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I am submitting herewith a thesis written by Joyce Spires Harris entitled "Hypertrophy of Myometrial Smooth Muscle Cells in the Pregnant Uterus of the Guinea Pig." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Zoology.

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HYPERTROPHY OF MYOMETRIAL SMOOTH MUSCLE CELLS

IN THE PREGNANT UTERUS OF THE GUINEA PIG

A Thesis

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ABSTRACT

The purpose of this study was to isolate a single myometrial cell from the uterus of the guinea pig and to make a quantitative study of growth changes in the pregnant myometrium by measuring the change of myometrial smooth muscle cells per unit area (SMC/A), to measure changes in smooth muscle cell width (SMCW), and to observe the change in percent smooth muscle cell area (%SMCA = (Area occupied by SMC/total area of micrograph) $\times$ 100) in various segments of the uterus during the estrous cycle and throughout gestation.

Single myometrial cells were isolated from the uteri of the pregnant and nonpregnant guinea pig during various stages of the estrous cycle and with various gestation durations. After incubation of myometrial tissue pieces in various collagenase media for 3-5 hr. Aliquots of the supernatant were placed on a slide and observed periodically with a Nikon-Suke Phase Contrast microscope to check for the presence of isolated myometrial cells. Photomicrographs were made with a Nikon micrographic camera to collect data for the study of cell configurations and for the calculation of the change in area of cell profile of myometrial smooth muscle during the last half of gestation.

The results from the isolated single myometrial smooth muscle cell studies indicate that the most successful technique requires treatment of myometrial segment with 2 mM EGTA, pH 7.4 for 1 hr. with subsequent incubation in a crude collagenase medium for 3-5 hr. with an optimal yield of cells isolated at week 7 of gestation. A two fold

increase in area of cell profile was observed in isolated smooth muscle cells during the last half of gestation.

The major finding of this research was that %SMCA and SMCW changes with the reproductive state of the uterus as well as with gestation duration. These responses were not uniform throughout the entire myometrium, but a differential response was observed among the various uterine sites. A 70% increase in SMC/A was observed in both upper and lower uterine horns during the first two weeks of gestation. However, no change in SMC/A is observed in the caruncle and intercaruncle sites at any stage of gestation.

A cross sectional study is limited in providing data about growth changes of myometrial smooth muscle cells since it does not provide accurate measurement of length. An accurate assessment of cell growth was not possible during this study since changes in SMCW might be related to factors other than growth changes.

The isolated single myometrial cell may provide a useful model for studying and testing the combined effects of hormones, stretch and other physiological and/or physical factors on the morphological changes of the myometrium.

Data obtained from the measurements of quantitative changes of smooth muscle cells in the various uterine sites in the pregnant and nonpregnant uterus provides a statistical basis for future investigations on the distribution of hormonal and neurotransmitter receptor sites in the myometrium during gestation. In future studies, there is a need for techniques which will permit more accurate measurements of

quantitative growth changes in myometrial smooth muscle cells during gestation with known physiological changes in uterine activity at partuition.

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I. INTRODUCTION

Gross Anatomy and Histology of the Guinea Pig Uterus

The guinea pig has a duplex type uterus which is identified by two separate horns with two separate cervixes that open independently into two separate cervical canals and has little or no uterine body present.

The uterus consists of three layers which from the outermost layer inwardly are the: (1) serosa or perimetrium, (2) muscularis or myometrium and (3) mucosa or endometrium (Copenhaver et al., 1978). The perimetrium has the structure of a serous membrane and covers the uterus and a portion of the cervix. The myometrium is a massive layer of muscle which consists of smooth muscle fibers held together by connective tissue (Copenhaver et al., 1978; Jacob et al., 1974). The endometrium is the mucous membrane which forms the inner layer of the uterine wall and consists of epithelial lining and connective tissue which supports tubular epithelial glands that open into the uterine lumen (Copenhaver et al., 1978; Jacob et al., 1974).

The myometrium is made up of three layers which are not always well defined. The three layers are named from the innermost layer subjacent to the endometrium to the outermost in the following order: (1) longitudinal muscle, (2) circular muscle, and (3) longitudinal layer (Weiss, 1977). The muscle layers of the myometrium consist of large and long smooth muscle cells embedded in an abundance of fibrous

connective tissue and elastic fibers (Rhodin, 1974; Dessouky et al., 1968, 1969; Laguens et al., 1964).

Electron microscopic studies reveal that myometrial smooth muscle cells in the nonpregnant uterus of the guinea pig are arranged in small bundles. The individual smooth muscle cells within these bundles are fusiform in shape and are oriented with their long axes parallel to each other and are connected to other cells at their tapering ends. Cells in adjacent bundles are usually arranged at various angles to each other (Dessouky, 1968, 1969). During gestation, smooth muscle cells of the myometrium increase in size and length. The cells become more widely separated from each other due to an increase in the amount of intercellular substance (Dessouky, 1968). A more detailed account of the changes observed in myometrial smooth muscle cells in the pregnant and nonpregnant uterus will be discussed in detail in another section of this thesis.

Rationale for Myometrial Smooth Muscle Separation Technique

The isolated single myometrial smooth muscle cell has great possibilities in becoming a valuable test model for the study of growth changes in the uterus. Changes in cell diameter can be affected by numerous variables other than growth factors. The single isolated cell offers one advantage when compared to cross-sectional studies because it provides information about changes in length as well as diameter. By considering both length and diameter of myometrial smooth muscle cells, a more accurate estimate of cell growth can be calculated because the typical smooth muscle cell is spindle shaped rather than spherical.

Statement of the Problem

Recent studies indicate that maternal influences within the pregnant myometrium might play an important role in triggering parturition. One of the most visible changes that takes place in the uterus during gestation is the enormous increase in myometrial smooth muscle tissue. Growth in myometrial smooth muscle is achieved by both an increase in cell number and also by the growth of existing smooth muscle fibers (Laguens, 1964; Maibenco, 1964; Dessouky, 1969; Althoff, 1970). Many detailed studies have been made concerning the changes in morphology, biochemical activity, electrical activity and physiological activity of the myometrium during gestation. However, due to the enormous amount of connective tissue, blood vessel, nerves, and hormonal influences on the myometrium, it is difficult to observe and describe the true electrophysiological properties of uterine smooth muscle and to correlate these events with the morphological and growth changes that accompany gestation (Marshall, 1973). A better understanding of the physiology of uterine smooth muscle may be achieved by studying the physiological activity and properties of single isolated myometrial smooth muscle cells. However, no successful technique for separating myometrial smooth muscle cells into single functional cells had been reported in the literature at the time that the present study was begun.

In past light microscopic and electron microscopic studies, researchers assumed that structural changes that took place during gestation were uniform in all segments of the myometrium without considering the local effects of ovarian hormones, especially on the

uppermost part of the uterine horn, the local effect of placental and fetal hormones at the implantation sites, and the influence of chronic stretch on local uterine sties. In this study, I decided to develop a technique for isolating a single myometrial cell from the myometrium of guinea pig uterus. A measure of cell size using the area occupied by a cell in a micrograph will be used to compare cell sizes in uteri having various gestation durations.

A preliminary quantitative study of growth changes in four different segments of the myometrium will be made to determine if growth changes of myometrial cells is uniform throughout all stages of gestation by using methods described in another section of this thesis.

In the nonpregnant duplex uterus, spontaneous activity in the ovarian or uppermost part of the uterine horn is most pronounced but the polarity of uterine activity is reversed at parturition and the lowermost portion of the uterine horn becomes most active (Reynolds, 1949; Assali, 1968). In the rabbit, uterine activity in the placental areas is inhibited shortly after implantation and they remain quiescent until late pregnancy (Reynolds, 1949). Based on these observations, I propose to observe the morphology of smooth muscle cells in these specific uterine sites for changes which might accompany the electrophysiological changes.

II. MATERIALS AND METHODS

Care and Management of Breeding Colony

The guinea pig, Cavia porcellus, is closer to man in its metabolic processes than any other rodent (Dessouky, 1968) and requires little in the way of special attention for care and management of a healthy breeding colony (Mosesson et al., 1968). Sexually mature guinea pigs (Ancare Corporation, New York) weighing between 400-500 grams were housed under laboratory conditions and fed Purina Chow guinea pig pellets supplied by Agriculture Feed & Supply Company, Knoxville, Tennessee, supplemented by green or root vegetables. Hay was provided for bedding for pregnant females near term and provided gnawing materials (Hafez, 1970; Dolensek et al., 1976).

The guinea pigs were housed in groups of 4-5 male or female animals in separate cages except in breeding experiments, where only one virgin nulliparous (400-500 grams in weight) was housed in a cage. A sexually mature male (500-800 grams in weight) was introduced to the female during estrus at the appropriate period which shall be described later in this thesis. This housing arrangement of female guinea pigs used in breeding experiments aided in staging the precise time of mating for each individual female guinea pig.

The use of the term estrous will be used in this thesis to describe the interval from one estrus or "heat" period to the onset of the next estrus period in a normal cycling female which repeats itself every sixteen days (Turner et al., 1971).

Staging Female Guinea Pigs at Estrus

Daily vaginal smears were taken from each female guinea pig used in the breeding experiment, stained with 1% giemsa stain and observed with a Nikon light microscope to study the change in architecture and cellular consistency of the vaginal fluid of the female guinea pig during the estrous cycle. This technique was reliable for establishing the regularity and length of the estrous cycle in female guinea pigs in this laboratory facility (Stockard, 1971; Hafez, 1970).

Each female guinea pig was examined daily to determine the exact time that each individual female came into estrus. One sexually mature male was placed into the cage of each female guinea pig at the first sign of the opening of the vaginal orifice or at the time of the estrus period prior to ovulation in the guinea pig (Stockard, 1917; Hafez, 1970).

Techniques Used for Staging Gestation Duration

The length of the gestation period in the guinea pig varies from 63-70 days (Hafez, 1970). However, it was necessary to find a reliable method for staging guinea pigs which were killed at various stages of pregnancy prior to parturition. An ejaculatory plug formed by the male during coitus at the vaginal orifice or its lodging in the dropping tray is evidence of mating (Hafez, 1970). During breeding experiments during this investigation, records were kept on each individual female guinea pig where ejaculatory plugs were found either in the vagina or dropping tray and the exact date on which it was found and dated as Day 0 of pregnancy (Heap et al., 1967; Dessouky, 1968; Hafez, 1970).

Preparation of Uterine Tissue for
Separation Technique Studies

Female guinea pigs weighing 400-800 grams in the nonpregnant animal and 800-100 grams in the pregnant animal were stunned with a blow to the head and bled until dead by severing both arteries. The uteri were removed from abdominal cavity and placed into cold Tyrode's solution (Lockwood, 1961; Hale, 1965) with the following composition in grams per liter; NaCl, 7.80; KCl, .035; NaHCO_3 , 1.37; $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.189; equilibrated with 95% O_2 and 5% CO_2 , pH 7.4.

After removing embryos and placental membranes, each uterine tract was weighed separately on a Mettler balance to the nearest one hundredth of a gram. These weights were recorded in a summary table. The uterine horns were excised at the cervical ends to separate them into right and left horn. Horn A was used for histological sections for Phase Contrast microscopic studies. The techniques used for tissue preparation for this study will be discussed in the next section of this thesis.

Portions of Horn B was placed into a small beaker containing Tyrode's solution and cut with fine dissecting scissors into small pieces of tissue which were then transferred to collagenase dispersion media modified from that used by Bagby et al., 1971, which will be referred to as collagenase medium A. Collagenase medium A consisted of 1 mg. per ml. of collagenase (Sigma, Type II) dissolved in Cold Tyrode's solution with CaCl_2 (0.0025) and MgCl_2 (0.001 M); pH 7.4.

The second technique used to isolate myometrial smooth muscle cell was modified from the technique of Small (1974) which consisted of pretreatment of uterine tissue fragments with 0.002 MEGTA dissolved in 0.05 M Tris Cl Buffer, pH 7.4 for 1-3 hours with subsequent incubation in Collagenase medium B which consisted of 1 mg. per ml of Crude collagenase CLS45D006 (Worthington Biochemicals, Freehold, N.J.), dissolved in 0.05 M Tris Cl Buffer with 0.005 M CaCl_2 added to the medium. The tissue fragments were agitated periodically to free cells by forcing tissue and collagenase suspension through a pasteur pipette. Aliquots of the cell suspensions were examined by a Nikon Phase Contrast Microscope with the 20x Objective. Photomicrographs were made using a Nikon camera back with Tri-X film (ASA 400) for studying structural details of isolated myometrial cells obtained from uterine tissue of the nonpregnant and pregnant uterus during various stages of gestation.

Individual isolated myometrial cells and mature red blood cells were cut out of photomicrographs at the same magnification and weighed on a Mettler balance to the nearest hundredth mg. The change in area of cell profile of myometrial smooth muscle cell was calculated by comparing the ratio of smooth muscle cell weight (mg) to that of a red blood cell (mg) multiplied by the volume of a mature mammalian red blood cell (SMC weight (mg)/RBC weight (mg) $\times$ volume of RBC (microns³). Volume of the RBC was calculated by $V = 3.14 \times r^2 h = 3.14 \times (3.75 \text{ microns})^2 \times 1.9 \text{ microns} = 8.4 \text{ (microns)}^3$.

Preparation of Tissue for Phase Contrast Studies

Samples of uterine tissue were taken from the myometrium of the pregnant and nonpregnant uterus of the guinea pigs which had been previously described in another section of this thesis. The upper and lower ends of the uterine horn were compared in the pregnant and nonpregnant guinea pig to determine if any significant changes took place in SMCW, SMCA, and SMC/A. "Upper end" refers to the uppermost one third end of the uterine horn nearest to the ovaries, "lower end" refers to the most caudal end of the lower one third of the horn adjacent to the cervix, "caruncle area" refers to the placental sites and "inter-caruncle area" refers to the uterine tissue located between the placental sites.

The guinea pigs were divided into four groups; Group I represented the Control animals for the experiment and consisted of 3 animals in estrus (estrogen dominated uterus) and 3 animals in diestrus (progesterone dominated uterus). Group II represented the animals which were in the early stages of gestation (1-5 wk.) and consisted of 4 animals. Group III represented the animals in late pregnancy (6-9 wk.) and consisted of 4 animals.

