous investigators have studied the effects of uterine removal on ovarian function, particularly luteal function in several species. The results have been reviewed by several authors including Bland and Donovan (1966) and Anderson (1973; 1977). Briefly, while in primate species, including the human being, the ovarian function continues uninterrupted after removal of uterine tissue, in non-primates, the effects of uterine removal vary between species and according to the reproductive state of the animals. In the cyclic mice, rat, and rabbit, for example, hysterectomy does not interfere with ovarian function, but it can prolong the ovarian function close to that of normal pregnancy if it is conducted after a sterile mating. In contrast, in guinea pigs, removal of the uterine horn at day 5 of the cycle causes a prolongation of progesterone secretion for more than eight months. The persistence of this progesterone secretion, however, will be reduced for a period of equivalent to gestation (65 - 70 days) if only the uterus is removed on day 10 of the cycle. In those species in which uterine removal prolongs the ovarian function, there is a relationship between the functional length of the ovary and the amount of uterine tissue remaining after operation (Bland and Donovan, 1966; Anderson, 1973; 1977).

Although luteal regression (luteolysis) can be caused by the withdrawal or inadequacy of luteotrophic support, in many species it can also be caused by active production of a luteolytic factor that brings about normal luteal regression (Bland and Donovan, 1966; Anderson, 1973; Anderson, 1977; Johnson and Everitt, 1995).

Luteal function can be prolonged in many mammals, with the notable exception of primates, either by hysterectomy or by ligating the tissues, including the blood vessels, between the uterus and the ovary; however if the endometrium of the excised uterus is homogenised and injected, luteolysis occurs (see Johnson and Everitt, 1995). Both the ovary and the endometrium may be sources of lutolytic factors, depending on the species and stage of the cycle. Hysterectomy results in regression of the CL only in the contralateral ovary in the ewe, and if the whole uterus is transplanted elsewhere in the body, luteolysis is prevented unless the ovaries are transplanted with the uterus as a unit (Hansel *et al.*, 1973; Johnson and Everitt, 1995). In addition, a small segment of uterine horn remaining after partial hysterectomy in the sow is able to induce an earlier luteal regression in the ovary ipsilateral to that uterine segment than in the contralateral ovary (see Anderson, 1977). These results demonstrate that there is a humoral factor which passes from the endometrium to the ovary and causes luteolysis, that the luteolytic effect of the uterus is exerted locally and that the ovary must be in close proximity for luteal regression. The endometrial substance responsible for CL luteolysis has now been established in the ewe, sow (Hansel *et al.*, 1973; Heap and Flint, 1984), cow (Hansel *et al.*, 1973; Heap and Flint, 1984; Johnson and Everitt, 1995), sheep, guinea-pig and horse (Heap and Flint, 1984; Johnson and Everitt, 1995) as $PGF_{2\alpha}$. $PGF_{2\alpha}$ passes from the endometrium into the uterine vein, and then by a local counter-current transfer it passes to the ovarian artery, and thence to the ipsilateral ovary (Hansell *et al.*, 1973; Heap and Flint, 1984; Johnson and Everitt, 1995).

The production of $PGF_{2\alpha}$ is stimulated by the oxytocin secreted from the CL, and if oxytocin is neutralised, then luteolysis is delayed (Johnson and Everitt, 1995).

4.1.2 Uterine removal and progesterone levels

The effect of uterine removal (or hysterectomy) on progesterone concentration has been reported by several investigators in mice (Critser *et al.*, 1982), rats (Rothchild *et al.*, 1973), guinea pigs (Rowlands and Short, 1959; Heap *et al.*, 1967) and in sheep (Southee *et al.*, 1988). Hysterectomised pseudopregnant mice had plasma progesterone concentrations at day 6, 8, 10, 12 after mating which were greater than those of intact pseudopregnant but lower than those of intact pregnant mice (Critser *et al.*, 1982). In hypophysectomised rats, progesterone secretion continues for about 3 days after removal of the uterine horn at day 12 of pregnancy (Rothchild *et al.*, 1973). Although the level of progesterone found by Rothchild *et al.* (1973) was lower than that of either the intact or of the day 12 hypophysectomised pregnant rats, this finding is consistent with the finding of Macdonald *et al.*, (1970) who reported that the luteolytic effect of LH can be reduced by removing uterine tissue of the hypophysectomised rats. In non-pregnant guinea pigs, removal of the uterine horn results in both a prolongation of luteal function and an increase in secretory capacity (Rowlands and Short, 1959; Heap *et al.*, 1966). Prolongation of the progesterone secretion after hysterectomy was also found in the anoestrous ewe which implies that premature regression of CL can be prevented by removing the uterine tissue (Southee *et al.*, 1988).

Luteal regression of the cycling beef heifer (Anderson *et al.*, 1962) or pseudopregnant rat (Labhsetwar, 1967) is dependent upon a stimulus from the uterus. Hysterectomy of cycling heifers results in an extension of the luteal function to a period equivalent to or exceeding that of pregnancy (Anderson *et al.*, 1965). Hysterectomy failed to affect significantly the hypophysial levels of gonadotrophins during the period studied by Labhsetwar (1967) and Christian *et al.* (1968). This allows the possibility that luteolytic factors secreted by the endometrium act directly on CL without interfering with pituitary function. Thus elimination or deletion of these factors (for example, by hysterectomy) would allow the CL to remain active to secrete progesterone for longer than normal.

Hysterectomy at earlier days of the cycle also prolongs luteal function and increases the luteal content of progesterone in the ewe (Anderson, 1973). Both luteal content and peripheral progesterone concentration are raised after hysterectomy, which suggests maintenance of the CL in this species. In goats, hysterectomy after mating decreases jugular progesterone concentration over a period of 5 to 10 days, remains relatively constant for a variable length of time, and then decreases either gradually or abruptly to anestrous levels (<0.5 ng/ml). This indicates that maintenance of progesterone secretion

occurs in hysterectomised goats, although at a depressed level for a variable length of time after mating (147 to 184 days) (Currie and Thorburn, 1974).

Masuda *et al.*, (1967) reported that either the presence of embryos in the uterus or the absence of the uterus caused an increase in progesterone production as early as Day 14 of pregnancy, when compared with the same day of the cycling pig. Therefore, it may be that, as in mice (Critser *et al.*, 1980), luteal maintenance during pregnancy in the pig can be exerted either by suppression of the luteolysin factors or by secretion of the luteotrophic factors. However, a contradictory finding was reported by Belt *et al.* (1970), who found that the luteal content of progesterone of a pig hysterectomised on Day 40 and 60 of pregnancy was generally similar to that of intact animals during comparable stages. This was then confirmed by the finding of similarities in the fine structure of granulose lutein cells in the CL in pregnant and hysterectomised animals (Belt *et al.*, 1970). It is likely that the trophic influences controlling the CL may be similar during pregnancy and after hysterectomy.

Though hysterectomy shortens the anestrous interval, it has no effect either on progesterone, estradiol, prolactin, and growth hormone concentrations in cyclic dogs (Hoffmann *et al.*, 1992).

In guinea pigs, arterial concentration of progesterone is low during the normal cycle, after hypophysectomy and hysterectomy in the cyclic animals, and in the first 2 weeks of pregnancy (Heap *et al.*, 1967). During gestation the placenta and ovaries contribute to the progesterone content of the peripheral blood, but placental production is relatively more important near term (Heap *et al.*, 1973). Rowlands (1961) reported that hysterectomy in cyclic animals resulted in prolongation of luteal function, noting that the stage of the cycle at hysterectomy was critical. The earlier hysterectomy is performed in the cycle, the longer the CL will be functional. Even removal of the uterine horn very late in the estrous cycle (Day 15) had a detrimental effect on the life span of the CL. Secretory activity of CL in pregnant hysterectomised guinea pigs is similar to that of the estrous cycle (Heap *et al.*, 1973).

The peripheral serum concentrations of progesterone and the ovarian tissue levels of 20α-OH-SDH show similar patterns during prolonged pseudopregnancy in hysterectomised rats and in rats bearing decidual tissue (Pepe and Rothchild, 1974). Hysterectomy either before or after inducing pseudopregnancy in rats prolongs the CL life span until days 18 - 25, though the CL is smaller than those found during

pseudopregnancy (Anderson, 1973). Progesterone concentration after hysterectomy will be maintained at a level comparable to that found on day 6 of intact pseudopregnant animals for about one week longer than normal pseudopregnancy, but in the last week these levels are much lower than during a similar period of pregnancy (Heap *et al.*, 1973). In pseudopregnant, deciduomata-bearing pseudopregnant, and lactating animals, hysterectomy combined with hypophysectomy regressed the CL but progesterone secretion was maintained for about 3 days after day 12 of pregnancy in the absence of pituitary and placenta (Rothchild *et al.*, 1973). Since the progesterone concentration detected after operation is significantly lower than that found in intact or in Day 12 hypophysectomised rats (Rothchild *et al.*, 1973), this suggests that in the rat the placental luteotrophin seems to increase the rate of progesterone secretion in the absence of LH.

Little is known of the effects of removal of the uterus and/or the ovary on progesterone levels during the subsequent pregnancy. The following section provides data on this topic.

4.2 Objectives

As described above, several investigators have examined the concentration of progesterone in maternal blood during various stages of gestation in intact mice. However, few examinations on the effect of unilateral hysterectomy to maternal hormone concentration during gestation have been made. The purpose of the present study was to characterise progesterone concentration in maternal plasma after removal of one uterine horn, with or without its ipsilateral ovary, at several different stages of pregnancy. Besides that, this study was also undertaken to provide details of the histologic changes, if any, in the remaining uterine horn (especially in the epithelial cells lining the endometrium) and in the ovary(ies) (especially in the luteal cells) of the mice after surgery. Surgery was carried out on alternate sides of the uterine horn in successive animals.

4.3 Methods

4.3.1 Progesterone Measurement

a. Blood sampling

Blood samples were collected at several specified days of gestation and on the day of parturition. Under light anesthesia, the mouse was exsanguited by directly puncturing the heart with a 26G needle. The blood (0.8 - 1.5 ml/mice) was sucked out by using

heparinised disposable plastic syringes, placed in heparinised tubes and centrifuged. The plasma was then harvested and kept in a freezer (-20°C) until progesterone assay (see Chapter 2). All blood samples were collected between 0900 and 1100 h in the morning or no longer than 4 hours after parturition. Blood samples were collected from 210 pregnant mice used for the embryonic growth experiment as described previously in Chapter 3.

b. Progesterone assay

The radioimmunoassay (RIA) procedure used to measure plasma progesterone concentrations was similar to that of Jones and Rose (1992). This procedure involved a separation (extraction) of progesterone from plasma and an assay of the progesterone concentration by radioimmunoassay. The antibody used in this RIA was kindly provided by Dr. Susan Jones of the Zoology Department, University of Tasmania.

Separation of progesterone from the plasma:

Plasma was extracted with iso-octane (Ajax, A.R. grade) for progesterone. Glass pasteur pipettes were packed with approximately 1 ml of 60-80 mesh chromosorb (Chromosorb W) mixed with 25% *(w/w)* ethylene glycol (made by 24 g chromosorb + 6 g ethylene glycol). Approximately 5 mm acid-washed sand was added on top of the chromosorb to avoid chromosorb disturbation when the sample or solvent was added. Columns were wetted with 1 ml (2 x 0.5 ml) iso-octane and flushed with 3 ml (6 x 0.5 ml) of the same solvent to remove impurities from the column. Fifty microlitre plasma samples were added to each column and progesterone was eluted with 3 ml (6 x 0.5 ml) iso-octane. All 3 ml of eluent, containing progesterone, from each column were collected into glass tubes for assay. Percent recovery of progesterone was 93%.

Progesterone assay:

A set of duplicate standard solutions containing 0, 25, 50, 100, 200, 400, and 800 pg progesterone in 50 µl ethanol was prepared. These were prepared by a 1:1 *(v/v)* serial dilution of a stock solution of 800 pg progesterone/ml in ethanol. Two extra tubes with zero standard for non-specific binding (NSB) were also prepared. Fifty microlitres of [^{3}H]-progesterone in ethanol was added to each standard, NSB, and sample tubes. All of these tubes were then dried under air in a warm water bath. One hundred microliters prepared anti-progesterone antiserum solution were added to each standard and sample tube, while the NSB tubes received 100 µl of assay buffer G. All tubes were then vortexed, covered with aluminium foil, and incubated overnight at 4°C.

Free molecules of progesterone were separated from antibody-bound progesterone by the addition of 0.5 ml dextran coated-charcoal suspension (5 mg charcoal + 0.5 mg dextran T.70 + 0.2 mg thiomerosal/ml 0.2 M phosphate buffer). All tubes were vortexed and then incubated at 4°C for 15 minutes and centrifuged at 1500 *g* and 4°C for 15 minutes to sediment the dextran coated-charcoal. One hundred and fifty microlitres of supernatant were taken from each tube and added to a scintillation vial containing 2.5 ml scintillant (Eco-scint, National Diagnostics) for radioactive counting. All vials were then shaken well to avoid separation phase. Radioactivity was measured in a Beckman LS 5801 liquid scintillation spectrometer. The absolute amount of progesterone for each sample was determined automatically, as a computer print-out, using a radioimmunoassay program. The intra- and inter-assay coefficients of variation were 14.21% (n = 10) and 10.25% (n = 8), respectively.

4.3.2 Histological Procedure

a. Tissue collection

Organs required for histological examination were the ovaries and uterine tissue with placental tissues (the latter tissue denoted as implantation site) which were obtained from the animals examined for the previous experiment (Chapter 3). Four animals per group were chosen as a source of the tissue. On Day 18 of pregnancy, the pregnant animals were killed by cervical dislocation. The remaining uterine horn was opened and fetuses with their attached membranes were carefully removed without disturbing other tissue. The placenta remained intact and its contact with the uterine tissue was not disturbed. In order to avoid any tissue curving during fixation period, the uterine tissue (implantation site) was spread in the petri dish. Bouin's fixative was immediately poured slowly over the surface of the tissue until the whole tissue was evenly soaked (2 - 3 minutes). During this pre-fixation treatment, care was taken to avoid excessive physical stretching. The tissue was then sliced transversely and fixed in Bouin's solution for 48 hours.

The ovarian tissue was directly fixed in Bouin's solution after all the excess fat covering the ovary was removed. The duration of fixation period was 48 hours. Two kinds of ovarian tissue were obtained from the UX group. The first kind was an ovary where its ipsilateral uterine horn remained intact (denoted as O+), and the second was an ovary where its ipsilateral ovary was removed (denoted as O-).

b. Tissue processing

Following the removal of the yellow stain from the picric acid by using a saturate solution of lithium carbonate in 70% alcohol, each tissue was dehydrated in a series in which alcohol content was gradually increased from 70% (four changes for 6 hours each), 80% (for 3 hours), 90% (for 3 hours) to absolute alcohol (two changes for one hour each).

Before embedding, the dehydrated tissues were then directly transferred from absolute alcohol to xylene until transparent (for about 12 hours), transferred to a wax-xylene mixture (50:50) for 30 minutes at 40°C, and infiltrated with wax by transferring them into two changes of pure wax (for 30 minutes each) in the vacuum embedding oven. Each tissue was then embedded in wax (Paraplast).

Sectioning was made transversely (for implantation site) and longitudinally (for ovarian tissue) at 6 µm. Serial sectioning of both implantation site and ovarian tissue was made. Sections were then mounted on the slides and stained with hematoxylin and eosin.

c. Microscopic Examination

The diameter of large luteal cells and their nuclei and the length of the epithelial cells lining the endometrial uterine were measured using the NIH Image computer program v. 1.56 (written by Wayne Rasband, the U.S National Institutes of Health, available from the Internet by anonymous FTP from *zippy.nimh.nih.gov*) running in the Macintosh machine.

From each animal, four slides were chosen for examination. Four sections from each slide were chosen randomly for examination, and five cells from each section were randomly measured. Examination was made under 50x magnification of a light microscope. The tissue image was captured by the video camera connected to the Macintosh machine. The NIH Image program allows an image to be captured and measured. Before measuring. the unit of measurement was calibrated from pixell to micrometer. The measurement results (in micrometer) were then sent to the statistical Systat program for analysis. The number of CL and Graafian follicles were also counted during the microscopic examination.

4.3.3 Statistical analysis

Results are expressed as mean ± SEM, except where otherwise indicated. Two way analysis of variance, if appropriate, was applied to access the effect of treatments. The effect of operation on maternal progesterone concentrations for each group of Day factor were accessed by using the one way analyses of variance. Multiple comparisons between groups were made by the Tukey HSD-test. Paired samples t-test was also applied if appropriate.

4.4 Results

4.4.1 Progesterone Concentrations

Progesterone concentrations (ng/ml plasma) measured during the period of one day after operation to the parturition day for each group are presented in Figure 4.1. In the animals operated on, on day 2 of pregnancy (Figure 4.1A), the UX and SOP groups showed a small increase in plasma progesterone from 43.26 ± 10.93 and 48.52 ± 10.65 (mean ± SEM) on day 3 to 53.11 ± 9.93 and 62.84 ± 14.49 for the UX and SOP respectively on day 7 before reaching a constant level until day 11. In the same period, progesterone concentration in the UOX group remained constant, valued between 40.80 ± 8.80 on day 3 and 44.77 ± 9.52 on day 11. All groups reached a peak concentration of 113.73 ± 21.59 for the UX, 69.15 ± 19.22 for the UOX, and 104.98 ± 12.44 for the SOP group on day 15 before dropping abruptly to the lowest level on day 18 of pregnancy, except for the SOP group where the lowest level was reached on the parturition day. At the day of parturition, progesterone concentrations rose again in the UX and UOX groups. During the period from day 3 to day 15 of pregnancy progesterone concentration of the UOX group tended to be lower (but not significantly) than those of either the UX or the SOP group. At day 18 of pregnancy, however, plasma progesterone concentration of the UX (6.95 ± 1.75) was similar to those of the UOX groups (4.85 ± 2.19) which were significantly ($p < 0.001$) lower than that of the SOP group (23.15 ± 2.03).

The pattern of progesterone concentration was altered when the operation was performed after day 2 of pregnancy (Figure 4.1B, C, and D). The peak levels of progesterone on day 15 of pregnancy, as can be observed in all groups of animals operated on, on day 2 of pregnancy, were never reached by the UX and UOX if the operation was performed on day 6 (Figure 4.1B), day 10 (Figure 4.1C), and day 14 of

pregnancy (Figure 4.1D). Statistical analysis revealed that at day 15 of pregnancy the progesterone concentration

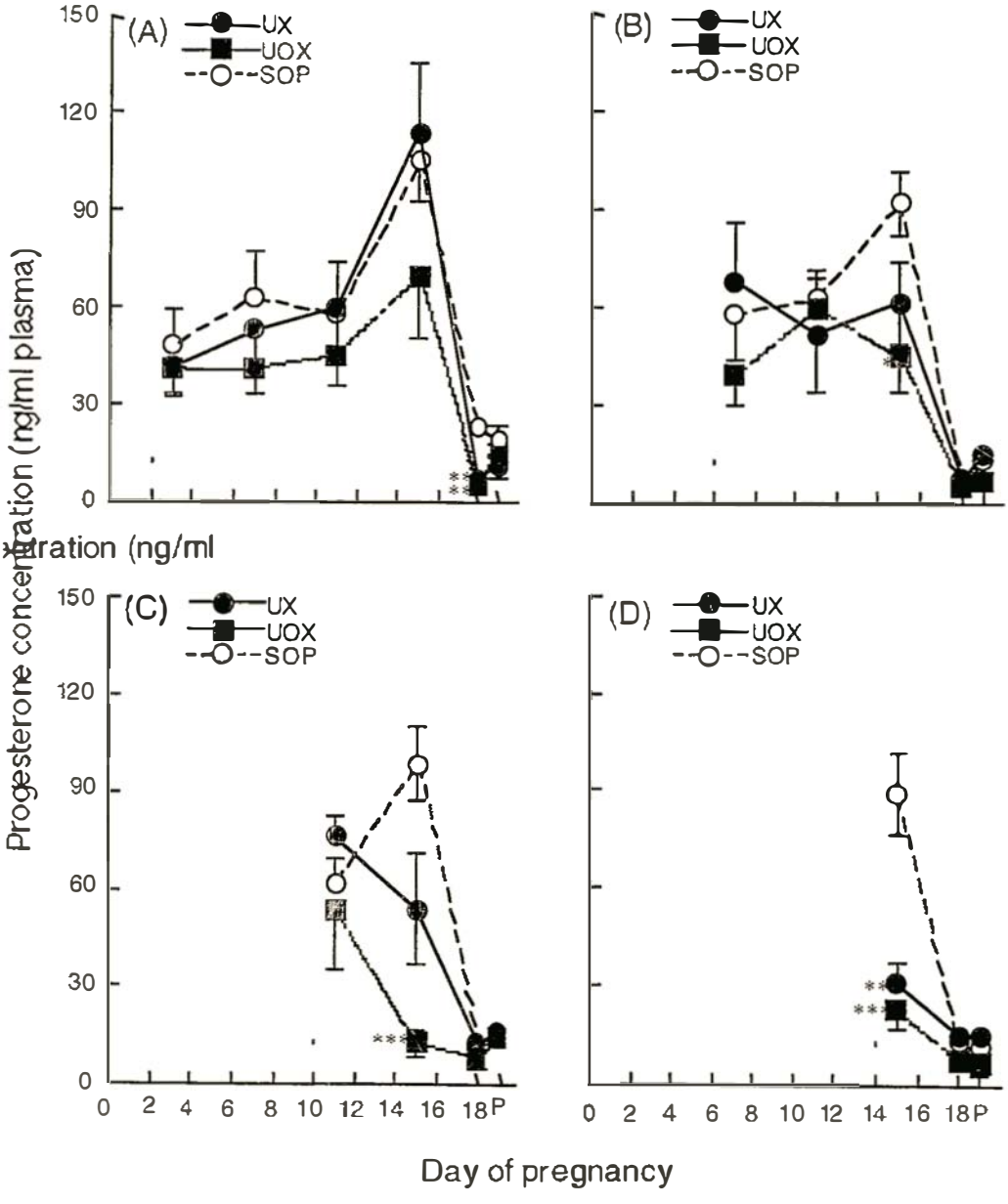

Figure 4.1 Mean (± SEM) plasma progesterone concentration for the UX, UOX and SOP

of the UOX group, but not the UX group, was significantly lower than the SOP group if they were operated on, on, on day 6 ($p < 0.05$) (Figure 4.1B) or day 10 of pregnancy ($p < 0.001$) (Figure 4.1C). If the operation was performed on day 14 of pregnancy, both the UOX ($p < 0.001$) and the UX group ($p < 0.01$) showed a significant decrease in their progesterone levels (Figure 4.1D).

At all days of observation, except at day 11 for the animals operated at day 6 of pregnancy (Figure 4.1B), progesterone concentrations of the UX group were higher than those of the UOX group. However, this difference was not significant. At parturition day, all groups of animals operated on, on day 2, day 6, and day 11, but not on day 14, showed an increase in their plasma progesterone concentrations except for the SOP group operated on, on day 2 of pregnancy.

A two way analysis of variance was performed in order to determine whether the Day factor and the Operation factor affected the plasma progesterone concentrations in combination or separately. The analysis revealed that the interaction effect of the Day x Operation factor on the progesterone concentration was only detected on day 18 of pregnancy. The SOP groups operated on, on after day 2 of pregnancy, in general, showed a higher level in their progesterone concentration on day 18 of pregnancy than the UX and UOX groups. Results of the Tukey HSD-test for this interaction effect are presented in Table 4.1.

