The Fimbrial Ovarian Attachment in Cynomolgus Macaques

by

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ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
C	centigrade
Ci	curie (2.22×10^{12} DPM)
DPM	disintegrations per minute
E	estradiol
FOA	fimbrial ovarian attachment
mg	milligram(s)
ml	milliliter(s)
mm	millimeter(s)
mmol	millimole(s)
n	number
ng	nanogram(s)
p	probability
P	progesterone
pg	picogram(s)
S.E.M.	standard error of the mean
TCA	trichloroacetic acid
TSH	thyroid-stimulating hormone (thyrotropin)

uCi	microcurie(s)
ug	microgram(s)
ul	microliter(s)
um	micron(s)
U.S.P.	United States Pharmacopeia

Introduction

Fallopius (1561) gave the first accurate anatomical description of the tube named after him. The following is an excerpt from his poetic report on the oviduct:

> "The slender and narrow seminal passage arises very white and sinewy from the uterine horn, but after it has passed outward a little way it becomes gradually broader and curls like the tendrils of a vine until near the end when the tendril-like curls spread out, and it terminates in a very broad ending which, because of its reddish color appears membranous and fleshy. This ending is quite shredded and worn, as if it were the fringe of a worn piece of cloth, and it has a broad opening which always lies closed because of the apposition of those fringed ends. However, if they be carefully opened and spread apart they form, as it were, the bell-like mouth of a bronze trumpet. Consequently since, where the tendril-like curls may be removed or even added to this classical instrument the seminal passage will extend from its head even to its uttermost ending, and so it has been designated by me the trumpet of the uterus."

DeGraaf (1672) was the first to note that the fimbriated end of the oviduct was attached to the superior pole of the ovary:

> "The extremities of the tubes furthest from the uterus, after their sudden gathering together, spilt into many parts, some of which attach themselves to the `testicles' (ovaries). Hence Fallopia and other anatomists seem to us, in claiming that the tubes have no common link at all with the `testicles', to have wandered from the road of truth."

Richard (1851) more fully described the gross relationships of this attachment and it was later named the fimbria ovarica by Henle (1866). Wislocki (1932) described the connection between the ovary and

oviduct in various nonhuman primates. Recently, Okamura (1977a) has demonstrated smooth muscle cells within the connection between the ovary and the fimbriae in women. Nevertheless, today, as in DeGraaf's era, many anatomists are unaware (Tortora, 1980) that the tubes have a common link with the ovaries. In this thesis I will describe the attachment in nonhuman primates and will suggest physiological roles it may play in the female reproductive tract.

Anatomy of the Oviduct

The oviduct is supported in the upper free border of the broad ligament. The part of this ligament between the oviduct, the ligament of the ovary and the mesovarium is termed the mesosalpinx. In nonhuman primates the oviduct is situated just inferior to the free border of the mesosalpinx, creating a superior, as well as an inferior mesosalpinx. There are several recent descriptions of the anatomy and physiology of the oviduct (Hafez and Blandau 1969; Woodruff and Pauerstein 1969; and Johnson and Foley, 1974). The emphasis of our research is on the distal portion of the fallopian tube where the tube proper expands into a trumpet-shaped infundibulum. The thin walled infundibulum with its outer longitudinal and inner circular muscle layers, contains the abdominal ostium of the tube, which communicates with the general abdominal cavity, and is surrounded by mucosal folds called fimbriae.

The simple columnar fimbrial epithelium is composed of ciliated and secretory cells that are present in approximately equal numbers in the late follicular phase (Brenner et al., 1974; Verhage et al., 1979). Underlying the fimbrial epithelium is the lamina propria, a loose connective tissue composed of collagen fibers, blood vessels, lymphatics, macrophages, mast cells and fibroblasts.

Branches from the ovarian and uterine arteries supply blood to the primate oviduct (Ginther et al., 1974; Eddy and Pauerstein, 1980). In the rhesus monkey the uterine artery supplies 91 to 100 percent of the arterial blood to all segments of the reproductive tract except during late pregnancy when the ovarian artery supplies most of the blood to the ovaries and oviducts (Wehrenberg et al., 1977). The venous drainage of the oviduct follows the arterial supply (Eddy and Pauerstein, 1980).

The classic work on the lymphatics of the fallopian tube was done by Anderson (1927) in the sow. She observed that lymph vessels in the tubal portion of the oviduct were larger in the follicular phase than in the luteal phase. Little work has been done on the lymphatics of the primate oviduct. Sampson (1937) concluded that in women the fimbrial mucosa is richly supplied with lymphatics which drain into lymph vessels in the wall of the infundibulum. Three distinct lymphatic networks drain the mucosa, muscularis, and serosa portions of the oviduct. When they emerge from within the oviduct those three systems combine to enter

the mesosalpinx and ultimately drain into the paraaortic nodes (Beck and Boots, 1974; Eddy and Pauerstein, 1980).

The fallopian tube is innervated by both the parasympathetic and sympathetic portions of the autonomic nervous system. There is a rich adrenergic innervation to the isthmus, whereas only a few adrenergic nerves are found in the ampulla (Brundin, 1969). The distal portion of the tube is innervated with sympathetic nerves derived from the eleventh thoracic to the upper lumbar nerves, and with parasympathetic vagal fibers from the ovarian plexus (Eddy and Pauerstein, 1980).

Hormonal Regulation of the Oviduct Epithelium

Ovarian steroids induce dramatic cyclic variations in the oviductal epithelium. This phenomenon was originally noted by Allen (1927) in rhesus macaques. Several recent reviews (Brenner and West, 1975; Hafez and Blandau, 1969; and Johnson and Foley, 1974) discuss the role of hormones in regulating growth and differentiation in the oviductal epithelium of various species. In nonhuman primates estradiol induces growth and differentiation in the oviductal epithelium, as indicated by an increase in cell height, mitosis, ciliogenesis, nuclear swelling, and the appearance of secretory granules. In the late follicular phase the epithelium reaches its maximum differentiation, containing fully developed ciliated and secretory cells. After

ovulation, under the influence of progesterone, the epithelium begins to regress as evidenced by scattered epithelial cell death, the presence of macrophages, a decline in cell height, deciliation, nuclear wrinkling, and atrophy of the secretory cells. These effects of estradiol and progesterone on oviductal epithelium have been demonstrated in rhesus macaques (Brenner et al., 1974), pig tail macaques (Rumery et al., 1978; Odor et al., 1980), and baboons (Odor and Augustine, 1979).

Women also exhibit cyclic changes in their oviductal epithelium (Novak and Everett, 1928). However cyclic changes are less dramatic in women than in other primates. During each menstrual cycle in women the secretory cells undergo a complete cycle of differentiation and dedifferentiation, while only 10-12% of the ciliated cells lose and regenerate cilia (Verhage et al., 1979). Unlike the rhesus monkey, the human menstrual cycle is characterized by a luteal rise in plasma estradiol levels. The higher level of estradiol in the luteal phase may protect the human oviductal epithelium from the dramatic deciliation that occurs in nonhuman primate oviducts (Brenner and West, 1975).

The cyclic changes in oviductal epithelium are correlated with levels of ovarian steroids in the plasma. These changes are regulated in the target cell through steroid hormone receptors. Receptor levels for estradiol and progesterone, in the human oviduct epithelium, change throughout the menstrual cycle in response to levels of ovarian hormones in the plasma (Pollow et al., 1981; Punnonen and Lukola, 1981; and

Pino et al., 1982). Estrogens stimulate the synthesis of estrogen, progestin, and androgen receptors, while progesterone antagonizes these effects (Brenner and West, 1975).

In spayed rhesus macaques treated with estradiol and progesterone to induce artificial menstrual cycles, estradiol treatment alone increased estrogen receptor levels in the oviduct, however those levels were decreased to castrate levels when progesterone treatment was added to the estradiol treatment (Brenner et al., 1974). In the oviducts of cycling cynomolgus macaques nuclear and cytoplasmic estrogen receptor levels were two-fold higher in the follicular compared to the luteal phase (West et al., 1979; West and Brenner, 1983).

Evidence of cyclic changes in estradiol and progesterone receptor levels in the oviducts of women is less clear. Pollow et al. (1981) demonstrated the highest estradiol and progesterone receptor levels in the proliferative phase, and the lowest receptor levels after ovulation. Robertson et al. (1975) reported similar results regarding the estradiol receptor. However, Flickinger et al. (1977) did not find any difference in levels of estradiol receptors between the follicular and luteal phases in women.

Mechanisms of Ovum Pick-Up

A modern concept of ovum pick-up, based on observations at laparoscopy, is that the fimbriae are brought into contact with the ovary, at the time of ovulation, by contraction of the smooth muscle of the mesosalpinx and tubo-ovarian ligaments (Decker, 1951; Pauerstein and Eddy, 1979) and that pickup of the ovum is facilitated by the ciliary activity of the fimbriae (Blandau, 1969).

Westman observed movements of the oviduct and ovary at the time of ovulation through an abdominal window in the rabbit (1926) and through a laparoscope in the monkey (1937). In both species he observed muscular activity in the mesenteries at the time of ovulation, that brought the fimbriae and ovary into contact. Since then several investigators have studied the muscular arrangement in the primate fallopian tube and its mesenteries (Wislocki, 1932; Horstsmann, 1952; and Hansen, 1957). Stange (1952) described a `tubal attracting muscle' in women that originates at the tubal end of the fimbria ovarica and terminates at the cranial pole of the ovary. Okamura et al. (1977b) verified the presence of smooth muscle in the fimbria ovarica of women and demonstrated that its highest contractile activity occurred during the ovulatory phase. Both authors postulated that the muscle associated with the fimbria ovarica could bring the fimbriated end of the oviduct to the ovary at the time of ovulation.

Cohen and Katz (1978) found that the length of the fimbria ovarica in fertile women was significantly shorter than in some patients with patent, but convoluted oviducts. On the basis of this evidence Cohen (1980) developed a surgical method for shortening abnormally long fimbria ovaricae in infertile women. His results indicated that these women had increased fertility following the restoration of their fimbrial ovarian proximity. In contrast, Eddy and Laufe (1983) found a 60% pregnancy rate in rhesus monkeys who had their fimbriae and ovary microsurgically dissociated. They concluded that ovum-pick up could be successful with a healthy fimbriae located in proximity to the ovary, but not necessarily directly attached to it or in contact through a `given arrangement of mesenteries'.

There have been recent studies of the role of ovarian hormones in tubal motility. Halbert and Conrad (1975) demonstrated that smooth muscle in the rabbit tubal mesentery (mesotubarium superius) decreased contractile activity 60 days postovariectomy, while a dramatic increase in activity occurred 12 hours postovariectomy, and at the time of ovulation. Boling and Blandau (1971) also found that contractions in the smooth muscle of the rabbit oviduct and mesenteries were gentle under estrogen dominance, and vigorous after estrogen withdrawal or progesterone injection in the estrogen-primed animal. They suggested that the vigorous contractility in the rabbit oviduct around the time of ovulation was triggered in some manner by progestins. All investigators agreed that oviductal contractility was reduced in the castrate.

The degree to which the mesenteries of the oviduct enclose the ovary in an ovarian bursa, the anatomical relationship of the ovary to the oviduct, and the extent of coiling of the oviduct have been used by Beck (1972) to describe eight anatomical classes of oviducts in mammals. Classes one to three are represented by primates, and no ovarian bursa is formed. In classes four through six, which include pigs and rabbits, an open ovarian bursa is formed by the oviductal mesenteries. In rodents, classes seven and eight, a complete ovarian bursa is formed. Forty two species of mammals were examined, representing ten orders. In classes with an open ovarian bursa, or no bursa, there was always an attachment between the ovary or mesovarium and the fimbriated end of the oviduct. However, in classes with a complete ovarian bursa the infundibulum was within the bursa, close to the ovary, but was not attached. This suggests that an attachment between the ovary and oviduct has evolved, in those species without a complete ovarian bursa, to retain the ovarian fimbrial proximity.

Blandau (1969) has demonstrated that the ovum in the cumulus mass is transported from the ovarian surface, to the fimbriae, and into the oviduct ostium by means of ciliary action. Odor and Blandau (1973) examined rabbit fimbriae to establish the role of hormones in ovum pick-up, and found that fimbriae with less than 44% ciliated cells failed to transport eggs, while fimbriae that had at least 60% ciliation

or higher transported the eggs. Blandau and Boling (1973) also found that eggs denuded of their sticky cumulus mass were not transported efficiently.

Although the evidence indicates that cilia are responsible for the transport of the ovum from the ovarian surface to the oviduct, there are case reports of pregnancies in women with the "immotile cilia syndrome" (Jean et al., 1979; Rott, 1979; and Afzelius et al., 1978). On the other hand Brosens and Vasquez (1976) have presented evidence that a reduced number of cilia in the fimbriae may impair fertility. Norwood and Anderson (1980) suggested that since conception is possible with immotile cilia, but too few cilia impairs fertility, cilia may be required for some phase of ovum transport that does not involve their ability to beat. Several investigators have observed a specialized glycocalyx in the tip region of oviductal cilia in the mouse (Dirksen and Satir, 1972) and rabbit (Anderson and Hein, 1977). In rabbits this glycocalyx contains a high density of anionic sites (Anderson and Hein, 1977). Norwood and Anderson (1980) demonstrated that administration of polycations to the rabbit oviduct blocked the ability of the oviduct to pick up the cumulus mass in situ. They concluded that the tips of the rabbit oviductal cilia contain adhesive sites that are more critical to ovum pick-up than ciliary beat. Such adhesive sites have not been demonstrated in oviductal cilia of primates.

Fimbriectomy was considered a safe and easily performed method of sterilization in some hospitals for a number of years. Kroener (1969) reviewed over 200 cases of fimbriectomy in which no pregnancies had ensued. He credited this success to an interruption of the fimbrial-ovarian relationship. Recently however, two to three percent failure rates have been reported with fimbriectomies (Oskowitz et al., 1980; Metz, 1978). Oskowitz et al. (1980) postulated that accessory ostia proximal to the fimbriae or lack of resection of all of the fimbriae were the cause of fimbriectomy failure and concluded that this method of sterilization was unacceptable. Metz (1978) suggested that the success of fimbriectomy was more dependent on complete ampullary occlusion than on the absence of the fimbriae. Novy (1980) confirmed this conclusion after he successfully reversed nine fimbriectomies by everting the endosalpinx of the ampulla to create a neofimbria.