The uteri were removed from the body cavity and freed of embryos by the method described in Chapter III of this thesis. The uterine horns were placed into small petri dishes containing Cold Tyrode's solution (Lockwood, 1961; Hale, 1965). Small myometrial strips were cut from the individual uterine sites which have been described for the nonpregnant and pregnant uterus. Each myometrial strip was cut

into 1-2 mm. segments and placed into separate vials containing 2.5% glutaraldehyde in 0.1 M Cacodylate buffer and fixed for 3 hr., 4°C, pH 7.4. Uterine tissue segments were transferred to vials containing 0.1 M Cacodylate buffer, pH 7.4 and rinsed overnight, postfixed in 2% Osmium tetroxide in 0.1 M Cacodylate buffer, pH 7.4, 4°C for 2 hr. Using graded series of acetone (25%, 50%, 90%, 100%) concentrations, the uterine segments were then dehydrated and embedded in Araldite Resin 506. Thick sections, white in color were obtained from each site by using a Porter Blum MT-1 Ultra-Microtome. Sections were mounted on a clean slide, dried, and covered with a drop of oil and coverslip and observed with a Nikon Suke Phase-Contrast Microscope. The circular layer of the myometrium was observed and studied carefully to identify any differences in cell configuration, SMCW, SMCA or SMC/A in the myometrium. Photomicrographs were made using a Nikon camera back with high contrast copy film (ASA 64) at 40x magnification.

Measurement and Analysis of Cell Growth

Photomicrographs were used to collect data on changes in SMCW in the upper uterine horn, lower uterine horn, intercaruncle areas and caruncle area, and for different reproductive stages of the nonpregnant and pregnant uterus as described previously in this thesis. A random sample was taken to determine the change in SMCW by measuring the widest portion of 5 cells in each of the four corners of the micrograph plus 5 cells taken from the center of the micrograph totaling a sample size of 25 cells.

The change in SMC/A was compared to each of the four uterine sites with varying gestation durations by blocking off areas in the micrograph one centimeter square, cutting out the space occupied by cells in the micrograph and weighing it separately from the space within the square which did not contain cells or any parts of cells. A mettler balance was used to weigh the paper contained in each area to the nearest 1/10 of a milligram. Each individual weight for each square centimeter was expressed as a percentage of the total weight for each square (sum of the weight of the paper contained in SMCA area plus the weight of paper in the intercellular area). The sampling method was the same technique used for selecting cells to determine changes in SMCW which has been previously described in this thesis with a sampling size of ten.

The change in %SMCA was determined by counting the number of parts of cells located within each of ten squares (which measured one centimeter square) and recorded each separately.

The data was analyzed separately for changes in SMCW, %SMCA, and $\text{SMC}/100 \text{ micron}^2$ in the pregnant and nonpregnant uterus by a one-way analysis of variance to determine the effect of gestation length on each of these factors in the pregnant and nonpregnant uterus.

A two-way analysis of variance was also used to test the combined effect of gestation duration and uterine site on cell diameter, change in percent cell area and cell number. A posteriori test, the Student-Newman Keul test, was used to compare individual group means from the samples used in the analysis of variance and to determine exactly what stages of gestation and/or uterine sites showed significant growth changes.

The effects of gestation duration on morphological changes of smooth muscle cells in the various uterine sites were determined by analyzing photomicrographs for the presence or absence of protoplasmic processes and/or interfiber junctions as viewed microscopically in the pregnant and nonpregnant uterus.

III. RESULTS

Data Obtained from Breeding Experiments

The mean length of the estrous cycle for female guinea pigs used during this investigation was 15 days, 9 hours (Table 1). These findings are in agreement with the findings of Stockard et al. (1917), and Hafez (1970) who report a mean estrous cycle of 15-16 days which varied among individual females, with a slightly shorter estrous cycle occurring during the warm months. It was observed in breeding experiment during this study that 60% of the female guinea pig were fertile after mating. Hafez (1970) reported values which showed that 90% of the female guinea pigs were fertile after one ejaculation.

During the summer months between June and August, female guinea pigs failed to show a typical estrous cycle. This behaviour has been reported in the sheep (Turner, 1971) and mice (Long et al., 1922). Autopsies performed on the guinea pigs which showed an irregular estrous cycle revealed atrophic changes of the uterus and vagina which is a common characteristic of long term castrated female animal.

Pseudopregnancy was observed in females which showed infertile matings. This was confirmed by removing the uterus from female guinea pigs which had been mated and showed all of the signs of true pregnancy; increased development of the mammary glands and hypertrophy of the uterus and vagina, except that no embryos were present. Similar results have been reported in the bitch (Marshall, 1922), rats (Turner, 1971) and laboratory mice (Long et al., 1922).

Table 1. Variations in the length of the estrous cycle of the guinea pig

Animal Number	Body Weight (gms)	Month Observed			Length of Estrus Cycle/Days
		March	June	August	
2	733		x		19
4	870		x		17
5	547		x		17
6	700		x		15
13	704	x		x	12, 19
14	652	x			14
15	662	x			15
16	670			x	19
17	838	x			14
20	543	x			14
21	640	x			14
22	390	x			14

Delayed partuition was observed in several female guinea pigs near term which failed to deliver, resulting in death. It has been reported in the literature that the public symphysis fuses in female guinea pigs mated for the first time after 85 days old (Mosesson et al., 1968). In some cases, female guinea pigs have been reported to instinctively fail to deliver if there was no nesting material available (Hafez, 1970).

Changes in Gross Uterine Weight during Gestation

Implantation takes place in the guinea pig Day 6 postcoitus (Deanesly, 1960). According to the data in Table 2, no increase took place during the first two weeks of gestation. These results are in agreement with the findings of Reynolds (1949).

Gross uterine weight values increased in the guinea pig with gestational duration (Table 2, Figure 1). Uterine weight/body weight ratios when plotted against gestation; duration showed a linear increase with maximum uterine weight values observed near term (Figure 2).

SMC/A Observed in Myometrial Smooth Muscle in the Pregnant and Nonpregnant Uterus

The number of cell per 100 sq. micrometers observed in the nonpregnant uterus showed no significant variation between the upper and lower uterine horns (Table 3, Figure 3). During the first two weeks of gestation a 70% increase in the number of SMC/A are observed in both upper and lower horns (Figure 3). Similar results have been reported

Table 2. Litter size, body weights, and uterine weights observed in female guinea pigs with various gestation durations

Female No.	Gestation Age	Body Weight (gm)	Uterine Weight (gm)	Litter Size
8	2 wk.	494.00	1.624	3
31	4 wk.	933.00	10.870	5
5	5 wk.	701.00	13.620	4
2	7 wk.	1056.70	21.990	5
15	8 wk.	1122.50	26.200	4
14	9 wk.	952.6	37.764	2
17	Postpartum	810.00	16.451	2
	(3-12 hr)			
6	Postpartum	939.30	13.680	3
	(3-12 hr)			
12	Control (estrus)	492.89	1.461	-
16	Control (estrus)	968.00	2.650	-
24	Control (estrus)	428.80	1.220	-
19	Control (diestrus)	538.00	1.590	-
23	Control (diestrus)	725.40	0.800	-
25	Control (diestrus)	632.80	0.800	-

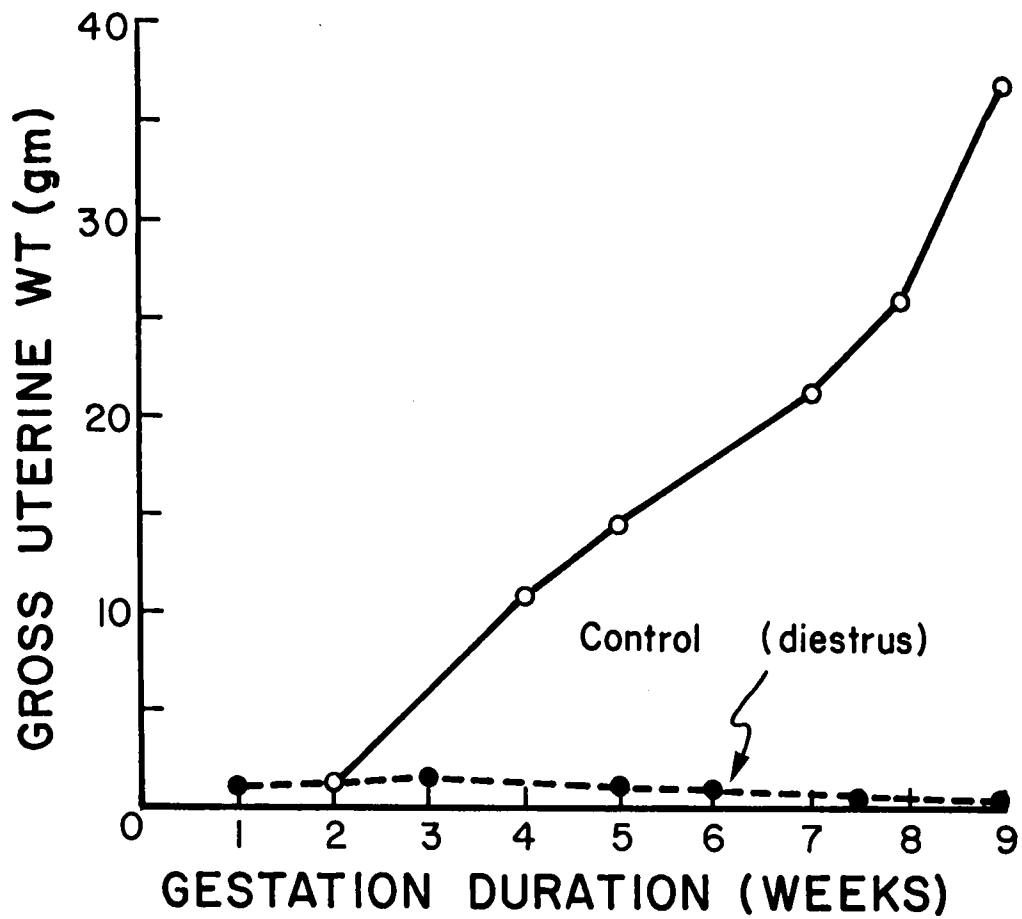


Figure 1. The effect of gestation duration on changes in gross uterine weight of the pregnant guinea pig.

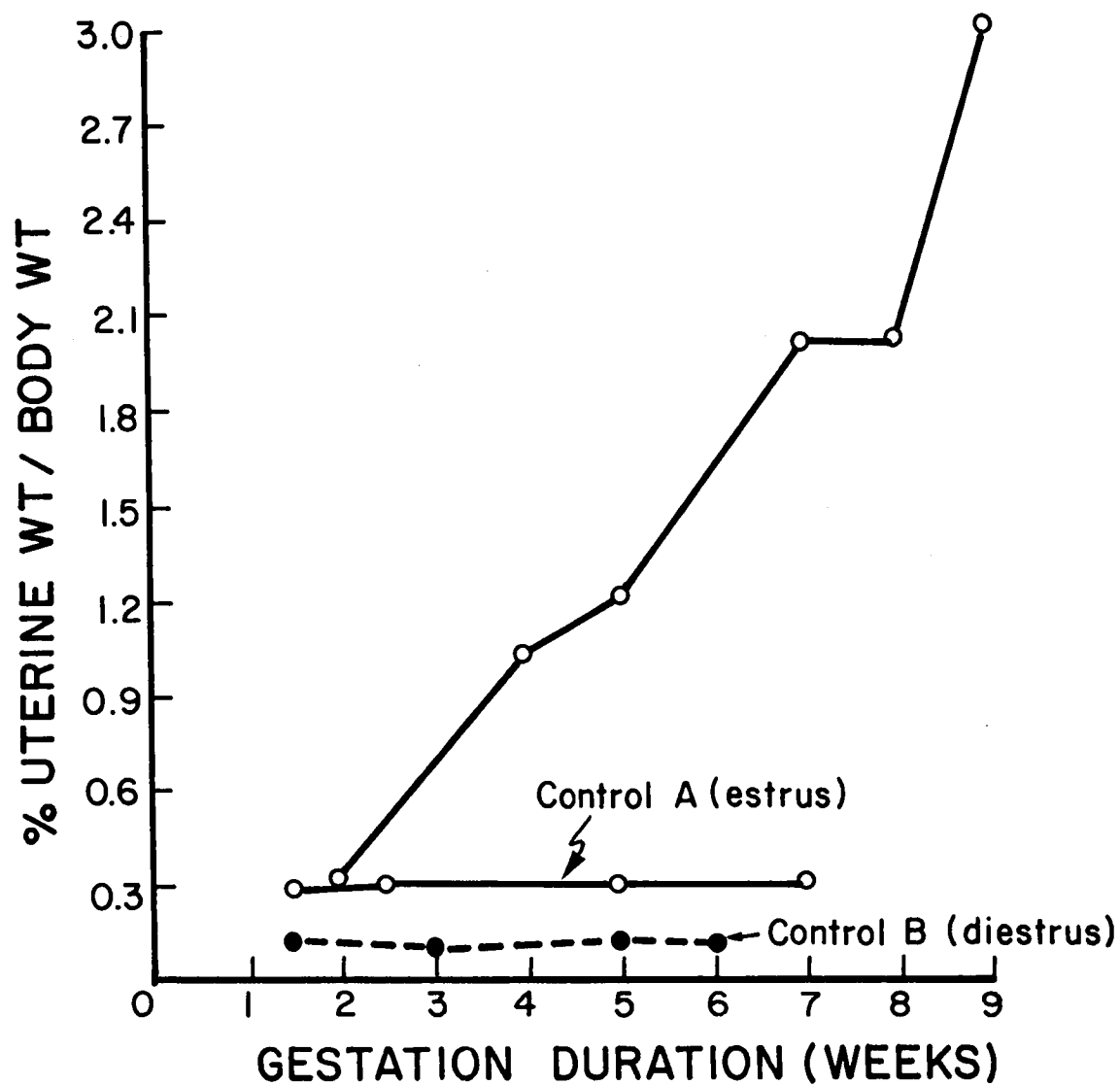


Figure 2. Changes in % uterine weight/body weight ratios with gestation duration in the pregnant guinea pig.

Table 3. Mean values for the effect of gestation duration on SMC/100 microns² in the upper and lower horn of the guinea pig uterus

Gestation Length	Uterine Site	
	Upper cell number/micron ²	Lower cell number/micron ²
<u>Controls</u>		
Estrus	3.6 ± 0.9 S.D.	3.4 ± 1.3 S.D.
Diestrus	3.6 ± 1.0 S.D.	4.6 ± 1.2 S.D.
<u>Early pregnancy</u>		
2 weeks	6.1 ± 1.8 S.D.	5.8 ± 1.7 S.D.
4 weeks	6.7 ± 1.3 S.D.	6.1 ± 1.0 S.D.
<u>Late pregnancy</u>		
6 weeks	4.8 ± 1.2 S.D.	4.9 ± 1.2 S.D.
8 weeks	5.0 ± 1.1 S.D.	6.8 ± 1.5 S.D.
9 weeks	6.3 ± 0.83 S.D.	4.9 ± 1.1 S.D.