The independent effects of the Day factor and Operation factor on progesterone concentration after operation are presented in Figure 4.2. Regardless of the Operation factor, at day 15 of pregnancy the concentration of the animal treated on day 2 was significantly higher than that of animals treated on either day 6, day 10 or day 14 of pregnancy (Figure 4.2A). Significant differences between groups in the progesterone concentration also occurred on day 18 of pregnancy. Progesterone concentration in the animals operated on, on day 6 was significantly lower than that of the animals operated on, on either day 2, day 10 or day 14. Although the progesterone concentration in both the day 10 and day 14 groups was significantly higher than that of the day 6 group, their concentrations did not differ greatly from the day 2 group. Regardless of the Day factor, progesterone concentration in the UOX group (whose uterine horn and ovary had been removed unilaterally) was lower than that of both the UX and SOP groups (in which the two ovaries were left intact) at all days of pregnancy observed (Figure 4.2B). However, these differences were only significant at day 15 and day 18 of pregnancy.

Table 4.1 Multiple comparison of Tukey-HSD test results for plasma progesterone concentration of females autopsied on day 18 of pregnancy.

	Significance (p)*											
Groups	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
2UX (1)	-	ns	ns	<0.05	<0.001	ns	ns	ns	ns	ns	ns	ns
2UOX (2)		-	ns	ns	<0.05	ns	ns	ns	ns	ns	ns	ns
2SOP (3)			-	ns	ns	ns	ns	ns	ns	ns	ns	<0.05
6UX (4)				-	ns	ns	ns	ns	<0.05	ns	ns	<0.01
6UOX (5)					-	<0.01	ns	ns	<0.001	ns	ns	<0.001
6SOP (6)						-	ns	ns	ns	ns	ns	ns
10UX (7)							-	ns	ns	ns	ns	ns
10UOX (8)								-	<0.05	ns	ns	<0.01
10SOP (9)									-	ns	ns	ns
14UX (10)										-	ns	ns
14UOX (11)											-	ns
14SOP (12)												-

*ns, not significant

Table 4.2 Mean (±SEM) ovarian weight (g), number of corpora lutea, and number of Graafian follicles of unoperated (O+) and operated (O-) sides of ovary at day 18 after removal of one uterine horn at days 2, 6, 10, and 14 of pregnancy.[1]

	Ovarian weight (x10^{-2} g)		Number of corpora lutea		Number of Graafian follicles[2]	
Group	O+	O-	O+	O-	O+	O-
By day and operation factors:						
	(5)	(5)	(5)	(5)	(4)	(4)
2UX	1.39 ± 0.05	1.26 ± 0.02†	8.26 ± 0.37	7.60 ± 0.87	5.25 ± 0.49	6.75 ± 0.49
2UOX	1.48 ± 0.03	-	7.80 ± 0.58	-	7.25 ± 0.49	-
2SOP	1.42 ± 0.01	1.47 ± 0.05	7.20 ± 0.20	7.20 ± 0.37	6.50 ± 0.65	6.50 ± 1.19
6UX	1.40 ± 0.06	1.27 ± 0.03†	8.20 ± 1.11	8.60 ± 0.69	4.75 ± 1.25	6.25 ± 0.63
6UOX	1.50 ± 0.06	-	10.40 ± 0.93	-	6.25 ± 0.25	-
6SOP	1.43 ± 0.01	1.44 ± 0.03	7.80 ± 0.58	7.80 ± 0.86	6.00 ± 0.71	5.75 ± 0.48
10UX	1.50 ± 0.03	1.34 ± 0.03†	9.20 ± 0.86	8.80 ± 1.02	6.25 ± 0.48	7.25 ± 0.75
10UOX	1.44 ± 0.06	-	8.60 ± 0.51	-	7.75 ± 0.63	-
10SOP	1.45 ± 0.03	1.45 ± 0.04	7.60 ± 1.03	7.40 ± 0.68	6.25 ± 0.48	6.25 ± 1.03
14UX	1.37 ± 0.06	1.48 ± 0.02	7.00 ± 0.55	7.40 ± 0.40	4.75 ± 0.75	5.25 ± 0.63
14UOX	1.46 ± 0.07	-	8.00 ± 0.45	-	7.00 ± 0.41	-
14SOP	1.39 ± 0.03	1.44 ± 0.03	7.80 ± 0.20	7.60 ± 0.93	5.00 ± 0.41	5.50 ± 0.50
By Operation factor:						
	(20)	(20)	(20)	(20)	(16)	(16)
UX	1.42 ± 0.03	1.34 ± 0.05	8.15 ± 0.39	8.10 ± 0.38	5.25 ± 0.39***	6.38 ± 0.37
UOX	1.47 ± 0.03	-	8.70 ± 0.38	-	7.06 ± 0.25	-
SOP	1.42 ± 0.01	1.45 ± 0.02	7.60 ± 0.28	7.50 ± 0.34	5.93 ± 0.29*	6.00 ± 0.39

[1]O+ and O-, for the UX group, represent the unoperated (ipsilateral uterine horn remained intact) and operated (ipsilateral uterine horn was removed) ovaries, and for the SOP group, represent the right and left ovaries respectively; [2]The number Graafian follicles was counted at microscopic examination.
†$p < 0.05$ (paired samples t-test), are different from their O+ sides; *$p < 0.05$ and ***$p < 0.001$ (Tukey HSD-test), are different from the UOX group (number in parenthesis denotes the sample size for each group).

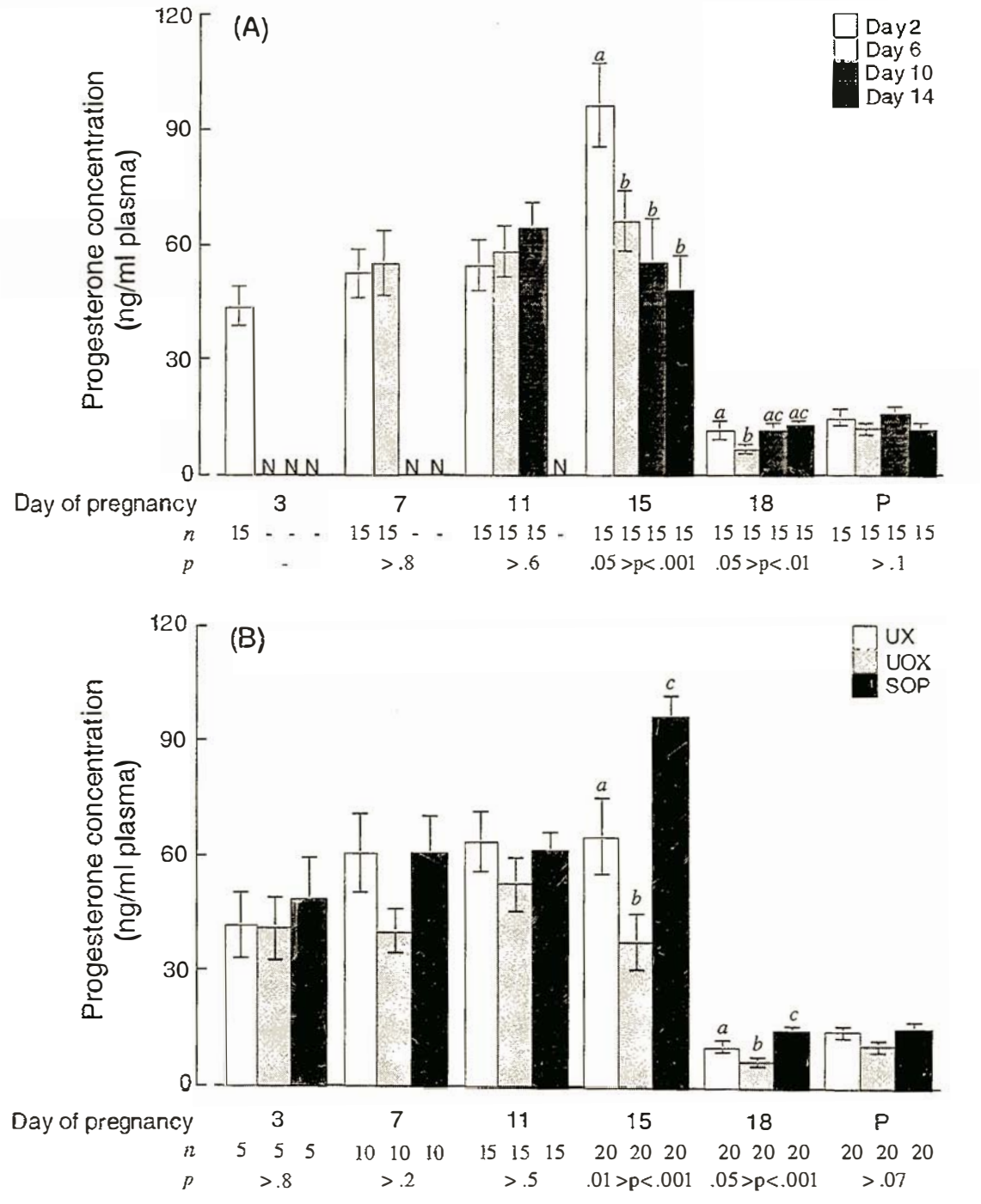

Figure 4.2 Mean (± SEM) plasma progesterone concentration in treated females measured from Day 3 of pregnancy until parturition day (P). Data were analysed separately on the base of both (A) the Day factor and (B) the Operation factor. No data was collected before the animals were operated (N). Different letter within the same day indicate significant difference (Tukey HSD-test; *n* and *p* at the bottom rows of the diagram denoted the number of animal per group and the level of significance respectively).

4.4.2 Histology

4.4.2.1 Ovarian histology

The ovary from the unoperated or intact side (O+) was significantly heavier than that from the operated side (O-) in the animals operated on, on days 2, 6, and 10 but not on day 14 of pregnancy (Table 4.2). The number of CL and Graafian follicles was not different for the O+ and O- ovaries (Table 4.2). However, two way analysis of variance revealed that the number of Graafian follicles of the O+ ovary from both the UX and SOP groups was significantly lower than that of the UOX group ($p < 0.001$ and $p < 0.05$ for the UX and SOP groups respectively; Tukey HSD-test).

Luteal cells from the O+ and/or O- ovaries from all groups were examined microscopically (Figure 4.3). The corpus luteum mainly consisted of large luteal cells which were characterised by a light staining of cytoplasm, and a large rounded and centrally located nucleus with a distinct nucleolus. This kind of cell was polyhedral in shape with an easily distinguished cell boundary. Vacuolated cytoplasm of large luteal cells was commonly observed in all groups. The CL also consisted of small luteal cells which were characterised by a small, spindle shape and darkly stained cytoplasm with an irregularly shaped nucleus. This type of cell was prominently observed in the O- ovaries from the UX females. Of these two kind of luteal cells, only the large luteal cell diameter was measured.

Two way analysis of variance revealed that both the Day factor and the Operation factors were affected the luteal cell diameter (LCD), luteal nuclear cell diameter (LND), and luteal nuclear to luteal cell diameter ratio (N/C) (Figure 4.4). The smallest and the largest LCD were found in the 6UX (23.49 ± 0.74; mean $\pm$ SEM, μm) and 10UOX (26.47 ± 0.46) groups (Figure 4.4(A)) respectively. The two values were significantly different from each other (Tukey HSD-test, $p < 0.05$), but were not different from the other groups of day and type of operation factors. The LND of the UX group tended to decrease, while both the UOX and SOP groups tended to increase with the day of operation (Figure 4.4(B)). Thus, it is likely that removal of one uterine horn on the earlier days of pregnancy, namely day 2 and day 6, caused an enlargement of the LND in the UX group. This enlargement, however, was only significant (Tukey HSD-test; $p < 0.05$) in the animals operated on, on day 2 of pregnancy (Figure 4.4(B)). The UX and UOX groups were significantly different in the N/C diameter ratio in the animals operated on, on day 2 and day 6 (Tukey HSD-test, $p < 0.05$) (Figure 4.4(C)). The N/C diameter variation between groups, however, tended to decrease with the day of pregnancy when the operation was performed.

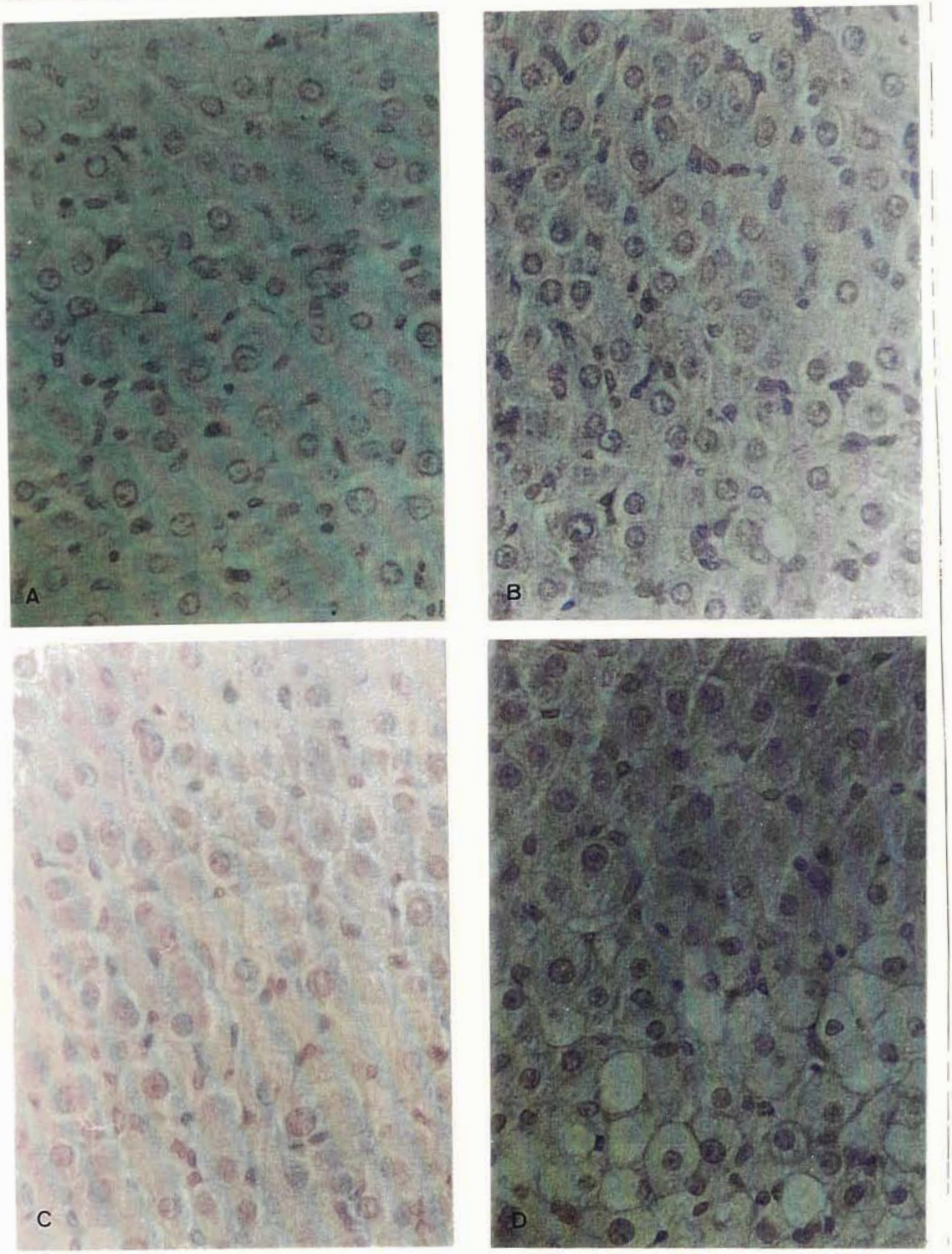

Figure 4.3 Microphotography of corpora lutea from 0+ (A) and 0- (B) ovaries of the UX and from the ovaries of the UOX (C) and SOP (D) groups. Females were operated on Day 2 of pregnancy and ovaries were removed for histological examination on Day 18 of pregnancy (mag x400)

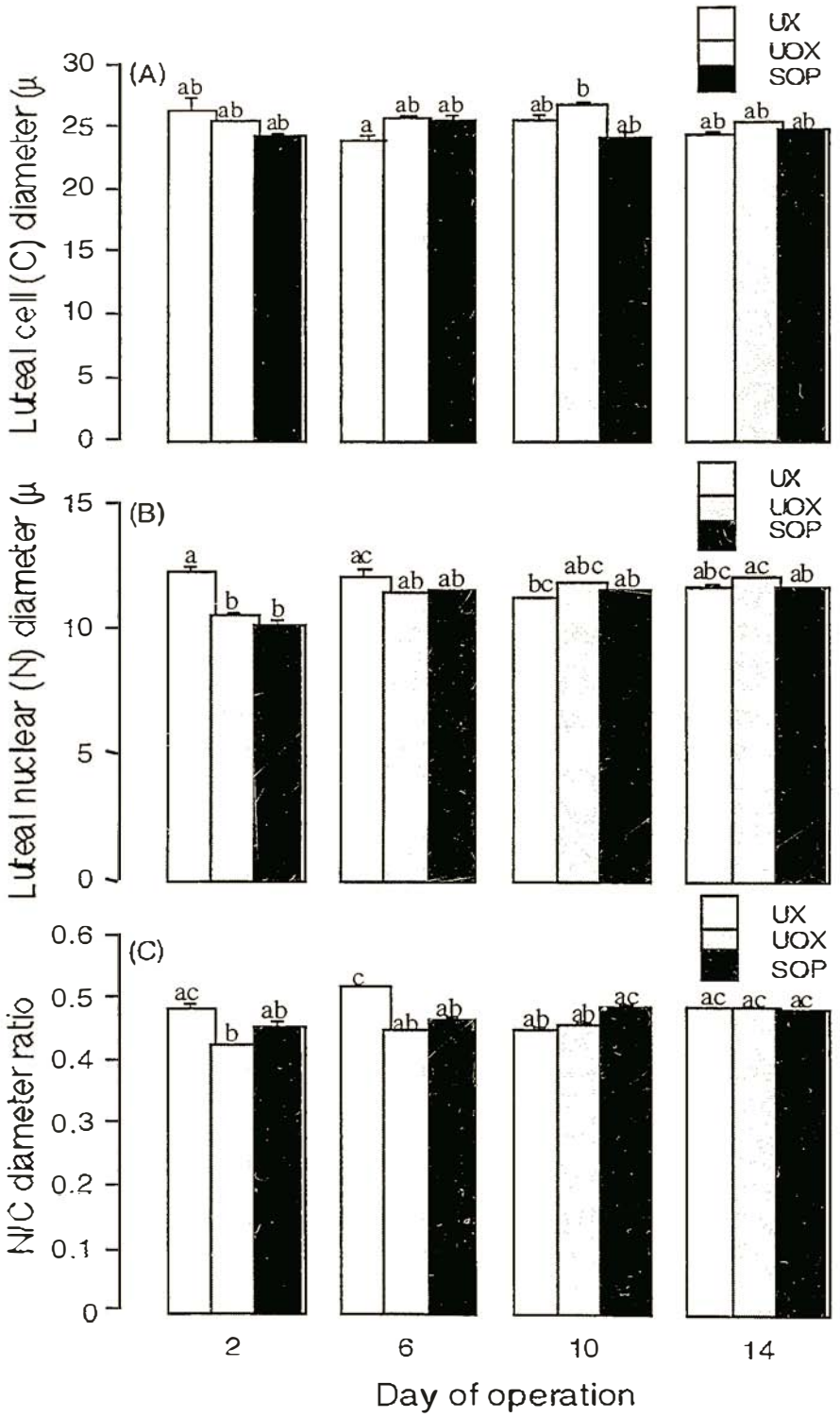

Figure 4.4 Mean (±SEM) luteal cell diameter (A), luteal nuclear diameter (B), and N/C diameter ratio (C) of the operated (UX and UOX) and unoperated (SOP) females at day 18 of pregnancy after removal of one uterine horn on days 2, 6, 10, and 14. Different letter between groups indicates significant difference (Tukey HSD-test, $p < 0.05$, $n = 4$).

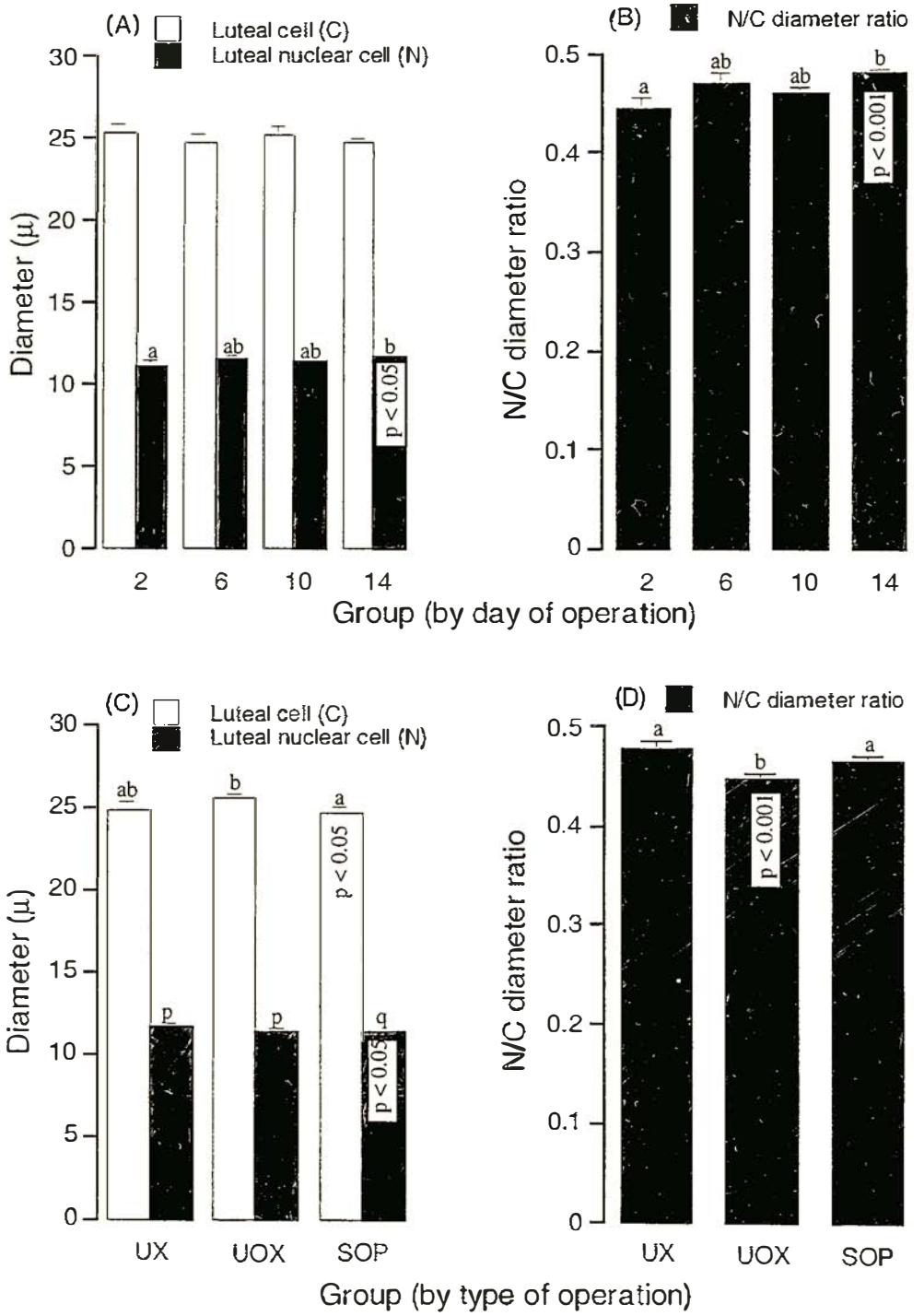

Figure 4.5 Mean (±SEM) luteal cell and luteal nuclear cell diameters and N/C diameter ratio of the animals at day 18 of pregnancy after removal of one uteine horn on days 2, 6, 10, and 14. Data from Figure 4.3 were pooled and analysed separately by both the day of operation (A and B) and the type of operation (C and D). Different letters within parameter indicate significant difference (Tukey HSD-test: p values are noted in the bar).

