Contraception: The Chemical Control of Fertility

CONTRACEPTION:

The Chemical Control of Fertility

Edited by DANIEL LEDNICER

Research Laboratories
The Upjohn Company
Kalamazoo, Michigan

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Preface

As recently as a decade ago, it was feasible for any biologist with a reasonable training in endocrinology to undertake work on oral contraceptives. Similarly, his chemical colleague needed no more than a working knowledge of steroid chemistry. These past ten years have, however, been of extreme importance to this field of fertility control. The introduction of a safe and efficacious oral contraceptive has spurred a mass of work to find new agents and to better understand the mechanism of action of existing drugs. This, in turn, has led to much research of a fundamental nature to outline the events of the normal mammalian reproductive process. At the same time, governmental agencies throughout the world have finally awakened to the implications of the so-called "population explosion." The sense of urgency felt in these circles has been reflected in financial support for research in reproduction.

While presently available contraceptives are completely satisfactory as far as efficacy and safety are concerned, there is no reason to think that they constitute the final answer to population control; the relatively rigid regime of administration makes their use unsuitable in precisely that sector where they are most needed, that is, in unsophisticated societies. The search for a means by which man can control his numbers is thus well started but by no means over.

There has, as a result of all this intensive effort, come into being a new field which for lack of a better term can be called antifertility research. The newcomer to the area is likely to be overwhelmed not

only by the wealth of published reports but also by the fact that these are likely to be scattered in the journals of disciplines as diverse as reproductive physiology and organic chemistry.

The goal of this volume is to provide the interested student with a critical overview of antifertility research, to acquaint the newcomer to the area, be he a biologist or chemist, with the literature and methods of the field. In the search for a coherent presentation, discussion of topics such as steroid metabolism and induction of ovulation were omitted from this volume. As should be apparent to even the most casual reader, this book deals with only half of any species; results of work on practical male contraception are as yet too scant to include in a volume of this nature.

Since contraception constitutes interference with a normal process, we have provided the reader with a description of that process in the mammalian female. Subsequent discussion can then be understood against that frame of reference.

Though there is no magical distinction between steroids and all other organic compounds, it is convenient to discuss both the chemistry and biology of these broad classes separately. The steroids used in the oral contraceptives are obtained largely from starting materials possessing the steroid nucleus obtained from plant sources by suitable chemical modification. The chemical reactions employed in steroid work thus tend to be those used to modify and transform functionality. Due to steric factors, considerable selectivity can be exercised among these functional groups; a considerable lore of steroid chemistry has thus accumulated. The biology of steroids involves both progestins and estrogens; no compounds of the former category can be found among nonsteroids.

The chemistry of the nonsteroidal agents is much more concerned with the total synthesis of molecules from simple starting materials—the so-called coal tar intermediates. Though there are at present no nonsteroid contraceptives marketed for use in humans, many of these compounds show profound and intriguing activities in experimental animals. A discussion of the biology of these compounds sheds much light on the reproductive process and may point the way to the design of a successful agent of this type.

Thus, in an attempt at comprehensiveness, the biology and chemistry of each class of compounds is discussed from the viewpoint of the biologist and chemist, respectively. Wherever possible, structures in the biological chapters are cross referenced by the number in the

companion chemical chapter the first time a given chemical entity is mentioned

The recent efforts in the field of antifertility research have led to the development of a number of screens and assays to uncover contraceptive agents. An understanding of the strengths and limitations of these assays is essential to a proper evaluation of literature reports. It is all too easy, for example, to class a compound as an antiestrogen. This classification tends to be almost meaningless without knowledge of the assays which lead to that description.

I would like to express my appreciation to the Upjohn Company for the encouragement which made this volume possible. Particular thanks are due to Miss Carolyn Jarchow whose skill in translating my cryptic notes to a neat typescript is unequaled; it goes without saying that the moral and typographical support of my wife cannot be fully compensated.

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CHAPTER 1

The Reproductive Cycle in the Female

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I. Introduction

Although facets of the reproductive process vary widely among different species, common to all is the integration of the endocrine and central nervous systems. The fine interrelationship of these systems results in the regulation of repetitive events with a high degree of accuracy, including the cyclic variation observed in the hypothalamus, pituitary, ovary, and reproductive tract.