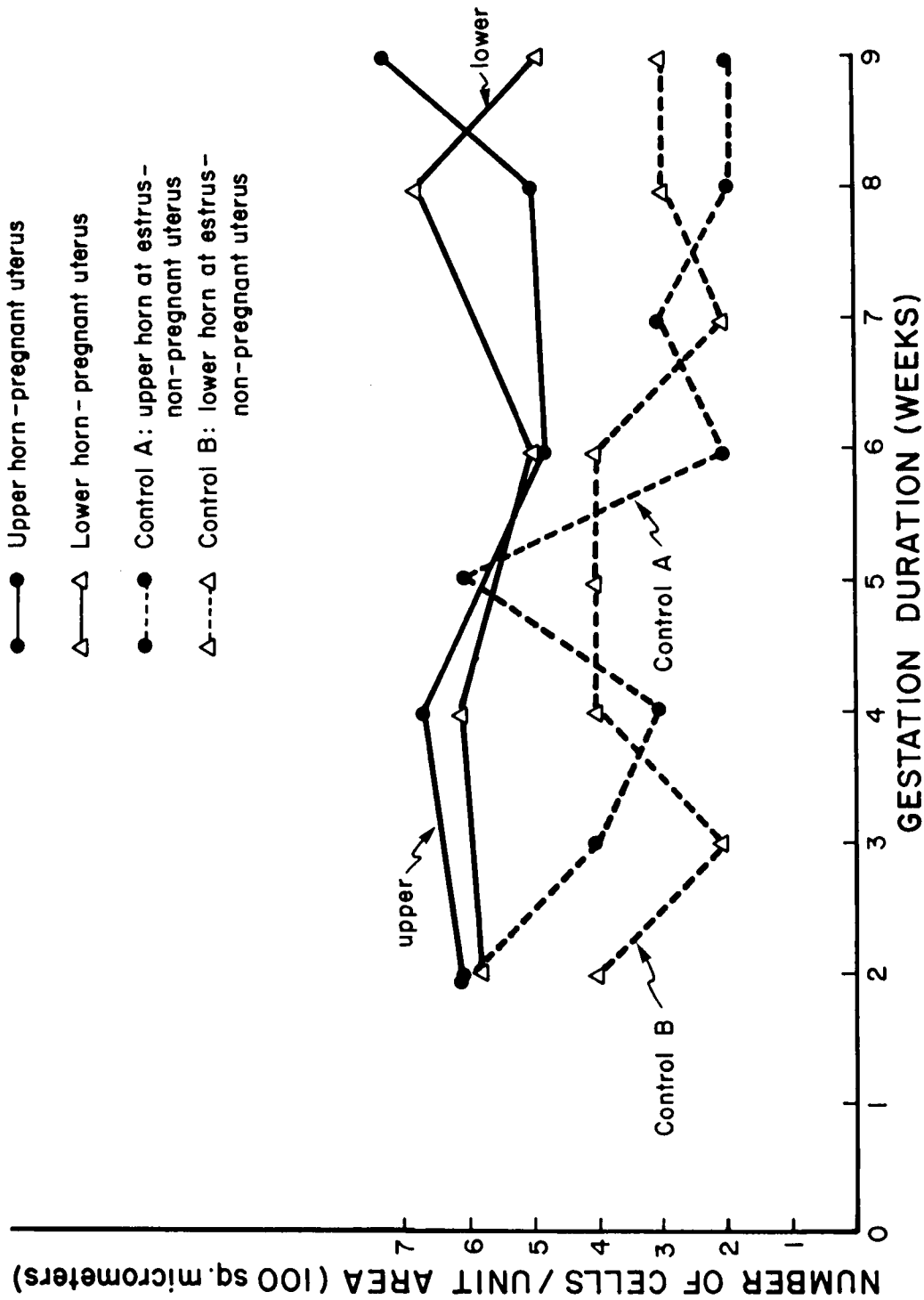


Figure 3. The effect of gestation duration on SMC/100 microns² in the upper and lower regions of the guinea pig uterus.

in the rat (Reynolds, 1949), in the guinea pig (Mehrotra et al., 1973) and in the mouse (Kang et al., 1975). No further significant change is observed in SMC/A in the upper and lower uterine horn (Table 3).

There was no significant change in the number of SMC/A in the caruncle and intercaruncle areas at any stage of gestation (Table 4, Figure 4).

Variation in Cell Width of Myometrial Smooth Muscle Cells

According to the data in the summary table for the one-way analysis of variance for the effect of gestation length on the change of SMCW (Table 5), no significant variation in size is observed between the upper and lower horns in the controls at estrus and diestrus. However, significant variation is observed in cell diameter among the uterine sites in the pregnant uterus at all stages of gestation except week 8 (Tables 5 and 6, Figures 5 and 6). A comparison of group means was made by the Student-Newman Keul test to determine which of the uterine sites contained SMC with widths which were significantly greater than the other. The results of the data is as follows:

1. At week 2, the SMCW in the upper and intercaruncle areas are significantly greater than those in the lower horn.
2. At week 4, the SMCW in the lower horn are significantly greater than SMCW in the upper and caruncle areas.
3. At week 6, SMCW in the upper, intercaruncle, caruncle area are significantly higher than the lower horn.

Table 4. Mean values for the effect of gestation duration on SMC/100 microns² in the caruncle and intercaruncle area in the uterus of the guinea pig

Gestation Length	Uterine Site	
	Caruncle cell number/100 microns ²	Intercaruncle cell number/100 microns ²
<u>Early pregnancy</u>		
2 weeks	-----	2.9 ± 1.7 S.D.
4 weeks	3.3 ± 1.4 S.D.	-----
5 weeks	3.7 ± 0.4 S.D.	3.5 ± 0.5 S.D.
<u>Late pregnancy</u>		
6 weeks	3.4 ± 0.96 S.D.	2.8 ± 1.3 S.D.
7 weeks	3.3 ± 1.0 S.D.	3.8 ± 0.87 S.D.
8 weeks	-----	2.7 ± 0.67 S.D.
9 weeks	2.8 ± 1.0 S.D.	2.6 ± 0.90 S.D.

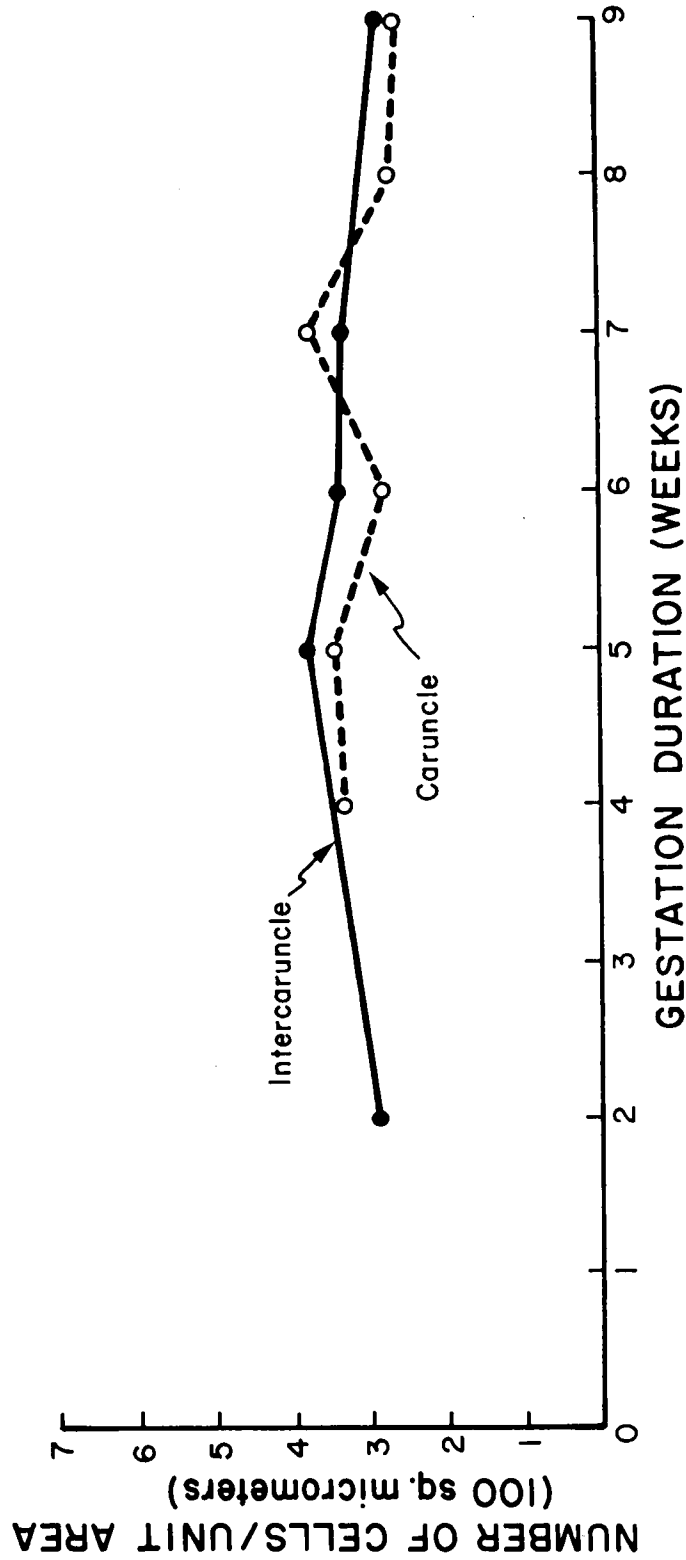


Figure 4. The effect of gestation duration on SMC/100 microns² in the caruncle and intercaruncle regions. Note that no significant changes occur in SMC/A is observed in both caruncle and intercaruncle regions at any gestation duration.

Table 5. Mean values for the effect of gestation duration on SMCW in the upper and lower uterine horns of the guinea pig uterus

Gestation Length	Uterine Sites	
	Upper Width (microns)	Lower Width (microns)
<u>Control</u>		
Diestrus	3.0 ± 0.3 S.D.	3.9 ± 0.6 S.D.
Estrus	4.3 ± 0.8 S.D.	3.6 ± 0.7 S.D.
<u>Early pregnancy</u>		
2 weeks	4.0 ± 0.6 S.D.	2.2 ± 0.4 S.D.
4 weeks	2.0 ± 0.2 S.D.	2.6 ± 0.5 S.D.
<u>Late pregnancy</u>		
6 weeks	4.8 ± 0.8 S.D.	5.6 ± 0.8 S.D.
8 weeks	3.2 ± 0.5 S.D.	2.8 ± 0.5 S.D.
9 weeks	2.9 ± 0.5 S.D.	4.4 ± 0.7 S.D.

Table 6. Effect of gestation duration on SMCW in the caruncle and intercaruncle regions of the guinea pig uterus[†]

Gestation Length	Uterine Sites	
	Caruncles Width (microns)	Intercaruncles Width (microns)
<u>Early pregnancy</u>		
2 weeks	-----	3.68 ± 0.3 S.D.
4 weeks	2.0 ± 0.6 S.D.	-----
5 weeks	4.0 ± 0.5 S.D.	5.60 ± 0.9 S.D.
<u>Late pregnancy</u>		
6 weeks	3.1 ± 0.7 S.D.	4.06 ± 0.7 S.D.
7 weeks	2.8 ± 0.5 S.D.	2.40 ± 0.3 S.D.
8 weeks	-----	2.56 ± 0.3 S.D.
9 weeks	2.9 ± 0.5 S.D.	4.40 ± 0.7 S.D.

[†]Data taken from micrograph.

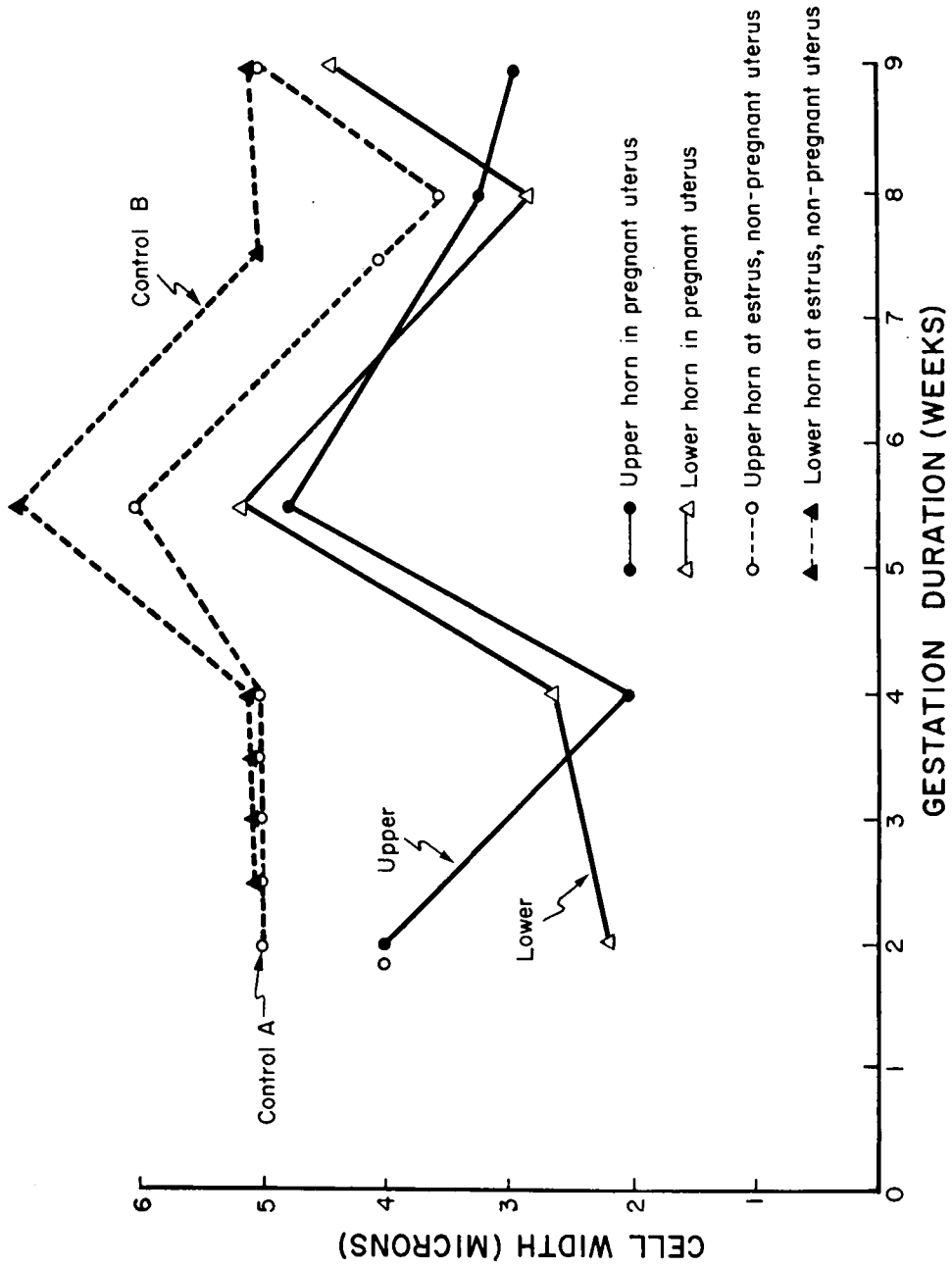


Figure 5. The effect of gestation duration on SMCW in the upper and lower uterine horns. Note the two-fold increase in SMCW between week 4-5.