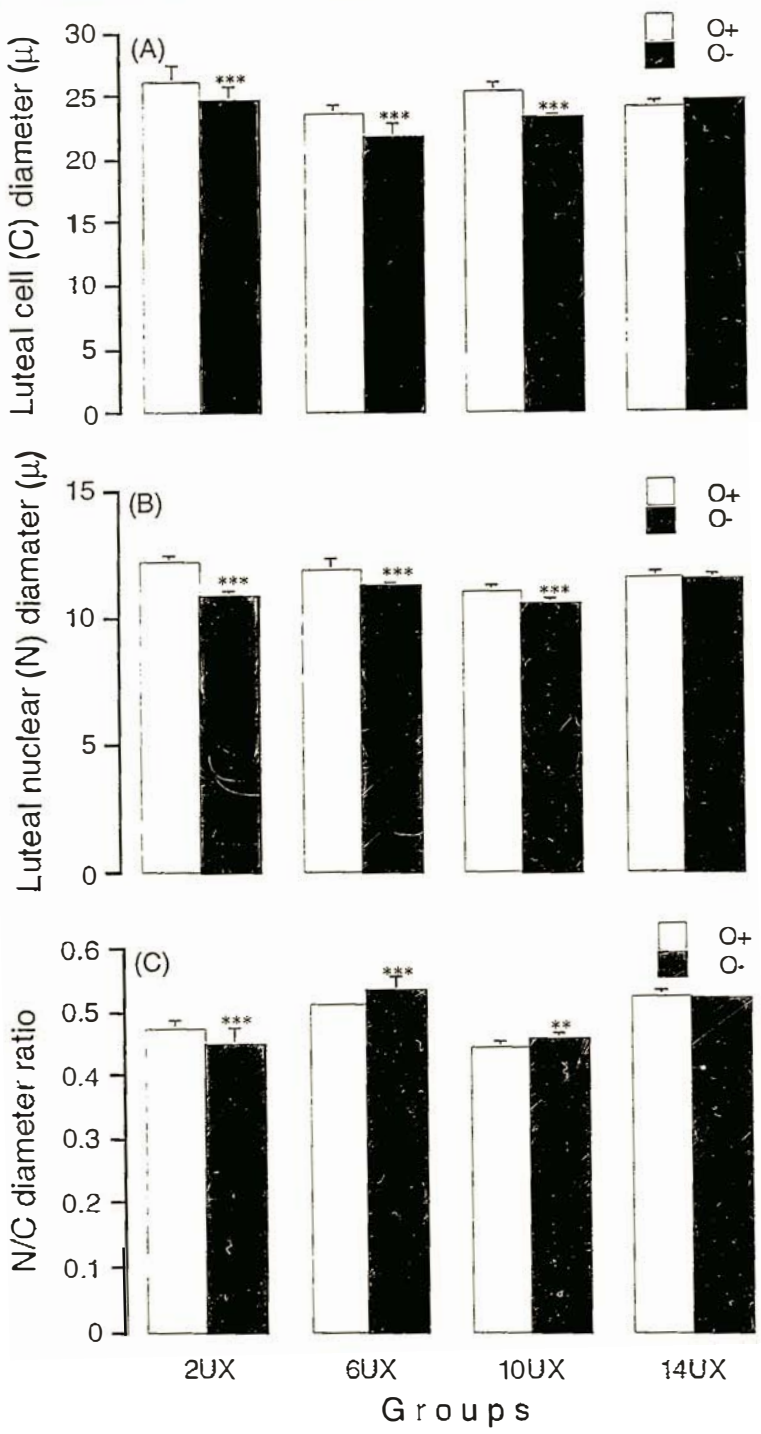

Figure 4.6 Mean (±SEM) luteal cell diameter (A), luteal nuclear diameter (B) and N/C diameter ratio (C) from intact (O+) and operated (O-) side of ovaries at day 18 of pregnancy after removal of one uterine horn (UX) on several different days of pregnancy (days 2, 6, 10, and 14). ** $p < 0.01$ and *** $p < 0.001$ are different from intact side (paired samples t-test; n for 2UX and 10UX are 240, and for 6UX and 14UX are 244).

When the data was grouped into the day of operation factor (Figure 4.5(A) and (B)), without considering the operation factor, it was found that: 1) The highest and the lowest LCD were found in the animals operated on, on day 2 (25.39 ± 0.42) and day 14 (24.47 ± 0.17) of pregnancy respectively, but no differences between groups were found; 2) The lowest value for LND was found in the animals operated on, on day 2 (11.15 ± 0.26) which is significantly different (Tukey HSD-test, $p < 0.05$) from the highest value obtained in the animals operated on, on day 14 (11.67 ± 0.10), but not significantly different from the other two groups (day 6 and day 10); 3) Consequently, the lowest value for N/C was found in the animals operated on, on day 2 (0.44 ± 0.01) which is significantly different (Tukey HSD-test, $p < 0.001$) from the highest value obtained in the animals operated on, on day 14 (0.48 ± 0.01), but not significantly different from the other two groups (day 6 and day 10).

When the data was grouped into the type of operation factor, without considering the day factor (Figure 4.5(C) and (D)), it was found that : 1) The highest values for LCD were found in the UOX group (25.54 ± 0.22), and this value was significantly different (Tukey HSD-test, $p < 0.05$) from the lowest value in the SOP group (24.57 ± 0.26) but not different from the UX group; 2) The SOP female was significantly different (Tukey HSD-test, $p < 0.05$) from both the UX and UOX females in luteal nuclear diameter, but was only significantly different from the UOX female in luteal cell diameter. No differences between the UX and UOX females were detected (Figure 4.5(C)); 3) The N/C ratio of the UOX group was significantly different (Tukey HSD-test, $p < 0.001$) from those of both the UX and SOP females (Figure 4.5(D)).

Statistical analysis on parameters from the O+ and O- ovaries of the UX groups indicated that luteal cell size and luteal nuclear diameters of the O- ovaries were significantly (paired samples t-test, $p < 0.001$) lower than those of the O+ ovaries (Figure 4.6(A) and (B)). The N/C diameter ratio of the O- ovary obtained from females operated on, on day 2 of pregnancy was significantly ($p < 0.001$) lower than that of the O+ ovary, but significantly higher than that of the O+ ovary if operations were performed on days 6 and 10 (Figure 4.6(C)). These parameters were not different in the animals operated on, on day 14 of pregnancy.

4.4.2.2 Uterine histology

The only quantitative data obtained from the uterine tissue was the length of the endometrial epithelial cells measured on day 18 of pregnancy (Figure 4.7). The length of

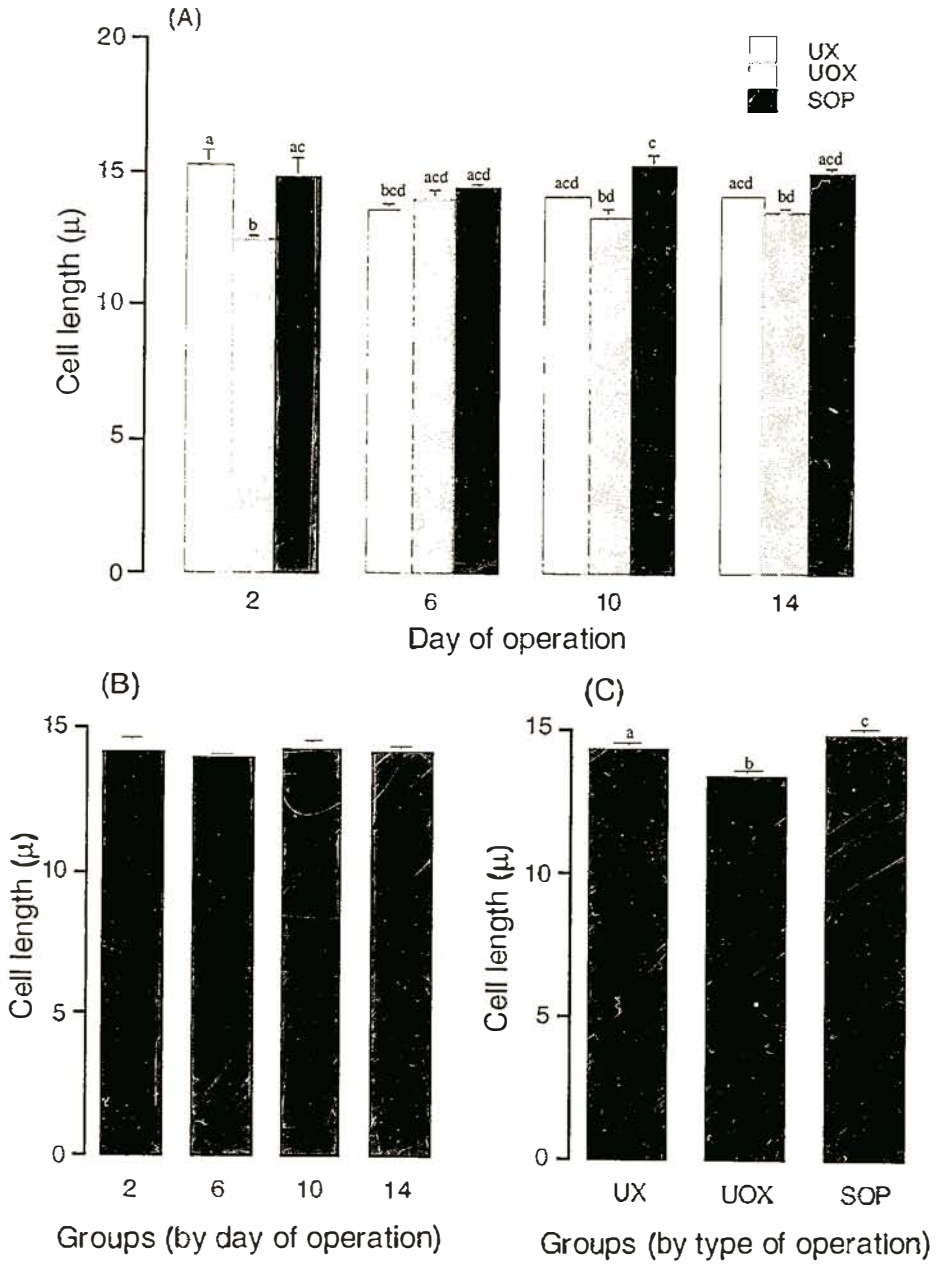

Figure 4.7 Mean (±SEM) endometrial epithelial cell length of females at day 18 of pregnancy after one of the uterine horns was removed on days 2, 6, 10, and 14. The interaction effect of day and type of operation factor is depicted in (A), and independent effect of either the day or the type of operation is depicted in (B) and (C) respectively. Different letters between groups indicates significant difference (Tukey HSD-test: (A), n = 4, $p < 0.05$; (C), n = 16, UX v UOX, $p < 0.001$; UX v SOP, $p < 0.05$; UOX v SOP, $p < 0.001$).

epithelial cells (mean ± SEM, μm) ranged between 12.39 ± 0.16 (the lowest value, in the 2UOX group) and 15.29 ± 0.40 (the highest value, in the 2UX group) (Figure 4.7(A) . Between the UX groups, the lowest value for the length of epithelial cells was found in females operated on, on day 6 of pregnancy (13.58 ± 0.21) which is significantly (Tukey HSD-test, $p < 0.05$) lower than that of females operated on, on day 2 but was not different from females operated on, on days 10 and 14. In contrast to this, the highest value for epithelial cell length of the UOX groups was found in the females operated on, on day 6 of pregnancy (19.93 ± 0.25) which is significantly lower than that of females operated on, on day 2 (Tukey HSD-test, $p < 0.05$). There were no significant differences found between the SOP groups in their epithelial cell length.

According to the statistical analysis, it was found that, the day factor had no significant independent effect on the length of epithelial cell (Figure 4.7(B)), while the Operation factor worked in either an independent way from (Figure 4.7(C)) or interaction with the day factor (Figure 4.7(A)). As can be seen in Figure 4.7(C), overall, the SOP group had the highest value for epithelial cell length while the UOX group had the lowest value, and values for the UX group were placed between the two values. These values were significantly different from one another (p values are depicted in Figure 4.7).

4.5 Discussions

4.5.1 Progesterone Concentrations

It has been well documented that the principal effect of total uterine removal is a persistence of the CL in a functional condition resulting in the prolongation of pseudopregnancy, the prolongation of high levels of progesterone secretion, the inhibition of ovulation, the cessation of the dioestrous cycle and the retention of the vaginal epithelium in a secretory state for periods equivalent to or longer than that of pregnancy (Rowlands and Short, 1959; Rowlands, 1961, 1962; Perry and Rowlands, 1961; Anderson *et al.*, 1962; Heap *et al.*, 1967; Macdonald *et al.*, 1970; Hausler and Malven, 1971; Kelley and Brinkley, 1971; Rothchild *et al.*, 1973; Currie and Thorburn, 1974; Critser *et al.*, 1982; Southee *et al.*, 1988). For extensive review, see Bland and Donovan (1966) and Anderson (1973; 1977). The focus of this study is to examine the effect on the progesterone concentration after the removal of one uterine horn, either with or without its ipsilateral ovary, at varying days of pregnancy.

The pattern of progesterone concentration in the sham-operated control (the SOP) group during the observation period is remarkably similar to that of normal pregnant female mice reported by McCormack and Greenwald (1974a), Murr *et al.*, (1974), and Virgo and Bellward (1974). However, there are minor differences between the results reported here and their results. The progesterone concentrations reported here are, in average, higher than those of McCormack and Greenwald (1974a) and Virgo and Bellward (1974), but similar to those of Murr *et al.*, (1974). These differences may be due to the strains of mice used.

Plasma progesterone concentration slightly increased between day 3 to day 7 of pregnancy in the UX and SOP, but not in the UOX groups operated on, on day 2 of pregnancy (Figure 4.1A). During this period several critical events for uterine preparation of pregnancy, i.e. implantation or induction and maintenance of nidation, and placentation, occur in the uterus. All these events require the presence of progesterone. Day 3 of pregnancy in the mouse is a starting point for luteal secretion of progesterone (Finn and Martin, 1971). Virgo and Bellward (1974) postulated that plasma progesterone changes during the period from day 2 to day 9 of pregnancy are related to the role of progesterone in inducing and maintaining decidualisation. In the rat, Wiest (1970) reported a high intra uterine concentration of progesterone, higher than that of peripheral blood concentrations, throughout early and mid pregnancy.

In the sham-operated control (SOP) group operated on, on day 2, but not in the SOP group operated on, on day 6, progesterone levels slightly decreased between day 7 and day 11 of pregnancy. This finding is consistent with the data reported by Murr *et al.*, (1974) and Virgo and Bellward (1974). This mid-pregnancy decrease in progesterone concentration correlates with the rapid phase in the growth of the CL. The CL of pregnancy increased sharply in size on day 8 of pregnancy, and this maximum growth persists until day 13 (see Hilliard, 1973). This period is also indicated by the transition period of pregnancy maintenance from the pituitary to the placenta, since hypophysectomy after day 10 can be conducted without terminating pregnancy (Choudary and Greenwald, 1969).

The increase in plasma progesterone concentration on day 11 to day 15 in all groups operated at day 2 might be caused by the additive effect of placental and pituitary sources of luteotrophin. It is well known that, in the mouse, the ovaries are the main sources of progesterone required for maintenance of pregnancy since ovariectomy or destruction of the CL results in termination of pregnancy (Hall, 1957). Hypophysectomy after day 10

of pregnancy is compatible with the maintenance of pregnancy (Choudary and Greenwald, 1969). During the second half of pregnancy the mouse placenta synthesises progesterone, although this placental contribution to the maternal progesterone is very low compared with that of the ovary and is not sufficient to maintain pregnancy (Deane *et al.*, 1962; Pointis *et al.*, 1981). In the rat, this placental progesterone production occurs throughout pregnancy and it may be independent of pituitary influences (Macdonald and Matt, 1984; Matt and Macdonald, 1984).

The trophic support of the corpus luteum during the second half of pregnancy in mice depends on the capability of the mouse placenta to produce a gonadotrophin, i.e. placental chorionic gonadotrophin (mouse chorionic gonadotrophin, mCG), which is similar to hCG (Rao *et al.*, 1982). This mCG reached peak levels on day 11 and day 16 *post coitum* (Wide and Wide, 1979). Another gonadotrophin secreted by the placenta that maintains progesterone production during the gestation period is mouse placental lactogen (mPL) which is positively correlated with litter size (Markoff and Talamantes, 1981). These two placental hormones, mCG and mPL, are believed to be involved in maintaining steroid production in the ovaries during the second half of pregnancy (Humpreys *et al.*, 1985). Recently, two mPL molecules have been characterised in mice as mPL-I and mPL-II (Soares *et al.*, 1982; Ogren and Talamantes, 1988). The mPL-I and mPL-II concentration peaks on day 10 and day 14 respectively (Soares *et al.*, 1982; Ogren *et al.*, 1989) and secretion of the mPL-II is modulated by both activin and inhibin proteins produced by both the decidual and placental tissues (Yamaguchi *et al.*, 1995). Recent work clearly indicates that the two mPL are produced by the trophoblast giant cells (Yamaguchi *et al.*, 1994), and that both are luteotropic and support progesterone production at mid pregnancy in the mouse (Galosy and Talamantes, 1995).

The finding that there is a sharp decline in plasma progesterone concentration between days 15 and 18 of pregnancy (Figure 4.1 and Figure 4.2) is consistent with the data previously reported by several researchers in mice (McCormack and Greenwald, 1974a; Murr *et al.*, 1974; Virgo and Bellward, 1974), in the rat (Morishige *et al.*, 1973; Grota and Eik-Nes, 1967), or in the golden hamster (Leavitt and Blaha, 1970). These data are consistent with the hypothesis that a high concentration of progesterone may prevent the onset of parturition and that progesterone withdrawal is required to permit parturition. In many animals, the decrease in progesterone concentration one day before parturition is caused by the regression of corpus luteum function. The decrease in progesterone concentration coincides with the increase in LH during the second half of gestation in the rat (Morishige *et al.*, 1973), and one of the major factors causing decreasing plasma

progesterone is fetal and placental capabilities to metabolise progesterone into 3α and 3β hydroxy derivatives which have no progestational properties (Sanyal and Villee, 1973). The relationship between progesterone decrease and the initiation of parturition during the last period of gestation has clearly been described by Thorburn (1991). Progesterone decrease in the last period of gestation is largely caused by the increase in placental 17α-hydroxylase activity due to the increase in the cortisol release by the fetus. The decrease in the progesterone concentration in turn causes an increase in the maternal estrogen to progesterone concentration ratio. The latter will induce an increase in $PGF_{2\alpha}$ synthesis in and release from the maternal placenta, which directly causes an increase in the uterine activity and allows parturition to occur. The presence of a local signal sent by the gravid horn to the ovary for the onset of parturition in rats is reported by Cavaille and Maltier (1978).

An increase in progesterone concentration found in all groups (except in the SOP group operated on, on day 2 and day 14) at parturition day is in agreement with the data of Grota and Eik-Nes (1967) assaying progesterone in the peripheral blood of the pregnant rat. Grota and Eik-Nes (1967) reported an increase in progesterone levels within 6 hours after delivery; from 10 ng/ml plasma at day 22 to 43 ng/ml plasma. Due to difficulties in collecting the blood samples exactly at the time of parturition, the samples from the delivered mothers were collected around 3 to 4 hours after parturition. Pregnant females were checked for parturition in a 3 to 4 hour period.

Removal of one uterine horn on day 2 of pregnancy (either followed by the removal of its ipsilateral ovary or not) did not alter the pattern of maternal progesterone secretion except a slight difference (but highly significant) between operated females (the UX and UOX groups) and the control (SOP) group at day 18 (Figure 4.1A). However, while the UX and SOP groups are approximately the same in their progesterone concentration during the period from day 3 to day 15, the UOX group shows a moderate reduction compared with those of both the UX and UOX groups. This suggests a compensatory effect of the surviving ovary or extra-ovarian origin in progesterone secretion might occur after the operation. It has been well documented that during the gestation period, progesterone synthesis occurs in the ovary (McCormack and Greenwald, 1974a; Murr *et al.*, 1974; Virgo and Bellward, 1974), adrenal cortex (Butterstein and Hirst, 1977; Pointis *et al.*, 1981; Macdonald and Matt, 1984), and in the placenta (Pointis *et al.*, 1981; Macdonald and Matt, 1984; Matt and Macdonald, 1984). In an earlier study on the rat, Wiest (1970) reported the capability of the uterine tissue to concentrate progesterone during early and mid pregnancy periods. More recently it was found that the high

concentration of progesterone in the uterus is not only caused by the capability of the uterus to concentrate progesterone but also by its capability to produce the progesterone itself (Bonnamy *et al.*, 1992). It is likely that the ovaries are the main source of progesterone during pregnancy since bilateral ovariectomy results in the termination of pregnancy (Hall, 1957; Bronson *et al.*, 1966).

Removal of one uterine horn or removal of one ovary automatically reduces the number of conceptuses or CL of intact animals by up to one half. Unilateral ovariectomy after implantation (at days 8.5, 11.5, and 14.5 of pregnancy) results in compensatory changes in the remaining ovary with respect to the number of Graafian follicles, with no changes in follicular size, in mice (Ross and Beaumont, 1974). In unmated mice or rats, there was also a compensatory growth of the surviving ovary after unilateral ovariectomy (McLaren, 1963; Peppler and Greenwald, 1970; Gibson *et al.*, 1979; Gosden *et al.*, 1989) although the weight of the ovary which remained intact was not equal to the total weight of the two ovaries in the control group (McLaren, 1963). This compensatory growth was greater if the unilaterally ovariectomised females were allowed to mate and became pregnant because the remaining ovary sheds nearly twice as many eggs as the normal number per ovary (McLaren, 1963). Rahima and Bruce (1987) also reported that unilateral ovariectomy before and after mating in rats caused an increase in the rate of progesterone and 20α-dihydroprogesterone secretion in the surviving ovary. In contrast to this, there was no extra ovulation in guinea pigs after unilateral ovariectomy at day 4 or day 5 *post coitum* unless all of the CL in the remaining ovary were also removed at the time of operation (Deanesly, 1971). After ovariectomy at day 28 *post coitum* in guinea pigs, the concentration of progesterone in the systemic blood was reduced up to half the values of intact animals which are characterised by a high concentration in uterine veins but low in placental tissue (Heap and Deanesly, 1966).

In the UOX's females operated on in the later days of pregnancy, i.e. day 6 (Figure 4.1B), day 10 (Figure 4.1C), and day 14 (Figure 4.1D) the differences were significant at day 15 of pregnancy. In fact, progesterone concentration of the UX group was also significantly lower than the SOP group when the operation was performed on day 14 of pregnancy (Figure 4.1D). As a consequence of this reduction in progesterone concentration, both the UX and UOX groups which were operated after day 2 of pregnancy never reached a peak level on day 15 of pregnancy as in the SOP group. Thus, it is likely that the later the operation is performed the greater the effect of the uterine removal on the progesterone concentration of both the UX and UOX groups.

4.5.2 Ovarian and Uterine Histology

In mice, the ovary is necessary until the last 2 days of pregnancy (Hillard, 1973). The CL of pregnancy continue to grow until it reaches a maximum size on day 13 and there are no significant changes that take place until day 18 when the CL form fibrous masses and accumulate large fat globules and gradual shrinking begins (Fekete, 1941; Rugh, 1967; Hillard, 1973). In the cyclic animal the effect of uterine removal on the CL function is both species- and time-dependent. The effect is species dependent since in species with a long metestrus phase, uterine removal can prolong the CL function but not in species with a short estrous cycle; or time dependent since whether the CL will regress normally or not depends on the stage of cycle at which the operation is performed (Moor *et al.*, 1970; Finn and Porter, 1975).