Strong evidence exists that muscle contractions of the oviduct and its mesenteries move the fimbriae over the ovary at the time of ovulation, and that the ciliated cells of the fimbriae can hold and move the ovum in its cumulus mass to the ostium of the tube. However, evidence also indicates that ovum pick-up can occur in the absence of the fimbriae. In rare cases the contralateral fimbriae can capture the ovum (First, 1954; Doyle et al., 1966). This latter evidence indicates that the fimbriae are not indispensable to fertility. However, the highly specialized structure of the fimbriae, including the attachment region, has been conserved by evolutionary processes in all primates,

and we can conclude that these structures probably favor reproductive success in these species.

Asymmetry in the Female Reproductive Tract

Although both ovaries in primates are perfused with the same peripheral blood, only one ovary typically ovulates in each cycle. This asymmetry is reflected in the concentrations of steroid hormones in the ovarian venous blood. DiZerega et al. (1980) demonstrated asymmetrical estradiol levels in ovarian venous blood in the rhesus macaques as early as day three of the cycle, and by day seven there was a significant difference in estradiol levels between the two ovaries, even though a dominant follicle was not yet visible. In the late follicular phase in humans (Mikhail, 1970) and rhesus monkeys (Channing and Coudert, 1976) the ovary bearing the preovulatory follicle secretes more estradiol than the contralateral ovary. Prior to ovulation in nonhuman primates (Resko et al., 1975; diZerega et al., 1980) both ovaries secrete preovulatory progestin, while after ovulation the ovary bearing the corpus luteum secretes large amounts of progesterone, and the contralateral ovary contributes little progesterone (Marut and Hodgen, 1982). Marut and Hodgen (1982) also demonstrated in nonhuman primates that the ovary bearing the corpus luteum secreted more estradiol than the contralateral ovary in the early and mid luteal phases, and that this asymmetry

vanished in the late luteal phase as the contralateral ovary again contributed estradiol.

Levels of ovarian steroids are higher in the ovarian venous plasma than in peripheral plasma in humans (Mikhail, 1970; Lloyd et al., 1971) and rhesus monkeys (diZerega et al., 1980). Lloyd et al. (1971) concluded that since there was a higher concentration of ovarian steroids at the local level, the fallopian tube and uterus may be exposed to a different hormonal environment than more peripheral target organs. They postulated direct local access to ovarian hormones through lymphatics or vascular anastomosis. Since ovarian hormones have been shown to dramatically effect the morphology and physiology of the fallopian tube, asymmetrical ovarian hormone secretion could result in local effects in the oviduct adjacent to the more active ovary.

There are several examples in the literature of unilateral reactions in the female reproductive tract. Maas et al. (1976) found a consistent unilateral rise in oxygen tension in response to insertions of a probe within the oviductal lumen in a rhesus macaque only on the side where ovulation had occurred. They postulated that the ovum entering the oviduct triggered a similar response as the probe to provide a well-oxygenated environment for gamete survival, fertilization and embryo development. Contractile activity in the fallopian tubes of women (Sica Blanco et al., 1970) and estrous rabbits (Salomy and Harper, 1971) are independent of each other. The authors theorized that the

reason for asymmetry in tubal motility could be a local difference in ovarian hormone secretion. Spilman et al. (1978) studied this phenomenon further in the rabbit and found no significant correlation between oviductal motility and ovarian vein steroid concentrations, and concluded that changes in ovarian steroid secretion and changes in oviductal motility may be out of phase, where steroid surges may induce delayed changes in oviductal motility. The long tongued bat (Rasweiler, 1972), the short tail fruit bat (Bonilla and Rasweiler, 1974), the little bulldog bat (Rasweiler, 1978), and the little sac-winged bat (Rasweiler, 1982) all exhibit unilateral oviductal reactions. In these bats the oviduct ipsilateral to the ovary bearing a mature follicle or new corpus luteum contains more glycogen, exhibits greater cytoplasmic vacuolation, and frequently is more dilated with fluid than the contralateral oviduct.

Side differences in oviductal estradiol receptor concentrations have been reported. West and Brenner (1983) found a significantly lower level of estradiol cytosol receptor in the oviduct ipsilateral to a functional corpus luteum. Flickinger et al. (1977) also demonstrated a significantly lower level of estradiol cytosol receptor in the fimbriae adjacent to an active corpus luteum, but he found no side differences in receptor levels in the ampulla or isthmus.

Von der Borch (1963) demonstrated that in the brush possum, a monovular and polyestrous marsupial, the uterine horn adjacent to the

ovary bearing a corpus luteum is heavier than the contralateral uterine horn. Marshall (1953) reported that in the giant fruit bat following fertilization, the glands of the adjacent uterine horn undergo progestational changes, whereas the opposite horn retains its estrous appearance, and that the site of this progestational change is confined to the distal end of the horn, where uterine and ovarian tissues lie in close proximity.

Several explanations have been proposed for the phenomenon of unilateral reactions in the female reproductive tract. Ginther (1974b; 1976) has demonstrated a local utero-ovarian pathway for the uterus induced regression of the corpus luteum in sheep, cattle, and guinea-pigs. Since in these animals the ovarian artery coils around the uterine vein, he suggested that a luteolysin in the uterine vein could be transmitted to the ovary by diffusion through the vessel walls to the adjacent ovarian artery and transported directly to the ovary. Similar local concentrating mechanisms have been demonstrated for testosterone in the pampiniform plexus of rats (Free and Jaffe, 1975), monkeys (Dierschke et al., 1975), and men (Bayard et al., 1975). Walsh et al. (1979) have shown that infusion of ^{3}H-progesterone into the uterine vein of ewes increased the concentration of ^{3}H-progesterone in ovarian arterial plasma, but not in mesenteric arterial plasma. They suggested that countercurrent exchange of steroid hormones provides the reproductive organs with a means of maintaining steroid concentrations independent of the peripheral circulation. Countercurrent exchange of

hormones has not been demonstrated in primates, although the extensive surface contact between veins and arteries in the ovarian pedicle and uterus in rhesus monkeys (Ginther et al., 1974a) and women (Bendz, 1977) suggests that it may occurr. Wimsatt (1979) suggested that asymmetry may imply either a differential sensitivity to hormonal stimulus, or a difference in the efficiency of hormone delivery on opposite sides of the tract. Rasweiler (1978) suggested the following possible mechanisms for unilateral oviductal and uterine reactions: 1) Direct diffusion of hormones from the ovary into the ipsilateral oviduct and uterus via ligaments connecting these organs; 2) Transport of hormones from the ovary to its adjacent oviduct and uterus via blood vessels or lymphatics; 3) Countercurrent exchange of hormones between the ovarian venous and or lymphatic drainage, and the oviductal or uterine arterial supply; 4) Local passage of hormones between the ovary and oviduct or uterus via arteriovenous anastomses; and 5) unilateral neural arcs.

A complicating factor in unilateral oviductal reactions is the possible presence of ovarian steroids in the peritoneal fluid surrounding the oviduct. Recent studies on ovarian steroids in human peritoneal fluid indicate that it is primarily formed by ovarian exudation (Koninckx et al., 1980b), since volume is higher in cycling women than in women with suppressed ovarian activity. Koninckx et al. (1980a) demonstrated in women that progesterone concentrations were higher in peritoneal fluid than in peripheral plasma throughout the menstrual cycle, while during the follicular phase estradiol

concentrations were comparable in peritoneal fluid and plasma, and only increased sharply after ovulation. They also showed that peritoneal fluid levels of estradiol and progesterone, for at least one week after ovulation, were much higher than peripheral plasma levels. Since the fallopian tube is bathed in peritoneal fluid, the presence of large amounts of estradiol or progesterone could counteract any local ovarian influence on the oviduct, particularly following ovulation.

Hormonal Regulation of Nuclear Shape

It is well established that cell nuclei can undergo functional enlargment in response to various stimuli, including hormones. O'Leary (1931) demonstrated that in the endometrium of women the epithelial nuclei were largest in the ovulatory phase and during menstruation. Stromal nuclei of the spayed rhesus monkey endometrium hypertrophy under the influence of estradiol, as well as during sequential estradiol-progesterone administration. They decrease in size when progesterone is administered after estradiol treatment (Pfeiffer and Hooker, 1944). Klarner (1955) found that ACTH increased nuclear volume in the zona fasciculata cells of the rat adrenal gland. TSH was shown to have the same effect in the rat thyroid gland (Alfert et al., 1955). Recently, Jacobi et al. (1982) showed that estrogen increased the mean nuclear diameter in pituitary cells of male rats. Salvator (1950) demonstrated that estrogens caused a doubling of nuclear volumes in the

endometrial epithelial cells of rat uteri, while progesterone alone caused no increase. He suggested that the nuclear volume increase was due to duplication of the chromosomes. Alfert and Bern (1951) also found that estrogens increased nuclear volumes in epithelial cells of the rat endometrium, and measured protein and DNA content in single nuclei to test Salvator's hypothesis. They found that estrogen treatment induced a doubling of the protein content in uterine gland nuclei, while the DNA content remained constant. In a later study, estradiol was shown to increase nuclear volumes in the rat uterus even when DNA synthesis and mitosis were inhibited (Gelfant et al., 1955). Gelfant and Clemmons (1955) also determined nuclear volumes, DNA content (cytophotometric measurements), and total organic mass (historadiographic measurements) of individual nuclei in rat endometrial cells. They found that estradiol caused a doubling in nuclear volume in gland cell nuclei and a tripling in volume of the surface epithelial cell nuclei, while the DNA content remained constant. However, this increase in nuclear volumes was accompanied by a substantial increase in total organic mass, which they attributed to an increase in the non-histone protein fraction of the nucleus.

Although hormones have been known to dramatically affect the histology of the fallopian tube in primates since Allen's work in 1927, I have found only one study where oviductal nuclei have been measured during the cycle. Lehto (1963) measured nuclear volumes in the epithelium of the human oviduct, and concluded that an increase in

nuclear volume took place from the beginning of the menstrual cycle towards ovulation. Nuclei remained large through the secretory phase and showed a further increase in size at the end of the cycle. It is difficult to interpret these results, because of the way the tubes were dated, and the area of the tube examined. Fallopian tubes were grouped according to the day of the menstrual cycle, but no accompaning hormone levels were presented. The variablity in individual menstrual cycles requires hormone data to more accurately date the time in the cycle (Brenner et al., 1983). In addition, Lehto used the border between the isthmus and ampulla in the fallopian tube to assess nuclear changes. In macaques, this section of the oviduct is the least responsive to hormonal changes (Brenner et al., 1974; Brenner et al., 1983). Lehto also noted a decrease in nuclear volume during pregnancy and suggested that this was due to lack of functional demand. Her conclusion that the epithelial nuclei in the fallopian tubes of women are responsive to hormones is appropriate, but further study of this phenomenon in the human oviduct is warranted.

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Although the fimbrial ovarian attachment was described in the seventeenth century by de Graaf, only a few modern anatomists are familiar with this structure. This research was designed to describe the histology of the connection, and to explore its physiologic role.

Morphological examination of the attachment was carried out in four ways. First, ovaries and fimbriae were observed with a dissecting microscope, and drawings were made to detect the location of the attachment in six species of nonhuman primates. Secondly, whole ovaries and their attached fimbriae were embedded in glycol methacrylate and serial-sectioned at two microns. Thirdly, the attachment region was embedded in araldite and sectioned at 0.7 microns for high resolution light microscopy, or at 1000 angstroms for electron microscopy. Fourthly, the vasculature of the connection was examined by histochemistry (alkaline phosphatase reaction), and vascular injection techniques (latex, gelatin, and Microfil).

The presence of a direct connection between the ovary and fimbriae, and reports of asymmetry in the oviduct (see literature review), led us to examine the possibility that the ovary could preferentially influence the adjacent oviduct. Our initial study was designed to investigate whether small molecules injected into one ovary would be preferentially concentrated in the adjacent oviduct, and if so,

whether cutting the fimbrial ovarian attachments would abolish the preferential concentration.

Positive results from that study led us to postulate that ovarian steroids could preferentially influence the adjacent oviduct. We found morphological evidence of asymmetry in the oviduct, and in a separate study we developed the use of cross-sectional nuclear area and form factor (a dimensionless number indicating roundness) measurements to quantitate oviduct morphology.

Nuclear area and form factor were established as reliable indicators of hormone action in the oviduct. Our final study was designed to assess morphological side differences in the nonhuman primate oviduct epithelium.

PAPER ONE

The Fimbrial Ovarian Attachment in Cynomolgus Macaques

ABSTRACT

In primates, there is a small region of attachment between the fimbriated end of the fallopian tube and the superior pole of the ovary. We have examined the relationships between the ovary and the adjacent fimbriae in cynomolgus macaques by light microscopy, intravascular Microfil injection, and radioisotope infusions. In these animals, the attachment is a firm, short stalk, lined by a low cuboidal mesothelium which is continuous at one end with the ciliated columnar epithelium of the fimbriae and at the other with the ovarian germinal epithelium. Within the stalk are collagen fibers, smooth muscle cells, blood vessels, and lymphatics. A fimbrial ovarian attachment was also found in each fetal and infant tract examined. Intravascular infusion with Microfil revealed small blood vessels within the fimbrial ovarian attachment. The histology of the stalk suggests that it could serve as a local pathway between the ovary and the ipsilateral fimbriae. To test this possibility, a 40 uCi bolus (1 uCi/ul) of ^{3}H-leucine was injected into the cortical region of one ovary. Biopsies of the ipsilateral and contralateral fimbriae were taken simultaneously at 30 minutes post-injection. In animals with an intact attachment (N=3) there was a 10 fold greater incorporation of tritium in the ipsilateral compared to the contralateral fimbriae. In three other animals, this side difference was abolished by cutting the fimbrial ovarian attachments before the injection of one ovary with ^{3}H-leucine.

These data suggest that the attachment region can provide a route for transport of molecules between the ovary and the fimbriae.