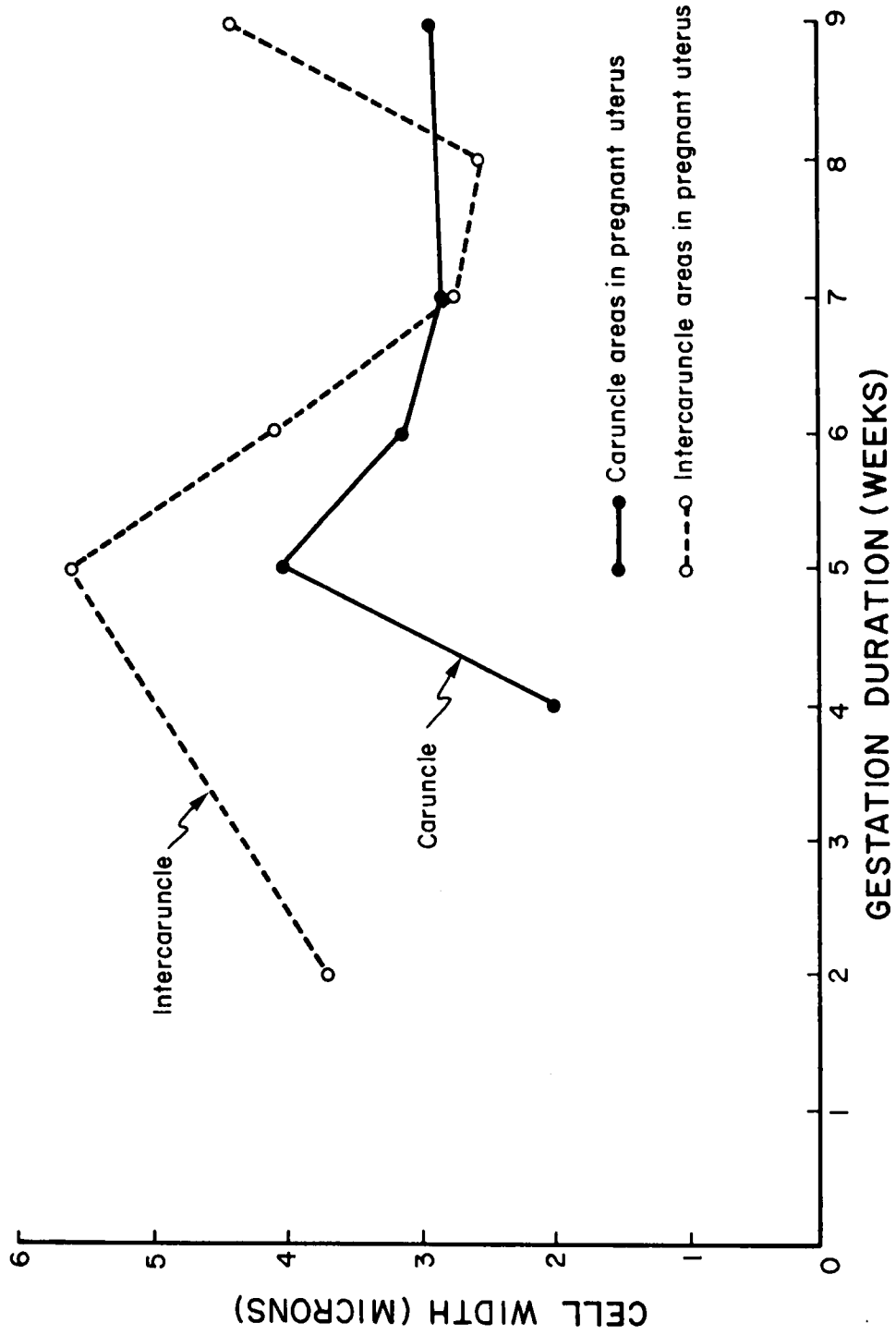


Figure 6. The effect of gestation on SMCW in the caruncle and intercaruncle regions. Note the increase in SMCW between week 4-5 in both caruncle and intercaruncle regions.

4. At week 9, SMCW in the lower horn and caruncle areas are significantly greater than SMCW in the upper and inter-caruncle areas.

When the combined effect of gestation and uterine site was analyzed by a two-way analysis of variance to test the effect on cell diameter of myometrial smooth muscle cells, the results were as follows:

1. During the first trimester of gestation, a significant decrease in SMCW is observed in both upper and lower uterine horns with values lower than the controls at estrus and diestrus.
2. During the second trimester of gestation (week 4-6), SMCW in both upper and lower horns show a sharp increase followed by a sharp decrease in SMCW which remains low in the upper horn until term but shows a sharp increase in the lower horn between week 8 and week 9.
3. No significant variation in SMCW was observed between the controls at estrus and diestrus. However, significant variation was observed between the upper and lower horn in one animal at estrus which might be caused by error in measurement.

Effect of Gestation Duration on SMCA in the Various Uterine Sites

A significant variation in the SMCA was observed among the controls at diestrus (Table 7). Comparison of group means for individual guinea pigs reveals that this variation represents significantly higher percentages of SMCA values in both upper and lower horn regions in

Table 7. A comparison of mean values for changes in % SMCA observed in the upper and lower horn of the uterus in the guinea pig

Gestation Length	Upper % Cell Area	Lower % Cell Area
<u>Control</u>		
Estrus	53.8 ± 12.9	55.6 ± 14.6
Diestrus	38.1 ± 16.2	56.3 ± 18.04
<u>Early pregnancy</u>		
2 weeks	42.1 ± 14.4	62.0 ± 10.3
4 weeks	42.9 ± 12.9	61.1 ± 11.02
<u>Late pregnancy</u>		
6 weeks	84.4 ± 8.8	44.7 ± 11.3
8 weeks	70.1 ± 16.08	64.1 ± 15.9
9 weeks	45.3 ± 16.7	55.0 ± 16.7

only one animal out of three which suggests variation due to error and/or individual variations.

The data obtained from the one-way and two-way analysis of variance indicates the following:

1. In the upper horn, %SMCA shows an increase during the second trimester of gestation (week 4-6) but decreases during the third trimester (Figure 7).
2. SMCA in the lower horn is reversed with a decrease in SMCA observed during the second trimester and an increase during the third trimester of gestation.
3. Changes in SMCA in the caruncle areas (Figure 8) are similar to that observed in the lower horn with an increase in SMCA during the second trimester and a decrease in SMCA during the third trimester of gestation while the changes in SMCA in the intercaruncle area is similar to that observed in the upper horn with increased values during the second trimester and decreased values during the third trimester.
4. SMCA observed in the upper horn is significantly greater than all other uterine sites during the second trimester of gestation while the percentage of SMCA is significantly greater in the lower horn during the third trimester. A gradual decrease is observed in SMCA in both horns until term with values at week 9 not significantly greater than values observed in the controls at estrus and diestrus.

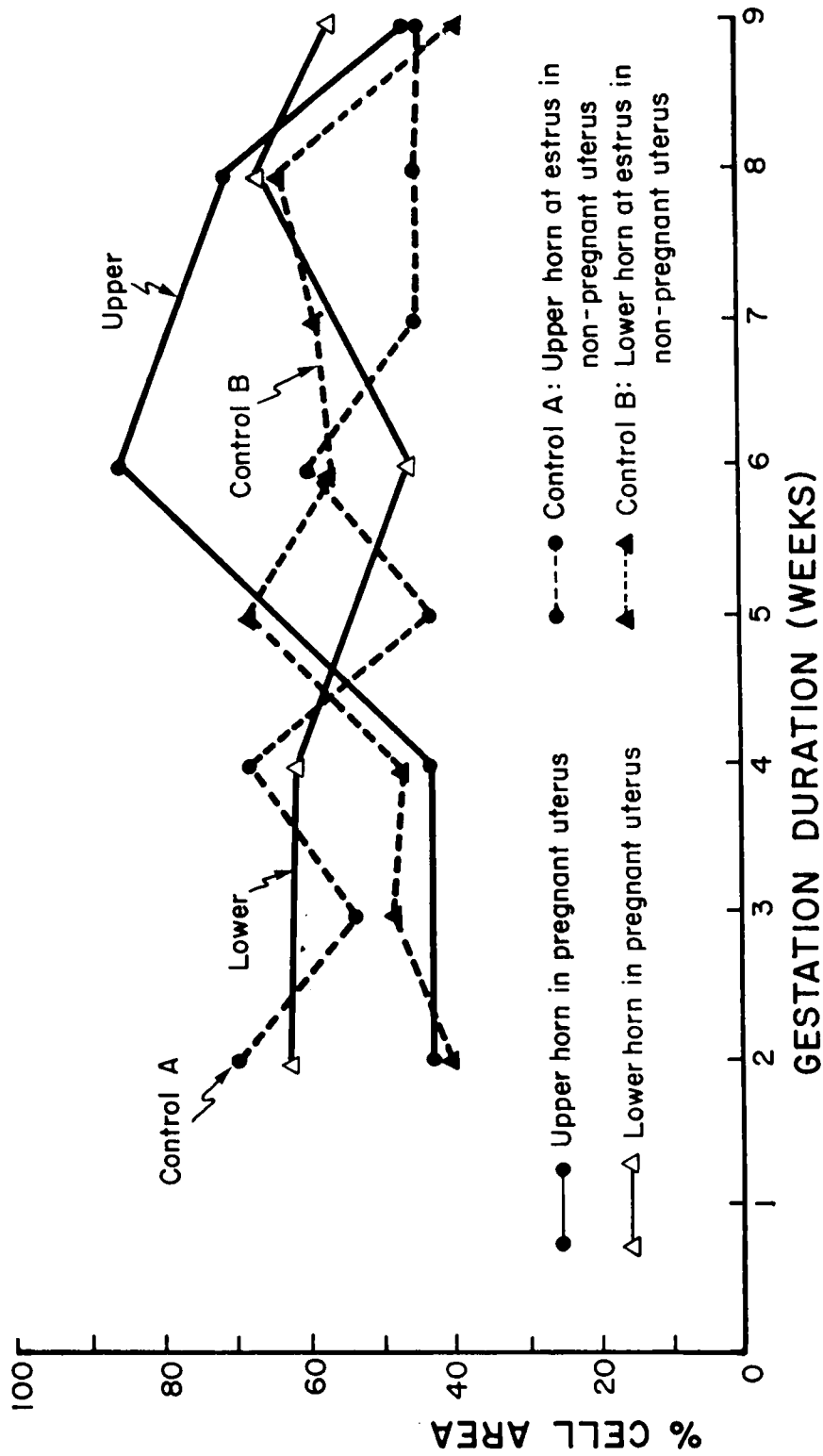


Figure 7. The effect of gestation duration on change in % SMCA in the upper and lower horn regions. Note the increase in % SMCA during the second trimester of gestation in both upper and lower uterine horns.

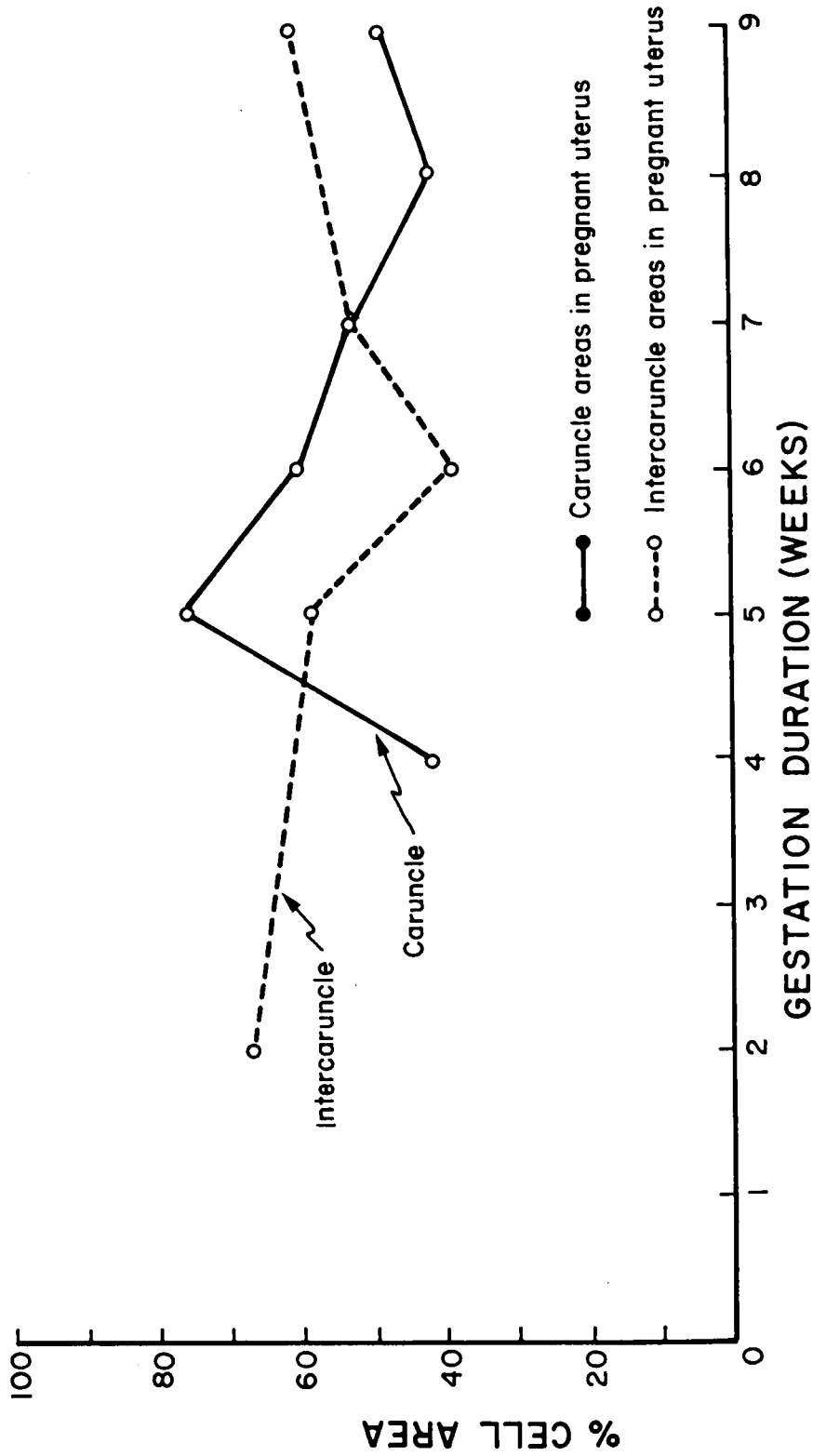


Figure 8. The effect of gestation duration on % SMCA in the caruncle and intercaruncle regions. Note that the increase in % SMCA observed between week 4-5 is not considered statistically significant.