Measurements on the ovarian weight on day 18 of pregnancy indicate that removal of one uterine horn in mice as early as day 10 of pregnancy results in a decrease in weight of the ovary ipsilateral to the operated horn faster than that of the contralateral side though both the CL and Graafian follicle contents of the two ovaries are not different. Histological evidence revealed that this regression in the ipsilateral ovary of the UX group might be due to the regression in the luteal cells. Unilateral utero-ovariectomy in pregnant females (in the UOX group) significantly induces a rapid growth of young follicles into Graafian follicles which, in turn, apparently affect the weight of the remaining ovary.

The non pregnant uterine horn is luteolytic; removal of uterine horn prolongs luteal function in the mouse (Hillard, 1973). During the course of pregnancy the life span of the CL of pregnancy is maintained by the conceptuses in addition to the trophic support of the uterus and pituitary. However, the trophic action of the pituitary is only dominant during the first half of the gestation period and then ceases after day 10 of pregnancy (Choudary and Greenwald, 1969; Gibori, 1993). The trophic support of the pituitary is then taken over by the placenta (Choudary and Greenwald, 1969; Kohmoto and Bern, 1970; Kato *et al.*, 1979; Gibori, 1993; Galosy, 1995), while by day 8 the conceptuses enhances the trophic action by inhibiting the uterine luteolytic mechanism and by actively producing luteotrophic substances (Critser *et al.*, 1980). Recently, *in vivo* and *in vitro* study revealed that mouse placental lactogen I (mPL-I) and mPL-II which are actively secreted by the placenta at midpregnancy are luteotrophic (Yamaguchi *et al.*, 1994;

Galosy and Talamantes, 1995). The secretion of mPL-II is regulated by the activin and inhibin secreted by both the uterine dicidua and placenta during pregnancy (Yamaguchi *et al.*, 1995).

The finding that the O+ ovary is heavier than the O- ovary in the UX group is apparently consistent with the theory that the uterus and conceptuses play an important role in maintaining CL function during the course of pregnancy, and that the trophic action of the uterus and conceptuses is exerted locally rather than systemically. Thus the size and endocrinological activity of the CL is dependent on the presence or absence of the gravid uterine horn. Implantation is initiated on day 5 and completed by day 6 of pregnancy in the mouse (Rugh, 1967; Abrahamsohn and Zorn, 1993), two days before the CL of pregnancy sharply increase in size (Hillard, 1973). The role of the mouse conceptuses in maintaining the CL function was also studied by Humphreys *et al.*, (1985) who reported that the number of CL is negatively related to mean CL volume and the number of conceptuses is positively related to mean CL volume per female. The same result was also reported by Kato *et al.*, (1979) in the rat.

The local effect of the uterus on the CL function has been reported by several authors in the hamster, guinea pig, sheep, pig and cow (but not in the rat or rabbit). When one uterine horn is removed the corpora lutea ipsilateral to the removed horn regresses, whereas those in the opposite ovary are maintained (for review, see Anderson, 1973). In addition, unilateral ovariectomy alone (the ipsilateral uterine horn remains intact) before mating results in CL hypertrophy and increases the rate of progesterone secretion in the remaining ovary; however, unilateral ovariectomy after mating decreases the rate of progesterone secretion though CL hypertrophy occurs in the remaining ovary (Rahima and Bruce, 1987).

Histological evidence also indicates that the removal of one uterine horn, whether this surgical procedure is followed by the removal of ipsilateral ovary or not, results in luteal cell enlargement although the only significant effect is detected in the unilaterally utero-ovariectomized females (the UOX group) (Figure 4.5(C)). Overall means (±SEM) of large luteal cell diameters were 24.74 ± 0.43, 25.54 ± 0.22, and 24.57 ± 0.26 μm, with the mean (±SEM) nuclear luteal cell being 11.68 ± 0.16, 11.33 ± 0.16 and 11.29 ± 0.10 μm for the UX, UOX and SOP groups respectively. This suggests that enlargement of luteal cells in the UOX group is solely caused by the abundant increase in the cytoplasm. Consequently, the N/C diameter ratio value for the UOX group is significantly lower than both the UX and SOP groups (Figure 4.5(D)). The normal size

of large luteal cells is 26 - 45 μm in the mouse (Galosy and Talamantes, 1995), 30 μm in the rat (Gibori, 1993) or 40-50 μm in the pig (Belt, 1970).

These findings are parallel with the finding that the UOX group had progesterone concentrations lower than those of both the UX and SOP groups as previously described (see Section 4.5.1). The enlargement of luteal cells in the UOX females might be due to the compensatory effect of the remaining ovary to fulfil progesterone needs during pregnancy. The rate of progesterone secretion by the ovary in rats is related to the mass of luteal tissue available (Elbaum *et al.*, 1975) which in turn is a function of the number and volume of the individual CL in the ovary. Because both the total number and the total DNA content of large luteal cells in the CL is 1.5 to 2-fold greater than the small cells (Nelson *et al.*, 1992), thus total steroidogenic activity of the large luteal cells is greater than the small cells (Nelson *et al.*, 1992; Gibori, 1993) and total steroid output of the CL should be determined by the large luteal cells rather than the small cells.

It was found that endometrial epithelial cells of the UOX group were significantly lower than those of both the UX and SOP groups and the value for the UX was significantly lower than that of the SOP group (Figure 4.7(C)). This finding indicates that the presence of the two ovaries or the presence of the counterpart horn are required for the growth and maintenance of the endometrial epithelial cells. However, whether the decrease in the endometrial epithelial cell size is caused by the surgical procedures or not remains unclear. Bilateral ovariectomy in the mouse results in the reduction of both the uterine weight and the size of the endometrial cells, but the weight of the uterus and morphological changes in the endometrium, which are comparable to those found in estrous or during the follicular stage of the menstrual cycle, will be observed soon if the ovariectomised animals are injected with estrogen (Finn and Porter, 1975).

Chapter 5
Effect on Postnatal Growth

5.1 Introduction

5.1.1 Postnatal Growth

Prenatal and preweaning periods are probably the most critical in determining adult body weight and reproductive efficiency. According to Hafez (1963), preweaning growth is determined by both internal and external factors. Internal factors include birth weight, genotype, and hormonal condition of the neonate. External factors include litter size, maternal environment (maternal milk production, behaviour, age, genotype, nutrition status), and other factors such as environmental physical condition, including temperature, humidity, and light. The effect of prenatal and postnatal maternal environments on body weight are varied with the age of pups (El Oksh *et al.*, 1967; Moore *et al.*, 1970).

Postnatal growth of individual mice generally follows a sigmoid curve, and the same curve is shown by the body content of fat and water if plotted against age (Cheek and Holt, 1963). In the rat postnatal growth occurs in two main stages, the first stage being pre-maturity growth and the second postmaturity growth. (Pahl, 1969). Prematurity

growth is marked by the growth of all parts of the animal and results in high relative growth rate, while the postmaturity growth is marked by an increase in body weight but is not accompanied by significant changes in any part of the animals.

Since the body content of fat and water shows a sigmoid curve, and since the fat free dry solids (proteins and mineral ash) of the body show a linear relationship with time (Cheek and Holt, 1963), it is likely that the growth of the neonate is primarily determined by the fat and water content of the body, especially during the second stage (post-maturity) growth (Pahl, 1969). Maximal growth rate occurs at about 37 days of age, the time when the first oestrus can be observed in females. After the 5th day after birth there is a progressive reduction in the rate of weight gain until the end of the weaning period and after the weaning period there is a decrease in the concentration of water and electrolyte per unit weight (Cheek and Holt, 1963).

The size of litter at birth has a strong negative and approximately linear effect on body weight at 100 days after birth in mice (Wahlsten and Bulman-Felming, 1987), thus the reduction of litter size at birth results in an increase in growth rate (Park and Nowosielski-Slepowron, 1971; Timson and Dudenhoeffer, 1985). After birth, the young are entirely dependent upon maternal care at first and thereafter are largely dependent for a considerable period. Some of the most important postnatal maternal care includes the milk supply available and maternal responses towards the young. Growth rate increases after the reduction of litter size during the suckling period, presumably as a result of the decreased competition between young for the limited amount of milk available.

5.1.2 The Growth of Mammary Glands During Pregnancy

In the mouse, the lobulo-alveolar system begins to grow and differentiate at day 5 of pregnancy, although the secretion of milk will not be initiated before parturition occurs (Sinha *et al.*, 1974). The alveolar system is well established at day 14 of pregnancy, while the secretory activity begins in alveoli near the nipple and progresses until day 17 when the whole alveoli are involved in anticipation of lactation (Rugh, 1964). The growth of the lobulo-alveolar system is stimulated by a complex of hormones which includes the lactogenic hormones (prolactin, growth hormones, and placental lactogen), steroid hormones (progesterone and oestrogen), adrenal corticoids and insulin. These hormones originate from the ovaries. adrenal cortex, or placenta (Cowie and Tindal, 1971; Cowie, 1984; Buttle, 1988; Wang *et al.*, 1990; Johnson and Everitt, 1995).

The action of the steroid hormones and adrenal corticoids and insulin occurs in a synergistic manner with the lactogenic hormones. The effect of steroid hormones, for example, is felt in two ways, either directly by affecting the growth of mammary parenchyma or indirectly by stimulating the pituitary gland to release prolactin (Cowie, 1984) eg. steroid hormones do not work effectively in the absence of the pituitary.

The important role of progesterone, and oestrogen as well, on the growth of mammary glands is well known. The effect of the steroid hormones on the growth and differentiation of the lobulo-alveolar system occurs in both synergistic and separate ways. Oestrogen induces the mammary epithelium to proliferate and causes little alveolar differentiation, while progesterone induces both epithelial proliferation and alveolar differentiation. The combination of the two hormones results in an additive action on both epithelial proliferation and alveolar differentiation (Rugh, 1964). Most recently, Wang *et al.* (1990) presumed that progesterone action on the epithelial proliferation is mediated by the oestrogen-dependent progesterone receptors.

5.1.3 Milk Production

Lactogenesis in the alveolar epithelium is blocked by circulating progesterone during pregnancy (Gandelman and Svare, 1975). This interpretation is supported by the fact that parturition is preceded by a decrease in plasma progesterone concentrations (Murr *et al.*, 1974), and that prolactin and placental lactogen increase in plasma throughout pregnancy, reaching their maximum level at parturition, although the mammary glands remain unresponsive to these hormones until progesterone levels drop after parturition (Johnson and Everitt, 1995). The inhibitory effect of progesterone on lactose synthesis in the mouse and rat has also been documented (Cowie, 1984). Since lactation will occur following hysterectomy as early as day 12 of pregnancy in mice (Gandelman and Svare, 1975), thus luteotrophic factors originating from the placenta, which is important in inducing ovarian progesterone secretion, also play an important role in inhibiting lactation during the course of pregnancy in the animals.

In mice, lactation usually proceeds for a period of 3 weeks and reaches its peak at 14 days after parturition (Rugh, 1967). During this period the ducts and alveoli are dilated with milk, but any unused glands or all the glands at the end of the lactation period will regress.

5.1.4 Maternal Behaviour

How the mother behaves towards the neonate after a normal gestation is an important factor in determining the growth of the neonate. Maternal behaviour to the pups soon after parturition includes cleaning (licking), warming (nest building), feeding (nursing) and protection (pup-retrieval) (Gandelman *et al.*, 1970; Sayler and Salmon, 1971; Pedersen, 1987). Hormonal mechanisms modulating maternal behaviour have been studied extensively in mice (Lisk *et al.*, 1969; Gandelman, 1973; Hauser and Gandelman. 1985) and show that hormonal events during pregnancy are responsible for the stimulation of maternal behaviour at parturition (Lisk *et al.*, 1969; Hauser and Gandelman, 1985). Virgin mice implanted with prolactin in the hypothalamus, for example, exhibit a full set of maternal behaviours (Lisk *et al.*, 1969). Studies on rats also show that the latency of the ovariectomised or ovariectomised-hysterectomised virgin females to behave maternally can be reduced by administration of a combination of progesterone, oestrogen, androgen, and prolactin (Zarrow *et al.*, 1971; Bridges *et al.*, 1978; Bridges and Russel, 1981).

Recently, the most intensive research on the role of hormones in the stimulation of maternal responsiveness has been focussed upon the involvement of steroids, primarily progesterone (Bridges, 1984; Crombie *et al.*, 1995; Wang *et al.*, 1992; Wang *et al.*, 1995). In the rat, the responsiveness of the mother to her young at birth is primed by progesterone and oestrogen during pregnancy (Bridges, 1984). Immunisation of mice against progesterone results in the aberrant or negative behaviour of the mother during the first 5 days of lactation (i.e. cannibalism, pup-retrieval and feeding failures, and pup rejection) towards her young after the first successful pregnancy following recovery of fertility (Wang *et al.*, 1992). Furthermore, Wang *et al.* (1995) revealed that the aberrant behaviour of the antibody-treated mother is not due to the lack of milk secretion nor to pup abnormality. More recently, it has been found that the occurrence of negative behaviour in the mother treated with anti-progesterone antibody or progesterone antagonist is due to the impairment of the imprinting mechanism associated with the establishment of normal maternal behaviour (Crombie *et al.*, 1995).

In Chapter 4, it was revealed that in mice operated on. on day 2 of pregnancy, the mean progesterone concentration of both the SOP and UX groups is higher than that of the UOX group. In mice operated after day 2 of pregnancy, progesterone concentration of the UX group is higher than that of the UOX but lower than that of the SOP groups. Although these differences are not significant in all days examined. this tendency

informs us that the reduction of corpora lutea and conceptuses or the reduction of conceptuses only up to a half of the normal numbers results in a reduction in the maternal concentration of progesterone.

As noted previously (Section 5.1.2.2), either the normal growth and differentiation of the mammary gland or the initiation of the milk secretion during gestation period is regulated hormonally where the role of progesterone is important. One study on mice showed that placental numbers and weight, but not fetal numbers or weight, are related to the development of mammary glands (Barkley *et al.*, 1979). Progesterone is also involved in the initiation of maternal responsiveness towards the pups after parturition (Bridges, 1984; Crombie *et al.*, 1995; Wang *et al.*, 1992; Wang *et al.*, 1995).

5.1.5 Objective

Since progesterone concentration of the SOP control group of mice was generally higher than that of both the UX and UOX groups (with the exception of the UX operated on, on day 2 of pregnancy) (Chapter 4) it was assumed that uterine removal might result in one or all of the following conditions: 1) an abnormality in the growth and differentiation of the mammary gland during gestation period which causes maternal incapability to nourish pups, 2) an impairment of post partum maternal behaviour towards the pups. However, since the gestation length of the SOP mice was significantly shorter than both the UX and UOX groups (Chapter 3), it was assumed that unilateral uterine transection would result in conditions which favoured the growth of neonates in the UX and UOX group. This study aims to examine the possible effect of unilateral uterine removal, with or without removal of its ipsilateral ovary, on the growth of neonates delivered by the operated females.

5.2 Methods

Mature virgin mice were used for examination of postnatal growth. Handling, mating, and surgery procedures and environmental conditions in the laboratory room are described in Chapter 2. After surgery, females were caged in group of two or three. Two or three days before the expected parturition day for the normal mice, pregnant animals were caged individually in small opaque plastic cages of 30 x 12 x 12 cm in size with a 0.5 cm thick dry pellet paper bedding. The procedure for birth checking is described in Chapter 3.

5.2.1 Maternal Behaviour

With minor modifications, the methods of Bridges (1984), Wang *et al.* (1992), Crombie *et al.*, (1995), and Wang *et al.*, (1995) were applied in the study of maternal behaviour testing (described in Chapter 2). In addition to the pellet paper bedding, toilet tissue paper strips were provided daily for a fresh nest after removing the old materials. Testing was carried out on the day of delivery and repeated for five consecutive days. Maternal response under testing was observed for about 15 minutes at 10.00 after all pups were moved out of the nest to the opposite side of the nesting cage. The following criteria were used to determine maternal post partum behaviour Bridges (1984), Wang *et al.* (1992), Crombie *et al.*, (1995), and Wang *et al.*, (1995).

Normal maternal behaviour:

Females which displayed the following response patterns were considered as behaving normally: (1) retrieving: the mother was observed to pick up all the pups and return them to the nest; (2) nest-building: the mother was observed to display one or more of the following behaviours - bringing material to the nest, nibbling materials, or assembling the material; (3) lactation position: the mother covered at least two pups with her body; and (4) licking: the mother was seen to lick pups.

Abnormal maternal behaviour:

Females who display one of the following response patterns were considered as behaving abnormally: (1) cannibalism: the mother attacked and killed one or more of her pups during the observation period; (2) isolation: the mother failed to return one or more pups to the nest site; (3) nursing failure: less than two pups were hovered over or tucked under the dam.

Rejection:

As a result of abnormal maternal behaviour, some pups were rejected, i.e. in the form of cannibalism, isolation (the failure of mother to return back the pups to the nest site or the transference of pups away from the nest site) or nursing failure.

5.2.2 The Growth of Neonates

At delivery, litter size was recorded, the pups were sexed, weighed individually and the mean weight of the whole litter was calculated. The number of stillborn and the

possibility that the mother had eaten her own pups (cannibalism) at parturition day was also recorded.

To reduce variation arising from the effects of litter size on postnatal growth, mothers were allowed to nurse a maximum of four pups. Thus, only a mother with four or more live pups, which consisted of at least 2 males and 2 females, was used for the next observation. In the case of the mother having more than 4 pups, two males and two females were selected from each litter after exclusion of obvious 'runts', while the mother having less than 4 pups was excluded. Only the mother with 4 pups remaining at the end of observation was included in the analysis.

Young mice were weighed daily at about 10.00 h until they reached 21 days of age, the first day when pups can be weaned without a detrimental effect on the pups (Rugh, 1964). The body weight of individual pups was recorded to the nearest 0.01 g by placing the mouse in a tared beaker glass on a Mettler direct reading balance.

5.2.3 The Mammary Gland Weight

To examine whether the removal of one uterine horn affects the growth of mammary glands, data of mammary gland weight was obtained from another 60 females used in the previous experimentation for the length of gestation (Chapter 3). The five pairs of mammary glands were carefully removed from the maternal skin at the day of parturition. Mammary glands were dissected in order from the thoracic to the inguino-abdominal position. The mammary glands on the left side were removed and weighed after those on the right position were finished. This procedure was important in order to determine whether the weight of mammary glands from the operated side (the side where the uterine horn was removed) were different from the unoperated side or not. The glands were weighed individually on a Mettler direct reading balance to the nearest 0.0001 g. This examination of the mammary gland was conducted in one to three hours after delivery following bleeding for progesterone analysis purposes.

5.3 Results

5.3.1 Maternal Behaviour

Normal maternal behaviour was observed in 60-80% of the UX and 80-100% in the SOP females, much higher than in the UOX females (Table 5.1) The mean percentages of the pups rejected by the mother during observation period were higher (but statistically not significant) in the UOX females compared to both the UX and SOP females. Since maternal rejection usually ended in neonatal death, the number of young at the end of observation (at day 5 post partum) was lower. Especially in the UOX groups the difference between initial (at delivery) and final (at day 5 post partum) number of pups was significant ($p < 0.05$; paired t-test). The weight of pups delivered either by the UX or UOX females was significantly higher than those delivered by the SOP females ($p < 0.05$ to $p < 0.001$; Tukey HSD-Test). However, the weight differences between groups became obscure as the lactation process proceeded to the 5th day.

If the data are grouped by Operation factor without considering the Day factor (Table 5.1, at the bottom rows) it can be seen that the overall mean of females maternally normal was 75% and 85% respectively for the UX and SOP females compared to only 35% for the UOX females. The mean percentages of pups rejected by the UOX mother (33.68 ± 6.97) was significantly higher than those of both the UX (11.01 ± 5.44) ($p < 0.05$) and the SOP ($3.85 \pm 2.26\%$) ($p < 0.01$) groups. There was no difference between the UX and SOP groups in their rejection of the pups although their litter size was significantly different at delivery ($p < 0.001$).

Although the overall incidence was small, pup rejections occurred as early as day 1 of the lactation period (Table 5.2). Rejection then increased drastically at day 2 and continued with a slight decrease at day 3 and became constant at day 4 and day 5 of lactation period. The UOX group showed a higher rejection rate than that of both the UX or UOX groups. Interestingly, while pup isolation and/or nursing failure occurred in both the UX and SOP groups, cannibalised rejection was a more common behaviour in the UOX groups.

Table 5.1 Post partum maternal behaviour towards young and the number of pups rejected by the mother during the first 5 days of the lactation period.

Group	n	Litter at birth		Normal maternal behavior (%)	Number of pups rejected (%)	Litter at day 5 *post partum*	
		Size	Mean weight (g)			Size	Mean weight (g)
By Day and Operation factors:							
2UX	5	8.4 ± 1.4*	1.65 ± 0.03*	4/5 (80)	20.00 ± 20.00	6.0 ± 1.8*	3.71 ± 0.08**
2UOX	5	5.8 ± 1.1**	1.61 ± 0.09*	2/5 (40)	38.00 ± 19.08	3.8 ± 1.2**	3.22 ± 0.09
2SOP	5	13.6 ± 0.9	1.42 ± 0.02	4/5 (80)	7.27 ± 7.27	12.8 ± 1.6	3.07 ± 0.12
6UX	5	7.6 ± 0.5*	1.77 ± 0.05***	4/5 (80)	6.67 ± 6.67	7.2 ± 0.9*	3.15 ± 0.08
6UOX	5	7.4 ± 0.9**	1.54 ± 0.05*	1/5 (20)	37.91 ± 12.79	5.0± 1.5**†	3.05 ± 0.19
6SOP	5	12.6 ± 0.7	1.38 ± 0.05	4/5 (80)	2.67 ± 2.67	12.2 ± 0.4	2.93 ± 0.09
10UX	5	5.4 ± 0.5***	1.71 ± 0.11	3/5 (60)	11.67 ± 7.27	4.8 ± 0.7**	3.03 ± 0.06
10UOX	5	7.6 ± 0.8**	1.63 ± 0.09	2/5 (40)	33.50 ± 15.32	5.4 ± 1.5**	2.87 ± 0.17
10SOP	5	12.4 ± 0.9	1.47 ± 0.06	4/5 (80)	5.45 ± 5.45	11.8 ± 1.2	2.89 ± 0.09
14UX	5	7.0 ± 0.8**	1.66 ± 0.05**	4/5 (80)	5.71 ± 5.71	6.6 ± 0.9**	3.09 ± 0.13
14UOX	5	7.2 ± 0.7**	1.59 ± 0.04*	2/5 (40)	25.32 ± 11.53	5.2 ± 0.7***	2.92 ± 0.16
14SOP	5	13.6 ± 1.5	1.39 ± 0.06	5/5 (100)	0.00 ± 0.00	13.6 ± 1.5	2.81 ± 0.08
By Operation factor:							
UX	20	7.1 ± 0.4***	1.69 ± 0.03***	15/20 (75)	11.01 ± 5.44*	6.2 ± 0.6***	3.22 ± 0.08*
UOX	20	7.0 ± 0.4***	1.59 ± 0.03***	7/20 (35)	33.68 ± 6.97	4.9 ± 0.6***§	3.04 ± 0.08**
SOP	20	13.1 ± 0.5	1.42 ± 0.02	17/20 (85)	3.85 ± 2.26**	12.6 ± 0.6	2.93 ± 0.05

All values are mean (± SEM) except for the number of normal maternal behaviours; *p<0.05, **p<0.01, and ***p<0.001, are different from the sham-operated control (SOP) group (except for the number of rejected pups, where both the UX and SOP groups are compared to the UOX group) (Tukey HSD-test); †p<0.05 and §p<0.001, are different from the initial number at delivery (paired samples t-test) Prefix number for each group denotes the day of operation.