INTRODUCTION

The attachment between the fimbriated end of the fallopian tube and the superior pole of the ovary in women is a well described, but generally unappreciated anatomical feature. Fallopius (1561) gave the first accurate anatomical description of the tube named after him, but he failed to notice the attachment between the fimbriated end of the fallopian tube and the ovary. He claimed that the "testicles" (ovaries) had no common link at all to the tube (DeGraaf, 1672). In the seventeenth century DeGraaf (1672) was the first to accurately describe the function of the fallopian tube in addition to noting the presence of an anatomical connection between the ovary and oviduct. Although the attachment was then more fully described by Richard in 1851, and was later named the fimbria ovarica by Henle (1866), few modern textbooks of histology or anatomy illustrate it clearly. In the early literature on the reproductive tract of nonhuman primates several authors (Allen, 1927; Wislocki, 1932) note the existence of the attachment, but there have been no recent studies in nonhuman primates of the histology of the attachment or its possible physiological role.

The nature of the attachment suggests it could play a role in female reproduction as an anchor for approximation of the ovary and

fimbriae at the time of ovulation. Okamura et al. (1977) found smooth muscle cells within the attachment in women. The spontaneous contractions of these cells in vitro were found to change throughout the menstrual cycle with the highest activity in the ovulatory phase. This suggests that the attachment may play a role in moving the fimbriated end of the fallopian tube closer to the ovary at the time of ovulation. Cohen and Katz (1978) found that the length of the fimbria ovarica in fertile women was significantly shorter than in some infertile patients with patent but convoluted oviducts. On the basis of this evidence Cohen (1980) developed a surgical method for shortening abnormally long fimbria ovaricas in infertile women. His results indicated that these women had increased fertility following the restoration of their fimbrial ovarian proximity. Beck (1972) found that in species, such as rodents, with a closed ovarian bursa (a mesenteric sac surrounding the ovary and fimbriated end of the oviduct) there was no attachment between the ovary and fimbriae. When there was an open ovarian bursa, as in carnivores, or no bursa, as in primates, there was always an attachment between the ovary and fimbriae. This suggests that the attachment is necessary to retain the ovarian fimbrial proximity which would be lost in those species without a complete ovarian bursa.

In the current paper we document the presence of a fimbrial ovarian attachment in adult and fetal cynomolgus and rhesus macaques, illustrate its histology, describe its vascular connections, and demonstrate an additional possible role of the attachment, namely, to

serve as a route for transport or diffusion of small molecules from the ovary to its adjacent oviduct.

MATERIALS AND METHODS

One hundred and forty two nonhuman primates, representing six species, were examined in the course of this study. The majority of the animals were cynomolgus macaques (Macaca fascicularis), ninety nine, and rhesus macaques (Macaca mulatta), thirty four. Other species represented are: five olive baboons (Papio anubis), two pigtail macaques (Macaca nemestrina), one Japanese macaque (Macaca fuscata), and one celebes black macaque (Macaca nigra). Six fetal reproductive tracts (three rhesus and three cynomolgus) were examined for the presence of an attachment between the ovary and the fimbriae. All tissue was freshly fixed after ovariectomies or other surgical procedures performed for several different investigators by the Department of Surgery at the Oregon Regional Primate Research Center over a period of three years.

Light Microscopy

For microscopic examination the ovary and attached fimbriae were fixed in a buffered glutaraldehyde-paraformaldehyde mixture (Sandow, et al., 1979). Ovaries with a large follicle or corpus luteum were fixed 24 hours, while smaller ovaries were fixed for 6 hours. The fixed tissue was stored at 4 degrees C in a mixture of 4.5% sucrose and 1 %

sodium cacodylate, then washed overnight in 1% sodium cacodylate at 37 degrees C, dehydrated in ethanol, and embedded in glycol methacrylate. Two micron sections were cut on a JB-4 microtome and stained with Gill's Hematoxylin and Lee's stain (Gill et al., 1974; Bennett et al., 1976).

Vascular Study

The vascularity of the attachment was examined with the techniques of histochemistry and vascular perfusion. For the demonstration of alkaline phosphatase in blood vessels, 80-100 um frozen sections (Roman, 1965) were fixed in buffered neutral formalin, incubated in 0.4% sodium glycerophosphate, 0.4% calcium chloride, 0.2% sodium barbital and 0.1% magnesium sulfate, sequentially immersed in 2% calcium chloride, 2% cobalt chloride and dilute (1:74) ammonium sulfide in distilled water, counterstained with paracarmine, dehydrated and mounted (Gomori, 1952).

A three-dimensional cast of the vascular connections within the attachment was made with Microfil (Canton Bio-Medical, Boulder, Colorado), a silicon rubber that can pass through the capillary bed, thus filling both the venous and arterial blood vessels. We injected approximately 70 mls of Microfil into the abdominal aorta, just above the bifurcation, in a heparinized (3,000 U.S.P. units) cynomolgus macaque. Blood was released by cutting the inferior vena cava at the beginning of the injection. The Microfil was allowed to harden for three hours at room temperature before the reproductive tract was

dissected out, cleared with glycerin, trimmed and displayed for photography.

Isotope Injection

To determine whether the fimbrial ovarian attachment could serve as a local pathway for diffusion of molecules in three periovulatory cynomolgus macaques, we injected a 40 uCi bolus (1uCi/ul) of ^{3}H-leucine (115.2 Ci/mmol; New England Nuclear) into the cortex of one ovary near the uterine pole (Figure 2). The injection was made into the ovary contralateral to a large follicle in two animals, and contralateral to a corpus luteum in one animal. After waiting 30 minutes, we took simultaneous biopsies of the ipsilateral and contralateral fimbriae. Half the biopsy was processed for autoradiography, the other half of the biopsy was prepared for liquid scintillation counting. Whole ovaries with their attached fimbriae were then removed and processed for autoradiography. A biopsy of the rectus abdominis muscle was taken prior to the injection and at the end of the procedure. These biopsies were then cut in half and processed with the fimbriae biopsies.

Fimbriae processed for autoradiography were fixed for one hour in a buffered glutaraldehyde-paraformaldehyde mixture. Whole ovaries with their attached fimbriae were fixed as described above. The tissue was stored at 4 degrees C in a mixture of 4.5% sucrose and 1% sodium

cacodylate, then washed overnight in 1% sodium cacodylate at 37 degrees C. Fimbriae to be embedded in araldite were postfixed for 30 minutes in an aqueous solution containing 1% OsO_4 and 1% $K_4Fe(CN)_6$ (by a modification of the procedure described in Karnovsky, 1971), dehydrated in ethanol, placed in propylene oxide, and embedded in araldite. Sections (0.7um) were cut on a Porter-Blum MT-2 ultramicrotome. Whole ovaries with their attached fimbriae were embedded and sectioned in glycol methacrylate as described above. Slides of fimbriae and ovaries were coated with a 1:1 dilution of Kodak NTB3 emulsion and distilled water and stored in dark boxes at 4 degrees C. Fimbriae were exposed for six months, and ovaries with their attached fimbriae for two months. Autoradiograms were developed in Kodak D-19, stained with toluidine blue (araldite sections) or Gill's Hematoxyline and Lee's stain (glycol methacrylate sections) and examined by light microscopy.

Fimbriae to be processed for liquid scintillation counting were weighed and then homogenized in 0.5 ml of Dulbecco's phosphate buffered saline (Gibco Laboratories, Grand Island, New York). 100 ul aliquots were placed on Whatman 3 mm filter papers that had been presaturated in 10% trichloroacetic acid. The filter papers with 100 ul aliquots of fimbriae homogenate were rinsed twice, 90 minutes total, in cold 10% trichloroacetic acid, washed in 100% ethanol for 10 minutes, and placed in vials with 1.0 ml Soluene 350 (Packard) and 200 ul distilled water for 12 hours. Finally, 10 mls of Dimilume 30 (Packard) were added to

the vials and radioactivity was determined by liquid scintillation counting.

In three other periovulatory animals the attachment was severed by cutting the fimbriae so one third of the fimbriae remained attached to the ovary and two thirds was lying free (Figure 1). Bleeding was controlled by cautery, and then ^{3}H-leucine was injected as described above. Whole ovaries with their attached fimbriae, and the detached ipsilateral and contralateral fimbriae were sampled and processed as described for the intact animals. The injection was made into the ovary ipsilateral to a follicle in one animal and contralateral to a corpus luteum in the other animal. In the third animal the right ovary was injected; no large follicle or corpus luteum was present in either ovary.

RESULTS

Every animal we examined had an attachment between the fimbriated end of the fallopian tube and the ovary. Figure 3 is a drawing of the posterior view of the female macaque reproductive tract, illustrating the position of the attachment in relationship to the ovary and oviduct. The connection between the fimbriae and the ovary is a firm short stalk attached to the superior pole of the ovary, although it often contacts the superior lateral border of the mesovarium. The length of the stalk may vary from 0.1 to 4.0 mm, and the diameter from 0.5 to 3.0 mm.

Figure 4 is a fresh preparation of an ovary and fimbriae where a thread has been passed under the attachment to clarify its position and display its breadth. A definite fimbrial ovarian attachment was found in each fetal and infant reproductive tract examined. Figure 5 is a fresh preparation of the reproductive tract of an 80 day old cynomolgus fetus that demonstrates the attachment.

The attachment is a connective tissue stalk, lined by a low cubodial mesothelium which is continuous at one end with the ciliated columnar epithelium of the fimbriae and at the other with the ovarian germinal epithelium (Figure 6). This connective tissue stalk contains collagen fibers, smooth muscle cells, blood vessels and lymphatics (Figure 6).

A vascular cast of the female cynomolgus reproductive tract was obtained by Microfil injection of the vasculature. Examination and careful focusing on this cast through a dissecting scope revealed delicate blood vessels within the fimbrial ovarian attachment (Figure 7). Gomori (1941) has demonstrated localized alkaline phosphatase activity in capillary endothelium. A histochemical reaction for alkaline phosphatase revealed small blood vessels within the fimbrial ovarian attachment. The vascularity of the attachment region varied between animals. Fimbriae that were attached on or close to the mesovarium had larger vessels within the attachment region than fimbriae attached directly to the ovary.

The study of the ability of the fimbriae to incorporate ^{3}H-leucine after intra-ovarian injection demonstrated that in animals with an intact attachment (N=3) there was a 10 fold greater incorporation of tritium in the ipsilateral compared to the contralateral fimbriae. In three other animals, this side difference was abolished by cutting the fimbrial ovarian attachments before the injection of one ovary with ^{3}H-leucine. Figure 8 illustrates the difference between the three intact animals and the three cut animals in the incorporation of ^{3}H-leucine in their ipsilateral and contralateral fimbriae as determined by liquid scintillation counting. Statistical analysis of the data, with the Mann Whitney U test (Table 1), demonstrates that there is a significant difference (p=0.05) between the ipsilateral and contralateral fimbriae in intact animals. There was not a significant difference between the two fimbriae in animals where the fimbrial ovarian attachments had been cut. There was a significant difference (p=0.05) between the ipsilateral fimbriae in the intact and cut animals, however, no significant difference was found between their contralateral fimbriae. The autoradiograms of fimbrial epithelium in figure 9 illustrate these differences in incorporation of ^{3}H-leucine. The autoradiograms and liquid scintillation counting data of the muscle biopsies, taken as a control, revealed no uptake of ^{3}H-leucine.

The autoradiograms of the fimbriae indicate that the epithelial cells incorporated more ^{3}H-leucine per cell than stromal or smooth

muscle cells. In animals where the fimbrial ovarian attachment had been cut the autoradiograms of the fimbriae which remained attached to the injected ovary showed uptake of ^{3}H-leucine, while the detached fimbriae showed almost none.

The autoradiograms of the attachment confirm that ^{3}H-leucine was present in the stalk. Figure 10 illustrates the incorporation of ^{3}H-leucine in: epithelial cells lining the attachment; smooth muscle cells; stromal cells; and blood vessels. Figure 10 (A and B) shows an artery and a vein in close approximation within the attachment. At higher magnification the vein, as indicated by the thin wall and the presence of blood cells within the lumen, reveals increased incorporation of ^{3}H-leucine in the endothelial cells and in a white blood cell within the lumen (figure 10E). At higher magnification the neighboring artery, as indicated by the thick smooth muscle wall and the internal elastic lamina, exhibits less incorporation of ^{3}H-leucine than the nearby vein (figure 10D).

Autoradiograms of the ovaries indicate that ^{3}H-leucine did not diffuse uniformly throughout the injected ovary. Instead the ^{3}H-leucine remained close to the injection site in the ovarian cortex, while the side of the ovary farthest from the site of injection had much less incorporation of ^{3}H-leucine. This suggests that the dense stroma of the ovary retarded the diffusion of ^{3}H-leucine. As was expected, the injected ovary incorporated ^{3}H-leucine, even

though unevenly, and the contralateral ovary showed very little incorporation. An interesting finding was that the luteal cells of an active corpus luteum always incorporated large amounts of ^{3}H-leucine, even when the corpus luteum was in the non-injected ovary. This is possibly due to the high rate of blood flow to an active corpus luteum and to cross-circulation between ovaries.

DISCUSSION

The fimbrial ovarian attachment is a regular anatomical feature in the female primate reproductive tract. It was present in all fetuses, infants, and adults examined in the present study. These results confirmed the observations by Wislocki (1932), vanWagenen and Simpson (1965), Beck (1972), and Okamura et al. (1977) of a fimbrial ovarian attachment in various primates, and extends these observations to include pig-tailed macaques, Japanese macaques, celebes black macaques, and adult and fetal cynomolgus macaques.

The presence of blood vessels and lymphatics within the attachment suggests that the attachment can serve as a bridge for transport of molecules between the ovary and fimbriae. To test this we injected ^{3}H-leucine into one ovary and then compared the incorporation of ^{3}H-leucine in the ipsilateral and contralateral fimbriae. ^{3}H-leucine was chosen since it has been shown that oviduct epithelial cells incorporate leucine for the synthesis and later

secretion of proteins (Brenner, unpublished), and also because an amino acid that is incorporated into many different proteins would favor the opportunity to detect it in the fimbriae. In future studies it would be important to use a physiologically important hormone, such as progesterone, but for the initial studies we chose a tag more easily detectable by autoradiography. Periovulatory animals were chosen for the isotope injection study, since the oviductal epithelium is highly differentiated, and was presumed to have a high rate of protein synthesis at that time in the cycle.