5. No significant difference is observed between SMCA between the caruncle and intercaruncle areas at any stage of gestation (Table 8).

A careful study of Tables 9-20 will provide further information about the effects of gestation on SMC/A, %SMCA and SMCW in the four different uterine sites.

Morphological Changes in Smooth Muscle Cells Observed in the Pregnant and Nonpregnant Uterus

In the nonpregnant uterus morphological changes can be observed in the lower uterine horn (Figure 9) at estrus when compared to the same region of the horn at diestrus.

In the upper uterine horn at week 4 of gestation (Figure 10a) protoplasmic processes have begun to form and interdigitate with neighboring cells at week 6 (Figure 10b) and at week 9 (Figure 10d). One can no longer distinguish between individual smooth muscle cells. The entire smooth muscle area appears as an amorphous mass of tissue.

A similar pattern is observed in the lower uterine horn with individual smooth muscle cells widely separated at week 2 (Figure 11a), interdigitation of neighboring cells at week 6 (Figure 11b), and an amorphous mass of tissue with no clear line of demarcation between individual cells at week 9 (Figure 11c).

In the intercaruncle regions, the pattern is similar to that observed in the upper and lower uterine horns except that at week 8 (Figure 12c) protoplasmic processes appear to disintegrate and cells are more widely separated at week 2 (Figure 12a) and at week 9 (Figure 12d).

Table 8. Effect of gestation on % SMCA located in the caruncle and intercaruncle areas of the pregnant guinea pig uterus

Gestation Length	Upper Cell Area	Lower Cell Area
<u>Early pregnancy</u>		
2 weeks	-----	67.8 ± 13.7
4 weeks	41.9 ± 10.7	-----
5 weeks	75.2 ± 6.9	57.4 ± 15.8
<u>Late pregnancy</u>		
7 weeks	51.7 ± 18.1	52.2 ± 15.0
8 weeks	-----	40.5 ± 10.2
9 weeks	59.4 ± 19.4	47.6 ± 12.8

Table 9. One-way analysis of variance for the effect of gestation on SMC/100 micron² on micrograph area in four different sites of the guinea pig uterus

Source of Variation	D.F.	S.S.	M.S.	F
<u>Control</u>				
<u>Estrus (1,2)</u>				
Among groups	1	0.2	0.2	0.125
Within groups	18	28.8	1.6	
Total	19	29.0	1.8	0.125
<u>Diestrus (1,2)</u>				
Among groups	1	5.0	5.0	3.97
Within groups	18	22.8	1.26	
Total	19	27.8	6.26	3.97
<u>2 weeks (1,2,3)</u>				
Among groups	2	62.47	31.28	12.56**
Within groups	27	63.40	2.49	
Total	29	125.87	33.77	12.56
<u>4 weeks (1,2,4)</u>				
Among groups	2	65.0	32.50	20.96**
Within groups	27	41.97	1.55	
Total	29	106.97	34.05	20.96
<u>5 weeks (3,4)</u>				
Among groups	1	0.2	0.2	0.54
Within groups	18	6.6	0.37	
Total	19	6.8	0.57	0.54
<u>6 weeks (1,2,3,4)</u>				
Among groups	3	32.47	10.82	10.4*
Within groups	36	37.50	1.04	
Total	39	69.97	11.86	10.4
<u>7 weeks (3,4)</u>				
Among groups	1	1.25	1.25	1.27
Within groups	18	17.70	0.98	
Total	19	18.95	2.23	1.27
<u>8 weeks (1,2,3)</u>				
Among groups	2	84.47	42.24	14.72**
Within groups	27	77.70	2.87	
Total	29	162.17	45.11	14.72
<u>9 weeks (1,2,3,4)</u>				
Among groups	1	1.25	1.25	1.27
Within groups	18	17.70	0.98	
Total	19	18.95	2.23	1.27

The number in parentheses represents the following uterine sites: 1 = upper horn, 2 = lower horn, 3 = intercaruncle area, 4 = caruncle areas.

**Significant at the .001 confidence level.

*Significant at the .050 confidence level.

Table 10. One-way analysis of variance for the effect of gestation duration on SMCW in four different uterine sites in the uterus of the guinea pig

Source of Variation	D.F.	S.S.	M.S.	F
<u>Control</u>				
<u>Diestrus (1,2)</u>				
Among groups	1	0.98	0.98	2.45
Within groups	48	19.60	0.40	
Total	49	20.58		
<u>Control</u>				
<u>Estrus (1,2)</u>				
Among groups	1	0.83	0.83	
Within groups	48	14.88	0.31	
Total	49	15.71		
<u>2 weeks (1,2,3)</u>				
Among groups	2	64.99	32.50	38.28**
Within groups	72	61.16	0.85	
Total	74	126.05		
<u>4 weeks (1,2,4)</u>				
Among groups	2	15.39	7.69	16.66**
Within groups	72	33.28	0.46	
Total	74	48.67		
<u>5 weeks (3,4)</u>				
Among groups	1	54.08	54.08	31.81**
Within groups	48	81.84	1.70	
Total	49	135.92		
<u>6 weeks (1,2,3,4)</u>				
Among groups	3	130.75	43.58	53.15
Within groups	96	79.00	0.82	
Total	99	209.75		
<u>7 weeks (3,4)</u>				
Among groups	1	5.78	5.78	10.50**
Within groups	48	26.60	0.55	
Total	49	32.38		
<u>8 weeks (1,2,3)</u>				
Among groups	2	6.48	3.24	1.84
Within groups	72	127.00	1.76	
Total	79	133.48		
<u>9 weeks (1,2,3,4)</u>				
Among groups	3	62.56	20.65	16.55**
Within groups	96	121.00	1.26	
Totals	99	183.56		

The numbers in the parentheses represent the following uterine sites:
1 = upper horn, 2 = lower horn, 3 = intercaruncle, 4 = caruncle areas.

**Significant at the .001 confidence level.

*Significant at the .05 confidence level.

Table 11. One-way analysis of variance for the effect of gestation duration on % SMCA in the uterus of the pregnant guinea pig

Source of Variation	D.F.	S.S.	M.S.	F
<u>Control</u>				
<u>Estrus (1,2)</u>	1	162.00	162.00	0.85
Among groups	1	162.00	162.00	0.85
Within groups	18	3414.00	189.66	
Total	19			
<u>Control</u>				
<u>Diestrus (1,2)</u>				
Among groups	1	1656.20	1656.20	5.90*
Within groups	18	5742.00	319.00	
Total	19	7398.20		
<u>2 weeks (1,2,3)</u>				
Among groups	2	3633.80	1816.90	10.07**
Within groups	27	4868.50	180.30	
Total	29	8502.30		
<u>4 weeks (1,2,4)</u>				
Among groups	2	2335.97	1167.98	6.74*
Within groups	27	4678.00	173.26	
Total	29	7013.97		
<u>5 weeks (3,4)</u>				
Among groups	1	1584.20	1584.20	7.53*
Within groups	18	3788.00	210.44	
Total	19	5372.20		
<u>6 weeks (1,2,3,4)</u>				
Among groups	3	12625.30	4208.43	28.85**
Within groups	36	5252.20	145.89	
Total	39	17877.50		
<u>7 weeks (3,4)</u>				
Among groups	1	1.25	1.25	N/A
Within groups	18	5849.70	324.98	
Total	19	5850.95		
<u>8 weeks (1,2,3)</u>				
Among groups	2	4897.07	2448.53	10.58**
Within groups	27	6248.24	231.40	
Total	29	11145.31		
<u>9 weeks (1,2,3,4)</u>				
Among groups	3	1278.88	426.29	1.49
Within groups	36	10283.78	285.66	
Total	39	11562.66		

The number in parentheses represents the following uterine sites:
1 = upper horn, 2 = lower horn, 3 = intercaruncle, 4 = caruncle areas.

**Significant at the .001 confidence level.

*Significant at the .05 confidence level.

N/A = Not Significant.

Table 12. Two-way analysis of variance for the combined effect of gestation duration and uterine site on SMC/100 microns² in the upper and lower uterine horns

Source of Variation	D.F.	S.S.	M.S.	F
<u>Control (Estrus)</u>				
Subgroup	3	8.4	2.8	
A	1	1.6	1.6	1.15
B	1	3.6	3.6	2.59
A × B	1	3.2	3.2	2.30
Within	36	50.0		
<u>Early pregnancy</u>				
Subgroup	5	99.89	19.97	
A	1	0.82	0.82	0.38
B	2	97.44	48.72	22.22**
A × B	2	1.62	0.81	0.37
Within	54	116.30	2.19	
<u>Late pregnancy</u>				
Subgroup	7	95.29	13.61	
A	1	0.61	0.61	0.34
B	3	68.74	22.91	12.54**
A × B	3	25.94	8.65	4.73*
Within	72	131.60	1.83	

**Significant at the .001 level.

*Significant at the .001 level.

Table 13. Two-way analysis of variance for the combined effect of gestation duration and uterine site on SMC/100 micron² in the caruncle and intercaruncle areas

Source of Variation	D.F.	S.S.	M.S.	F
Subgroup	7	14.19	2.03	
A	1	0.31	0.10	0.03
B	3	10.74	3.58	3.56*
A × B	3	3.14	1.04	1.04
Within	72	72.30	1.00	

*Significant at the .05 confidence level.

Table 14. Two-way analysis of variance for the combined effect of gestation duration and uterine site on the % SMCA in the caruncle and intercaruncle areas of the uterus

Source of Variation	D.F.	S.S.	M.S.	F
Subgroups	7	8107.35	1158.19	1.96
A	3	3200.45	1066.82	1.80
B	1	1754.95	1754.95	2.97
A × B	3	3151.95	1050.65	1.78
Within	72	42604.20	591.73	

No significant variation was observed in percent smooth muscle cell area in the caruncle and intercaruncle area during any stage of gestation.

Table 15. Two-way analysis of variance for the effect of gestation duration on % SMCA in upper and lower uterine horn

Source of Variation	D.F.	S.S.	M.S.	F
<u>Control (Diestrus)</u>				
Subgroup	7	11388.29	1626.89	
A	3	632.81	210.94	0.85
B	1	8408.54	8408.54	33.75**
A × B	3	12346.94	782.31	3.14
Within	72	17935.10	249.09	
<u>Control (Estrus)</u>				
Subgroup	5	3306.54	661.30	
A	1	540.00	540.00	2.23
B	2	2784.14	1392.07	5.77*
A × B	2	17.60	8.80	0.04
Within	54	13031.20	241.31	
<u>Early pregnancy</u>				
Subgroup	7	5894.29	842.04	
A	3	4219.51	1406.50	6.39*
B	1	585.64	585.64	2.66
A × B	3	1088.85	362.95	1.65
Within	72	15838.70	219.98	
<u>Late pregnancy</u>				
Subgroup	9	6987.06	776.34	
A	4	201.64	50.41	0.14
B	1	5870.34	5870.34	16.54
A × B	4	915.08	228.77	0.65
Within	90	31868.58	354.09	

**Significant at the .001 level.

*Significant at the .05 level.

Table 16. Two-way analysis of variance for the combined effects of gestation duration and uterine site on SMCW located in the upper and lower uterine horn

Source of Variation	D.F.	S.S.	M.S.	F
<u>Diestrus</u>				
Subgroup	5	63.36	12.67	
A	1	24.00	24.00	1.54
B	2	18.22	9.11	0.58
A × B	2	21.14	10.57	0.68
Within	144	2247.64	15.61	
<u>Estrus</u>				
Subgroup	5	42.16	8.43	
A	1	16.66	16.66	11.03**
B	2	9.12	4.56	3.01
A × B	2	16.21	8.10	5.37
Within	144	217.68	1.51	
<u>Early pregnancy</u>				
Subgroup	7	227.26	32.43	
A	1	3.44	3.44	5.98**
B	3	151.06	50.35	87.47**
A × B	3	72.76	24.25	42.13*
Within	192	110.52	0.58	
<u>Late pregnancy</u>				
Subgroup	9	385.72	42.86	
A	4	26.44	6.56	7.14**
B	1	316.86	316.86	344.78**
A × B	4	42.62	10.65	11.59
Within	240	220.64	0.92	

**Significant at the .001 confidence level.

*Significant at the .05 confidence level.