Table 5.2 Distribution of rejected pups on the day of observation and the type of rejection.

Group	No. of abnormal mother[1]	No. of rejected pups[2]	Distribution on the day of observation and type of rejection[3,4]										Total	
			Day 1		Day 2		Day 3		Day 4		Day 5			
			C	I+NF	C	I+NF	C	I+NF	C	I+NF	C	I+NF	C	I+NF
By Day and Operation factors:														
2UX	1/5	12/42	0	2	1	4	0	3	0	0	1	1	2	10
2UOX	3/5	10/29	0	1	3	1	0	0	0	2	1	2	4	6
2SOP	1/5	4/68	0	0	0	1	0	0	1	2	0	0	1	3
6UX	1/5	2/38	0	0	0	0	0	2	0	0	0	0	0	2
6UOX	4/5	12/37	0	2	3	0	2	1	1	1	1	1	7	5
6SOP	1/5	2/63	0	0	0	1	0	0	0	1	0	0	0	2
10UX	2/5	3/27	1*	0	0	0	0	1	0	0	0	1	1	2
10UOX	3/5	11/38	0	2	5	1	0	1	0	0	2	0	7	4
10SOP	1/5	3/62	0	0	0	1	0	0	0	0	1	1	1	2
14UX	1/5	2/35	0	0	0	0	0	2	0	0	0	0	0	2
14UOX	3/5	10/36	0	0	3	0	1	2	2	1	1	0	7	3
14SOP	0/5	0/68	0	0	0	0	0	0	0	0	0	0	0	0
By Operation factor:														
UX	5/20	19/142	1	2	1	4	0	8	0	0	1	2	3	16
UOX	13/20	43/140	0	5	14	2	3	4	3	4	5	3	25	18
SOP	3/20	9/261	0	0	0	3	0	0	1	3	1	1	2	7

[1]Total number of abnormal mother/total number of female group; [2]Total number of rejected pups/total number of pups per group; [3]Days *post partum*; [4]Cannibalised (C), isolated (I) and/or nursing failed (NF) pups; *Decapitated head of one pup was found on the day of delivery. Prefix number for each group denotes the day of operation.

5.3.2 The Growth of Neonates

Removal of one uterine horn in both the UX and UOX groups significantly reduced the size of the litter (Table 5.3). As expected, total litter weight at birth rose with litter size. Consequently, total litter weight to maternal weight (TLW/MW) ratio at delivery was also significantly reduced in both the UX and UOX groups. It was likely that the reduction in litter size allowed the fetuses at the remaining uterine horn to grow at a more rapid rate during the gestation period since mean birth weight of pups delivered by both the UX and UOX groups was significantly ($p < 0.01$ to $p < 0.001$) higher than that of the SOP groups. However, sex ratio of the pups surviving in the remaining uterine horn was not altered by unilateral uterine transection. There was no difference between the UX and UOX group in relation to either the size of litter, or both total and mean weight of pups per litter or to the TLW/MW ratio at delivery. Two way analysis of variance revealed that none of those parameters was affected by the day of operation (the day factor). Table 5.3 also showed that maternal body weight at parturition or weaning was significantly higher than that at mating (day 0 of pregnancy) in the UX or UOX or SOP group.

Mean body weight at birth and at weaning and calculated growth rate for each group after the reduction of litter size to four pups (2 males plus 2 females) per litter are presented in Table 5.4 (for completed daily body weight records, see Appendix 5.1). After allocation of pups into groups of four per litter at birth, mean weight of the pups from both the UX and UOX groups still remained higher than that of the SOP group as observed before selection (see also Table 5.3 for comparison to the original mean body weight of whole litter). At weaning (day 21 of lactation period), however, weight of pups from the UOX group was generally lower than that of the UX and was similar to the SOP group (with the exception of the animals operated on, on day 14 of pregnancy). One way analysis of variance revealed that the growth rate of both the UX and SOP groups was significantly lower than that of the UX group ($p < 0.01$; Tukey HSD-test). However this difference was only significant in the animals operated on, on day 2 and day 10 of pregnancy. Table 5.4 also shows that although the weight of selected pups at birth was not significantly different between males and females, the two sexes were significantly different both in their growth rate and final body weight (at weaning).

Table 5.3 Mean (±SEM) litter size, total and mean weight per litter, male sex ratio, maternal body weight at mating, delivery, and weaning, and total litter weight/maternal weight ratio at birth for each group.

	Litter at birth				Maternal body weight (MW) (g) at ...			TLW/MW
Group	Size	Total weight (TLW) (g)	Mean weight (g)	Male sex ratio[1]	Mating (day 0 of pregnancy)	Delivery	Weaning (day 21 *post partum*)	ratio at delivery
By Day and Operation factors (n = 5)[2]:								
2UX	7.8 ± 0.9*	12.99 ± 1.41*	1.68 ± 0.03**	0.52 ± 0.05	32.70 ± 0.85	38.00 ± 1.17††	41.50 ± 0.98†††	0.39 ± 0.04*
2UOX	7.6 ± 1.0**	13.18 ± 1.77*	1.74 ± 0.02***	0.51 ± 0.04	33.70 ± 0.44	39.60 ± 0.51††	44.00 ± 0.89†††	0.39 ± 0.05*
2SOP	12.8 ± 1.0	19.06 ± 1.51	1.49 ± 0.03	0.54 ± 0.03	33.00 ± 0.65	38.40 ± 0.58†††	39.90 ± 0.68†††	0.58 ± 0.04
6UX	6.8 ± 0.6*	12.36 ± 1.06*	1.76 ± 0.06***	0.53 ± 0.05	32.20 ± 0.66	39.50 ± 1.38††	45.60 ± 2.12†††	0.38 ± 0.03*
6UOX	7.2 ± 0.9*	11.26 ± 1.16*	1.58 ± 0.03**	0.53 ± 0.04	32.30 ± 0.64	36.60 ± 1.21††	41.40 ± 1.34††	0.35 ± 0.04**
6SOP	12.2 ± 1.6	19.76 ± 1.99	1.51 ± 0.04	0.51 ± 0.06	32.30 ± 0.66	38.80 ± 0.37††	41.30 ± 1.49††	0.56 ± 0.13
10UX	6.4 ± 0.8*	11.63 ± 1.15*	1.83 ± 0.05***	0.47 ± 0.11	33.00 ± 0.73	37.30 ± 0.66††	45.10 ± 1.60†††	0.35 ± 0.03*
10UOX	6.2 ± 0.6*	10.67 ± 0.75*	1.74 ± 0.07**	0.52 ± 0.05	32.30 ± 0.66	37.40 ± 1.26††	44.50 ± 1.66††	0.33 ± 0.02*
10SOP	12.0 ± 1.8	17.48 ± 2.27	1.48 ± 0.05	0.53 ± 0.03	32.10 ± 0.60	36.50 ± 0.50†	39.00 ± 1.30†††	0.55 ± 0.08
14UX	5.8 ± 0.7***	8.85 ± 0.71***	1.63 ± 0.14**	0.54 ± 0.04	32.30 ± 0.72	35.80 ± 0.75††	41.40 ± 1.25†††	0.27 ± 0.02**
14UOX	6.6 ± 0.5***	10.92 ± 0.83***	1.66 ± 0.02**	0.54 ± 0.05	32.60 ± 0.69	37.60 ± 1.41††	42.30 ± 1.83†††	0.34 ± 0.03*
14SOP	13.8 ± 1.2	20.24 ± 1.43	1.48 ± 0.04	0.52 ± 0.03	33.00 ± 0.42	40.40 ± 0.62†††	44.90 ± 1.55†††	0.61 ± 0.04
By operation factor (n = 20):								
UX	6.7 ± 0.4***	11.45 ± 0.63***	1.72 ± 0.03***	0.51 ± 0.03	32.55 ± 0.35	37.65 ± 0.56	43.40 ± 0.84	0.35 ± 0.02***
UOX	6.9 ± 0.4***	11.51 ± 0.59***	1.68 ± 0.03***	0.52 ± 0.02	32.73 ± 0.31	37.80 ± 0.58	43.05 ± 0.73	0.35 ± 0.02***
SOP	12.7 ± 0.7	18.75 ± 0.84	1.49 ± 0.02	0.52 ± 0.02	32.60 ± 0.29	38.53 ± 0.40	41.28 ± 0.79	0.58 ± 0.03

[1]Total number of males/total number of pups per litter; [2]Prefix number denotes the day of pregnancy when operation is performed; *p<0.05; **p<0.01; ***p<0.001, are different from sham-operated control (SOP) group (Tukey HSD-test); †p<0.05; ††p<0.01; †††p<0.001, are different from the weight at mating (paired samples t-test).

Table 5.4 Mean (±SEM) weight at birth and weaning (day 21 *post partum*) and growth rate after the reduction of litter size to 4 pups per litter

	Weight (g)[1]								
	Birth			Weaning (Day 21)			Growth rate (g/day)		
Group	Male	Female	Overall	Male	Female	Overall	Male	Female	Overall
By Day and Operation factors (n = 5)[2]									
2UX	1.70 ± 0.03	1.70 ± 0.03	1.70 ± 0.03$^{\#\#}$	14.38 ± 0.27	13.15 ± 0.34††	13.76 ±0.29	0.60 ± 0.01	0.55 ± 0.02††	0.58 ± 0.01
2U●X	1.76 ± 0.05	1.73 ± 0.06	1.74 ± 0.05$^{\#\#}$	13.09 ± 0.09	12.10 ± 0.31†	12.59 ± 0.21*	0.54 ± 0.01	0.49 ± 0.01†	0.52 ± 0.01**
2SOP	1.51 ± 0.03	1.50 ± 0.03	1.50 ± 0.03	12.43 ± 0.18	11.73 ± 0.28††	12.08 ± 0.22**	0.52 ± 0.01	0.48 ± 0.01††	0.50 ± 0.01**
6UX	1.80 ± 0.04	1.79 ± 0.04	1.79 ± 0.04$^{\#\#\#}$	13.46 ± 0.73	12.94 ± 0.64	13.19 ± 0.68	0.56 ± 0.04	0.53 ± 0.03	0.54 ± 0.03
6UOX	1.58 ± 0.03	1.58 ± 0.03	1.58 ± 0.03$^{\#\#}$	12.73 ± 0.74	11.89 ± 0.96††	12.31 ± 0.39	0.53 ± 0.02	0.49 ± 0.02††	0.51 ± 0.02**
6SOP	1.52 ± 0.04	1.52 ± 0.04	1.52 ± 0.04	12.09 ± 0.14	11.05 ± 0.13†††	11.58 ± 0.13	0.50 ± 0.01	0.45 ± 0.01†††	0.48 ± 0.03**
10UX	1.82 ± 0.05	1.82 ± 0.05	1.82 ± 0.05$^{\#\#}$	14.38 ± 0.63	13.58 ± 0.63††	13.98 ± 0.63	0.59 ± 0.03	0.56 ± 0.03††	0.58 ± 0.03
10UOX	1.72 ± 0.08	1.72 ± 0.08	1.72 ± 0.08$^{\#}$	12.18 ± 0.37	11.36 ± 0.47††	11.77 ± 0.41*	0.50 ± 0.02	0.46 ± 0.02††	0.48 ± 0.02**
10SOP	1.52 ± 0.04	1.44 ± 0.08	1.48 ± 0.05	12.89 ± 0.40	12.10 ± 0.30††	12.49 ± 0.35	0.54 ± 0.02	0.51 ± 0.01†	0.52 ± 0.02**
14UX	1.67 ± 0.03	1.66 ± 0.03	1.67 ± 0.03$^{\#\#}$	11.60 ± 0.54	10.34 ± 0.63††	10.97 ± 0.58	0.47 ± 0.03	0.41 ± 0.03††	0.44 ± 0.03
14UOX	1.68 ± 0.02	1.67 ± 0.02	1.67 ± 0.02$^{\#\#}$	12.23 ± 0.57	11.45 ± 0.60†††	11.84 ± 0.59	0.50 ± 0.03	0.46 ± 0.03†††	0.48 ± 0.03
14SOP	1.47 ± 0.05	1.47 ± 0.05	1.47 ± 0.09	11.74 ± 0.76	11.07 ± 0.57†	11.40 ± 0.66	0.49 ± 0.04	0.46 ± 0.03†	0.47 ± 0.03
By Operation factor (n = 20)									
UX	1.75 ± 0.02	1.74 ± 0.02	1.75 ± 0.02$^{\#\#}$	13.45 ± 0.37	12.49 ± 0.39†††	12.98 ± 0.38	0.56 ± 0.02	0.51 ± 0.02†††	0.56 ± 0.02
UOX	1.68 ± 0.03	1.67 ± 0.03	1.68 ± 0.03$^{\#\#}$	12.56 ± 0.19	10.67 ± 0.22†††	12.13 ± 0.21**	0.52 ± 0.01	0.48 ± 0.01†††	0.52 ± 0.01**
SOP	1.50 ± 0.02	1.48 ± 0.03	1.49 ± 0.02	12.29 ± 0.23	11.49 ± 0.19†††	11.89 ± 0.21*	0.51 ± 0.01	0.47 ± 0.01†††	0.51 ± 0.01**

[1]Litter size = 4 (2 males plus 2 females); [2]Prefix number for each group denotes the day of operation; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ are different from its SOP group (Tukey HSD-test); $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ are different from its UX group (Tukey HSD-test); $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ are different from their male littermates (paired samples t-test).

Quantitative relationship between pups' body weight (g) and age (days) is plotted in Figure 5.1 (complete data for each point in this figure is presented in Appendix 5.1); and the intercept *(a)*, slope *(b)* and coefficient of determination (r^2) values for each regression line are listed in Table 5.5. During the lactation period (from day 1 to day 21 post partum) pups weight was positively related to age in all groups. No statistical analyses were done to examine the differences between groups in either intercepts or slopes values, (the statistical comparision of a series of repeated measures on the same animals is a complex task) but it is clear from Figure 5.1, Table 5.4 and Table 5.5 that the pups from the UX group had a higher growth rate than both the UOX and SOP groups. except in the animals operated on, on day 14 of pregnancy (Figure 5.1 (D)). That is, some maternal factors rather than the number of pups suckled by the mother during lactation period affected the pups' growth rate.

The daily weight gain of pups from animals operated on, on several different days of pregnancy during lactation period is presented in Figure 5.2. The daily weight gain pattern was similar in all groups. Weight increased slightly during the first 6 days of lactation before remaining constant until day 12 to 13. The highest weight gain values were reached by all groups between day 13 to 14. After that, the pattern was slightly varied between groups. The pups delivered by the animals operated on, on day 2 or 14 of pregnancy showed a continuous decrease in their weight gain from day 14 to day 21. In contrast, the pups delivered by the animals operated on, on day 6 or 10 of pregnancy showed an increase in their weight gain during the last three days of the lactation period. Figure 5.2 also shows that during the first week of the lactation period, the weight gain of the SOP group was inferior to both the UX and UOX group (especially in the animals operated on, on day 2 (Figure 5.2(A)) and 6 (Figure 5.2(B)). However, during the last week of the lactation period the SOP group was similar or even superior to the UX and UOX groups. This figure also provides additional confirmation that in general daily mean weight gained by the UX groups was higher than that of both the UOX and SOP groups, especially in the first two weeks of the lactation period (pups delivered by females operated on, on day 14 of pregnancy was an exception to this generalisation).

5.3.3 The Mammary Glands Weight

There was no difference between operated and unoperated groups in their mean weight of mammary glands (Table 5.6). Also, in the same groups of operated animals, the weight of mammary glands from the operated side was not different from the glands from the unoperated side.

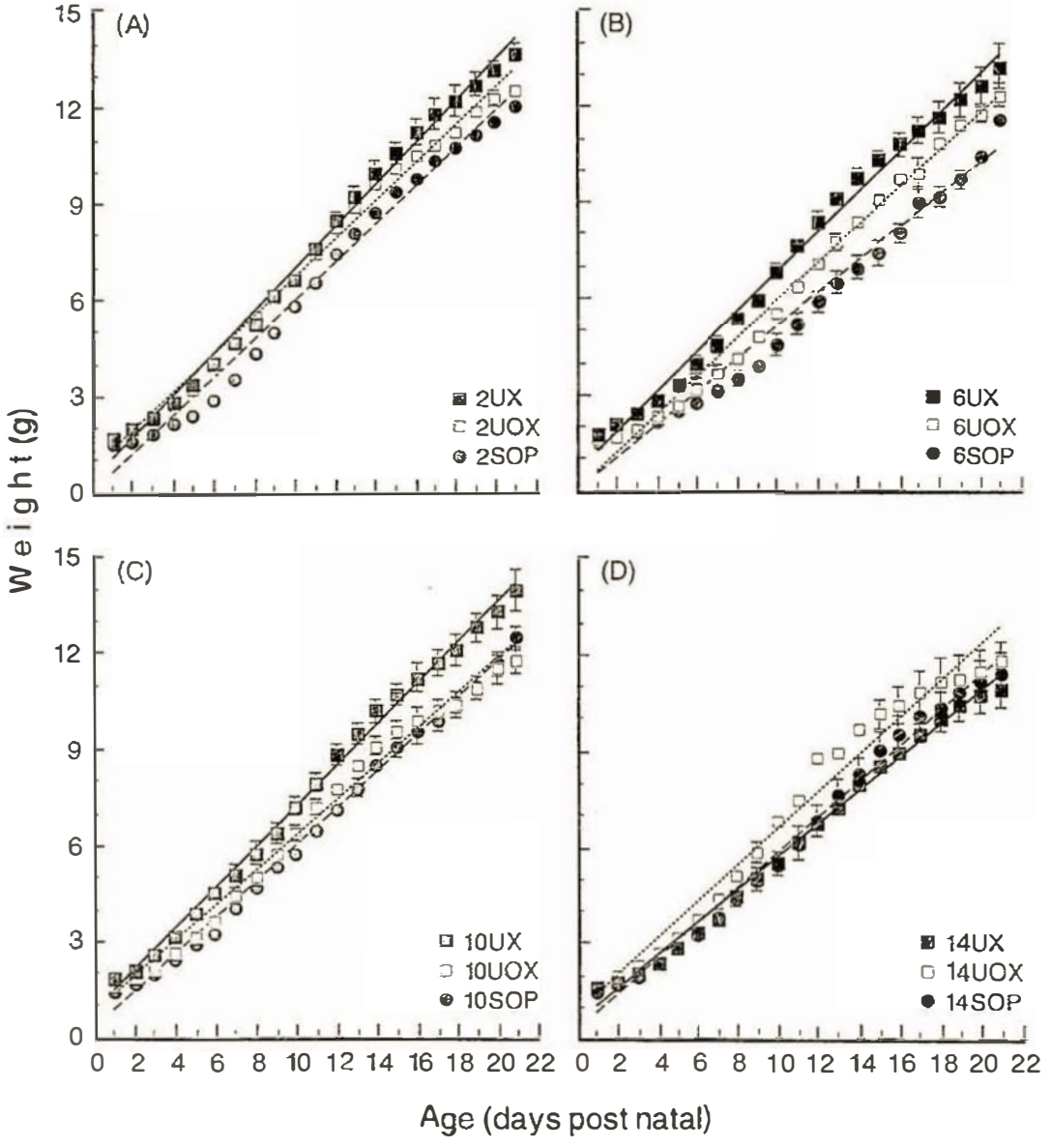

Figure 5.1 The relationship between mean (±SEM) weight (g) and age (days) of neonates during the first 21 days of lactation period from females operated on day 2 (A), day 6 (B), day 10 (C), and day 14 (D) of pregnancy. Five litters (each litter consisting of 2 males and 2 females) per group were examined during the observation period. Values for intercept, slope and coefficient of determination for each line are listed in Table 5.5.

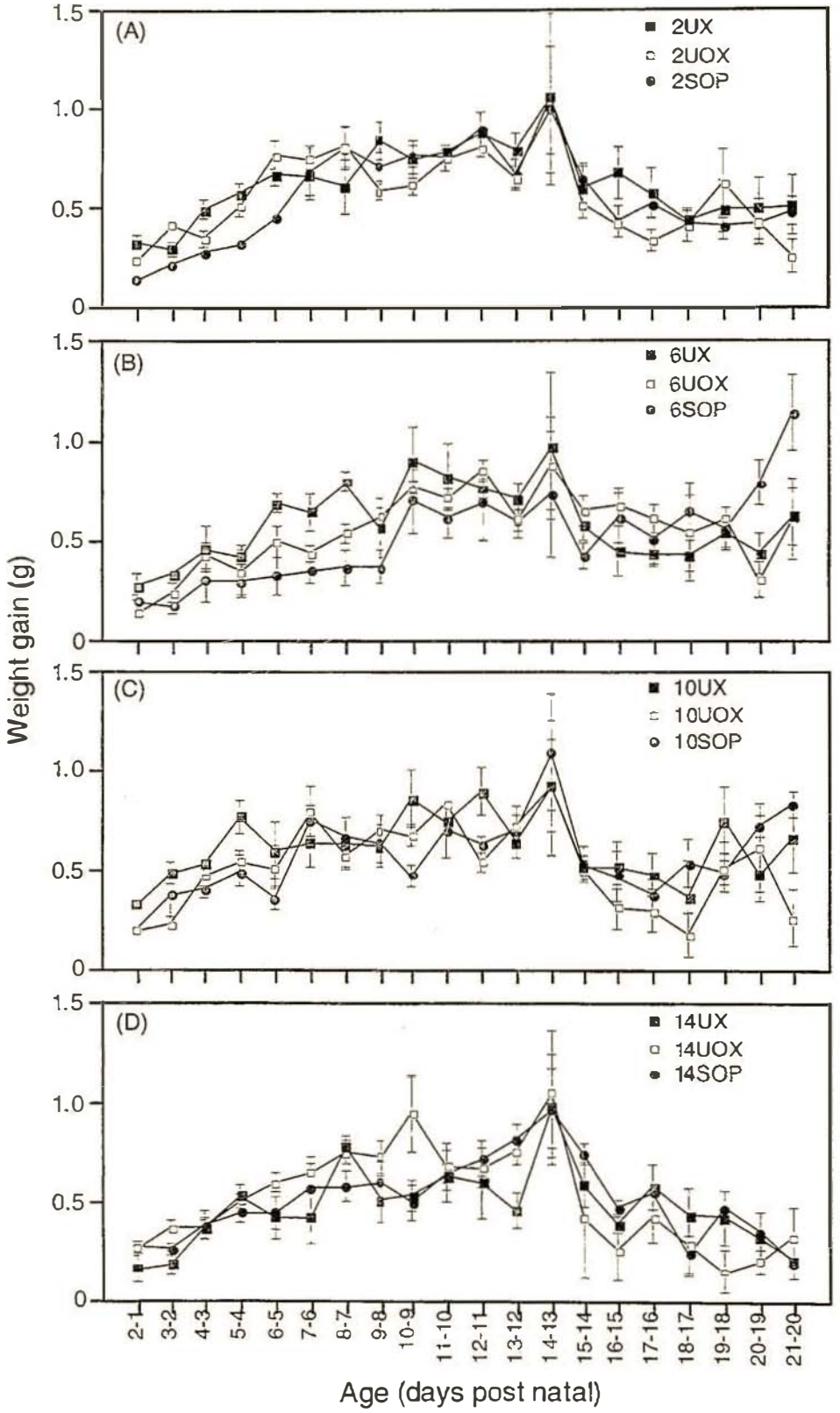

Figure 5.2 Mean (±SEM) daily weight gain (g) for pups nourished by operated (UX and UOX) and sham operated (SOP) females during the lactation period. Operation was performed on several different days of pregnancy, i.e. day 2 (A), day 6 (B), day 10 (C), and day 14 (D). Each point represents 5 females with 4 pups each (2 males plus 2 females).