Our results demonstrate that ^{3}H-leucine was preferentially concentrated in the ipsilateral fimbriae compared to the contralateral fimbriae in animals with an intact attachment. When the attachments were cut, preferential concentration was abolished and the ipsilateral and contralateral fimbriae were equivalent in their incorporation of ^{3}H-leucine. In animals where the attachments were cut, the segment of the fimbriae that remained on the injected ovary incorporated much more ^{3}H-leucine than the detached fimbriae. These results indicate that cutting the fimbriae did not disturb transport or diffusion of ^{3}H-leucine between the ovary and fimbriae. The lack of incorporation of ^{3}H-leucine by the rectus abdominis muscle indicates that the majority of the ^{3}H-leucine was incorporated in the ovary and oviduct. Some cross circulation was suggested since the contralateral fimbriae (and corpora lutea in the opposite ovary) incorporated some ^{3}H-leucine, while the skeletal muscle did not.

The Microfil vascular cast and the alkaline phosphatase histochemistry study revealed small blood vessels within the attachment, but the direction of blood flow in these vessels was not established by these techniques. The autoradiograms of the stalk revealed a greater incorporation of ^{3}H-leucine in the endothelial cells of the veins versus the endothelial cells of the arteries or lymphatics. This suggests that transport of ^{3}H-leucine from the ovary to its adjacent fimbriae occurred through veins within the stalk. Further quantitative studies of such autoradiograms would be required to more definitively assess the nature of the vascular route between the ovary and the fimbriae.

Our intraovarian ^{3}H-leucine injection study demonstrated that a small molecule from the ovary can be preferentially concentrated in the ipsilateral fimbriae. These results naturally raise the question of transport or diffusion of ovarian steroids to the adjacent oviduct through the attachment. Unilateral oviduct reactions could result if ovarian steroids from an ovary with a large follicle or corpus luteum were preferentially concentrated in the adjacent oviduct. There are several examples in the literature of asymmetry in the female reproductive tract. Maas et al. (1976) found a consistent unilateral rise in oxygen tension in response to insertion of a probe within the oviductal lumen only on the side where ovulation had occurred. Rasweiler (1977) has found, in the little bulldog bat, that the secretory cells of the oviduct ipsilateral to a mature follicle or a new

corpus luteum are more heavily vacuolated and hypertrophied than the contralateral side. DiZerega et al. (1980) have demonstrated asymmetrical estradiol levels in ovarian venous blood in the rhesus macaque as early as day three of the menstrual cycle, and by day seven there was a significant difference in estradiol levels between the two ovaries, even though a dominant follicle was not yet visible. Flickinger et al. (1977) found that in women there is a significantly lower level of estradiol cytosol receptor in the fimbriae ipsilateral to the corpus luteum than in the contralateral fimbriae. In our laboratory West et al. (1979) have confirmed in cynomolgus monkeys that there is a significantly lower level of estradiol cytosol receptor in the fimbriae ipsilateral to the corpus luteum than in the contralateral fimbriae. Since progesterone decreases estradiol receptor levels, these data suggest that progesterone may attain higher levels in the fimbriae adjacent to the corpus luteum than in the contralateral fimbriae. Further studies of both oviducts may reveal differences in hormone levels, biochemical processes, or morphology related to asymmetrical ovarian hormone secretion. Such studies are currently ongoing in our laboratory.

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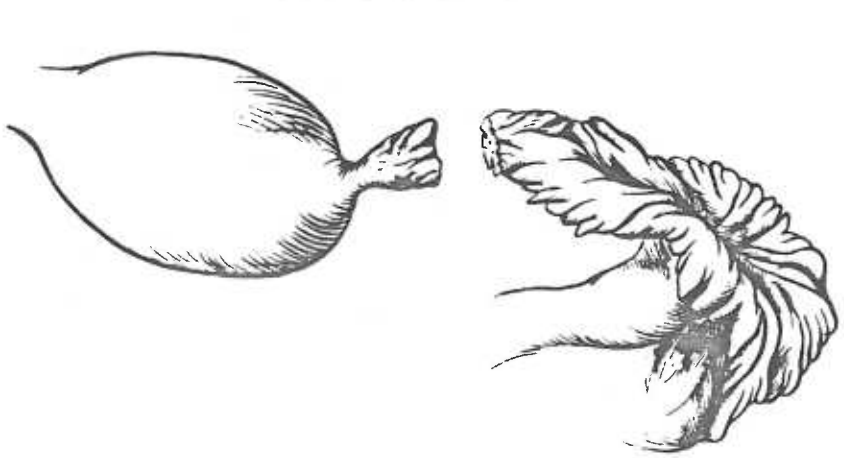


FIGURE 1: The attachment between the ovary and the fimbriae was severed by cutting the fimbriae so a portion of the fimbriae remained attached to the ovary and the remainder was lying free.

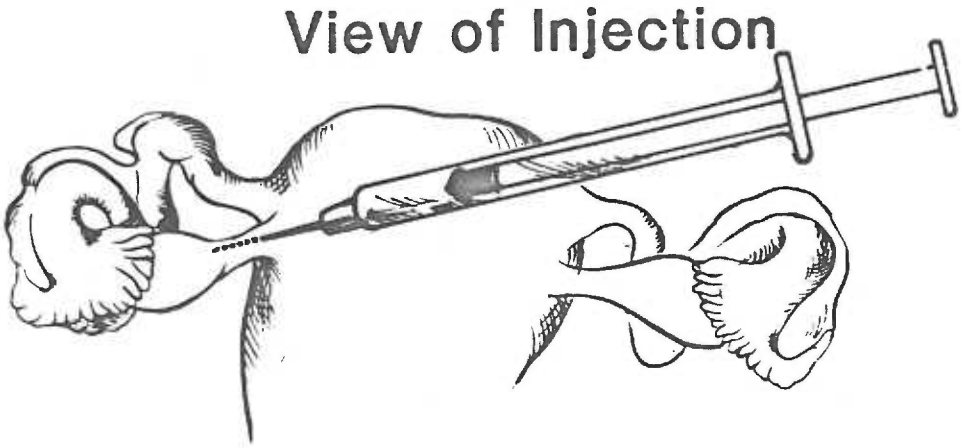


FIGURE 2: Method of injecting ^{3}H-leucine: The needle of the syringe was directed through the ovarian ligament to the ovarian cortex. At the uterine end of the ovary, opposite the tubal pole, 40 uCi (1uCi/ul) of ^{3}H-leucine were injected into the ovarian cortex. The needle was withdrawn through the ovarian ligament to avoid leakage of the isotope.

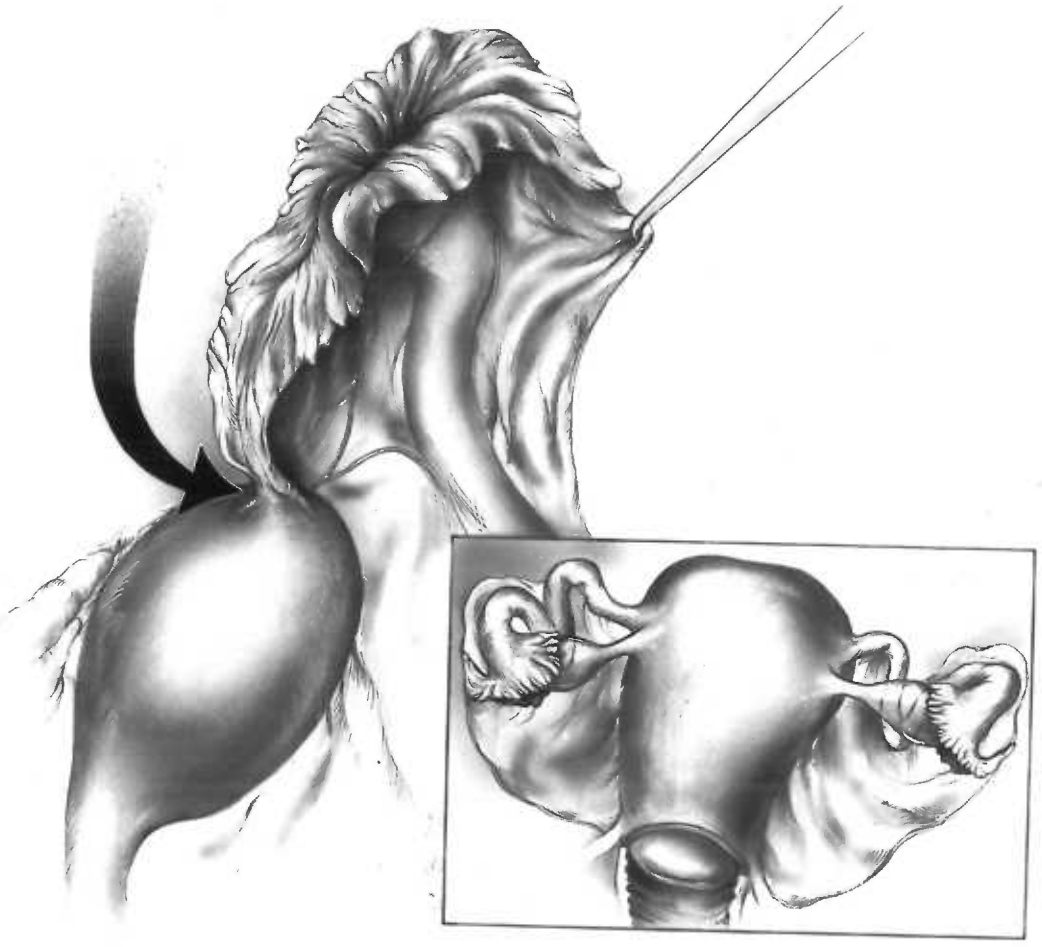


FIGURE 3: In the upper part of the figure the fimbriae is pulled away from the ovary to display the fimbrial-ovarian attachment (arrow), at the superior pole of the ovary. The inset is a drawing of the posterior view of the female macaque reproductive tract, illustrating the position of the attachment in relationship to the ovary and oviduct.

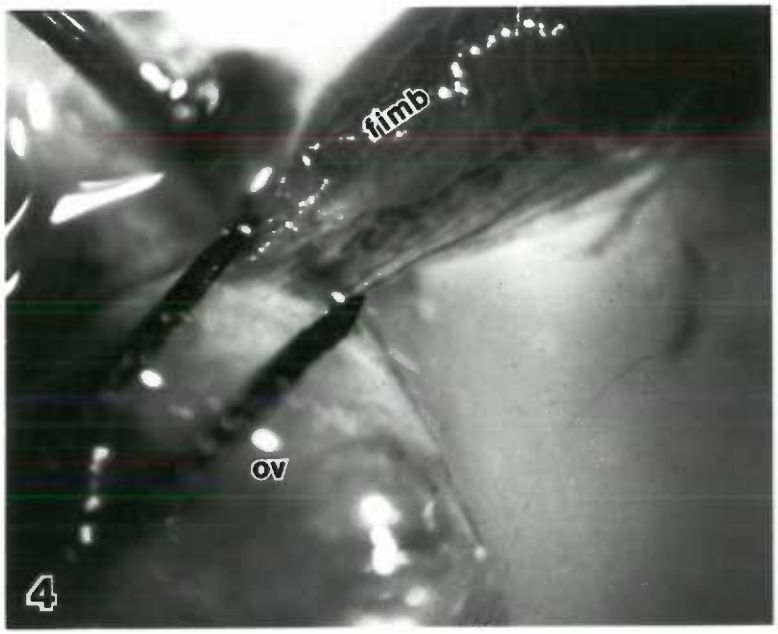
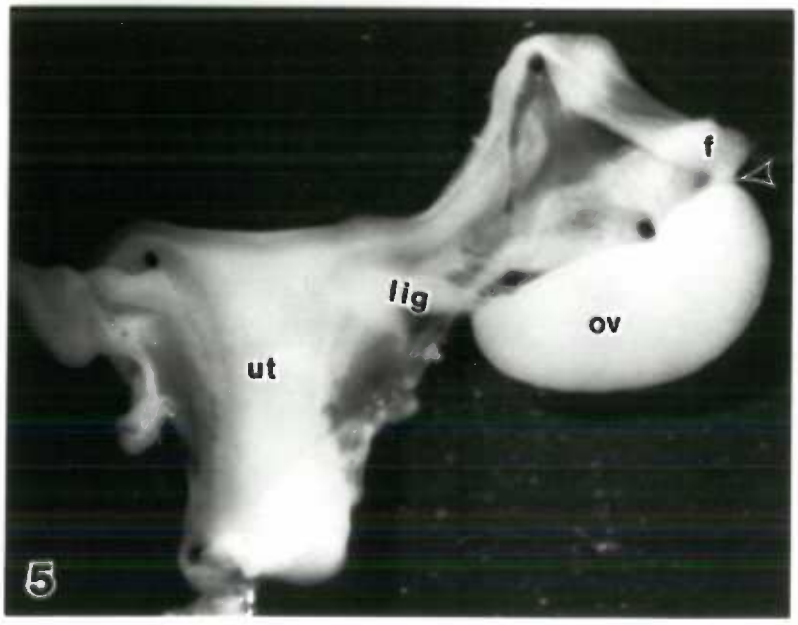


FIGURE 4: A fresh preparation of the ovary (ov) and fimbriae (fimb). We placed a thread under the attachment to demonstrate the breath of the connection.

FIGURE 5: A fresh preparation of the female reproductive tract of an 80 day old fetal cynomolgus macaque. There is an attachment (arrow) between the fimbriated end of the oviduct (f) and the superior pole of the ovary (ov). The uterus (ut) and ovarian ligament (lig) are also illustrated.