The availability of new techniques has greatly enhanced understanding of the endocrine aspects of reproductive physiology. These

new methods include microtechniques for steroid hormone determinations, procedures of radioimmunologic assay of pituitary hormones, and improved techniques of purification of new hormones as well as the study of the mechanism of action of hormones at the molecular level.

It is the purpose of this chapter to present a description of the reproductive cycle in the mammalian female, focusing particular attention on endocrinologic, physiologic, and autonomic aspects in the nonpregnant human cycle.

II. The Reproductive Cycle

The reproductive cycle in the human is the result of the interplay of the hypothalamus, pituitary, and ovary. The central nervous system participates through the secretion, by the hypothalamus, of humoral-releasing factors which in turn cause release of gonadotropins from the anterior pituitary. These pituitary hormones stimulate the process of ovulation.

Chronology of the Reproductive Cycle

1. *Ovulation*

Ovulation is the maturation and escape of an ovum from the vesicular ovarian follicle. Locally, the ovarian cycle involves three periodic reccurent phases (Fig. 1): the growth and ripening of a follicle (follicular phase), the rupture of this graafian follicle with discharge of an ovum (ovulatory phase), and following this, the formation of a corpus luteum (luteal phase).

In the embryo, as well as in the newborn, the great majority of primordial ovarian follicles form a thick layer under the tunica albuginea and are distinguished by the fact that the ova contained in them do not have a surrounding (vitelline) membrane. The ova are surrounded first by a simple layer of follicular cells (primary follicle) and then by multiple cell layers destined to form the granulosa layer of the more mature follicles. At this point they are called secondary follicles. Following this, a fluid-filled space, the antrum, is formed containing follicular liquor. Follicles with antra are known as tertiary follicles. The growth of a follicle that has reached the antrum stage of maturation marks the start of each cycle.

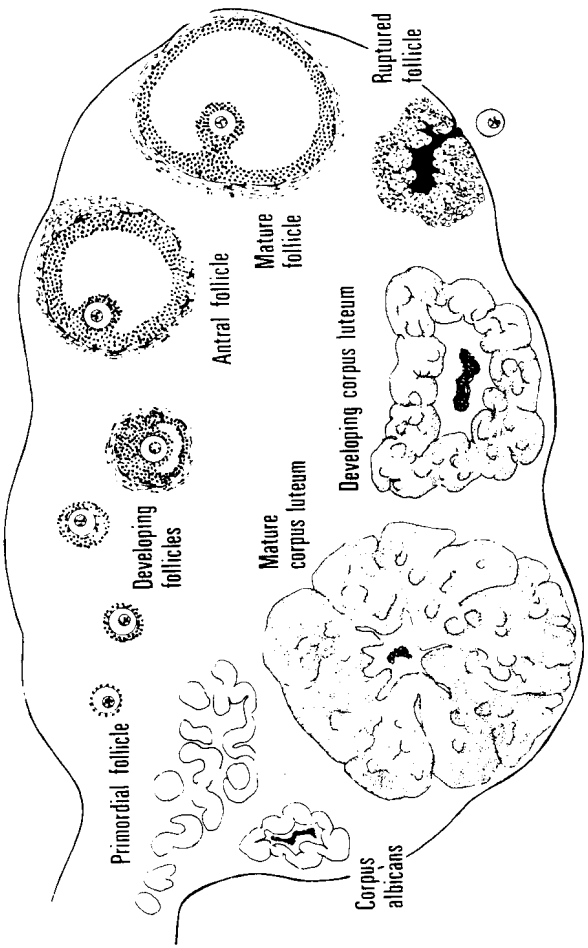

Fig. 1. Schematic illustration of maturation of ovarian follicle and corpus luteum. From M. R. Abell and J. R. G. Gosling, "Pathology of the Female Reproductive Tract," to be published.