Table 5.5. The intercept *(a)*, slope *(b)* and standard error (SE), coefficient of determination *(r^2)*, and probability *(p)* values for each linear regression line *($y = a + bx$)* are shown in Figure 5.1

	Regression			
Group	*a*	*b* (±SE)	r^2	$p <$
2UX	0.43	0.66 (0.01)	0.99	0.001
2UOX	0.75	0.59 (0.01)	0.99	0.001
2SOP	0.02	0.59 (0.01)	0.99	0.001
6UX	0.64	0.62 (0.01)	0.98	0.001
6UOX	0.05	0.59 (0.01)	0.99	0.001
6SOP	0.04	0.50 (0.01)	0.98	0.001
10UX	0.86	0.64 (0.01)	0.98	0.001
10UOX	0.79	0.55 (0.01)	0.98	0.001
10SOP	0.28	0.57 (0.01)	0.99	0.001
14UX	0.54	0.52 (0.01)	0.98	0.001
14UOX	0.82	0.58 (0.01)	0.97	0.001
14SOP	0.31	0.55 (0.01)	0.97	0.001

Table 5.6 Mean (±SEM) mammary gland weight of operated and unoperated females at the day of delivery.

		Mammary gland weight ($x10^{-4}$g)		
Group	n	Operated side	Unoperated side	Overall
2UX	5	15.00 ± 0.45	15.60 ± 0.40	15.60 ± 0.24
2UOX	5	15.40 ± 0.93	15.60 ± 0.51	15.60 ± 0.40
2SOP*	5	14.80 ± 0.37	15.40 ± 0.68	15.20 ± 0.37
6UX	5	15.60 ± 0.40	16.00 ± 0.63	15.80 ± 0.58
6UOX	5	15.20 ± 0.97	15.20 ± 0.37	15.40 ± 0.68
6SOP*	5	15.60 ± 1.12	15.60 ± 1.60	15.60 ± 0.81
10UX	5	15.00 ± 1.14	15.20 ± 0.86	15.00 ± 0.71
10UOX	5	17.00 ± 0.84	17.60 ± 0.75	17.20 ± 0.66
10SOP*	5	15.80 ± 0.86	17.20 ± 0.86	16.80 ± 0.58
14UX	5	15.80 ± 0.66	16.20 ± 1.24	16.00 ± 0.55
14UOX	5	16.40 ± 0.75	17.00 ± 1.05	16.40 ± 0.75
14SOP*	5	16.00 ± 0.89	15.80 ± 0.80	15.80 ± 0.73

*To simplify the presentation, the weight of mammary glands from the right side of the SOP animals is listed in the operated side and from the left side is listed in the unoperated side column although the removal of one uterine horn was made alternately on the right and left sides of the animals.

5.4 Discussion

5.4.1 Maternal Behaviour

This study begins with the observation on postnatal maternal behaviour towards pups after removal of one uterine horn on several different days of pregnancy. It was assumed that post operation changes in progesterone concentration might cause the incomplete mammary gland growth and differentiation during the course of pregnancy. It was also assumed that the decrease in the progesterone concentration after operation in pregnant females, especially in the UOX group (see Chapter 3), might result in maternal behaviour impairment. Based on these two assumptions, it was expected that uterine removal on several different days of pregnancy could have a negative effect on the growth of the neonate. These effects might be caused by the maternal incapability to nourish the pups due to an abnormality in the milk production and/or an abnormality in maternal behaviour towards pups.

In the present study, weight of the mammary glands of the operated animals (both the UX and UOX groups) was not different from the unoperated females (the SOP group). In the same animals, weight of mammary glands from the operated side was not different from the unoperated side (Table 5.6). However, data on postnatal maternal behaviour indicates that unilateral uterine transection followed by removal of its ipsilateral ovary can induce abnormality in maternal behaviour. Only one of five (20%) to two of five (40%) of the UOX females behaved normally towards their pups after delivery (Table 5.1). Twenty five percent to forty percent of pups were rejected by the UOX mother (Table 5.1), where cannibalism was the most frequent kind of maternal rejection observed (Table 5.2).

These findings suggest that unilateral uterine transection at several different days of pregnancy had no effect on the growth and differentiation of mammary glands. However, abnormal maternal behaviour after birth could be induced if the operation was followed by the removal of the ovary ipsilateral to the removed uterine horn. This impairment in maternal behaviour is likely not due to the reduction in litter size at delivery since the UX females, like the SOP females, remained maternally normal even though their litters were reduced up to a half of normal size.

The onset of normal maternal behaviour at birth is primed by the oestrogen and progesterone ratio during the course of pregnancy (Bridges and Russell, 1981; Fleming, 1986; Siegel, 1986). In the rat, for example, a low level of progesterone in the presence of oestrogen is needed to induce the onset of normal maternal behaviour while

maintenance of a high level of progesterone in the presence of oestrogen prevents the rapid onset of maternal behaviour (Bridges *et al.*, 1978; Bridges and Russell, 1981; Siegel, 1986). Fleming (1986) also reported that hormonal action not only works on the initiation of maternal behaviour at birth but also increases maternal attraction to pups and facilitates a close relationship between mother and pups. Furthermore, Wang *et al.* (1992) reported abnormal maternal behaviour in females previously treated with antibody against progesterone and suggested that partial withdrawal of progesterone at parturition, which results in hormonal imbalance, can cause abnormal maternal responses to pups. The findings in this study are in agreement with those of earlier studies described above. Progesterone concentration of the UOX group was lower than that of both the UX and SOP group (see Chapter 4). Thus the low levels of progesterone after operation in the UOX group was associated with a high incidence in the abnormality responses and maternal rejection in operated mothers towards their young (Table 5.1 and Table 5.2). The UX group showed moderate values for both maternal abnormality responses and rejection towards pups, lying between the UOX and SOP groups. It suggests that removal of one uterine horn alone, when the two ovaries remain intact, on several different days of pregnancy was not strong enough to induce abnormal postnatal maternal behaviour towards the pups.

In this study, cannibalism was the most frequent form of maternal rejection found in the UOX group over isolation or nursing failure, while the two latter forms are the most frequent found in the UX group. The significance of this finding and the underlying mechanisms are not known. One possible explanation is that progesterone, as proposed by Crombie *et al.*, (1995), has a broad spectrum of effects on the establishment of normal maternal behaviour. This may work in different ways in determining whether post partum maternal behaviour is manifested in isolation, nursing failure or cannibalism. According to Crombie *et al.*, (1995), cannibalism is an indicator of aberrant olfactory recognition which occurs without any impairment to maternal behaviour.

The weight of the mammary gland from the operated females (the UX and UOX groups) on the day of delivery did not differ from the unoperated females (the SOP group) (Table 5.6). This finding is not consistent with the finding of impairment in maternal behaviour as described above. It also contradicts the finding of Cerruti and Lyons (1960) in mice. By injecting placental extract directly into the area of skin covering one of the mammary glands of two month old virgin mice, Cerruti and Lyons (1960) revealed that mammogenic activities of the mid-gestational placenta work in both

a local and a systemic mode of action. It was expected that removal of one uterine horn, which in turn reduces the number of placenta up to a half of the normal size, would result in growth retardation or in a slower differentiation of mammary glands in either the operated side or in operated animals compared to the unoperated side or unoperated control animals.

It is known that the mammary gland development is progesterone dependent (Wang *et al.*, 1990) and ovary removal in pregnant animals results in the delay of both alveolar development during pregnancy and lactogenesis at the time of parturition (Buttle, 1988). Thus, the idea that abnormality in maternal responsiveness and pup rejection may derive from the effects of progesterone deprivation, or from partial inhibition of progesterone dependent mammary gland development (Wang *et al.*, 1990), leading to an inability to provide enough milk for the young, is not applicable in this study. However, this finding supports the result of Wang *et al.*, (1995) who reported that a higher frequency of pup rejection by the antibody-treated mother was not due to the lack of milk secreted by the mother or a result of defects in the pups.

5.4.2 The Growth of Neonates

These findings offer additional evidence that there is an inverse relationship between the number of young in the litter and the size of young at birth in polytocous mammals (Eckstein and McKeown, 1955; Healy, 1960) and that a reduction in litter size results in an increase in birth weight due to the reduction of prenatal competition for the limited nutrients provided by the mother (Eckstein and McKeown, 1955; McCarthy, 1967).

In this postnatal study, the variation arising from the effects of litter size on postnatal growth was eliminated by reducing the number of litter to four pups per litter (2 males plus 2 females). In such conditions it was assumed that postnatal competition between litter mates in obtaining both maternal environment (milk from maternal mammary glands and maternal responses) and physical environment of the cage (space and nest material), and the effect of sexes were minimised. It was expected, therefore, that the only source of variation in postnatal body weight would originate from the mother, namely the operated females (the UX and UOX groups) and the unoperated females (the SOP group).

Data on growth of neonates show that at the day of delivery, overall mean weights of selected pups from the UX and UOX groups were significantly higher than those of the

SOP groups, whether these animals were operated on, on day 2, day 6, day 10, or day 14 of pregnancy. At weaning, however, overall mean weight of pups from the UX group tended to higher than that of both the UOX and SOP group. This difference was significant in the animals operated on, on day 2 and day 10 of pregnancy. Consequently, the growth rate of pups from the UOX and SOP females was significantly lower than the UX group, especially in groups of animal operated on, on day 2, day 6, and day 10 of pregnancy (Table 5.4; Table 5.5; Figure 5.1; Figure 5.2).

These results agree with those of El Oksh *etal.* (1967) and Moore *et al.*, (1970) in that maternal postnatal effects caused significant differences in body weight of mice. El Oksh *et al.* (1967) reported that maternal postnatal environment accounted for 0, 51, 63, 52% of the total variation in body weight at birth and at day 7, day 14, and day 21 of the lactation period respectively. After that, this postnatal maternal effect decreases, while the effect of genotype factor increases with the age of young (El Oksh *et al.*, 1967).

Although the weight gained by the pups from the UX group tended to be higher than that from both the UOX and SOP groups, the pattern of the weight gain of these groups was similar over 21 days of lactation period. For all groups, the gain is low during the first 3 days, slightly increaseed until day 6 before reaching a nearly constant value until day 12 to 13 and reaching a maximum value on days 13 to 14 (Figure 5.2). This similarity in weight gain supports the finding for the mammary gland weight as previously described (see Section 5.4.1). Weight gain during the period of 7 to 14 days is a good criterion for measuring mammary gland activities (milk production) since weight gain during this period is most influenced by the milk supply (El Oksh, 1967).

What explains the difference in growth rate between the UX and either the UOX or SOP groups? The fact that the mammary gland weight was not different between groups, and that the percentages of abnormality in maternal behaviour are higher in the UOX than those of both the UX and SOP groups contradicts the assumption in this study that the growth rate (also the final weight) of the young is influenced by both the maternal production of milk and behaviour towards pups. The UX group was not different from the SOP group in either maternal behaviour or mammary gland weight. The two groups were also similar in that they have intact ovaries. However, the two groups differed in the pup weight at weaning and growth rate.

Possible explanations include: 1) the type of postnatal maternal behaviour towards pup might in some way affect postnatal growth, 2) single measurement of the mammary gland weight as conducted in this study might not be a good procedure to estimate milk production during the lactation period. Pup isolation and/or nursing failure were the most dominant form of rejection behaviour displayed by the UX group while cannibalism was specific for the UOX group although the form of isolation and/or nursing failure of the UOX group was higher than the UX group. However, this argument does not explain the difference in growth rate since the type and pattern of postnatal maternal behaviour between the UX and SOP groups is not different (see Table 5.2).

Maternal factors affecting preweaning growth of mammals includes genetic composition (genotype), nutrition, milk production, behaviour (maternal care), and age, while the other factors originate from the pups, including genotype, hormonal control, birth weight, and litter size (Hafez, 1963).

The difference between males and females in either body weight or growth rate (Table 5.4) is parallel with those of Pahl (1969), and Wahlsten and Bulman-Fleming (1987) who reported that the difference between males and females originated from intact animals in growth rate. At days 100 after birth for both male and female mice the weight differences between litters of 11 and 2 was about 3.7 g body weight. (Wahlsten and Bulman-Fleming, 1987). In the rat, the point of divergence of male and female growth curves begins 30 days *post partum* (Pahl, 1969). However, Eisen (1976) in his review of the growth curves of mice noted that maximum postnatal growth rate of the mouse is reached at about half its mature size, and postnatal growth rate of males is higher than that of females. The two sexes differ in the time taken to reach a sigmoidal growth curve, where an adequate time for males to fit the graph is age 70 days, and for females is age 105 days (Pahl, 1969).

Chapter 6
General Discussion

6.1 Experimental Design

This work is designed to examine the effect of removal of one uterine horn on fetal and neonatal growth of mice. Removal of one uterine horn was carried out on four different days of pregnancy, i.e. day 2, day 6, day 10, and day 14 with three different types of operation, i.e. uterine horn only was removed (UX), uterine horn and its ipsilateral ovary was removed (UOX), and neither the uterine horn nor the ovary was removed (sham-operated control) (SOP). The consequences of these procedures are as follows: 1) In the UX females, the size of litter is reduced up to a half of normal size in the condition of normal number of the CL; 2) In the UOX females, both the size of litter and the number of CL are reduced up to about a half of normal size; 3) In the SOP females, both the size of litter and the number of CL remain uninterrupted.

This design allows us to examine the effect of the reduction of litter size on several different days of pregnancy, whether it was followed by the reduction of CL number, on the length of gestation, prenatal growth, post operative plasma progesterone concentrations and ovarian and uterine histology, and post partum maternal

responsiveness towards young and neonatal growth during the first week of the lactation period.

6.2 Experimental Limitation

Due to the difficulty of separating the fetus from embryonic membranes in fresh preparation, uterine horn obtained from females on days 3, 7, 11, and 15 of pregnancy were fixed in Bouin's solution before examination was made. Hence values of uterine horn, placenta, and fetus weights and other parameters obtained from these females are based on Bouin-fixed preparation. In relation to this, it is possible that values of these data are slightly below the real values obtained from fresh preparation due to the dehidratory effect of the Bouin's solution used. Rugh (1967) suggests a formula to correct Bouin-fixed data by adding 24% and 6.5% of values to the original weights and lengths data respectively. On the other hand, it is also possible that those data are not different from, or even slightly more than, the real values obtained from freshly prepared since Bouin-fixed preparations were first rinsed gently with running tap water before examination. In such conditions, it was decided to analyse and present those data without applying the correction formula suggested by Rugh (1967).

6.3 Effects on Length of Gestation, Litter Size and Progesterone Concentrations

This study confirmed that the reduction of litter size by approximately 50 percent results in prolongation of the length of gestation up to about one day longer than normal litter size. However, the reduction of CL number due to the removal of one ovary following removal of one uterine horn had no effect on the length of gestation though it was apparently related to the post operative concentration of progesterone. These results suggest that it may be in this strain of mouse: 1) The length of gestation is regulated by the number of fetuses or conceptuses in the uterine horn rather than the number of CL in the ovaries; 2) Progesterone concentration during pregnancy is more affected by the number of CL rather than the number of conceptuses; 3) The reduction in progesterone concentration due to the reduction in CL number had no significant effect on pregnancy maintenance; 4) Progesterone concentration is just one of several, not the only, factors involved in regulating the length of gestation or in determining the onset of parturition.

Litter size and progesterone concentration and their relationships with the length of gestation can be explained by understanding the mechanism of the onset of parturition. The control of parturition is a complex process and initiated by several interacting factors. no single factor is solely responsible for the onset of parturition. In many mammals. the onset of parturition is determined by the fetus via secretions of cortisol from the adrenal cortex. Cortisol released by the fetus then induces an increase in placental 17α-hydroxylase activity which results in an increased in E/P ratio. Increasing in E/P ratio, via an oxytocin-dependent mechanism, will stimulate the uterine synthesis and release of $PGF_{2\alpha}$. and in turn, this prostaglandin will activate myometrial contractions and cervical ripening. As parturition proceeds, $PGF_{2\alpha}$ synthesis and release then enhanced by oxytocin (Thorburn, 1991; Johnson and Everitt, 1995). Thus, an increase in fetal number should be followed by an increase in fetal cortisol secretion, which in turn induces the onset of parturition earlier than that of a small litter. From this point of view it can be understood, therefore, why gestation length of both the UX and UOX females in the study reported here is longer than that of the SOP females.

This result of the inverse relationship between litter size and length of gestation in mice is in substantial agreement with those of Biggers *et al.* (1963), McLaren and Michie (1963), McLaren (1967), and Dewar (1968) who hypothesised that the effect of litter size operates systemically rather than locally. In the pig, the inverse relationship between litter size and gestation length (Omtvedt, 1965; Martin *et al.*, 1977) and the positive correlation between litter size and estrogen concentration during the last two weeks of gestation period, and the lack of relationships between CL number and gestation length, progesterone, and estrogen concentration (Martin *et al.*, 1977) have been documented. In relation to these previous results, Biggers *et al.* (1963) and Martin *et al.* (1977) claim that placental estrogen plays a significant role in initiation of parturition. However, the involvement of placental estrogen in regulation of parturition was no longer accepted since Harkness *et al.* (1964) failed to detect the presence of placental progesterone, and Dewar (1968) failed to induce prolongation of gestation length by injecting a physiological dose of estrogen.

Progesterone sources during pregnancy include ovaries, adrenal cortex and placenta. In addition to its progestational role it is well known that placenta also actively produce a luteotrophic hormone (Heap *et al.*, 1973; Linkie and Niswender, 1973; Kelley *et al.*, 1975; Galosy and Talamantes, 1995) which can take over the pituitary role in maintaining ovarian function. Although ovaries are the main source of progesterone during pregnancy, unilateral ovariectomy following the unilateral hysterectomy at any

stage of pregnancy in the present study apparently have no effect on pregnancy maintenance in the mouse. This finding is in accord with Pepe and Rothchild (1973) who reported that pregnancy can be maintained in association with serum progesterone levels that are approximately 75 to 80 percent lower than normal values.

In females operated on day 2 of pregnancy, surgical procedures failed to alter the pattern of progesterone secretion though mean values for the unilaterally utero-ovariectomised (UOX) females were lower, but not significantly, than those of both the UX and SOP females. By day 10 of pregnancy, however, surgical procedures succeeded in altering the pattern of progesterone secretion. Operation on day 6 of pregnancy apparently had an unpredictable effect on both the pattern and concentration of progesterone. These findings offer clear evidence that ovarian compensation (or maybe placental compensation also) in producing progesterone due to the surgical procedures can be induced if the operation is carried out as early as day 2 of pregnancy. Compensatory growth of follicles in the remaining ovary after unilateral ovariectomy has been reported in unmated mice (Biggers *et al.*, 1963; McLaren, 1963). Unilateral ovariectomy as early as day 8 of pregnancy also results in the CL hypertrophy in the rat, though plasma progesterone levels remain low compared either to the control or to the premating unilaterally ovariectomised females (Rahima and Bruce, 1987).

These findings also strongly support the theory that: 1) Ovaries are the main source of progesterone and uterine (or placental) origin of progesterone are very small (Hall, 1957; Elbaum *et al.*, 1975; Kensinger *et al.*, 1986); 2). Although placental capacity in producing progesterone is very small, after day 10 of pregnancy their overall contribution is very significant (Macdonald and Matt, 1984; Matt and Macdonald, 1984; Kato *et al.*, 1979). In mice, implantation is completed on day 6 of pregnancy (Rugh, 1967) and placentae grow sharply from day 8 and then, on day 10 they completely take over the pituitary role in maintaining pregnancy by producing gonadotrophin, mCG and mPL (Choudary and Greenwald, 1969; Markoff and Talamantes, 1981; Rao *et al.*, 1982; Soares *et al.*, 1982; Humphreys *et al.*, 1985; Ogren and Talamantes, 1988).

6.4 Effect on Prenatal Growth

In several polytocous species litter size is inversely related to birth weight. A small size of litter is usually related to the delayed parturition and a large litter is usually associated with early parturition. This litter size factor also affects the rate of fetal growth, both

locally and systemically, during gestation period, therefore, causing a variation in birth weight. Clapp (1989) suggested that placental and fetal growth are limited by the rate of utero-placental blood flow and a minor reduction in flow results in growth restriction with no evidence of metabolic abnormalities. Removal of one uterine horn results in the reduction of litter up to a half normal size and prolongation of gestation length (see Section 6.1.2).

The results from the prenatal growth study confirmed that:
1. The effect of unilateral uterine removal in the mouse was time dependent. Until day 15, uterine removal at any day of pregnancy had no effect on prenatal growth. However, if pregnancy was allowed to proceed until day 18, fetal weight from females operated on days 2 and 6, but not on days 10 and 14, was significantly higher than that from the SOP females.

These findings clearly indicate that: a) the last three or five days of pregnancy is the most critical period for prenatal growth when the uterine horn begins to exert its effect on prenatal growth b) the rate of prenatal growth is affected by the size of litter which works systemically, c) the effect of litter size on prenatal growth is exerted during the first half of the gestation period although the result of this effect can only be detected during the last three to five days of pregnancy due to the uterine limitation on fetal growth, and d) any effort directed to reducing the size of litter during the period of day 10 to term failed to induce a better prenatal growth of remaining fetuses.

These interpretations are parallel with Clapp's flow limited-prenatal growth theory that prenatal growth is limited by the blood flow reaching the uterine horn (Clapp, 1989) and also parallel with the uterine limitation theory suggested by McKeown (1976). In mice, implantation is initiated on day 4 and is then completed on day 5 of pregnancy (Rugh, 1967). The initial placental formation was associated with both dramatic growth of the uterine vasculature and an elevation in uterine blood flow (Garris, 1984; Edward and Milligan, 1987) and reduction in the uterine blood flow during early pregnancy has the greatest effect on placental growth (Garris, 1984; Clapp, 1989). Thus, removal of one uterine horn as early as day 2 or day 6 of pregnancy might result in the reconstruction and/or enlargement in uterine vasculature of the remaining uterine horn, especially in placental arteries, better than that of later days; and this, in turn could result in blood flow redistribution and provide more nutrients to the remaining uterine horn. These speculations to a large extent are supported by both placental and uterine vasculature data discussed below (point 2, 3, and 4).

In intact animals variation in blood supply to the uterine horn which may influence prenatal growth can originate from both the distension of the uterus and the decrease in blood pressure induced by pregnancy McCharty (1965). The distension of the uterine horn and the decrease in blood pressure may restrict the blood supply to the placenta and then affect the rate of fetal or placental growth. Both the degree of uterine distension and reduction in maternal blood pressure correlate with the number of implants. The effect of maternal blood pressure reduction on prenatal growth is exerted both locally and systemically. Whether prenatal growth in the strain of mice reported here is affected by the local effect or not was not examined. However, at least four reports on the presence of local effect on prenatal growth in mice have been documented (McCharty, 1965; 1967; McLaren, 1965; Bruce and Wellstead, 1992).

2. In all groups, placentae grow rapidly during the first half of gestation period before fetuses reached their maximal growth rate. The growth of fetuses reached a maximal rate in the second half of pregnancy when placenta have reached a plateau in their growth rate. However, the difference in placental weight between operated and unoperated females was only detected as early as day 11 of pregnancy. Placental growth, but not fetal growth, was significantly influenced by the day of operation. Therefore, placental weight of females operated on days 2 and 6 was significantly higher than that of females operated on day 14 of pregnancy.

These findings confirm that fetal growth is controlled by the placenta and that optimal growth of placentae is an important prerequisite for the growth of fetuses. Thus, the idea that the decline in fetal weight is associated with the decline in placental weight (mice: McLaren, 1965; pig: Knight *et al.*, 1977; rat: Norman and Bruce, 1979a; 1979b; guinea pig: Eckstein and McKeown, 1955a; 1955b; Eckstein *et al.*, 1955) and that intrauterine growth retardation is partly determined by the reduction in placental blood flow (Dawes, 1976) are in agreement with these findings. More recently, Robinson *et al.* (1995) reported that placental effect on prenatal growth is exerted by both metabolic and endocrine mechanisms (Robinson *et al.*, 1995).