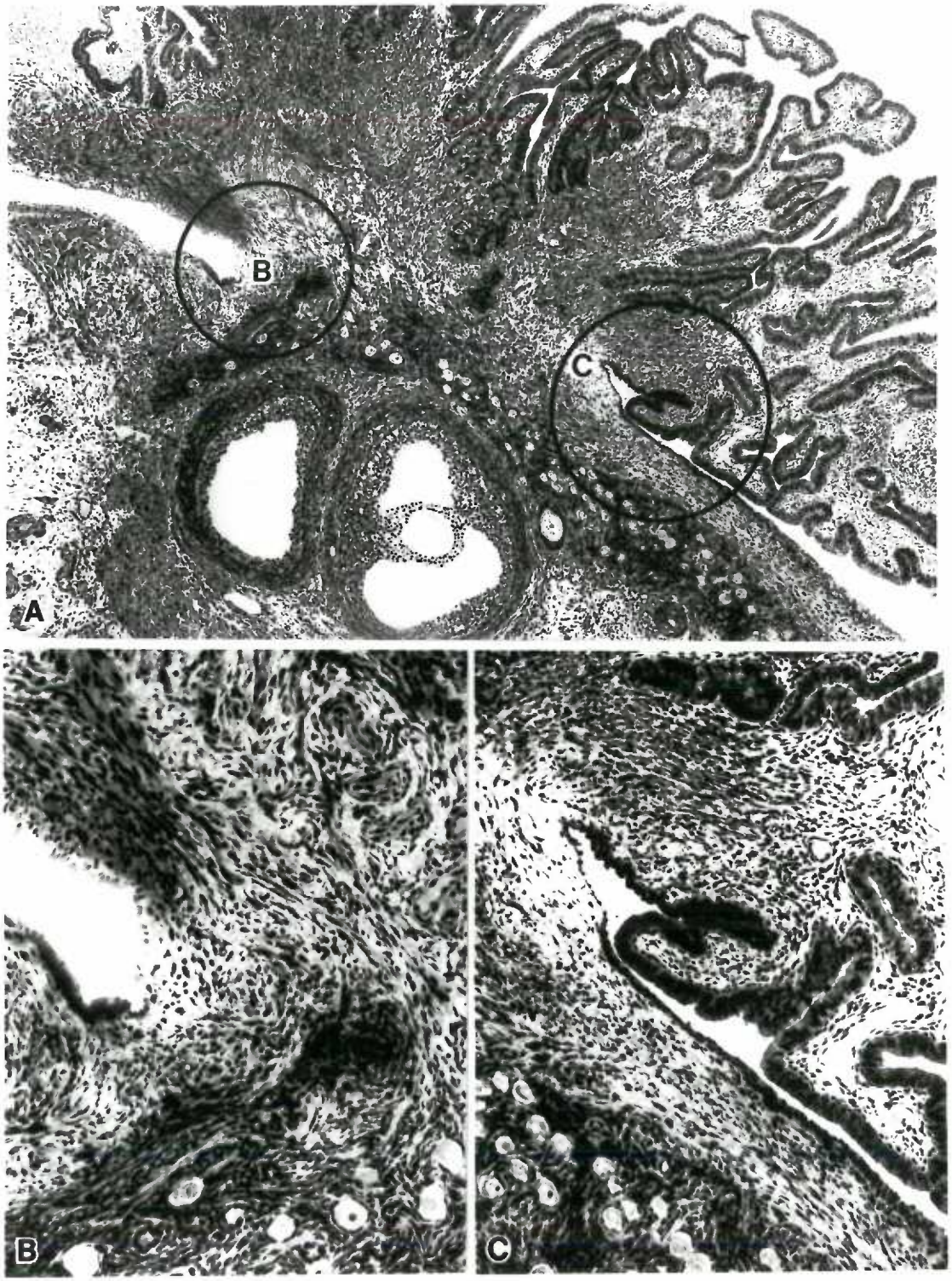


FIGURE 6: A) The fimbrial ovarian attachment in a cynomolgus macaque. Ovarian and fimbrial stroma intermingle across the attachment. (x 50) B) This connective tissue stalk contains collagen fibers, smooth muscle cells, blood vessels and lymphatics. (x 120) C) The attachment is lined by a low cuboidal mesothelium which is continuous at one end with the ciliated columnar epithelium of the fimbriae and at the other with the ovarian germinal epithelium. (x 120)

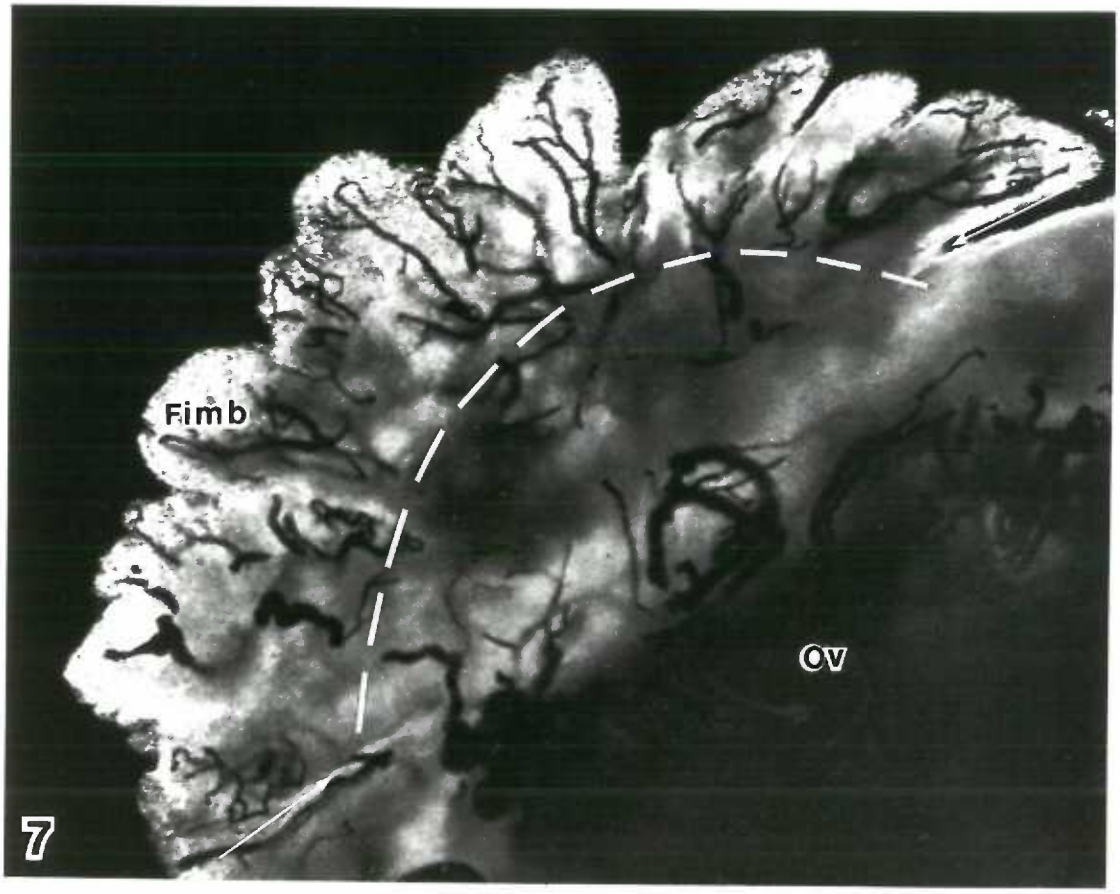


FIGURE 7: A section of a Microfil vascular cast of the fimbrial ovarian attachment in a cynomolgus macaque. The cast was sectioned by hand, with razor blades, at approximately 2 mm. The section was transilluminated from below, and photographed through a Zeiss stereomicroscope with an Olympus OM-2 35mm camera on Plus-x pan film (Kodak). The arrows indicate the lateral borders of the attachment and the dotted line shows the approximate extent of the connective tissue of the attachment. Small blood vessels are visible within the attachment between the ovary (Ov) and the fimbriae (Fimb). (x 300).

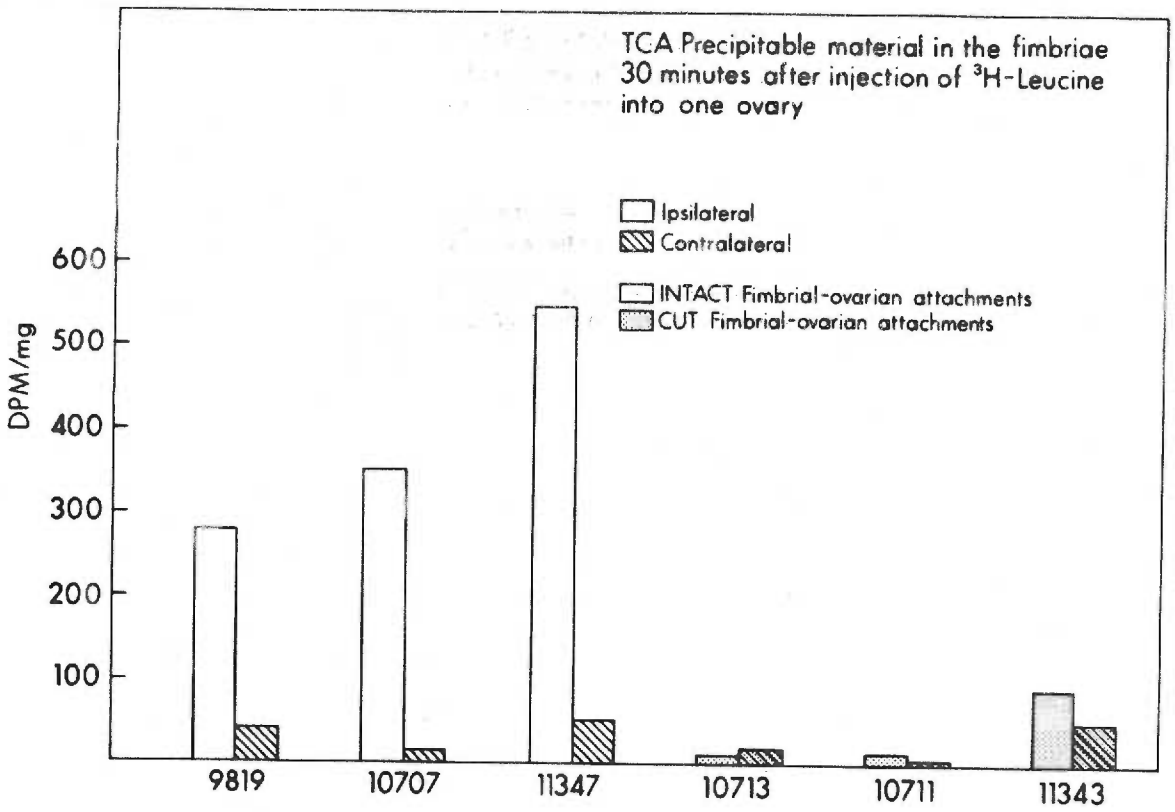


FIGURE 8: TCA precipitable material in the fimbriae of animals with intact (9819, 10707, 11347) fimbrial ovarian attachments (FOAs) and cut FOAs (10713, 10711, 11343). Thirty minutes after injection of 40 uCi (1uCi/ul) of ^{3}H-leucine into one ovary.

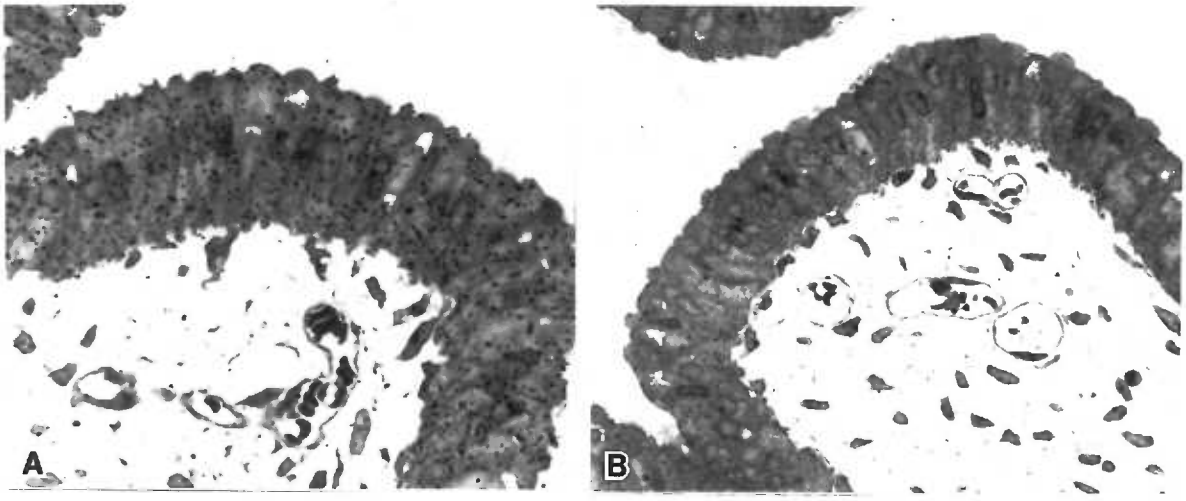


FIGURE 9: Autoradiograms of the ipsilateral (A) and contralateral (B) fimbrial epithelium 30 minutes after injection of ^{3}H-leucine into one ovary. The ipsilateral fimbriae incorporated more ^{3}H-leucine than the contralateral fimbriae. (x 500)