Table 17. Two-way analysis of variance for the combined effects of gestation duration and uterine site on SMCW in the caruncle and intercaruncle regions

Source of Variation	D.F.	S.S.	M.S.	F
Subgroup	7	274.63	39.23	
A	3	8.41	2.80	1.86
B	1	199.94	199.94	134.64**
A × B	3	66.29	22.09	14.88**
Within	192	129.25	1.49	

**Significant at the .001 confidence level.

Table 18. Comparison of group means by student Newman-Keuls test for the effect of uterine site on SMC/A at various gestation durations

Gestation Duration	Uterine Site (mean values ranked by magnitude of SMC/A)				Comparison of SMC/A in Uterine Sites (SMK)*
2 weeks	Upper 6.1	Lower 5.8	I. Car. 2.9		$U_1 = U_2 > U_3$
4 weeks	Upper 6.7	Lower 6.1	Car. 3.3		$U_1 = U_2 > U_4$
6 weeks	<u>Lower 4.7</u>	<u>Upper 4.8</u>	<u>I. Car. 3.4</u>	<u>Car. 2.8</u>	$U_2 = U_1 > U_3 = U_4$
8 weeks	Lower 6.8	Upper 5.0	I. Car. 4.9		$U_2 > U_1 > U_3$
9 weeks	Upper 6.3	Lower 4.9	Car. 2.8	I. Car. 2.6	$U_1 > U_2 > U_3 = U_4$

U_1 = Upper horn; U_2 = Lower horn; U_3 = Intercaruncle area;
 U_4 = Caruncle area.

Underlined values are not significantly different statistically.

Table 19. Comparison of group means by student Newman-Keuls test for the effect of uterine site on SMCW[†]

Gestation Duration	Uterine Site (mean values ranked by magnitude of SMCW in mm)				Comparison of Uterine Site SMCW (SNK test)
2 weeks	Upper <u>5.12</u>	I. Car. <u>4.64</u>		Lower 2.8	$U_1 = U_3 > U_2$
4 weeks	Lower 3.28		Upper <u>2.52</u>	Car. <u>2.2</u>	$U_2 > U_1 = U_4$
5 weeks	I. Car. 6.96		Car. 4.96		$U_3 > U_4$
7 weeks	Car. 3.52		I. Car. 2.88		$U_4 > U_3$
9 weeks	Lower <u>5.68</u>	Car. <u>5.48</u>	I. Car. 4.7	Upper 3.64	$U_1 = U_2 > U_3 > U_4$

[†]Values represent area measured in photograph.

U_1 = Upper horn; U_2 = Lower horn; U_3 = Intercaruncle area;
 U_4 = Caruncle area.

Underlined mean values are not significantly different statistically.

Table 20. Comparison of group means by the student Newman-Keuls test for the effect of uterine site on % SMCA at various gestation durations

Gestation Duration	Uterine Sites (mean values ranked by magnitude of % MSCA)				Comparison of Uterine Site SMCA (SNK test)
2 weeks	I. Car. 68%	Lower 62%	Upper 42%		$U_3 = U_2 > U_1$
4 weeks	Lower 61%	Upper 43%	Car. 42%		$U_2 > U_1 = U_4$
5 weeks	Car. 75%	I. Car. 57%			$U_4 > U_3$
6 weeks	Upper 84%	Car. 60%	Lower 44%	I. Car. 38%	$U_1 \quad U_3$ $U_1 = U_2 = U_4$ $U_2 = U_3 = U_4$
8 weeks	Upper 70%	Lower 64%	I. Car. 41%		$U_1 = U_2 > U_3$

U_1 = Upper horn; U_2 = Lower horn; U_3 = Intercaruncle area,
 U_4 = Caruncle area.

Underlined mean values are not significantly different statistically.

Figure 9. Morphology of myometrial smooth muscle cells in the nonpregnant uterus at (a) estrus in the upper horn regions, (b) at diestrus in the upper horn regions (at estrus in the lower horn regions), and (c) at diestrus in the lower horn regions. Note the change in morphology of myometrial smooth muscle cells in the lower horn when compared to the same region at diestrus, some cells appear to be more elongated and irregularly shaped while other cells show evidence of increased SMCW. Magnification 260 $\times$.

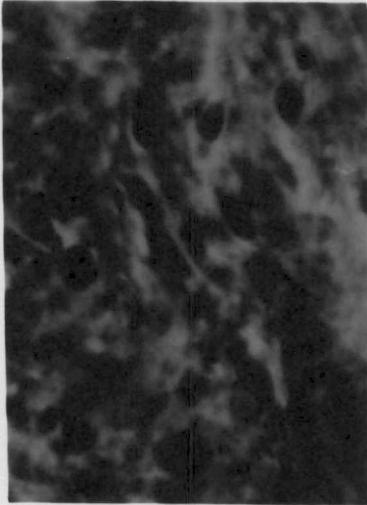
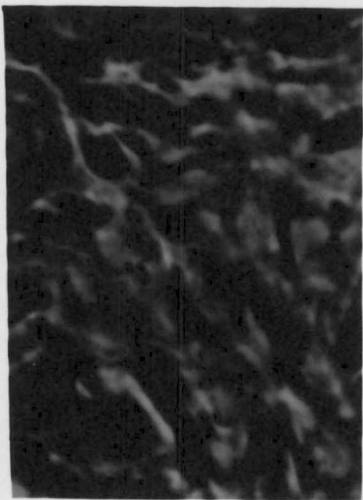
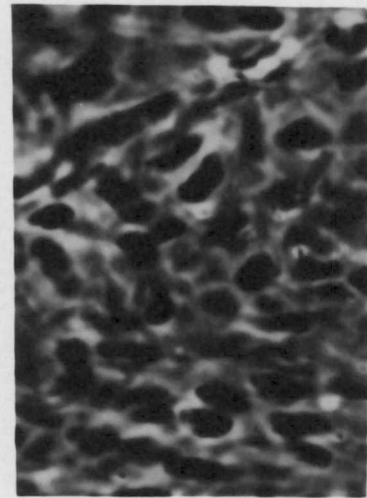
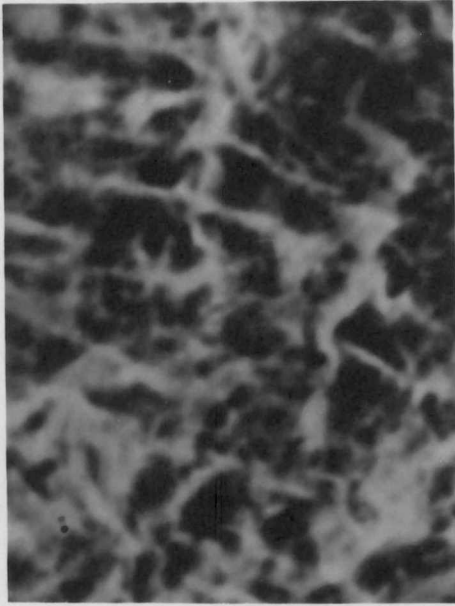
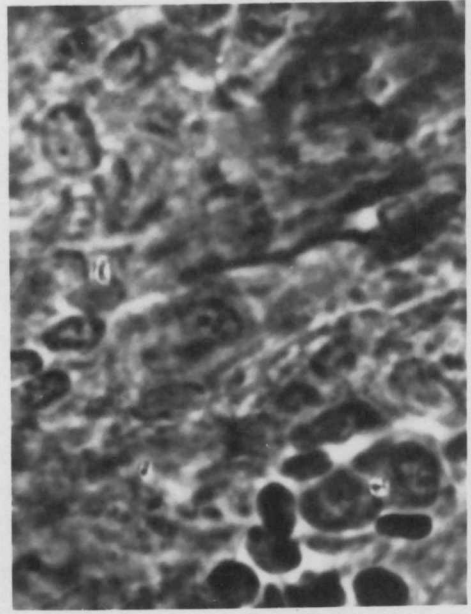
**a****b****c****d**

Figure 9.

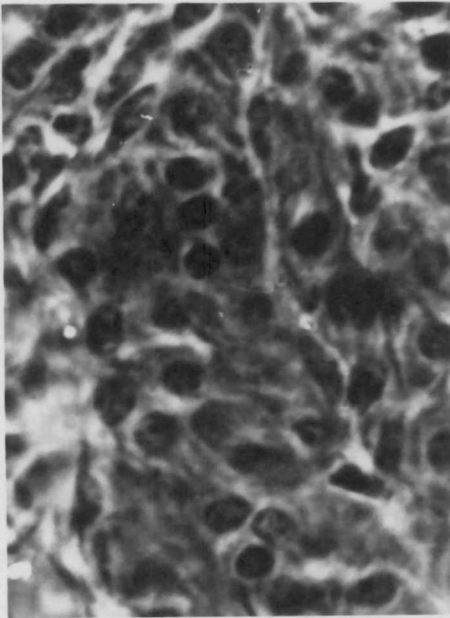
Figure 10. Change in % SMCA and morphology of smooth muscle cells observed in the upper uterine horn of the guinea pig myometrium throughout gestation at (a) 4 weeks, (b) 6 weeks, (c) 8 weeks, and (d) 9 weeks. Note the increase in SMCW from week 6 (Figure 10b) to week 8 (Figure 10c). Myometrial smooth muscle cells increase in SMCW when compared to week 6. There is also an observed increase in % SMCA at week 8 when compared to week 6 and week 9. Magnification 260 $\times$.



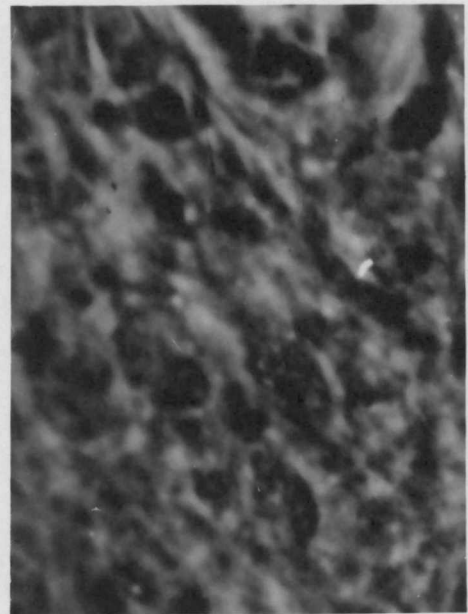
a



b



c



d

Figure 10.

Figure 11. Change in % SMCA observed in the lower uterine horn during early, middle, and late gestation at (a) 2 weeks, (b) 6 weeks, and (c) 9 weeks. Note that it is not possible to identify cell boundaries at week 9 (Figure 11c) when compared to week 2 (Figure 11a) and arrangement of myometrial smooth muscle cells appears as an amorphous mass (Figure 11c) which shows an increase in % SMCA at this stage of gestation. Magnification 260 $\times$.

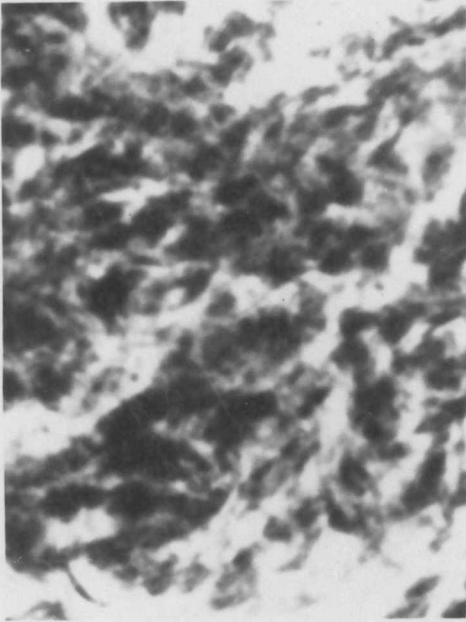
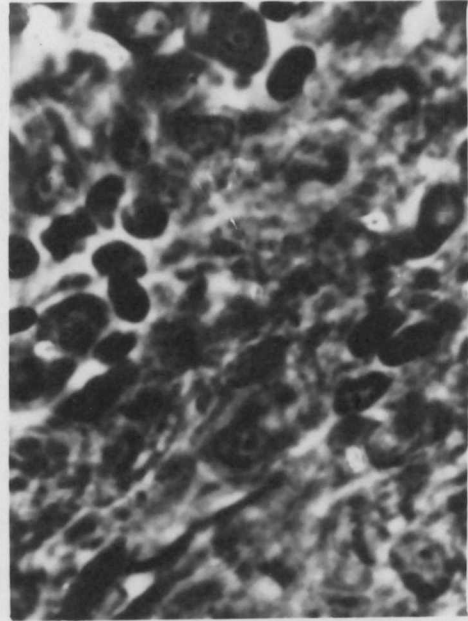
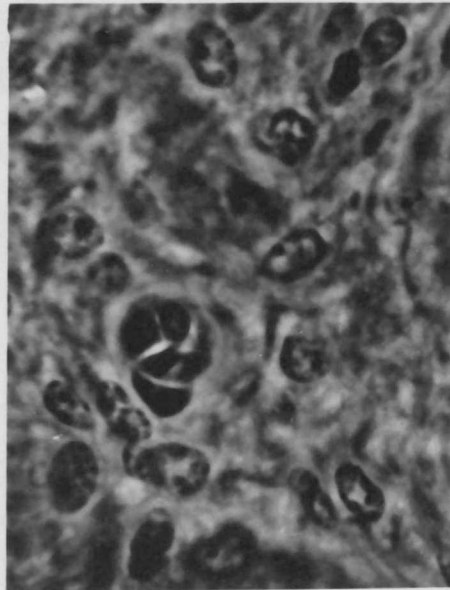
**a****b****c**

Figure 11.

Figure 12. Change in % SMCA observed in the intercaruncle regions of the guinea pig myometrium throughout gestation at (a) 2 weeks, (b) 6 weeks, (c) 8 weeks, and (d) 9 weeks. Note the change in intercellular space at week 8 (Figure 12c) which appears greater when compared to the same region at week 6 (Figure 12b) and week 9 (Figure 12d). Percent SMCA is observed to be greater at week 6 and week 9 than at week 8. Magnification 260 $\times$.