These findings also suggest that removal of one uterine horn firstly induces a maximum placental growth by facilitating it with more favourable conditions for growth. and this condition then prepares an optimum condition for the growth of fetuses in the remaining uterine horn. This finding is strongly supported by the findings in the present study on uterine vasculature which revealed that uterine vasculature undergoes a dramatic growth

early in the gestation period particularly between day 2 and day 6, and uterine removal during this period, but not after this period, results in vascular hypertrophy in the remaining uterine horn. The most dramatic increase was found in the placental artery. However, the critical stage of pregnancy for arterial development was different in different arteries.

3. Either placental weight or fetal:placental weight ratio was positively related to the fetal weight. Furthermore, placental efficiency increased dramatically with the age of pregnancy, but at the same age of pregnancy the ability of placenta to support fetal tissue was decreased as the weight of placenta increased either in the operated and unoperated females.

4. Mean weight of either the entire or empty uterus per horn of the operated females was significantly higher than that of the unoperated females when autopsied on days 15 and 18 of pregnancy. However, there was no evidence that females operated on, on earlier days of pregnancy had a uterine horn heavier than those operated on later days. Also, removal of one ovary following the removal of its ipsilateral uterine horn had no effect on the growth of the remaining uterine horn. In addition, removal of one uterine horn failed to alter the proportion of gravid components of the remaining uterine horn. On day 18 of pregnancy, almost 80% of the uterine component consisted of fetal contribution and the remaining components never exceed the value of 20%.

6.5 Effects on Postnatal Growth and Maternal Behavior

The size of litter at birth and total birth weight per litter were significantly lower, while both mean weight per litter and total litter weight to maternal weight ratio were significantly higher in the operated than the unoperated females. Removal of one uterine horn effectively induced abnormal maternal behavior when the ovary ipsilateral to the removed uterine horn was also removed. Only one of five (20%) to two of five (40%) of the UOX animals were maternally normal towards pups. Twenty five percent to forty percent of pups were rejected by the UOX mother where cannibalism was the most frequent kind of maternal rejection observed while isolation or nursing failure, were the most frequent found in the UX group.

Again, these findings strongly support the flow-limited theory of Clapp (1989) and uterine limitation theory of McKeown (1976). Based on these two theories, prenatal

growth, and therefore birth weight of young, are inversely related with litter size, thus the reduction in litter size results in an increase in birth weight which might be due to the reduction of prenatal competition for the limited nutrients provided by the mother (Eckstein and McKeown, 1955a; 1955b; McCarthy, 1967). However, the facts obtained in this study that the growth of mammary glands was not affected by the surgical procedures, and that the incidence of maternal behavior abnormality and rejection towards pups in the UX females is lower than that of the UOX but higher than that of the SOP females suggests that the abnormality of the UOX females is not solely due to the reduction in the size of litter at birth.

The onset of normal maternal behavior is primed hormonally by a combination of low level of progesterone in the presence of estrogen (Bridges *et al.*, 1978; Bridges and Russell, 1981; Fleming, 1986; Siegel, 1986). This ratio in progesterone and estrogen is in accordance with the hormonal condition during the last days of gestation period. This study found that maternal plasma progesterone concentration decreased abruptly from day 15 to day 18 of pregnancy and on most observation days plasma progesterone concentration of females from the SOP was higher than that of both the UX and UOX group though these differences were not always statistically significant (see Chapter 4). It is suggested that the decrease in progesterone concentration due to unilaterally uterine and ovarian loss during pregnancy can induce an abnormality in maternal behavior. This effect became weak if the ovary ipsilateral to the removed horn remained intact *in situ*. Wang *et al.* (1992) revealed that partial withdrawal of progesterone at parturition, which results in hormonal imbalance, can cause abnormal maternal responses to pups.

Regarding the effect of uterine removal on mammary gland growth, where mean weight of mammary glands from operated females (or from the operated side) were not different from unoperated females (or from unoperated side), these findings contradict with Cerruti and Lyons (1960) who found that mid-gestational placental extract had mammogenic activitiy which works both locally and systemically and with other authors (Wang *et al.*, 1990; Buttle, 1988) who reported the mammary growth dependency on progesterone and with the progesterone data reported here (see Chapter 4). It might be that the single measurement of mammary gland weight or the procedure applied to measure the mammary gland weight in this study are inappropriate.

Postnatal growth rate of pups from the UOX and SOP females was significantly lower than the UX group, especially in groups of animal operated on day 2, day 6, and day 10 of pregnancy. Consequently, at weaning overall mean weight of pups from the UX

group tended to be higher than that of both the UOX and SOP groups. These findings raised a big question on what is the factor or factors affecting postnatal growth in mice used in this study. The size of the litter during intrauterine growth period, which is inversely related to birth weight (Eckstein and McKeown, 1955a; 1955b; McKeown *et al.*, 1976), might affect preweaning growth and cannot be considered in this case since the UOX group is significantly different from the SOP, but not different from the UX group in litter size. To consider the maternal responsiveness towards young as a determinant factor in this case is not appropriate since the UOX and SOP groups are different in their post-partum behavior. Therefore, this question remains unanswered and needs a further study with a bigger sample size.

References

ABRAHAMSOHN, P.A. and Zorn, T.M.T. (1993) Implantation and decidualization in rodents. *The Journal of Experimental Zoology,* **266**: 603-628

AITKEN, R.J., Bowman, P., and Gauld, I. (1977) The effect of synchronous and asynchronous egg transfer on foetal weight in mice selected for large and small body size. *Journal of Embryology and Experimental Morphology,* **37**: 59-64

ALLEN, W.R. (1975) Endocrine function of the placenta. In *Comparative Placentation* (Ed.: D.H. Steven). Academic Press, London. pp. 214-267

ANDERSON, L.L. (1973) Effects of hysterectomy and other factors on luteal function. In *Handbook of Physiology, Section 7, Vol II* (Eds.: R.O. Greep and E.B. Astwood). American Physiological Society, Washington. pp. 69-86

ANDERSON, L.L. (1977) Uterine control of ovarian function. In *Biology of the Uterus* (Ed.: R.M. Wynn). Plenum Pres, New York. pp. 587-651

ANDERSON, L.L., Bowerman, A.M., and Melampy, R.M. (1965) Oxytocin on ovarian function in cycling and hysterectomized heifers. *Journal of Animal Science,* **24**: 964-968

ANDERSON, L.L., Neal, F.C., and Melaphy, R.M. (1962) Hysterectomy and ovarian function in beef heifers. *American Journal of Veterinary Research,* **23**: 794-802

BAIRD, T.D. (1984). The ovary. In *Hormonal Control of Reproduction. 2nd Ed.* (Eds.: C.R. Austin and R.V. Short). Cambridge University Press. Cambridge pp. 91-114

BARKLEY, M.S., Geschwind, I.I., and Bradford, G.E. (1979) The gestational pattern of estradiol, testosterone and progesterone secretion in selected strains of mice. *Biology Reproduction,* **20:** 733-738

BARKLEY, M.S., Michael, S.D., Geschwind, I.I. and Bradford, G.E. (1977) Plasma testosterone during pregnancy in the mouse. *Endocrinology,* **100**: 1472-1475

BARR, M., Jensh, R., Brent, R. (1970). Prenatal growth in the albino rat: effects of number, intrauterine position and resorptions. *Journal of Anatomy,* 128: 413-428

BARTKE, A. (1970) Influence of an IUD on the luteolytic content of the uterus and on the duration of pseudopregnancy in mice. *Journal of Reproduction and Fertility,* **23**: 243-247

BELT, W.D., Cavazos, L.F., Anderson, L.L., Kraeling, R.R. (1970) Fine structure and progesterone levels in the corpus luteum of the pig during pregnancy and after hysterectomy. *Biology of Reproduction,* **2**: 98-113

BIGGERS, J.D., Curnow, R.N., Finn, C.A., and McLaren, A. (1963) Regulation of the gestation period in mice. *Journal of Reproduction and Fertility,* **6:** 125-138

BLAND, K.P. and Donovan, B.T. (1966) The uterus and the control of ovarian function. In *Advances in Reproductive Physiology* (Ed.: A. McLaren). Academic Press, London. pp. 179-214

BONNAMY, P.J., Benhaim, A. and Leymarie, P. (1992) Changes of progesterone content of rat uterine flushings in relation to serum concentrations of progesterone during the oestrous cycle. *Journal of Reproduction and Fertility,* **96:** 233-239

BRIDGES, R.S and Russel, D.W. (1981) Steroidal interactions in the regulation of maternal behaviour in virgin female rats: Effects of testosterone, dihydrotestosterone, oestradiol, progesterone and the aromatase inhibitor, 1,4,6-androstatriene-3,17-dione. *Journal of Endocrinology,* **90**: 31-40

BRIDGES, R.S. (1984) A quantitative analysis of the roles of dosage, sequence, and duration of estradiol and progesterone exposure in the regulation of maternal behavior in the rat. *Endocrinology,* **114**: 930-940

BRIDGES, R.S., Rosenblatt, J.S., and Feder, H.H. (1978) Serum progesterone concentrations and maternal behavior in rats after pregnancy termination: behavioral stimulation following progesterone withdrawal and inhibition by progesterone maintenance. *Endocrinology,* **102**: 258-267

BRONSON, F.H., Dagg, C.P., and Snell, G.D. (1966). Reproduction. In *Biology of the Laboratory Mouse* (Ed.:E.L. Green). McGraw-Hill Book Co., New York. pp. 187-204

BRUCE, N.W. (1976) The distribution of blood flow to the reproductive organs of rats near term. *Journal of Reproduction and Fertility*, **46**: 359-362

BRUCE, N.W. and Abdul-Karim, R.W. (1973) Relationships between fetal weight, placental weight and maternal placental circulation in the rabbit at different stages of gestation. *Journal of Reproduction and Fertility*, **32**: 15-24

BRUCE, N.W. and Norman, N. (1975) Influence of sexual dimorphism on foetal and placental weights in the rat. *Nature*, **257**: 62-63

BRUCE, N.W. and Wellstead, J.R. (1992) Spacing of fetuses and local competition in strains of mice with large , medium and small litters. *Journal of Reproduction and Fertility*, **95**: 783-789

BRUMBY, P.J. (1960) The influence of the maternal environment on growth in mice. *Heredity*, **14**: 1-18

BUTCHER, R.L., Barley, D.A., and Inskeep, E.K. (1969) Local relationship between the ovary and uterus of rats and guinea pigs. *Endocrinology*, **84**: 476-481

BUTTERSTEIN, G.M. and Hirst, J.A. (1977) Serum progesterone and fetal morphology following ovariectomy and adrenalectomy in the pregnant rat. *Biology of Reproduction*, **16**: 654-660

BUTTLE, H. (1988) Role of the ovaries in inducing mammogenesis in pregnant pigs. *Journal of Endocrinology*, **118**: 41-45

CAVAILLÉ, F. and Maltier, J.P. (1978) A local signal from the gravid horn to the ovary for the onset of parturition in rats. *Journal of Reproduction and Fertility*, **54**: 227-231

CERRUTI, R.A. and Lyons, W.R. (1960) Mammogenic activities of the mid-gestational mouse placenta. *Endocrinology*, **67**: 884-887

CHAMPLIN, A.K. (1977) Strain differences in oestrous cycle and mating frequencies after centrally produced anosmia in mice. *Biology of Reproduction*, **16**: 513-516

CHEEK, D.B. and Holt, A.B. (1963) Growth and body composition of the mouse. *American Journal of Physiology*, **205**: 913-918

CHOUDARY, J.B. and Greenwald, G.S. (1969) Ovarian activity in the intact or hypophysectomyzed pregnant mouse. *Anatomical Record*, **163**: 359-372

CHRISTIAN, R.M., Yuan, L., and Rothchild, I. (1968) The effect of hysterectomy on parameters of luteinizing hormone secretion in pseudopregnant rats. *Acta Endocrinologica*, **58**: 57-66

CLAPP, J.F. 1989. Utero-placental blood flow and fetal growth. In *Fetal Growth* (Eds.: F.Sharp, R.B. Fraser, and R.D.G. Milner). Springer-Verlag, London. pp.235-244

COCKBURN (1989) Fetal growth: An overview. In *Fetal Growth* (Eds.: F.Sharp, R.B. Fraser, and R.D.G. Milner). Springer-Verlag, London. pp.133-138

COWIE, A.T. (1984) Lactation. In *Hormonal Control of Reproduction.* (Eds.: C.R. Austin and R.V. Short). Cambridge University Press, Cambridge. pp. 195-231

COWIE, A.T. and Tindal, J.S. (1971) Milk secretion. In *Physiology of Lactation* (Eds: A.T. Cowie and J.S. Tindal). Edward Arnold, London. pp. 137-184

CRITSER, E.S., Rutledge, J.J. and French, L.R. (1980) Role of the uterus and the conceptus in regulating luteal lifespan in the mouse. *Biology of Reproduction,* **23**: 558-563

CRITSER, E.S., Savage, P.J., Rutledge, J.J., and French, L.R. (1982) Plasma concentrations of progesterone and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ in pregnant, pseudopregnant and hysterectomyzed pseudopregnant mice. *Journal of Reproduction and Fertility*, **64**: 79-83

CROMBIE, D.L., Hayes, J.S., Heap, R.B., and Wang, M-W. (1995) Anti-progesterone effects on maternal recognition and behaviour imprinted during first pregnancy in mice. *Journal of Endocrinology*, **147**: 331-337

CSAPO, A.I. and Wiest, W.G. (1969) An examination of the quantitative relationship between progesterone and the maintenance of pregnancy. *Endocrinology,* **85**: 735-746

CURRIE, W.B. and Thornburn, G.D. (1974) Luteal function in hysterectomized goats. *Journal of Reproduction and Fertility,* **41**: 501-504

DAWES, G.S. (1968) *Foetal and Neonatal Physiology: A Comparative Study of the Changes at Birth.* Year Book Medical Pub., Inc. Chicago. pp. 42-59 (p247)

DAWES, G.S. (1976) The physiological determinants of fetal growth. *Journal of Reproduction and Fertility,* **47**: 183-187

DEANE, H.W., Rubin, B.L., Driks, E.C., Lobel, B.L., and Leipsner, G. (1962) Trophoblastic giant cells in placentas of rats and mice and their probable role in steroid-hormone production. *Endocrinology,* 70: 407-419

DEANESLY, R. (1971) The effects of corpora lutea removal by unilateral ovariectomy in the pregnant guinea-pig. *Journal of Reproduction and Fertility* **26**, 391-392

DEANESLY, R. (1973) Termination of early pregnancy in rats after ovariectomy is due to immediate collapse of the progesterone-dependent decidua. *Journal of Reproduction and Fertility,* **35**: 183-186

DELCAMPO, C.H. and Ginther, O.J. (1972) Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: guinea pigs, rats, hamsters, and rabbits. *American Journal of Veterinary Research,* **33**: 2561-2578

DEWAR, A.D. (1973) Effects of hysterectomy on corpus luteum activity in the cyclic, pseudopregnant and pregnant mouse. *Journal of Reproduction and Fertility*, **33**: 77-89

DEWAR, A.D. (1968) Litter size and the duration of pregnancy in mice. *Quarterly Journal of Experimental Physiology*, **53**: 155-161

DORFMAN, R.I. (1973) Biosynthesis of progesterone. In *Handbook of Physiology, Section 7, Vol II* (Eds.: R.O. Greep and E.B. Astwood). American Physiological Society. Washington. pp. 537-546

DWYER, C.M., Madgwick, A.J.A., Crook, A.R. and Stickland, N.C. (1992) The effect of maternal undernutrition on the growth and development of the guinea pig placenta. *Journal of Developmental Physiology*, **18**: 295-302

DZIUK, P.J. (1968) Effect of number of embryos and uterine space on embryo survival in the pig. *Journal of Animal Science*, **27**: 673

ECKSTEIN, P. and McKeown, T. (1955a) The influence of maternal age, parity and weight on litter size in the guinea-pig. *Journal of Endocrinology*, **12**: 115-119

ECKSTEIN, P. and McKeown, T. (1955b) Effect of transection of one horn of the guinea-pig's uterus on foetal growth in the other horn. *Journal of Endocrinology*, **12**: 97-107

ECKSTEIN, P., McKeown, T., and Record, R.G. (1955) Variation in placental weight according to litter size in the guinea-pig. *Journal of Endocrinology*, **12**: 108-114

EDWARDS, C. and Milligan, S.R. (1987) Uterine blood flow during the development and regression of the decidual cell reaction in ovariectomized, steroid-treated mice. EISEN, E.J. (1976) Results of growth curve analyses in mice and rats. *Journal of Animal Science*, **42**: 1008-1023

EL OKSH, H.A., Sutherland, T.M., and Williams, J.S. (1967) Prenatal and postnatal maternal influence on growth in mice. *Genetics*, **57**: 79-94

ELBAUM, D.J., Bender, E.M., Brown, J.M., and Keyes, P.L. (1975) Serum progesterone in pregnant rats with ectopic or in situ corpora lutea: correlation between amount of luteal tissue and progesterone concentration. *Biology of Reproduction*, **13**: 541-545

EENDO, H., Yamaguchi, M., Fransworth, R., Thordarson, G., Ogren, L., Alonso, F.J., Sakata, M., Hirota, K., and Talamantes, F. (1994) Mouse placental cells secrete immunoreactive growth hormone-releasing factor. *Biology of Reproduction*, **51**: 1206-1212

EVEN, M.D., Laughlin, M.H., Krause, G.F. and vom Saal, F.S. (1994) Differences in blood flow to uterine segments and placentae in relation to sex, intrauterine location and side in pregnant rats. *Journal of Reproduction and Fertility*, **102**: 245-252

FEKETE, E. (1941) Histology. In *Biology of the Laboratory Mouse* (Ed.: G.D. Snell). The Blakiston Co., Philadelphia. pp. 89-167

FINN, C.A. and Martin, L. 1971. The onset of progesterone secretion during pregnancy in the mouse. *Journal of Reprodution and Fertility*, **25**: 299-300

FINN, C.A. and Porter, D.G. 1975. *The uterus: Handbooks in reproductive biology, Vol.1.* Elek Science, London. pp. 96-99

FLEMING, A. (1986) Psychobiology of rat maternal behavior: How and where hormones act to promote maternal behavior at parturition. *Annals of the New York Academy of Sciences,* 474: 234-251

FOWDEN, A.L. (1995) Endocrine regulation of fetal growth. *Reproduction, Fertility and Development,* 7: 351-363

GALOSY, S.S. and Talamantes, F. (1995) Luteotropic actions of placental lactogens at midpregnancy in the mouse. *Endocrinology,* **136**: 3993-4003

GANDELMAN, R. (1973) Maternal behavior in the mouse: Effect of estrogen and progesterone. *Physiology and Behaviour*, **10**: 153-155

GANDELMAN, R. and Svare, B. (1975) Lactating following hysterectomy of pregnant mice. *Biology of Reproduction,* **12**: 360-367

GANDELMAN, R., Zarrow, M.X., and Denenberg, V.H. (1970) Maternal behavior: differences between mother and virgin mice as a function of the testing procedure. *Development and Phsycobiology*, 3: 207-214

GARRIS, D.R. (1983) Regional variations in guinea pig uterine blood flow during pregnancy: Relationship to intrauterine growth of the fetal-placental unit. *Teratology,* **27**: 101-107

GARRIS, D.R. (1984) Intrauterine growth of the guinea pig fetal-placental unit throughout pregnancy: regulation by utero-placental blood flow. *Teratology,* **29**: 93-99

GARRIS, D.R. and Dar, M.S. (1985) Decidua-associated changes in guinea pig uterine blood flow and volume: relation to norepinephrine concentration. *Anatomical Record,* **211:** 410-413

GARRIS, D.R. and Whitehead, D.S. (1981) Uterine blood flow and timing in blastocyst implantation in the guinea pig. *American Journal of Physiology,* **241:** E142-E145

GIBORI, G. (1993) The corpus luteum of pregnancy. In *The Ovary* (Eds.: E.Y. Adashi and P.C.K. Leung). Raven Press, New York. pp. 261-317

GIBSON, W.R., Ingram, B.W., and Lee, V.W.K. (1979) Can reduced consumption of gonadotrophins account for ovarian compensation in unilaterally ovariectomized immature mice injected whith gonadotrophins? *Journal of Reproduction and Fertility,* **57**: 209-218

GOSDEN, R.G., Telfer, E., Faddy, M.J., and Brook, D.J. (1989) Ovarian cyclicity and follicular recruitment in unilaterally ovariectomized mice. *Journal of Reproduction and Fertility*, **87**: 257-264

GROTA, L.J. and Eik-Nes, K.B. (1967) Plasma progesterone concentrations during pregnancy and lactation in the rat. *Journal of Reproduction and Fertility*, **13**: 83-91

GRUNEBERG, H. (1952) *The Genetics of the Mouse*. Martinus Nijhoff, The Hague. pp. 6-24

HAFEZ, E.S.E. (1963) Symposium on growth: Physiogenetics of prenatal and postnatal growth. *Journal of Animal Science*, **22**: 779-791

HALL, K. (1957) The effect of relaxin extracts, progesterone and oestradiol on maintenance of pregnancy, parturition and rearing of young after ovariectomy in mice. *Journal of Endocrinology*, **15**: 108-117

HAMMOND, J. (1934) The fertilisation of rabbit ova in relation to time. *Journal of Experimental Biology*, **11**: 140-161

HANSEL, W., Concannon, P.W., and Lukaszewska, J.H. (1973) Corpora lutea of the large domestic animals. *Biology of Reproduction*, **8**: 222-245

HANSEL, W. and Dowd, J.P. (1986) New concepts of the control of corpus luteum function. *Journal of Reproduction and Fertility*, **78**: 755-768

HARDING, J.E. and Johnson, B.M. (1995) Nutrition and fetal growth. *Reproduction, Fertility and Development*, **7**: 539-547

HARKNESS, R.A., McLaren, A., and Roy, E.J. (1964) Oestrogens in mouse placentae. *Journal of Reproduction and Fertility*, **8**: 411-413

HASHIMA, H. (1956) Studies on the prenatal growth of the mouse with special reference to the site of implantation of the embryo. *Tohoku Journal of Agricultural Research*, **6**: 307-312

HAUSER, H. and Gandelman, R. (1985) Lever pressing for pups: evidence for hormonal influence upon maternal behavior of mice. *Hormones and Behavior*, **19**: 454-468

HAUSLER, C.L. and Malven, P.V. (1971) Interaction between prolactin, LH, neurohypophysial hormones and hysterectomy on luteal function in rats. *Biology of Reproduction*, **5**: 262-269

HEALY, M.J.R. (1960) Foetal growth in the mouse. *Proceeding of Royal Society London*, **153**: 367-379

HEAP, R.B. and Deanesly, R. (1966) Progesterone in systemic blood and placentae of intact and ovariectomized pregnant guinea-pigs. *Journal of Endocrinology*, **34**: 417-423

HEAP, R.B. and Flint, A.P.F. (1984) Pregnancy. In *Hormonal Control of Reproduction.* (Eds.: C.R. Austin and R.V. Short). Cambridge University Press, Cambridge. pp. 153-194

HEAP, R.B., Perry, J.S., and Challis, J.R.G. (1973) Hormonal maintenance of pregnancy. In *Handbook of Physiology, Section 7, Vol II* (Eds.: R.O. Greep and E.B. Astwood). American Physiological Society, Washington. pp. 217-260

HEAP, R.B., Perry, J.S., and Rowlands, I.W. (1967) Corpus luteum function in the guinea-pig; arterial and luteal progesterone levels, and the effects of hysterectomy and hypophysectomy. *Journal of Reproduction and Fertility,* **13**: 537-553

HEBEL, R. and Stromberg, M.W. (1986). *Anatomy and Embryology of the Laboratory Rat.* BioMed Verlag. Worthsee. pp. 117-123