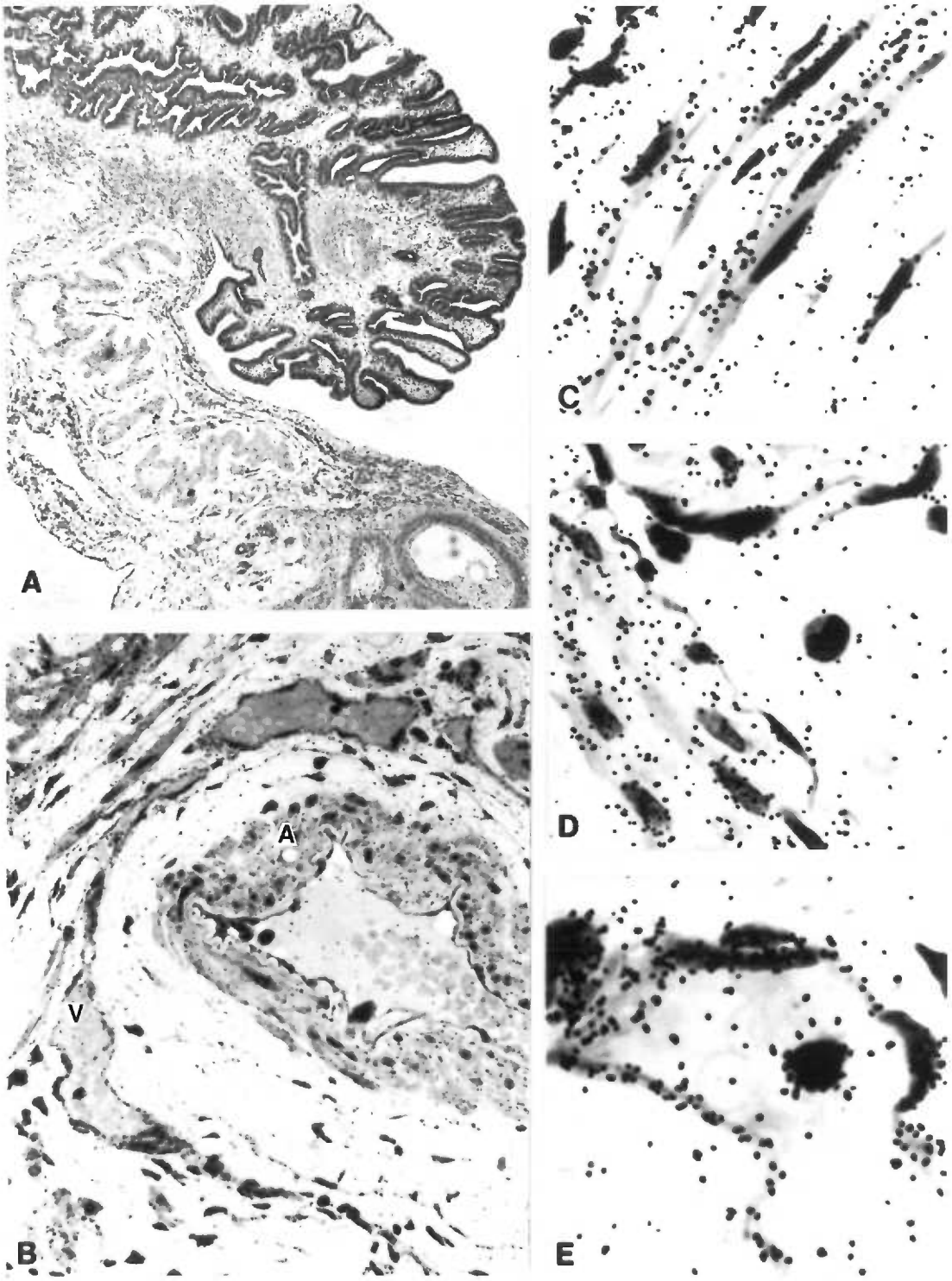


FIGURE 10: Autoradiograms of the fimbrial ovarian attachment 30 minutes after injection of ^{3}H-leucine into the ipsilateral ovary. A) Overview of the attachment. (x 30) B) Close approximation of an artery and vein within the attachment. (x 350) C) Smooth muscle cells within the attachment have incorporated ^{3}H-leucine. (x 1070) D) Higher magnification of the artery in A and B shows incorporation of ^{3}H-leucine in the tunica media. (1000) E) Higher magnification of the vein in A and B suggest there was more incorporation of ^{3}H-leucine in the tunica intima of the vein than in the same layer of the nearby artery. (x 1500)

TABLE 1. A comparison of differences in incorporation of ^{3}H-leucine between the contralateral and the ipsilateral fimbriae in intact FOA (n=3) and cut FOA (n=3) animals. The Mann Whitney U test was used for statistical analysis.

*FOAs INTACT	
ipsilateral fimbriae vs. contralateral fimbriae	p=0.05
FOAs CUT	
ipsilateral fimbriae vs. contralateral fimbriae	N.S.
IPSILATERAL FIMBRIAE	
intact FOAs vs. cut FOAs	p=0.05
CONTRALATERAL FIMBRIAE	
intact FOAs vs. cut FOAs	N.S.

*FOAs = Fimbrial Ovarian Attachments

PAPER 2

Hormonal Regulation of Nuclear Shape In Macaque Oviductal Epithelium

ABSTRACT

We have used the Zeiss image analyzer (MOP) to measure changes in cross-sectional nuclear area and form factor (deviation from roundness, where 1.00=a perfect circle) in the epithelium of oviductal fimbriae, under different hormonal conditions, in two macaque species. Spayed rhesus monkeys were treated with a sequential estradiol-progesterone regimen and sampled at intervals. Five fimbriae were sampled during the induced follicular and thirteen during the induced luteal phase. Cross-sectional nuclear areas were greater and nuclear form factors were larger in the follicular than the luteal phase of these animals. Subcutaneous estradiol implant treatment for 72 hours significantly increased cross-sectional nuclear area and form factor in the fimbrial epithelium of spayed cynomolgus monkeys. We also sampled fimbriae from cycling cynomolgus monkeys; 12 were measured during the follicular phase and 12 during the luteal phase. We again found that nuclei were larger and rounder in the follicular than the luteal phase. During the luteo-follicular transition cross-sectional nuclear area and form factor were significantly higher in the early follicular compared to the late luteal animals. Comparison of right and left sides revealed no significant differences in cross-sectional nuclear area or form factor. These data indicate that the size and shape of epithelial cell nuclei in the oviducts of macaques are hormonally modulated, that nuclear swelling

is one of the early effects of estrogen and that nuclear area and roundness measurements are useful tools to assess hormone action in the oviduct.

INTRODUCTION

The oviductal epithelium in nonhuman primates responds dramatically to ovarian steroids. Allen originally noted this phenomenon in rhesus macaques in 1927. More recently, Brenner et al. (1974) have demonstrated these cyclic changes in the oviduct of the rhesus macaque, and Odor et al. have demonstrated it in the pig tail macaque (1980) and baboon (1979). Similar changes occur in the dog (Verhage et al., 1973) and cat (Verhage and Brenner, 1975). Less dramatic, but comparable changes occur in the oviducts of women (Verhage et al., 1979). These investigators have shown that estradiol stimulates growth and differentiation in the oviductal epithelium as evidenced by mitosis, ciliogenesis, the appearance of secretory granules and an increase in cell height. Consequently, by midcycle the epithelium is fully differentiated with mature ciliated and secretory cells. After ovulation, under the influence of progesterone, regression begins in the oviductal epithelium, marked by cell death, loss of cilia, atrophy of the secretory cells, and a decrease in cell height so that at the end of the cycle the oviductal epithelium is maximally atrophied and dedifferentiated, resembling the spayed condition, particularly in the fimbriae.

Percent ciliation and cell height measurements have been used by many investigators in the past as quantitative measures of the morphological changes in the oviduct epithelium. However, percent ciliation measurements cannot reveal the earliest effects of estradiol in the oviductal epithelium since cilia do not appear until later in the cycle. We, as well as others, have noted that nuclear swelling is one of the earliest morphological effects of estradiol on the oviduct epithelium, but there has been no systematic documentation of this phenomenon. Therefore we measured cross-sectional nuclear area and form factor in order to quantitate early estrogen effects in the oviductal epithelium. In addition, we used such measurements to determine whether there were morphological differences between the right and left fimbriae of individual animals.

MATERIALS AND METHODS

Morphometric Analysis

Our measurements were made on a Wild compound binocular light microscope equipped with a drawing tube attachment, a rectangular mechanical stage, and a Wild 100x Fluotar oil immersion lens (numerical aperture = 1.30). Calculations were performed by the Zeiss image analyser (MOP), a minicomputer linked to a digitizing pad with a moveable cursor. A grain of wheat light bulb attached to the cursor just above the point on the cursor which interacted with the digitizing

tablet, was linked to a transformer to adjust the intensity of light. The light was superimposed over the microscopic field by means of the drawing tube, epithelial nuclei to be measured were brought into focus, and the perimeter of a nucleus was outlined with the lighted cursor. An automatic end-sensing feature terminated measurement when the cursor had returned to the initial point of measurement. Movement of the cursor was electronically sensed by the digitizer, which converted these impulses into digital readings that could be used by the computer to calculate the area and form factor of the nucleus. Form Factor is a dimensionless number between zero and one which indicates roundness, where form factor = 4πArea/(perimeter)^2. Readouts were in square microns for area, since a conversion factor, obtained by using a stage micrometer, was programmed into the MOP.

The MOP was interfaced to a Tektronics Graphics Systems 4051 minicomputer equipped with a 4051 E01 ROM expander, a 4907 flexible disc data storage unit, and a 4661 interactive digital plotter. Individual data from each nuclei were stored on flexible discs and filed under animal number, and right or left fimbriae. A statistical program calculated the mean, variances, standard deviations and ranges of individual or combined files. Another program was used to transform individual area readings to natural logarithms before statistical analysis.

Three criteria were used in selecting nuclei to measure to avoid measuring nuclei that were cut tangentially. These were that the base, luminal tip, and nuceolus of the cell all had to be visible in the plane of section. These three locators generally indicate a cross-section of a central region of the nucleus. If all three criteria were met the nucleus was measured.

Statistics

Cross-sectional nuclear areas were not normally distributed, therefore they were log transformed to correct for skewness and heteroscedasticity. Other statistical methods are reported in the results section.

Artificial Cycle

Two consecutive artifical menstrual cycles had been produced in spayed rhesus monkeys by a sequential estradiol-progesterone regimen (Brenner et al., 1974). Estradiol benzoate (Schering Progynon benzoate, 20 ug/day) and progesterone (Steraloids, Inc., 1.5 mg/day) had been dissolved in sesame oil and administered intramuscularly in two 0.5 ml doses at 7-8 AM and 4-5 PM daily. These animals had originally been used to correlate cyclic changes in oviduct morphology and residual cytoplasmic estradiol binding capacity with systemic levels of estradiol and progesterone. My measurements were made from tissue sections used

in that study. One hundred nuclei were measured from two different areas of fimbriae in each animal. One fimbriae biopsy was examined from a spayed, untreated animal, and eighteen other fimbriae were examined at the following biopsy times; where E represents days of estradiol only and P days of progesterone plus estradiol.

Cycle I: E-4, E-8, P-2, P-5, P-7, P-9, P-12, P-15, P-19.

Cycle II: E-4, E-8, E-14, P-2, P-4, P-7, P-11, P-15, P-21.

In a second group of animals biopsies had been taken at three key points during the artificial cycle by the same investigators. Animals had been treated with estradiol benzoate in sesame oil (20 ug/day) for 14 days (E14I, n=3), then with a combination of estradiol benzoate (20 ug/day) and progesterone (1.5 mg/day) in sesame oil for 21 days (P21, n=3) and finally with estradiol benzoate (20 ug/day) alone for 14 days (E14II, n=3). I measured cross-sectional nuclear area and form factor on sections from this study as well.

Spayed Cynomolgus Macaques

Cynomolgus macaques that had been spayed at least two months previously were used for this study. Three spayed monkeys were examined with no additional treatment. Four spayed monkeys were treated for 72 hours with an estradiol implant (Hess and Resko, 1973). One hundred

nuclei in two or three different areas of the fimbriae were examined. In all of the spayed, hormone treated animals and in one of the spayed, untreated animals right and left fimbriae were counted separately, for a total of 300 nuclei per side. Fimbriae, taken at laparotomy, were fixed in a buffered glutaraldehyde-paraformaldehyde mixture (Sandow et al., 1979) for one hour. The fixed tissue was stored at 4 degrees C in a mixture of 4.5% sucrose and 1% sodium cacodylate, then washed overnight in 1% sodium cacodylate at 37 degrees C, postfixed for 30 minutes in an aqueous solution containing 1% OsO_4 and 1% $K_4Fe(CN)_6$ (by a modification of the procedure described in Karnovsky, 1971), dehydrated in ethanol, placed in propylene oxide, and embedded in araldite. Sections (0.7 um) were cut on a Porter-Blum MT-2 ultramicrotome.

Cycling Cynomolgus Macaques

The fimbriae from 24 cycling cynomolgus macaques were analyzed; right and left sides were kept separate. This tissue had originally been used in another study, where estradiol and progesterone levels in the peripheral blood were determined by radioimmunoassay, and ovarian, oviduct (including percent ciliation and cell height measurements) and uterine morphology were described (Brenner et al., 1983). Twelve follicular and twelve luteal animals were examined and their oviducts were classified into seven groups based upon the degree to which their oviducts had progressed through the cycle. The criteria for distinguishing between these stages included percent ciliation, cell

height, mitotic activity, extent of ciliogenesis, nuclear shape, secretory cell tip extension, apoptosis, macrophage activity, and some others (Brenner et al., 1983). The groups were named as follows: preciliogenic-ciliogenic, ciliogenic-ciliated, ciliated-ciliogenic, ciliated-secretory, early regression, late regression, and full regression. These stages are roughly equivalent to phases of the cycle as follows: early, mid, and late follicular, periovulatory, and early, mid, and late luteal. One hundred nuclei from three different areas of each fimbriae were examined.

Fimbrial Ovarian Attachments (FOA) - Cut

In five cynomolgus macaques we detached their fimbriae from the ovary, and sutured them to the posterior abdominal wall. The animals were allowed to resume cycling. After at least two cycles, blood was drawn in the luteal phase, allowed to clot, and the serum was analyzed by a radioimmunoassay (RIA) for progesterone to indicate the presence of an active corpus luteum. The RIA technique was as previously described (Resko et al., 1974; 1975), except that a different antisera for progesterone (Surve et al., 1976) was utilized. If progesterone levels indicated the presence of a corpus luteum (Progesterone levels > 1.0 ng/ml), the ovaries and oviducts were removed in the early follicular phase of the following cycle. Three animals were oophorosalpingectomized on day three of the cycle, and two animals were taken on day four of the cycle. Fimbriae were processed as described

above. Right and left fimbriae were kept separate, and 100 nuclei from three different areas of each fimbriae were analyzed. Ovaries were fixed overnight in a buffered glutaraldehyde- paraformaldehyde mixture, cut in half and stored at 4 degrees C in a mixture of 4.5% sucrose and 1% sodium cacodylate, then washed overnight in 1% sodium cacodylate at 37 degrees C, dehydrated in ethanol, and embedded in glycol methacrylate. Sections (2 um) were cut on a JB-4 microtome and stained in Gill's hematoxylin and Lee's stain (Gill et al., 1974; Bennett et al., 1976).

RESULTS

There were cyclic changes in nuclear area and form factor during the rhesus artificial menstrual cycle that paralleled the cyclic changes in percent ciliation and cell height that had been previously described (Brenner et al., 1974). Figure 2 illustrates these cyclic changes of cross-sectional nuclear area and form factor in response to estradiol and progesterone. Both parameters increased with estradiol treatment and decreased when progesterone was administered. Cross-sectional nuclear areas were greater (51.49 ± 1.8 u^2 vs. 34.61 ± 2.0 u^2 ; $p<.001$) and nuclear form factors were larger (0.7182 ± 0.01 vs. 0.5661 ± 0.01; $p<.001$) when all the data from the animals in the artificial follicular phase were compared to those in the artificial luteal phase.

Table 1 demonstrates the cross-sectional nuclear area and form factor at three key points during the artificial menstrual cycle, and an analysis of variance (ANOVA) for statistical significance. Cross-sectional nuclear area and form factor in animals treated with 14 days of estradiol in the first and second cycle were significantly higher than in animals treated with 21 days of progesterone in addition to estradiol. There was not a significant difference in cross-sectional nuclear area or form factor between 14 days of estradiol in the first cycle and 14 days of estradiol in the second cycle.