It has been estimated that there may be as many as 500,000 primary follicles in both ovaries of the newborn. However, as the result of atresia, only about 400 ova are released during a woman's reproductive career. At the beginning of each cycle, several ova in both ovaries begin to ripen, but one outstrips the rest. The growth and maturation of each follicle may require a specific and increasing concentration of gonadotropins. It has been suggested that normally the amount of available hormone is enough to maintain only one follicle. Production of steroid hormones by the "major" follicle may inhibit further development of the others which then undergo atresia.

Further growth of the tertiary follicle results in the formation of a graafian follicle. Stimulated by pituitary gonadotropins, the graafian follicle matures and a blisterlike bulge appears on the surface of the ovary immediately prior to rupture. The follicle steadily increases in size, so that the thin bulging wall becomes distended, translucent, and, in one area, completely avascular. This area, called the stigma, is the site of rupture.

It is generally accepted that the rupture of the ovulatory follicle occurs in response to the action of luteinizing hormone (LH) and perhaps follicle-stimulating hormone (FSH). Several local factors have been implicated in this process. Rouget (*1*) suggested that the contraction of smooth muscles in the ovary produces an increase of follicular pressure which causes the follicle to rupture. Evidence of the existence of muscular activity has not been conclusively demonstrated (*2*). It has also been suggested that hypertrophy of the theca interna, the inner capsule of the graafian follicle, causes compromise of the space in which the follicular fluid is contained, and hence an increase in hydrostatic pressure (*3*). It has further been postulated that hypermia of blood vessels in the follicle (*4*) and increased resistance of lymphatic tissue (*5*) contribute to hydrostatic changes. Other authors have suggested different mechanisms of liquor folliculi increase. Smith and Kettering-ham (*6*) believed that the presence of an osmotically active substance produces the rise of liquor folliculi. Zachariae and co-workers (*7–9*) suggested an endosmotic process due to alteration of the blood–antral fluid barrier. Contradicting these hydrostatic hypotheses are several observations that the rupture of the graafian follicle may be either an explosive phenomenon or an oozing process (*10*). The latter cannot be explained on a hydrostatic basis. Mechanical pressure, applied externally or internally, may only occasionally result in rupture (*11*). Often the margins of the ruptured walls are smooth or

round, which suggests a mechanism other than explosion. Further, in some animals, considerable decrease in volume of the preovulatory follicle has been observed just prior to rupture. Finally, there is evidence that hydrostatic mechanisms are not brought into play in follicular rupture in fish, amphibia, birds, and certain mammals in which liquor folliculi is absent.

The tubal fimbriae do not seem to play a prominent role, as ovulation appears to occur normally in the pig and chicken even when the fimbriated ends and oviducts have been amputated (*12*). Digestive action of proteolytic enzymes has been suggested by Schochet (*13*) as leading to rupture. Several proteolytic enzymes have been found in liquor folliculi by other investigators (*14–16*). It is difficult to understand why enzymatic digestion should act only on the wall of the stigma and not at other sites. Perhaps the absence of vascularization in the stigma is of importance in this regard.

At present, there is still no single hypothesis which satisfactorily explains the entire mechanism of graafian follicle rupture.

Immediately following discharge of the ovum, profound changes occur in the wall of the follicle. The cyst collapses and the lining cells undergo luteinization. This process is characterized by imbibition and enlargement of the cells, during which time they become closely packed. Luteinization and proliferation occur in both the granulosa layer and in the theca interna. The total effect is the formation of a large structure, the corpus luteum. The life span of the corpus luteum during a nonpregnant cycle averages about eleven days. One of the major functions of this new structure is the formation of progesterone. Estrogens continue to be secreted during the luteal phase of the cycle. The trophic control of endocrine activity of the corpus luteum in cycles in which pregnancy does not supervene is poorly understood. Its life is brief unless it is exposed to massive trophic stimulation from the products of conception in the form of chorionic gonadotropin (HCG) and/or human placental lactogen (HPL). Within a few days of corpus luteum degeneration, a new follicular phase commences and the process is repeated.

2. *Changes in the Endometrium*

The above described cyclic pattern in the ovary results in cyclic production of steroid hormones during the menstrual cycle (Fig. 2, see p. 14 and Fig. 4, p. 15). Active estrogenic hormones are

produced in the pre-ovulatory follicular phase. During the postovulatory luteal phase, marked progesterone production also occurs.