HILLIARD, J. (1973) Corpus luteum function in guinea pigs, hamster, rats, mice and rabbits. *Biology of Reproduction,* 8: 203-221

HOFFMANN, B., Hoveler, R., Hasan, S.H., and Failing, K. (1992) Ovarian and pituitary function in dogs after hysterectomy. *Journal of Reproduction and Fertility,* **96**: 837-845

HOLINKA, C.F., Tseng, Y.C., and Finch, C.E. (1978) Prolonged gestation, elevated preparturitional plasma progesteron and reproductive aging in C57BL/6J mice. *Biology of Reproduction,* **19**: 807-816

HUGGETT, A.S.G., and Hammond, J. (1964) Physiology of the placenta. In *Marshall's Physiology of Reproduction, Vol II* (Ed.: A.S. Parkes). Longmans, Green and Co. Ltd., London. pp. 312-397

HUMMEL, K.P., Richardson, F.L. and Fekete, E. (1966). Anatomy. In *Biology of the Laboratory Mouse* (Ed.: E.L. Green). McGraw-Hill Book Co., New York. pp. 247-307

HUMPHREYS, E.M., Ghione, R., Gosden, R.G., Hudson, B.M., and Wide, L. (1985) Relationship between corpora lutea or fetal number and plasma concentration of progesterone and testosterone in mice. *Journal of Reproduction and Fertility*, **75**: 7-15

JAITLY, K.D., Robson, J.N., Sullivan, F.M., and Wilson, C. (1966) Hormonal requirement for the maintenance of gestation in hypophysectomized mice. *Journal of Endocrinology,* **34**: iv

JOHNSON, M.H. and Everitt, B.J. (1995) *Essential Reproduction. 4th ed.* Blackwell Science Ltd. Oxford. pp. 25-44, 60-78; 309-335

JOHNSON, D.R. (1971) Genes and genotypes affecting embryonic fluid relations in the mouse. *Genetical Research, Cambridge,* **18**: 71-79

JOHNSON, M.R., Abbas, A. and Nicolaides, K.H. (1994) Maternal plasma levels of human chorionic gonadotrophin, oestradiol and progesterone in multifetal

pregnacies before and after fetal reduction. *Journal of Endocrinology* **143**, 309-312

JJONES, C.T. (1976) Fetal metabolism and fetal growth. *Journal of Reproduction and Fertility*. **47**: 189-201

JONES, S.M. and Rose, R.W. (1992) Plasma progesterone levels in the pregnant rat-kangaroo *(Bettongia gaimardi). General and Comparative Endocrinology,* **87**: 178-182

KATO, H., Morishige, W.K., and Rothchild, I. (1979) A quantitative relation between the experimentally determined number of conceptuses and corpus luteum activity in the pregnant rat. *Endocrinology,* **105**: 846-850

KELLEY, H.E. and Brinkley, H.J. (1971) Effect of hysterectomy on luteinizing hormone-induced corpora lutea of pseudopregnant rabbits. *Biology of Reproduction,* **5**: 1-4

KELLEY, P.A., Shiu, R.P.C., Robertson, M.C., and Friesen, H.G. (1975) Characterization of rat chorionic mammotropin. *Endocrinology,* **96**: 1187-1195

KENSINGER, R.S., Collier, R.J., Bazer, F.W., and Kraeling, R.R. (1986) Effect of number of conceptuses on maternal hormone concentrations in the pig. *Journal of Animal Science,* **62**: 1666-1674

KIHLSTROM, J.E. (1972) Period of gestation and body weight in some placental mammals. *Comparative Biochemistry & Physiology,* **43A**: 673-679

KNIGHT, J.W., Bazer, F.W., and Wallace, H.D. (1973) Effects of superovulation and unilateral ovariectomy-hysterectomy on porcine uterine protein secretions. *Journal of Animal Science,* **36**: 61-65

KNIGHT, J.W., Bazer, F.W., Thatcher, W.W., Franke, D.E., and Wallace, H.D. (1977) Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: Interrelations among hormonal status, placental development, fetal fluids and fetal growth. *Journal of Animal Science,* **44**: 620-637

KOHMOTO, K. and Bern, H.A. (1970) Demonstration of mammotrophic activity of the mouse placenta in organ culture and by transplantation. *Journal of Endocrinology,* **48**: 99-107

LABHSETWAR, A.P. (1967) Hysterectomy and hypophysial FSH and LH levels in the rat. *Journal of Reproduction and Fertility,* **13**: 169-171

LEAVITT, W.W. and Blaha, G.C. (1970) Circulating progesterone levels in the golden hamster during the estrous cycle, pregnancy, and lactation. *Biology of Reproduction,* **3**: 353-361

LINZELL, J.L. and Heap, R.B. (1968) A comparison of progesterone metabolism in the pregnant sheep and goat: sources of production and an estimation of uptake by some target organs. *Journal of Endocrinology,* **41**: 433-438

LISK, R.D., Pretlow, R.A, and Friedman, S.M. (1969) Hormonal stimulation necessary for elicitation of maternal nest-building in the mouse *(Mus musculus)*. *Animal Behaviour*, **17**: 730-737

LUKASZEWSKA, J., Wilson, L., and Hansel, W. (1972) Luteotropic and luteolytic effects of prostaglandins in the hamster. *Proceedings of the Society for Experimental Biology and Medicine,* **40**: 1302-1307

MACDONALD, G.J. (1978) Factors involved in maintenance of pregnancy in the rat. The temporal need for estrogen, pituitary prolactin and the ovary. *Biology of Reproduction,* **19**: 817-823

MACDONALD, G.J. and Matt, D.W. (1984) Adrenal and placental steroid secretion during pregnancy in the rat. *Endocrinology*, **114**: 2068-2073

MACDONALD, G.J., Tashjian, A.H. and Greep, R.O. (1970) Influence of exogenous gonadotropins, antibody formation, and hysterectomy on the duration of luteal function in hypophysectomized rats. *Biology of Reproduction*, **2**: 202-208

MAKOWSKI, E.L. (1977) Vascular physiology. In *Biology of the Uterus* (Ed.: R.M. Wynn). New York, Plenum Press. pp. 77-100

MALISON, J.A., Swanson, P. and French, L.R. (1982) Effects of the uterus and mid-pregnancy conceptus on luteal lifespan of the rat. *Journal of Reproduction and Fertility,* **64**: 25-32

MALVEN, P.V., Hansel, W. and Sawyer, C.H. (1967). A mechanism the luteotrophic action of exogenous prolactin in the rat. *Journal of Reproduction and Fertility,* **13**: 205-212

MARTIN, L., Finn, C.A. and Carter, J. (1970) Effects of progesterone and oestradiol-17β on the luminal epithelium of the mouse uterus. *Journal of Reproduction and Fertility*, **21**: 461-469

MARTIN, P.A., Bevier, G.W., and Dziuk, P.J. (1977) The effect of number of corpora lutea on the length of gestation in pigs. *Biology of Reproduction*, **16**: 633-637

MARTIN, P.A., Bevier, G.W., and Dziuk, P.J. (1978) The effect of disconnecting the uterus and ovary on the length of gestation in the pig. *Biology of Reproduction,* **18**: 428-433

MASUDA, H., Anderson, L.L., and Henricks, D.M. (1967) Progesterone in ovarian venous plasma and corpora lutea of the pig. *Endocrinology,* **80**: 240-246

MATT, D.W. and Macdonald, G.J. (1984) In vitro progesterone and testosterone production by the rat placenta during pregnancy. *Endocrinology,* **115**: 741-747

MAUVAIS-JARVIS, P. (1983). Progesterone and progestine: A general overview. In *Progesterone and Progestins* (Eds.: C.W. Bardin, E. Milgrom, and P. Mauvais-Jarvis). Raven Press, New York. pp.1-16

MCCARTHY, J.C. (1967) Effects of litter size and maternal weight on foetal and placental weight in mice. *Journal of Reproduction and Fertility,* **14**: 507-510

MCCAR HY, J.C. (1965) Genetic and environmental control of foetal and placental growth in the mouse. *Animal Production,* **7**: 347-361

MCCORMACK, J.T. and Greenwald, G.S. (1974a) Progesterone and oestradiol-17β concentrations in the peripheral plasma during pregnancy in the mouse. *Journal of Endocrinology,* **62**: 101-107

MCCORMACK, J.T. and Greenwald, G.S. (1974b) Evidence for a preimplantation rise in oestradiol-17β levels on day 4 of pregnancy in the mouse. *Journal of Reproduction and Fertility,* **41**: 297-301

MCK OWN, T. and Record, R.G. (1953) The influence of placental size on fetal growth according to sex and order of birth. *Journal of Endocrinology,* **10**: 73-81

MCKEOWN, T., Marshall, T., and Record, R.G. (1976) Influences on fetal growth. *Journal of Reproduction and Fertility.* 47: 167-181

MCLAREN, A. (1963) Mechanism of ovarian compensation following unilateral ovariectomy. *Journal of Reproduction and Fertility,* **6**: 321-322

MCLAREN, A. (1965) Genetic and environmental effects on foetal and placental growth in mice. *Journal of Reproduction and Fertility,* **9**: 79-98

MCLAREN, A. (1967) Effect of foetal mass on gestation period in mice. *Journal of Reproduction and Fertility,* **13**: 349-351

MCLAREN, A. and Michie, D. (1960). Control of pre-natal growth in mammals. *Nature.* **187:** 363-365

MCLAREN, A. and Michie, D. (1963). Nature of the systemic effect of litter size on gestation period in mice. *Journal of Reproduction and Fertility,* **6:** 139-141

MCLAREN, A., Renfree, M.B., and Hensleigh, H.C. (1976) Influence of conceptus number and weight on the amount of foetal fluid in the mouse. *Journal of Embryology and Experimental Morphology,* **35:** 191-196

MELAMPY, R.L., Anderson, L.L. and Kragt, C.L. (1964). Uterus and life span of rat corpora lutea. *Endocrinology,* **74:** 501-504

METCALF, M.G., Braiden, V. and Livesey, J.H. (1992) Retention of normal ovarian function after hysterectomy. *Journal of Endocrinology,* **135:** 597-602

MICHAEL, S.D., Geschwind, I.I., Bradford, G.E., and Stabenfeldt, G.H. (1975) Pregnancy in mice selected for small litter size. Reproductive hormone levels and effect of exogenous hormones. *Biology of Reproduction,* **12**: 400-407

MOOR, R.M., Hay, M.F., Short, R.V., and Rowson, L.E.A. (1970) The corpus luteum of the sheep: Effect of uterine removal during luteal regression. *Journal of Reproduction and Fertility,* **21**: 319-326

MOORE, R.W., Eisen, E.J., and Ulberg, L.C. (1970) Prenatal and postnatal maternal influences on growth in mice selected for body weight. *Genetics,* **64**: 59-68

MORISHIGE, W.K., Pepe, G.J., and Rotchild, I. (1973) Serum luteinizing hormone, prolactin and progesterone levels during pregnancy in the rat. *Endocrinology,* **92**: 1527-1530

MURR, S.M., Stabenfeldt, G.H., Bradford, G.E., and Geschwind, I.I. (1974) Plasma progesterone during pregnancy in the mouse. *Endocrinology,* **94**: 1209-1211

MURR, S.M., Bradford, G.E. and Geschwind, I.I. (1974) Plasma luteinizing hormone, follicle stimulating hormone and prolactin during pregnancy in the mouse. *Endocrinology,* **94**: 112-116

NEIL, J.D., Johanson, E.D.B., and Knobil, E. (1969). Failure of hysterectomy to influence the normal pattern of cyclic progesterone secretion in the rhesus monkey. *Endocrinology,* **84**: 464-465

NELSON, R.E. and Robison, O.W. (1976) Effects of postnatal on litter size on reproduction of female mice. *Journal of Animal Science,* **42**: 824-830

NELSON, S.E., McLean, M.P., Jayatilak, P.G., and Gibori, G. (1992) Isolation, characterizaton, and culture of cell subpopulations forming the pregnant rat corpus luteum. *Endocrinology,* **130**: 954-966

NISWENDER, G.D. and Nett, T.M. (1988) The corpus luteum and its control. In *The Physiology of Reproduction* (Eds.: E. Knobil and J.D. Neill). Raven Press, Ltd., New York. pp. 489-525

NORMAN, N.A. and Bruce, N.W. (1979a) Fetal and placental weight relationships in the albino rat near term. *Teratology,* **19**: 245-250

NORMAN, N.A. and Bruce, N.W. (1979b) Fetal and placental weight relationships in the rat at days 13 and 17 of gestation. *Journal of Reproduction and Fertility,* **57**: 345-348

NUTI, K.M., Ward, W.F. and Meyer, R.K. (1971) Embryonic survival and development in unilaterally ovariectomized rats. *Journal of Reproduction and Fertility,* **26**: 15-24

OGREN, L. and Talamantes, F. (1988) Prolactins of pregnancy and their cellular source. *International Review of Cytology,* **112**: 1-65

OGREN, L., Southard, J.N., Colosi, P., Linzer, D.I.H., and Talamantes, F. (1989) Mouse placental lactogen-I: RIA and gestational profile in maternal serum. *Endocrinology,* **125**: 2253-2257

OMTVEDT, I.T., Stanislaw, C.M. and Whatley, J.A. (1965) Relationship of gestation length, age, and weight at breeding, and gestation gain to sow productivity at farrowing. *Journal of Animal Science*, **24**: 531-535

OWENS, J.A. (1991) Endocrine and substrate control of fetal growth: Placental and maternal influences and insulin-like growth factors. *Reproduction Fertility and Development*, **3**: 501-517

PAHL, P.J. (1969) Growth curves for body weight of the laboratory rat. *Australian Journal of Biological Science*, **22**: 1077-1080

PARK, A.W. and Nowosielski-Slepowron, B.A.J. (1971) The effects of litter size on rat body growth. *Acta Anatomica*, **79**: 15-26

PAYNE, G.S. and Deuchar, E.M. (1972) An in vitro study of functions of embryonic membranes in the rat. *Journal of Embryology and Experimental Morphology*, **27**: 533- 542

PEDERSEN, C.A. (1987) Maternal behaviour: Principles and findings. *Neuroendocrinology Letters*, **9**: 162

PEPE, G.J. and Rothchild, 1. (1973) Serum progesterone levels in ovariectomized rats injected with progesterone and estrone: relation to pregnancy maintenance and growth of decidual tissue. *Endocrinology*, **93**: 1193-1199

PEPE, G. and Rothchild, 1. (1974) A comparative study of serum progesterone levels in pregnancy and in various types of pseudopregnancy in the rat. *Endocrinology*, **95**: 275-279

PEPPLER, R.D. and Greendwald, G.S. (1970) Effect of unilateral ovariectomy on ovulation and cycle length in 4- and 5-day cycling rats. *American Journal of Anatomy*, **127**: 1-8

PERRY, J.S. and Rowlands, I.W. (1961) Effect of hysterectomy on the ovarian cycle of the rat. *Journal of Reproduction and Fertility*, **2**: 332-340

RAHIMA, A and Bruce, N.W. (1987) Effects of unilateral ovariectomy before and after mating on growth of corpora lutea and peripheral progestagen concentration in pregnant rats. *Biology of Reproduction*, **37**: 277-281

RAO, B., Pointis, G., and Cedard, L (1982) Presence of a chorionic gonadotrophin-like factor in mouse placental cultures during the second half of gestation. *Journal of Reproduction and Fertility*, **66**: 341-348

RAZIANO, J., Ferin, M., Van de Wiele, R.L. (1972) Effects of antibodies to estradiol-17β and to progesterone on nidation and pregnancy in rats. *Endocrinology*, **90**: 1133-1138

RENFREE, M.B., Hensleigh, H.C., and McLaren, A. (1975) Developmental changes in the composition and amount of mouse fetal fluids. *Journal of Embryology and Experimental Morphology*, **32**: 435-446

ROBINSON, J., Chidzanja, S., Kind, K., Lok, F., Owens, P., and Owens, J. (1995) Placental control of fetal growth. *Reproduction, Fertility and Development,* **7**: 333-344

ROSS, M.H. and Beaumont, H.M. (1974) Unilateral ovariectomy during pregnancy in mice. *Journal of Reproduction and Fertility,* **41**: 235-239

ROTHCHILD, I., Billiar, R.B., Kline, I.T., and Pepe, G. (1973) The persistence of progesterone secretion in pregnant rats after hypophysectomy: A comparison with pseudopregnant, deciduomata bearing pseudopregnant, and lactating rats. *Journal of Endocrinology,* **57**: 63-74

ROTHCHILD, I. (1983) Role of progesterone in initiating and maintaining pregnancy. In *Progesterone and Progestins* (Eds.: C.W. Bardin, E. Milgrom, and P. Mauvais-Jarvis). Raven Press, New York. pp. 219-229

ROWLANDS, I.W. (1961) Effect of hysterectomy at different stages in the life cycle of the corpus luteum in the guine-pig. *Journal of Reproduction and Fertility,* **2**: 341-350

ROWLANDS, I.W. and Short, R.V. (1959) The progesterone content of the guinea-pig corpus luteum during the reproductive cycle and after hysterectomy. *Journal of Endocrinology,* **19**: 81-86

ROWLANDS, I.W. (1962) The effect of oestrogens, prolactin and hypophysectomy on the corpora lutea and vagina of hysterectomyzed guinea-pigs. *Journal of Endocrinology,* **24**: 105-112

RUGH, R. (1967). *The mouse, its reproduction and development.* Burgess Pub. Co., Minneapolis. pp. 1-6; 7-43

RYAN, K.T. (1973) Steroid hormones in mammalian pregnancy. In *Handbook of Physiology, Vol. II, Sect. 7.* (Eds.: R.O. Greep and E.B. Astwood). American Physiological Society, Washington. pp. 285-293

SALOMON, D.S. and Sherman, M.I. (1975) The biosynthesis of progesterone by cultured mouse midgestation trophoblast cells. *Developmental Biology,* **47**: 394-406

SANYAL, M.K. and Villee, C.A. (1973) Production and physiologic effects of progesterone metabolites in pregnant and pseudopregnant rats. *Endocrinology,* **92**: 83-93

SAYLER, A. and Salmon, M. (1971) An ethological analysis of communal nursing by the house mouse *(Mus musculus). Behaviour,* **40**: 62-85

SIEGEL, H.I. (1986) Abnormal basis of maternal behavior in the rat. *Annals of the New York Academy of Sciences,*474: 203-215

SIMMER, H. (1968) Placental hormone. In *Biology of Gestation Vol. I* (Ed.: N.S. Assali). Academic Press. New York. pp. 290-354

SINHA, Y.N., Selby, F.W., and Vanderlaan, W.P. (1974) Relationship of prolactin and growth hormone to mammary functions during pregnancy and lactation in the C3H/St mouse. *Journal of Endocrinology,* **61**: 219-229

SNELL, G.D. (1941). Reproduction. In *Biology of the Laboratory Mouse* (Ed.: G.D. Snell). The Blakiston Co., Philadelphia. pp. 55-88

SNOW, M.H.L. (1989) Effect of genom on size at birth. In *Fetal Growth* (Eds.: F.Sharp, R.B. Fraser, and R.D.G. Milner). Springer-Verlag, London. pp.3-13

SOARES, M.J. and Talamantes, F. (1983) Genetic and litter size effects on serum placental lactogen in the mouse. *Biology of Reproduction,* **29**: 165-171

SOARES, M.J., Colosi, O., and Talamantes, F. (1982) Development and characterization of a homologous radioimmunoassay for mouse placental lactogen. *Endocrinology,* **110**: 668-670

SOUTHEE, J.A., Hunter, M.G., Law, A.S. and Haresign, W. (1988) Effect of hysterectomy on the short life-cycle corpus luteum produced after GnRH-induced ovulation in the anoestrous ewe. *Journal of Reproduction and Fertility,* **84**: 149-155

THORBURN, G.D. (1991) The placenta, prostaglandins and parturition: a review. *Reproduction, Fertility and Development,* 3: 277-294

TIMSON, B.F. and Dudenhoeffer, G.A (1985) Effect of increased growth rate during the suckling period on subsequent body weight and muscle weight to body weight ratio. *Growth,* 49: 455-469

VIRGO, B.B. and Bellward, G.D. (1974) Serum progesterone levels in the pregnant and postpartum laboratory mouse. *Endocrinology,* **95**: 1486-1490

VOM SAAL, F.S. and Dhar, M.G. (1992) Blood flow in the uterine loop artery and loop vein is bidirectional in the mouse: Implications for transport of steroids between fetuses. *Physiology and Behavior,* **52**: 163-171

WAHLSTEN, D. and Bulman-Fleming, B. (1987) The magnitudes of litter size and sex effects on brain growth of BALB/c mice. *Growth,* **51**: 240-248

WANG, M-W., Crombie, D.L., Mais, D.E., Hayes, J.S., and Heap, R.B. (1995) Anti-progesterone antibody administration and the impairment of postpartum maternal care in mice. *Journal of Endocrinology,* **145**: 363-369

WANG, M-W., Heap, R.B., and Taussig, M.J. (1992) Abnormal maternal behaviour in mice previously immunized against progesterone. *Journal of Endocrinology,* **134**: 257-267

WANG, S., Counterman, L.J., and Haslam, S.Z. (1990) Progesterone action in normal mouse mammary gland. *Endocrinology,* **127**: 2183-2189

WANG, H.S. and Chard, T. (1991) The role of insulin-like growth factor-I and insulin-like growth factor-binding protein-1 in the control of human fetal growth. *Journal of Endocrinology,* **132**: 11-19

WEBEL, S.K. and Dziuk, P.J. (1974) Effect of stage of gestation and uterine space on prenatal survival in the pig. *Journal of Animal Science,* **38**: 960-963

WEBEL, S.K., Reimers, T.J., and Dziuk, P.J. (1975) The lack of relationship between plasma progetsterone levels and number of embryos and their survival in the pig. *Biology of Reproduction,* **13**: 177-186

WEEMS, C.W., Vincent, D.L., and Weems, Y.S. (1992) Roles of prostaglandins (PG) $F_{2\alpha}$, E1, E2, adenosine, oestradiol-17β, histone-H2A and progesterone of conceptus, uterine or ovarian origin during early and mid pregnancy in the ewe. *Reproduction, Fertility and Developemnt,* 4: 289-295

WIDDOWSON, E.M. (1981) The role of nutrition in mammalian reproduction. In *Environmental Factors in Mammals Reproduction* (Eds.: D. Gilmore and B. Cook). MacMillan Publisher, Ltd., London. pp.145-159

WIDDOWSON, E.M. 1968. Growth and composition of the fetus and new born. In *Biology of Gestation Vol. II : The Fetus and Neonate* (Ed.: N.S. Assali). Academic Press, New York. pp. 1-49

WIDE, L. and Wide, M. (1979) Chorionic gonadotrophin in the mouse from implantation to term. *Journal of Reproduction and Fertility,* **57**: 5-9

WRIGHT, L.J., Feinstein, A., Heap, R.B., Saunders, J.C., Bennett, R.C., and Wang, M.Y. (1982) Progesterone monoclonal antibody blocks pregnancy in mice. *Nature,* **295**: 415-417

YAMAGUCHI, M., Ogren, L., Endo, H., Soares, M.J., and Talamantes, F. (1994) Co-localization of placental lactogen-I, placental lactogen-II, and proliferin in the mouse placenta at mid pregnancy. *Biology of Reproduction,* **51**: 1188-1192

YAMAGUCHI, M., Tasaka, K., Ogura, K., Sakata, M., Mizuki, J., and Miyake, A. (1995) Activin inhibits but inhibin activates mouse placental lactogen-II secretion. *Journal of Endocrinology,* **146**: 469-474

ZARROW, M.X., Gandelman, R. and Denenberg, V.H. (1971) Prolactin: Is it an essential hormone for maternal behavior in the mammal? *Hormone and Behaviour,* **2**: 343-354