Table 2 shows that cross-sectional nuclear area and form factor, of spayed animals, are significantly increased by treatment with estradiol for 72 hours.

For convenience of graphic presentation the naturally cycling cynomolgus macaques were arranged in the seven groups previously described. Figure 3 illustrates cyclic changes in nuclear area and form factor during the natural menstrual cycle. Nuclear areas increased until the mid-follicular stage and then began to decline, falling sharply after the early luteal phase. The cyclic pattern for form factor, also demonstrated in figure 3, is different from that of nuclear area. Form factor was highest in the early follicular phase and steadily declined through the luteal phase. We again found the nuclei were larger (37.45 ± 1.06 u^2 vs. 27.40 ± 1.49 u^2; $p<.001$) and

rounder (0.6358 ± 0.01 vs. 0.5326 ± 0.01; $p<.001$) when all the animals in the follicular phase were compared to all those in the luteal phase.

The repetition of group 1 after group 7 on these graphs illustrates the transition from the end of one cycle to the beginnning of the next. Brenner et al. (1983) have termed this time period the luteo-follicular transition. Figure 1 illustrates the nuclear swelling that occurs in the nuclei of the fimbrial epithelium during this transition. To study this transition, animals in groups 1 and 2 (n=8, early follicular) were compared to animals in groups 6 and 7 (n=7, late luteal). Peripheral levels of estradiol were not significantly different between the two groups, however, peripheral levels of progesterone were significantly lower in the early follicular animals compared to the late luteal animals (Table 3). Cross-sectional nuclear area and form factor were significantly higher in the early follicular compared to the late luteal animals (Table 3).

Side Differences

Comparisons of the right and left sides among the cycling cynomolgus macaques was calculated by a pooled within animal variance-covariance matrix among six measurements (percent ciliation, cell height, cross- sectional nuclear area, form factor, and nuclear and cytoplasmic estradiol receptor levels) and conversion of this matrix into a correlation matrix. From the correlation matrix the multiple

correlation coefficient for side versus all other variables was calculated (Morrison, 1967). We found that any combination of the measured characteristics did not differ between the sides within animals. Paired comparison t-tests between fimbriae ipsilateral or contralateral to an old corpus luteum (Table 4) in intact animals (cycle days 2-4), FOA cut animals (cycle days 3-4) and spayed hormone treated animals revealed no significant differences between the sides.

DISCUSSION

Our data demonstrates that the size and shape of epithelial cell nuclei in the oviducts of macaques are hormonally modulated. The epithelial nuclei become maximally shrivelled and wrinkled in the spayed condition, or in the cycle under the influence of progesterone. During natural or induced cycles, once progesterone levels fall, and estradiol action is unopposed, these nuclei become swollen and round.

A review of the literature indicates that various trophic hormones are responsible for the enlargement of the nucleus in a variety of tissues. Klarner (1955, as cited by Lehto, 1963) demonstrated that ACTH increased nuclear volume in the zona fasciculata cells of the rat adrenal gland, TSH was shown to have the same effect in the rat thyroid gland (Alfert et al., 1955), and Jacobi (Jacobi et al., 1982) found that estrogen (diethylstilbestrol dipropionate) increased the mean nuclear diameter in the pituitary cells of male rats. Salvator (1950) and

Alfert and Bern (1951) both demonstrated that estrogens caused a doubling of nuclear volumes in the endometrial epithelial cells of rat uteri. Pfeiffer and Hooker (1944) showed nuclear hypertrophy in macaque endometrial stroma cells induced by estradiol. Gelfant et al. (1955) found that estradiol increased nuclear volumes in the rat uterus even when DNA synthesis and mitosis were inhibited. Gelfant and Clemmons (1955) studied rat endometrial cells by determining nuclear volumes, DNA content of individual nuclei (cytophotometric measurements) and total organic mass of individual nuclei (historadiographic measurements). They found that estradiol caused a doubling in nuclear volumes in gland cell nuclei and a tripling in volume of the surface epithelial cell nuclei, while the DNA content remained constant. However, this increase in nuclear volumes was accompanied by an increase in total organic mass, which they attributed to an increase in the non-histone protein fraction of the nucleus.

Our measurements in the fimbrial epithelial nuclei of cycling cynomolgus macaques demonstrated the different cyclic patterns of cross-sectional nuclear area and form factor. Form factor was highest during the preciliogenic and ciliogenic phases of the oviduct cycle when the nuclei were plump and swollen, however, as the cell matured the nuclei became more wrinkled, even though the nuclear area remained large. In the ciliated-secretory phase the nuclei of the mature cells were large, yet the nuclear membrane was puckered. After ovulation, under the influence of progesterone, the nuclei decreased in size and

their nuclear membrane became wrinkled. At the end of the cycle, in the full regression phase, the nuclei were maximally shrivelled and wrinkled.

The luteo-follicular transition is characterized by progesterone levels falling dramatically, estradiol levels changing little, and nuclear and cytoplasmic estradiol receptor levels increasing sharply (West and Brenner, 1983). Percent ciliation has been used to quantify oviduct morphology, however this measurement cannot be used to quantify oviduct morphology during the luteo-follicular transition since very few cilia are present in the late luteal or early follicular phases of the cycle. Because nuclear swelling is prominent during the early follicular phase, cross-sectional nuclear area and form factor measurements can easily quantify oviduct morphology during the luteo-follicular transition.

The existence of an attachment between the fimbriated end of the oviduct and the superior pole of the ovary in primates, the demonstration that small molecules can pass from the ovary to the fimbriae through the attachment (Paper one), and asymmetrical ovarian hormone secretion all suggest that ovarian hormones could preferentially influence the adjacent oviduct. Reports of significantly lower levels of estradiol cytosol receptor in the fimbriae ipsilateral to the corpus luteum than in the contralateral fimbriae in women (Flickinger et al., 1977) and cynomolgus macaques (West et al., 1979) also suggest a local

ovarian influence on the adjacent oviduct. Extensive microscopic observations of fimbrial epithelium, led us to conclude that there might be morphological differences between the right and left fimbriae in the early follicular phase, and that one fimbriae might be more advanced than the other in its progress towards the ciliated-secretory state. In a majority of these animals the advanced fimbriae was contralateral to the old corpus luteum. Criteria for analysis of these fimbriae included percent ciliation, cell height, mitotic activity, extent of ciliogenesis, nuclear shape, and a number of other morphological features that are very difficult to quantify, such as staining intensity of the cytoplasm, regularities of the apical cell surface, abundance of glycogen and granules in the apices of secretory cells, and degree of orderly alignment of basal bodies in ciliogenic cells. From these kinds of observations we determined that nuclear swelling was one of the first signs of estrogen action in the oviduct, and that measurements of this swelling would provide a new tool for quantifing oviduct morphology. We wondered whether such quantitation would also reveal oviductal side differences. The results of this paper indicate that cross-sectional nuclear area and form factor measurements are useful tools for quantifing hormonal effects on oviduct morphology, but significant oviductal side differences in these measurements were not found. There are three possible reasons for this lack of detection, first of all there may be no side differences, secondly there may be differences we were unable to demonstrate due to small sample sizes, or thirdly there are side differences, but we did not measure the right characteristic.

The subtle morphological differences we initially observed may be a sluggish indicator of the underlying biochemical differences between oviducts. Measurements of rates of protein synthesis or activation of specific enzymes are needed to determine whether side differences really exist.

In conclusion, we have demonstrated that nuclear swelling is one of the early effects of estrogen, that cross-sectional nuclear area and roundness measurements are useful tools to assess hormone action in the oviduct, and that no oviductal side differences in these measurements were found under various physiologic conditions.

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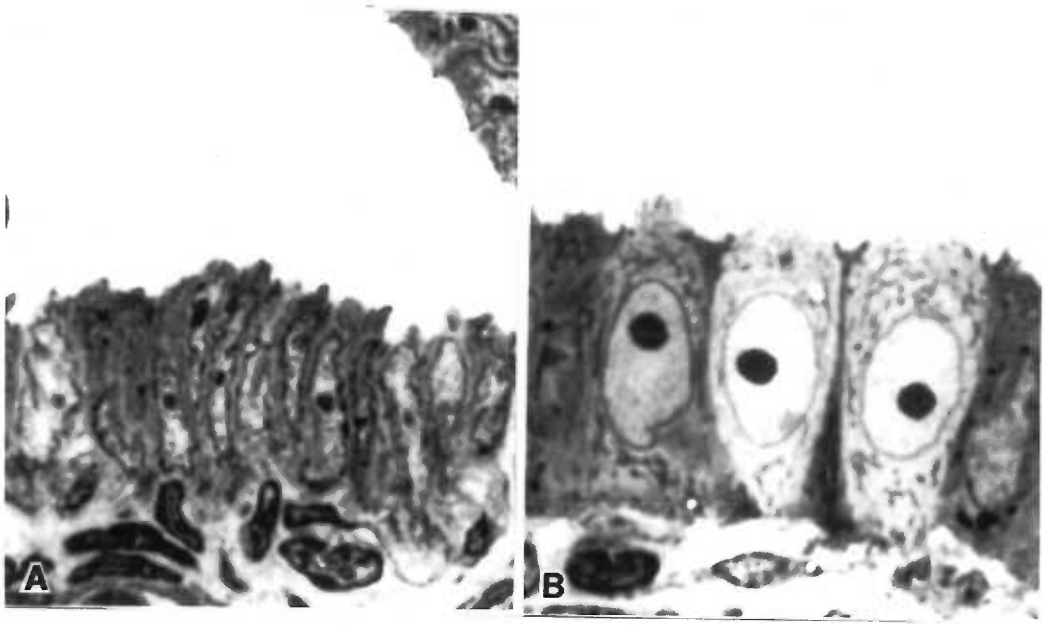

FIGURE 1: Fimbrial epithelium. A) Late Luteal phase; the nuclei are shrivelled and wrinkled. (x1800) B) Early follicular phase; the nuclei are swollen and round. (x1800)

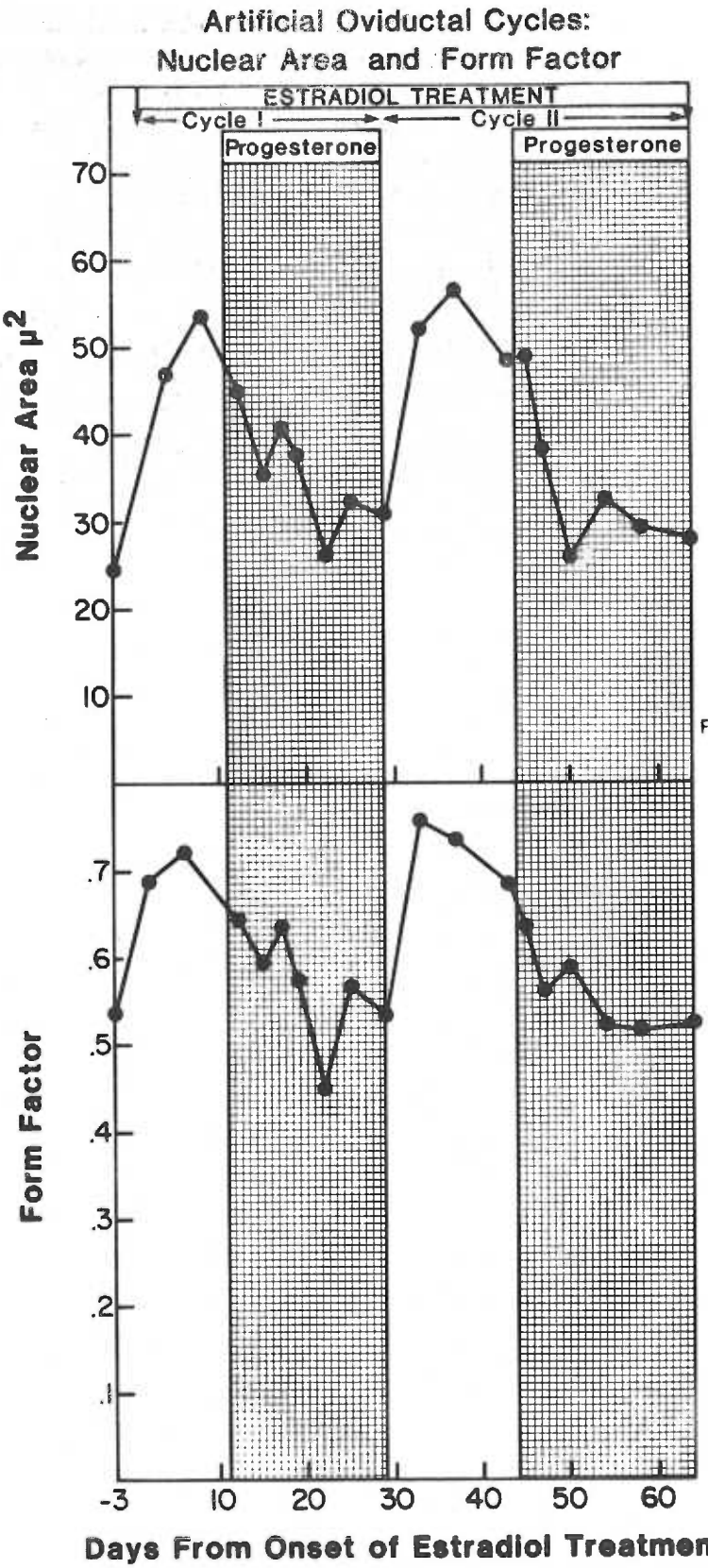

FIGURE 2 The changes in cross-sectional nuclear area and form factor in the fimbriae of spayed rhesus monkeys induced to cycle by the administration of a sequential estradiol-progesterone regimen.