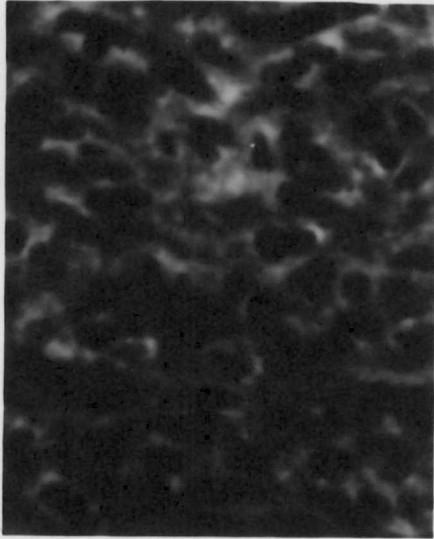
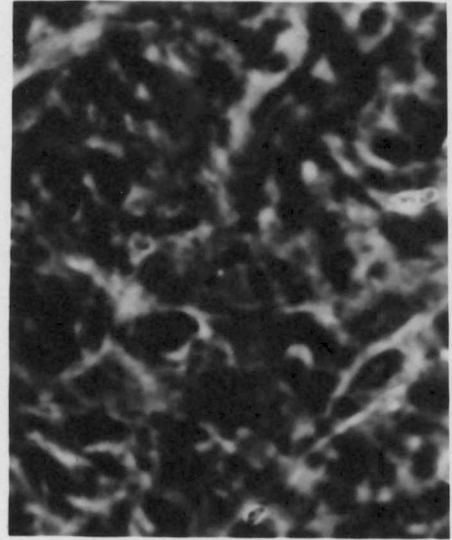
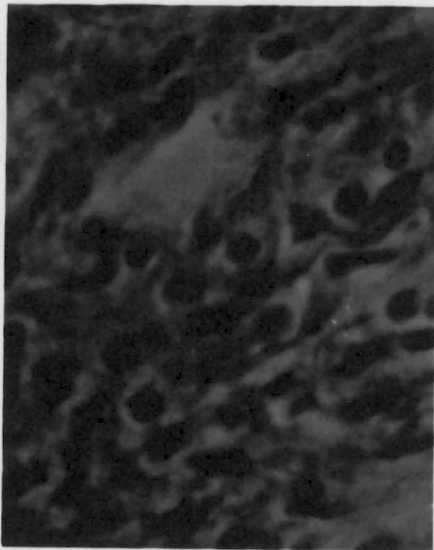
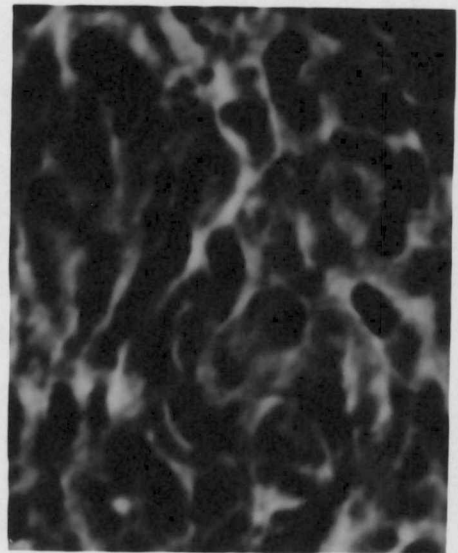
**a****b****c****d**

Figure 12.

The existence of protoplasmic processes are observed in the caruncle regions at all stages of gestation (Figure 13).

Myometrial Smooth Muscle Cells Harvested under
Different Environmental Conditions

Isolated single myometrial cells from tissue which had been incubated in collagenase Medium A at 31 degrees, pH 7.4 for 3-5 hours showed varying degrees of contraction among individual cells. This observation is in agreement with the findings of Small (1974) in which collagenase treatment of the guinea pig taenia coli and vas deferens tissue caused isolated cells to "round up." Observation of the intact tissue incubating in collagenase showed that cells had begun to shorten prior to dispersion. Isolated myometrial cells were more relaxed when incubated with calcium or pretreated with EGTA but the density of cells was small for all stages of the estrous cycle and in the nonpregnant animal as well as at various gestational durations in the pregnant animal. However, the density of dispersed cells harvested from the pregnant uterus at week 7 and from the early post-partum uterus (3-12 hours) is believed to have been greater.

A study of the graph in Figure 14 reveals a more than two-fold increase in area of cell profile at week 9 when compared to the area of cell profile in the nonpregnant isolated myometrial smooth muscle cell. These measurements are in agreement with those of Dessouky (1969). According to the data in Table 21 the greatest increase of change in area of cell profile occurred between week 5 and week 7 (Table 21, Figure 14).

Figure 13. Percent SMCA observed in the caruncle regions of the guinea pig myometrium throughout gestation at (a) week 4, (b) week 6, (c) week 7 and (d) week 9. Note that protoplasmic processes exist among myometrial smooth muscle cells in the caruncle regions at all stages of gestation observed in this study (Figures 13a-d) with no observable change in % SMCA. Magnification 260 $\times$.

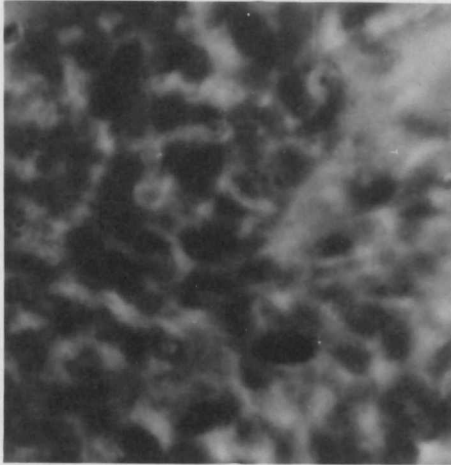
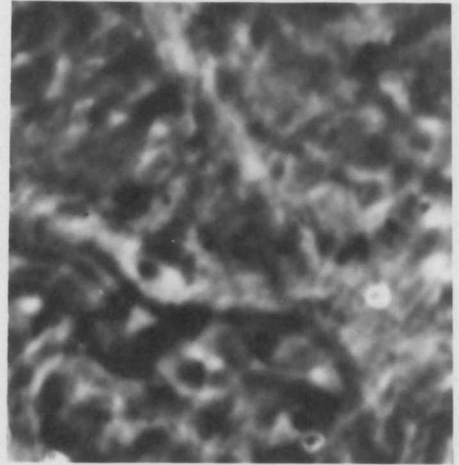
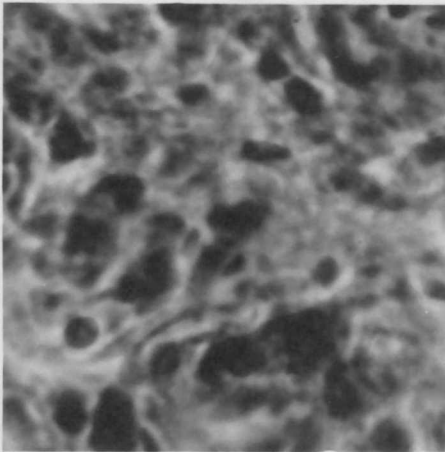
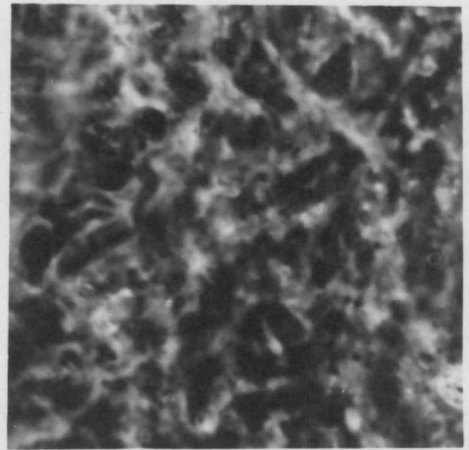
**a****b****c****d**

Figure 13.

Table 21. Changes in area of cell profile in single myometrial smooth muscle cells (SMC) isolated from the pregnant uterus

Gestation Duration	Conversion Factor SMC/RBC	Volume of RBC/Micron ³	Area of Cell Profile SMC/Micron ³
Control	29/1	84	2.4×10^3
5 weeks	33/1	84	2.8×10^3
7 weeks	60/1	84	5.0×10^3
9 weeks	64/1	84	5.3×10^3
Postpartum	64/1	84	5.3×10^3

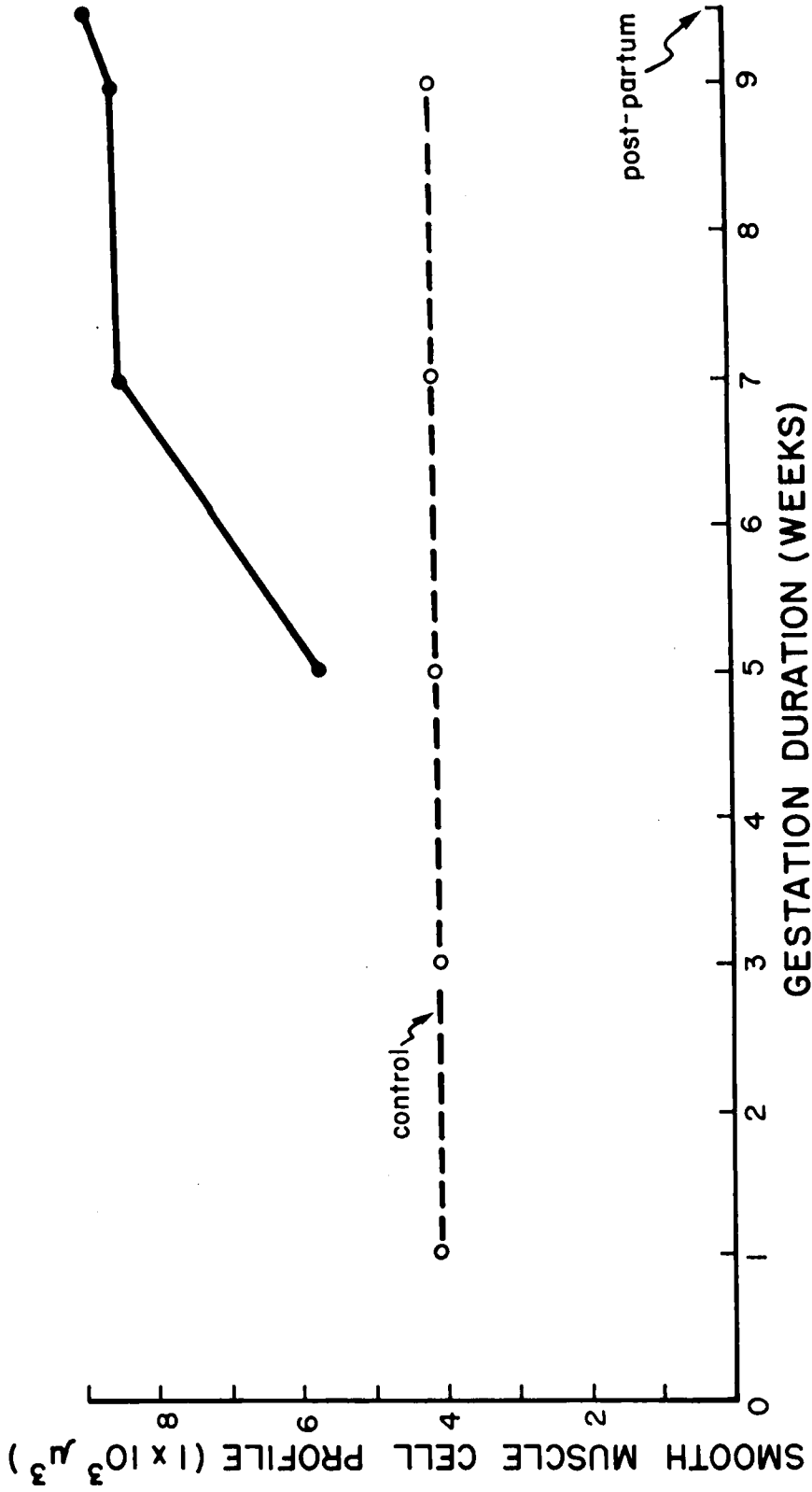


Figure 14. Changes in area of cell profile of myometrial smooth muscle cells isolated from the guinea pig uterus during the last half of gestation (data obtained from a photograph). Note the two-fold increase in area of cell profile observed between week 5-7.

A detailed description of cell filaments or membranes cannot be made because of the enormous amount of connective tissue which covered the dispersed myometrial smooth muscle cells (Figure 15).

Figure 15. Change in cell configurations of smooth muscle cells isolated from the pregnant and nonpregnant uterus: (a) nonpregnant uterus, (b) 5 weeks of gestation, (c) 7 weeks of gestation, (d) 9 weeks of gestation, and (e) postpartum uterus. Note that all cells are spindle-shaped in all stages of gestation but appear larger at week 5 and week 7 (Figures 15b,c) and taper more gradually toward each end than in the nonpregnant uterus (Figure 15a). Observe the undulations of cell membranes in smooth muscle cells at week 9 (Figure 15d) and the more contracted state observed following partuition (Figure 15e).

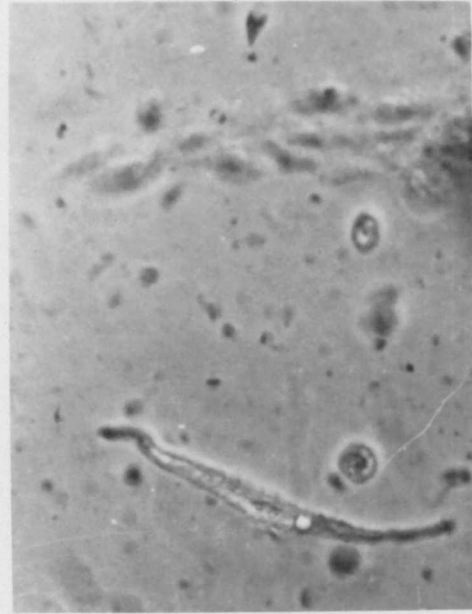
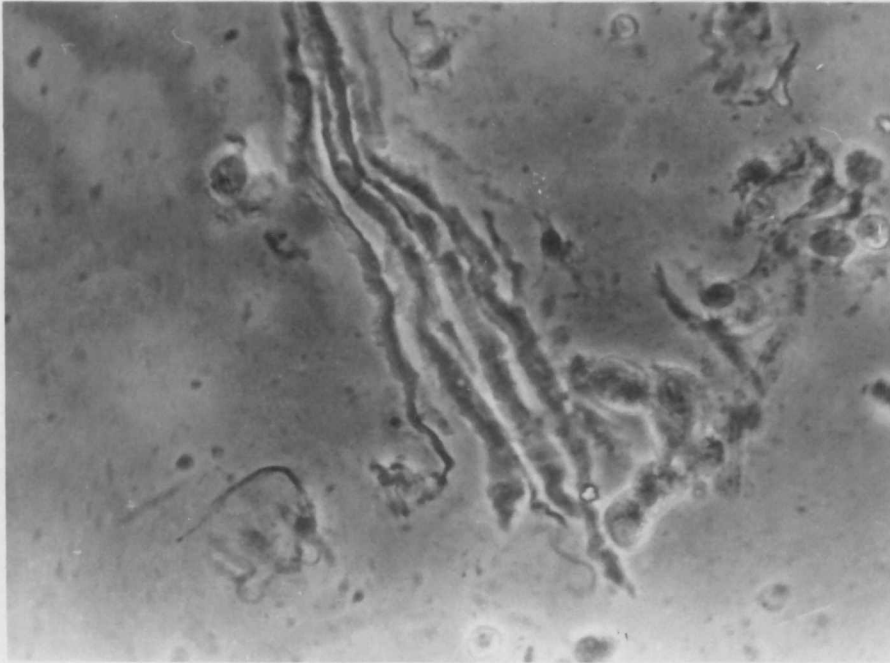
**a****b****c**

Figure 15.



d



e

Figure 15 (continued).