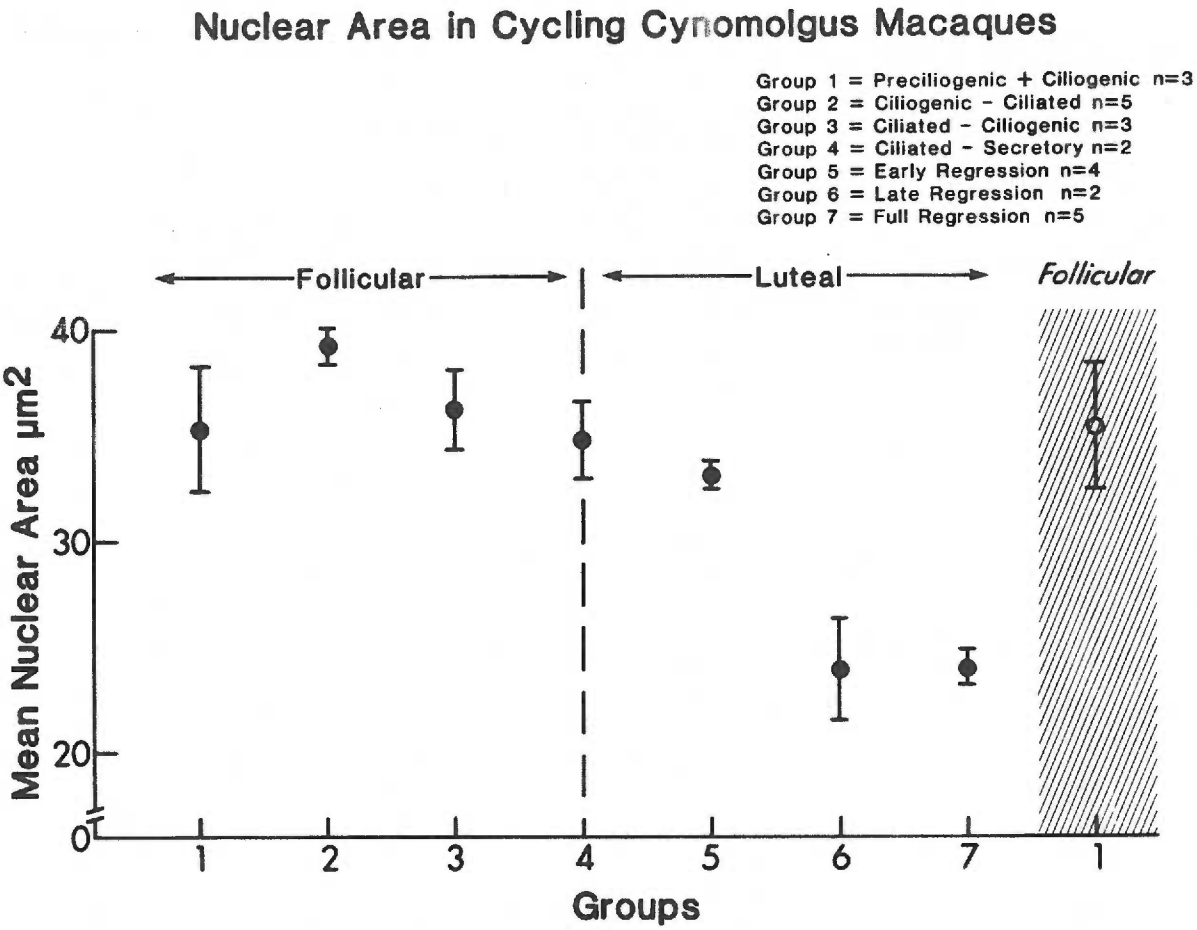

FIGURE 3: The changes in cross-sectional nuclear area in the fimbriae of cycling cynomolgus macaques arranged into seven groups based on their oviduct morphology, serum steroid levels, and ovarian and uterine histology.

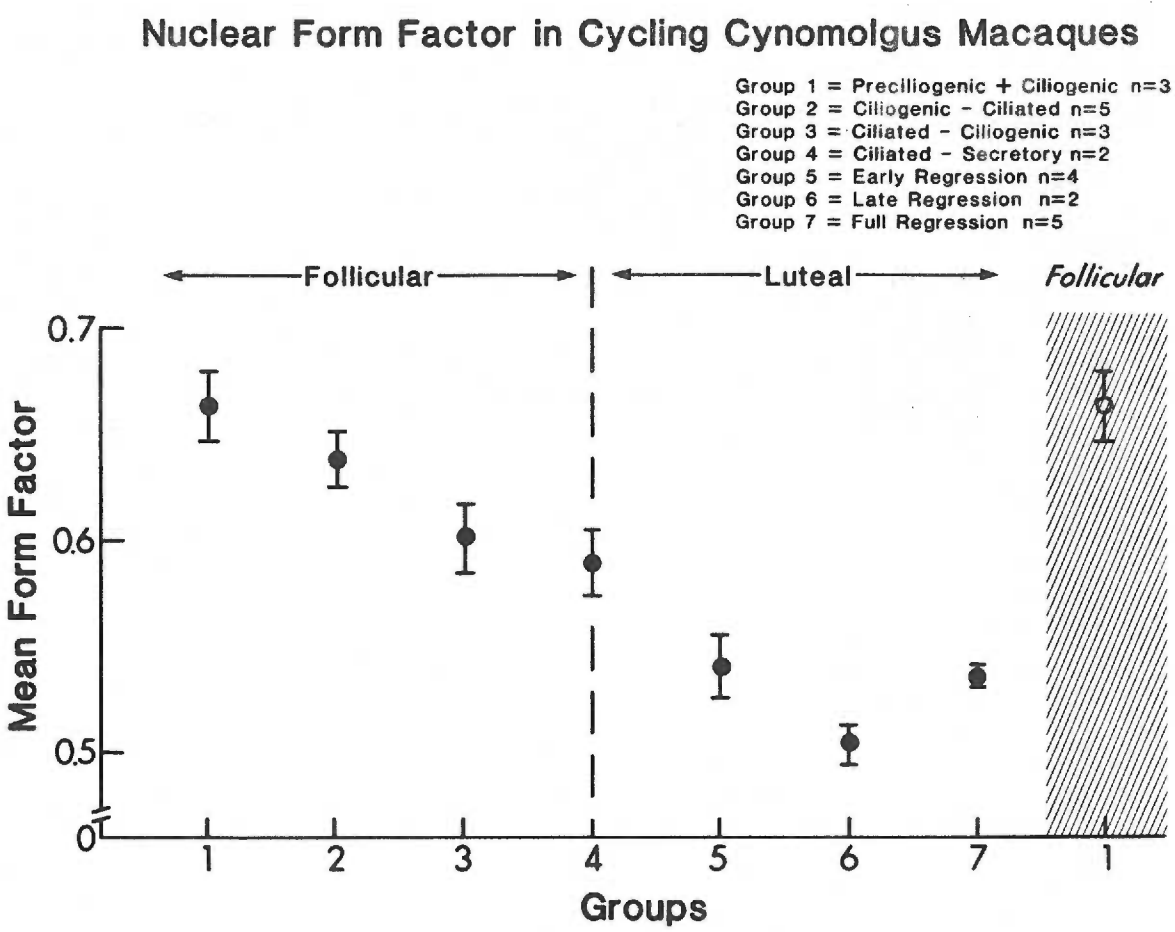

FIGURE 4: The changes in nuclear form factor in the fimbriae of cycling cynomolgus macaques arranged into seven groups based on their oviduct morphology, serum steroid levels, and ovarian and uterine histology.

TABLE 1. The effects of estradiol and progesterone on cross-sectional nuclear area and form factor in the fimbriae of spayed rhesus monkeys[a]

Time Sampled	# of Animals	Nuclear Area[c] (μm^2)	Nuclear Form Factor
E 14 I[b]	3	45.45 ± 1.7	0.632 ± 0.01
P-21[b]	3	27.30 ± 2.1[d]	0.559 ± 0.02[d]
E 14 II[b]	3	43.42 ± 0.6	0.606 ± 0.01

a. All data expressed as means ± standard errors

b. E 14 I : estradiol benzoate, 20 ug/day for 14 days

P 21 : Same as above, plus 21 additional days of progesterone (1.5 mg/day) and estradiol benzoate (20 ug/day)

E 14 II : Same as above plus 14 additional days of estradiol benzoate (20 ug/day)

c. Nuclear area measurements were transformed to natural logarithms for statistical testing (ANOVA)

d. Significantly different from E 14 I and E 14 II, $p < .001$ (E 14 I and E 14 II were not significantly different from each other)

TABLE 2. Effects of estradiol on epithelial nuclei of the fimbriae of spayed cynomolgus macaques[a]

Measurement	Spayed (n=3)	Spayed + 3 days E_2[b] (n=4)	P Students t
Nuclear Area[c] (μm^2)	24.16 $\pm$ 1.3	41.68 $\pm$ 1.7	< .001
Form Factor	0.532 $\pm$ 0.03	0.725 $\pm$ 0.01	< .001

a. All data expressed as means $\pm$ standard errors

b. Estradiol treatment: 72 hours with a silastic implant filled with estradiol benzoate

c. Nuclear area measurements were transformed to natural logarithms for statistical testing

TABLE 3. The luteo-follicular transition in the fimbrial epithelium of cycling cynomolgus macaques[a]

	Stage of the Menstrual Cycle		P Students t
	Late Luteal n=7	Early Follicular n=8	
Serum Steroids			
E_2 (pg/ml)	79 ± 12	100 ± 18	NS
P (ng/ml)	4.5 ± 1.6	0.41 ± 0.1	<.05
Morphological Criteria			
Nuclear Area (μm^2)[b]	24 ± 0.8	38 ± 1.3	<.001
Nuclear Form Factor	0.53 ± 0.01	0.65 ± 0.01	<.001

a. All data expressed as means ± standard errors

b. Nuclear area measurements were transformed to natural logarithms for statistical testing

TABLE 4. Local effects of ovarian steroids on oviductal nuclei[a]

Experimental Groups[d]	Nuclear Area (um^2)[b]	Form Factor
Intact (n=5)[c]		
ipsilateral to old CL	37.12 ± 1.3	0.6520 ± 0.02
contralateral to old CL	39.81 ± 1.7	0.6527 ± 0.02
FOA cut (n=5)[c]		
ipsilateral to old CL	37.01 ± 0.9	0.6527 ± 0.02
contralateral to old CL	37.95 ± 1.0	0.6594 ± 0.02
Spay + 3 days E_2 (n=4)		
right side	41.48 ± 1.5	0.7109 ± 0.02
left side	40.40 ± 2.1	0.7180 ± 0.01

a. All data expressed as means ± standard errors

b. Nuclear area measurements were transformed to natural logarithms for statistical testing

c. Cycle days 2-4

d. Paired comparison t-test revealed no significant differences between sides in any of the groups

SUMMARY

There is a small region of attachment between the fimbriated end of the oviduct and the superior pole of the ovary in primates. I have described the morphology of the attachment in cynomolgus macaques, and suggested several physiologic roles it may play. Six species of nonhuman primates were examined, and all had an attachment. Histological studies revealed that the attachment in cynomolgus and rhesus macaques is a firm short stalk, lined by a low cuboidal mesothelium which is continuous at one end with the ciliated columnar epithelium of the fimbriae, and at the other with the ovarian germinal epithelium. Within the stalk were collagen fibers, smooth muscle cells, blood vessels, and lymphatics. The presence of the stalk in fetal reproductive tracts, as early as day 80 of gestation, and the consistent morphology of the connection, indicates that the attachment is a normal anatomical feature of the nonhuman primate female reproductive tract. Vascular casts of the reproductive tract revealed delicate blood vessels within the fimbrial ovarian attachment. These vessels, and the anatomical continuity between the ovary and oviduct suggested that the attachment could serve as a bridge for transport or diffusion of molecules between the ovary and oviduct. I demonstrated that small molecules (^{3}H-leucine) injected into one ovary could be preferentially concentrated in the ipsilateral fimbriae, and that

cutting the fimbrial ovarian attachments before injection, abolished the preferential concentration.

Two physiologic roles have been postulated for the attachment. First, the nature of the attachment suggested that it could be an anchor for approximation of the ovary and the fimbriae at the time of ovulation. A review of the literature (see mechanisms of ovum pick-up) suggests this hypothesis is correct. Secondly, the results of the isotope injection study suggested that ovarian steroids could be transported or diffused through the attachment to the adjacent oviduct. Preferential concentrations of ovarian steroids in the adjacent oviduct would be expected to lead to unilateral oviductal reactions since the oviduct responds dramatically to ovarian steroids (see hormonal regulation of the oviduct epithelium). A review of the literature (see asymmetry in the female reproductive tract) indicates that many animals, including primates exhibit varying degrees of asymmetry in their reproductive tracts.

I have also outlined the development and use of cross-sectional nuclear area and form factor in the measurement of fimbrial epithelial nuclei of cynomolgus and rhesus macaques, as indices of hormonal modulation. I found that these nuclei became maximally shrivelled and wrinkled in spayed monkeys, or in intact monkeys under the influence of progesterone. During natural or induced cycles, once progesterone levels fell, and estradiol action was unopposed, these nuclei became

swollen and round. Nuclear swelling was shown to be one of the earliest morphological effects of estrogen on the nuclei of fimbrial epithelial cells.

Having established the validity and usefulness of the morphological indices of estrogen action, I then determined whether there were oviductal side differences in such measurements in individual macaques at different times during the menstrual cycle. No statistically significant side differences in cross-sectional nuclear area or form factor of fimbrial epithelial nuclei were found. I concluded that there may indeed be oviductal side differences of various kinds, but that these cannot be detected by the morphometric methods I used in this study.

Several questions remain to be answered about the fimbrial ovarian attachment in primates. These questions include; 1) Does the anatomical relationship between the ovary and oviduct change at different stages of the primate menstrual cycle, especially during ovulation? 2) Does fertility decrease if the fimbrial ovarian attachments are cut and sutured to the posterior lateral body wall? 3) Would injection of one ovary with ^{3}H-progesterone or ^{3}H-estradiol result in preferential concentration in the adjacent oviduct? 4) Would measurements of rates of protein synthesis or

activation of specific enzymes reveal oviductal side differences? Answers to such questions should lead to a better understanding of the physiologic role of the fimbrial ovarian attachment.

I wish to express my deepest appreciation for my mentor, Dr. Robert Brennner, who contributed to this research and my education, countless hours of his time in guidance and encouragement. I would also like to thank the various members of the staff at the Oregon Regional Primate Research Center for their assistance in surgery, the library, data processing, and animal handling. I am especially thankful to Mr. Joel Ito, and Ms. Katie Simon for help with the artwork, Mr. Harry Wohlsein for his photographic assistance, Dr. John Senner for assisting me with computer programming and statistics, and Ms. Jean McKinnon for her excellent secretarial assistance.

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