IV. DISCUSSION

Infertility and Irregularity of Estrous Cycle Observed in Breeding Colony

The findings of Long et al. (1922) and Hafez (1970) in the rat support the findings of this research that some females observed with vaginal plugs during estrus prove to be infertile. Some rats and guinea pigs mate at fairly long intervals without ever becoming pregnant due to an infection which blocks the lumen of the uterine tube but does not prevent ovulation (Long, 1922; Hafez, 1970). Our records of unfertile matings in our breeding colony occurred during late summer between the months of June and August. Cessation of the estrous cycle followed a pseudopregnant condition which was recognized by an edematous swelling of the uterus and vagina but lacked embryos. Pseudopregnancy may be caused by sterile matings or mechanical stimulation of the cervix which causes the next estrous cycle to be delayed because the corpus luteum continues to secrete progesterone for a prolonged period of time (Long et al., 1922; Young et al., 1939). Other factors which influence the estrous cycle length are environmental factors such as light and temperature fluctuations as well as hormonal imbalances.

The guinea pig reaches sexual maturity at the early age of one month but the most fertile matings occur when the female is 80 to 85 days old (Mosesson et al., 1968; Hafez, 1970). However, fertility of both women and laboratory animals decreases as maternal age increases (Hafez, 1970).

Patterns of Uterine Growth in the Guinea Pig
Myometrium during Gestation

Three periods of uterine growth have been observed in the pregnant uterus (Reynolds, 1949). Phase A takes place during the first week of gestation when estrogen and progesterone stimulates hyperplasia of the endometrium and myometrium but cause little or no increase in uterine weight. Phase B is the period of uterine enlargement which begins after implantation and proceeds concurrently with the products of conception (Reynolds, 1949; Ivy, 1949). Phase C is the period of uterine stretching which is characterized by marked decrease in uterine growth because of the conceptus that takes place during late gestation (Reynolds, 1949; Bradin, 1949; Ivy, 1942).

The data obtained from this study indicates that very little growth takes place in the whole organ during the first two weeks following conception (Figures 1 and 2, pages 17 and 18) but show a four-fold increase in gross uterine weight between week 2 and week 8 of gestation (Figure 1). A look at the change in area of cell profile of individual myometrial cells isolated from the pregnant uerus at various gestation durations reveals that the period of most active growth is between week 5 and week 7 (Figure 14, page 56). This pattern of growth is similar to that reported in the human myometrium (Laguens et al., 1964) in which the greatest increase in growth occurs during the last half of gestation and occurs in the guinea pig during the last third of gestation (Dessouky, 1968).

The peak values of growth activity in myometrial cells in the pregnant uterus corresponded in this study with peak values of plasma unconjugated estrogens which is observed in this species between day 31 and day 56 of gestation (Challis et al., 1971). The role of estrogen and its influence on the growth of myometrial smooth muscle will be discussed in another section of this thesis.

Some Factors Which Influence Growth of Myometrial Smooth Muscle during Gestation

Recent studies show that hyperplasia of myometrial smooth muscle cells is one of the main factors which contributes to the growth of uterine smooth muscle during gestation (Reynolds, 1949; Kang et al., 1975). However, there is no agreement as to the specific periods of gestation to which it is limited. In the guinea pig (Mehrotra, 1973) and in the rabbit (Reynolds, 1949; Gander, 1930), mitosis is restricted to the 5-6 day period after mating. However, it has been reported in the rat (Frobose, 1932) that smooth muscle cell mitoses can be observed in the caruncle and intercaruncle regions at all stages of gestation. The data obtained from this study indicates a 70% increase in the number of SMC/A during the first two weeks of gestation (Figure 3, page 20). In order to obtain an accurate measurement of SMC/A, a mitotic index of each uterine site or measurement of the DNA content per mg of protein per uterine site would be required.

Hyperplasia of uterine smooth muscle cells is hormonally controlled by estrogen and progesterone which play an important role in stimulating

mitotic division of smooth muscle and its associated connective tissue (Crandall, 1938; Kang et al., 1975; Mehrotra, 1973). When ovariectomized rabbits were injected with a single dose of estrogen (50 I.U.), the incidence of mitotic figures was significantly higher than in untreated animals (Crandall, 1938) with little or no effect on the smooth muscle cell mitoses. However, ovariectomized rabbits treated with daily doses of progesterone (0.2 mg) show a significantly higher number of mitoses in the smooth muscle than in connective tissue (Crandall, 1938). Recent studies show that both estrogen and progesterone are required to stimulate mitosis in the myometrium of the mouse (Finn et al., 1967) and in the guinea pig (Mehrotra et al., 1973). The role of progesterone as a requirement for stimulating smooth muscle cells mitosis is also supported by the finding of Mehrotra et al. (1973), who reported that few mitoses are observed in the myometrium of the ovariectomized guinea pig after a single injection of progesterone (2.5 mg.). However, the combination of estrogen (100 mg. of 17 β -estradiol) plus the progesterone (2.5 mg.) produced a significant increase in the number of mitoses.

A second factor which has been shown to stimulate mitosis in myometrial smooth muscle cells is stretch. When paraffin pellets were inserted in the myometrium of ovariectomized rabbits a stimulation in smooth muscle cell mitoses were observed (Allen, 1937; Reynolds, 1949). However, this hyperplasia was quite different histologically from the growth that resulted from estrogen and progesterone stimulation since the vascular effects were much less pronounced and there was no evidence of presecretory changes in the glandular epithelium such as found in the estrogen treated rabbit (Reynolds, 1949; Mehrotra, 1973).

As pregnancy progresses, myometrial smooth muscle cells increase in size rather than in absolute numbers (Reynolds, 1949) due to an increase in the number and size of the cell's major organelles (Laguens, 1964; Maibenco, 1964; Dessouky, 1969; Althoff, 1970) and increased amounts of contractile proteins (Maibenco, 1964; Michaels et al., 1969; Schofield et al., 1963). When ovariectomized rats are administered a single injection of estrogen dipropionate (10 ug in 0.1 ml of cottonseed oil), an increase has been measured in the number of glycogen granules, ER, mitochondria, and ribosomes (Bo et al., 1968; Dessouky et al., 1968; 1969; Frederici et al., 1968; Ross et al., 1967), enzymatic activity (Bo et al., 1968; Noteboom et al., 1963), contractile proteins and high energy phosphates (Needham et al., 1968; Csapo, 1971) and the rate of turnover of cellular components (Bo et al., 1968).

Evidence in the literature supports the theory that chronic stretch also stimulates hypertrophy of myometrial smooth muscle during gestation due to the gradual increase in intrauterine volume (Marshall, 1973). When balloons were introduced into the uterine cavity of ovariectomized rabbits and inflated to volumes of the conceptus at various gestational durations, hypertrophy of myometrial smooth muscle cells was induced as a function of the amount and duration of stretch (De Clue et al., 1969; De Mattos et al., 1967). When stretch was combined with estrogen and progesterone, hypertrophy was accentuated (De Clue et al., 1969; De Mattos et al., 1967; Marshall, 1973). However, estrogen treatment alone reduces the growth response to distention when compared to that of the nontreated ovariectomized rabbits (Reynolds, 1965).

Changes in Cell Width Observed in Myometrial
Smooth Muscle Cells during Gestation

The changes observed in SMCW during this investigation cannot be considered a reliable technique for measuring cell growth since the width of smooth muscle cells change with cell configuration (Bagby et al., 1971; Lane, 1965), state of contraction (Fay et al., 1972) angle at which the knife cuts the cell (Lane, 1965), hormonal state of the uterus (Kang et al., 1975) and the amount and duration of stretch imposed upon it (Csapo et al., 1965; De Mattos et al., 1967; Michaels, 1969). I failed to fix tissue for each uterine site at in situ length which would have provided basis for making more valid comparisons.

Data obtained from this investigation reveals significant variation in SMCW among the four uterine sites at various gestational durations (Table 16, page 40). SMCW ranged from 2.2 to 5.6 microns in diameter in the pregnant guinea pig. Similar values were reported by Dessouky for this species while higher values are reported in the pregnant myometrium of the rabbit (Crandall, 1938). Peak values for greatest increase in SMCW is during the second trimester of gestation for all uterine sites followed by a sharp decrease in SMCW during the third trimester of gestation (Figures 5 and 6, pages 26 and 27). It was observed that the first uterine site to show a marked decrease in SMCW was the intracaruncle region (Figure 6) which are sites located between individual embryos which suggest that chronic stretch may have induced positive SMCW changes during the second trimester of gestation and negative SMCW changes during the third trimester of gestation caused

by increased cell length and tension changes due to an alteration of the connective tissue and contractile proteins (Reynolds, 1949).

Increased Myometrial Smooth Muscle Area—
Relationship to Myometrial Contractions
during Gestation

Smooth muscle cells of the pregnant uterus do not only increase in cell size but also in total surface area by increasing the number and size of the protoplasmic processes and outpouchings of the cytoplasm which permits greater exchange of substances between the cell and its extracellular environment (Dessouky, 1969). Electron microscopic studies of the pregnant guinea pig myometrium reveal that the myofilaments of myometrial smooth muscle cells become more closely packed together during late pregnancy which is believed to be caused by the crowding of cellular organelles which are greatly increased during gestation (Dessouky, 1968). According to the data obtained during this investigation, it was revealed that SMCA showed a significant decrease in the upper horn and caruncle regions near partuition but showed a significant increase in SMCA in the lower horn and intercaruncle regions which may suggest that the improved propagation of muscle contraction observed in the lowermost portion of the duplex type uterine horn at partuition (Reynolds, 1949) is achieved by increased number of interfiber junctions (Ichikawa et al., 1970). This theory is supported in the literature which suggests that the uterus is electrically quiet when few cellular junctions exist between individual myometrial smooth muscle cells. These interfiber junctions are stimulated by and maintained by the action of estrogen (Bergman, 1968) while progesterone treatment reduces the

number of cellular junctions and impairs propagation of muscle contraction (Ichikawa et al., 1970).

The influence of placental and fetal hormones on uterine contractility was observed by Wood (1972) who suggested that placental sites block the propagation of contraction waves during gestation and the electrical activity is more evident at the antiplacental sites (Wood, 1972). These experimental results were observed in the rabbit in which myometrial contractions show a decrease in frequency and amplitude throughout gestation but show a drastic increase in both frequency and amplitude just prior to partuition. However, in the guinea pig, myometrial contractions show only a slight variation in frequency with no change in amplitude (Greig, 1939) which could suggest that at some specific area scattered along the contraction wave, slight impairment of the rate of propagation is caused. In this study, interfiber junctions existed among the myometrial smooth muscle cells in the caruncle regions (placental sites) at all gestational durations (Figure 13, page 53). However, interfiber junctions are not present at all gestational durations in the intercaruncle regions (Figure 12, page 50). This may suggest that increased SMCA evolves in the intercaruncle areas just prior to partuition and is the last segment of the pregnant myometrium to show synchronized activity in the guinea pig.

The Isolated Myometrial Smooth Muscle Cell— A Test Model for Uterine Growth Studies

In order to develop an effective technique for the isolation of single myometrial cells, a series of preliminary tests should be

designed to determine the exact chemical composition and physiological environment of the collagenase dispersing agent needed to give the highest yield of cells from this organ. Both organ and specie specificity have been observed in collagenolytic activity under the same environmental conditions. Organs such as bone (Peck, 1974) produce high yields of isolated single cells when tissue fragments are incubated in 1-3 mg/ml of collagenase, pH 6.8 at 31-37 degrees for 1-5 hours. Other organs such as the toad stomach, Bufo marinus (Bagby et al., 1971) heart cells (Vahouny et al., 1967); chicken gizzards (Small, 1974) require pretreatment of tissue fragment with trypsin or chelating agents (Small, 1974) prior to incubation in collagenase media to obtain a maximum yield of isolated cells. During this investigation, some variation was observed in the ability of collagenase to disperse isolated myometrial cells even following pretreatment with EGTA, pH 7.4, for 1-3 hr. Similar variation in response of individual animals has been reported when tissue has been obtained from old subjects (Keech, 1955, Mandl, 1970; Hilfer, 1973) which was caused by cross-linkage of the collagen molecule (Mandl, 1970).

There is evidence in the literature that collagenolytic activity is controlled by the reproductive state of the uterus. When progesterone (1×10^{-6} M) or its potent analog, Provera (1×10^{-6} M) was added to in vitro cultures of postpartum uterine fragment, collagenolytic activity was blocked if added within 20 hr. after delivery but activity was not inhibited if progesterone was added later (Goodall, 1966; Jefferey et al., 1971). During this investigation, an accurate cell count for comparison

in the effectiveness of collagenase to disperse myometrial cells for various reproductive and hormonal states of the myometrium was not made. However, the most successful experiment which yielded the greatest density of isolated myometrial cell occurred between week 7 and week 8 of gestation which corresponds to a gestational duration in which plasma progesterone levels are low in the guinea pig (Joshi et al., 1973). The density of isolated myometrial cells was very low for all other stages of gestation and the estrous cycle was low which might suggest the presence of some endogenous inhibitor of collagenolytic activity in the tissue.

This investigation will serve as a basis for developing more effective techniques for isolation of single myometrial cell under optimal physiological and environmental conditions to produce the highest yield of isolated cells which shall provide a model for making quantitative growth changes in the uterus by more accurate and sensitive methods than were possible during this investigation.

V. SUMMARY

1. Growth of uterine smooth muscle in the whole organ showed peak values during the last half of gestation.

2. It was observed that no uniform growth response occurred in the four different uterine sites with less variation observed in the caruncle and intercaruncle areas. More sensitive techniques are required to measure actual growth changes and to distinguish these from artifacts which resemble growth changes.

3. Single isolated myometrial cells were isolated from the nonpregnant and pregnant uterus by collagenase digestion with prior treatment with 2 mM EGTA. This is the first time that single isolated cells have been dispersed in this organ in any species. The difficulty in isolating myometrial cells from the uterus varied among individual guinea pigs. This may be related to the age and reproductive state of the uterus.

4. Measurement of isolated guinea pig myometrial cells from the pregnant uterus showed a two-fold increase in cell profile between Day 35 and Day 49 of gestation with a gradual increase until partuition.

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VITA

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