Since the mucosal lining of the uterus (endometrium) and vagina are target tissues for the action of ovarian hormones, these also demonstrate cyclic variations.

In response to increasing amounts of estrogens from the developing graafian follicle, endometrial repair and growth begins and proceeds rapidly. This, the proliferative phase, is characterized by total endometrial reconstruction. Within two or three days, the endometrium, which was necrotic, disorganized, and devoid of surface epithelium at the end of the previous menstrual cycle, now demonstrates an intact surface, the growth of new vessels from the stumps of the old, and reformed glands and stroma. Mitotic activity is common in epithelial and stromal cells. During the initial portion of the proliferative phase, the endometrial glands are narrow, tubular, and lined by cuboidal epithelium. The epithelium then becomes columnar, with basal nuclei and uniform eosinophilic cytoplasm resembling that of the glands. The stroma is still relatively dense, compact in the basal layer but becoming looser toward the surface. Small blood vessels run obliquely toward the surface.

By the time of ovulation, the glands have increased in size, are arranged perpendicular to the surface, and begin to demonstrate a convoluted pattern. Basal vacuolation begins to appear. In the glandular epithelium, large amounts of alkaline phosphatase and ribonuclease are present. The stromal vessels are more coiled during the proliferative phase. The endometrium doubles or trebles in thickness: From 1 mm after menstruation it attains a thickness of 2–3 mm by the fourteenth day of the cycle.

Following ovulation the secretory, or luteal, phase commences. The endometrial glands increase in size and become actively secretory under the influence of progesterone and estrogens. They demonstrate progressively more elaborate convolutions, becoming increasingly tortuous. Because of infoldings resulting from this growth, the glandular ephithelium presents first a "corkscrew" and then a "sawtooth" appearance in cross section. During the twenty-first or twenty-second day of the cycle, the glandular secretions enter the gland lumen. This secretion, rich in glycogen, is thought to have a nutritive function for the potential fertilized ovum which may reach the uterus. In the epithelium, as the nuclei move downward in the cells, basal vacuoles appear. Glycogen, acid phosphatases, and lipid material are contained

in the endometrial cells. In the mucosa, three layers can be distinguished: a superficial layer, still very compact because of engorged capillaries and venules, a middle, spongy layer, composed of dilated and tortuous glands, and the deepest nonfunctional layer, which contains the inactive fundi of the glands and remains almost unchanged in structure. The stroma becomes edematous in the basal layer as progesterone production begins to wane. Four or five days before menstruation, endometrial growth ceases. Increasing infiltration with leukocytes produces an inflammatory picture. Within one or two days shrinkage is apparent. This occurs as a result of dehydration of stroma, decreased blood flow, and discharge of the glands' secretions. Heralded by extravasations of serum and blood, menstruation occurs. The necrotic endometrium is fragmented and the surface epithelium breaks; this is carried out in the blood-containing discharge, the menstrual flow. The sloughing process continues until the only remaining element is the basal layer with its straight arteries, compact stroma, and simple glands.

3. *Changes in the Vaginal Tract*

Proliferative and luteal phase changes are also reflected in the vaginal tract. Under the influence of estrogen production during the proliferative phase, marked mitotic activity, resulting in increased numbers of cells, causes an increase in the number of layers of the vaginal epithelium. As the cells' distance from their blood supply increases, some form a mature squamous type of epithelium (cornification) and they then are shed (desquamation). In this superficial layer, acidophilia (red staining with hematoxylin and eosin) of the cytoplasm and pyknosis or fragmentation of the nuclei is observed, representing signs of maturation.

After ovulation, the superficial layer becomes thinner, because of both desquamation and a decrease in individual cell volume. The cytoplasm appears rich in glycogen granules and the nuclei are large and vesicular. Polymorphonuclear leucocytes appear in the vaginal stroma, as well as in the endometrium.

The pattern described above is often reflected in the vaginal smear. Here, in the unstimulated vagina, small basal cells with intact nuclei are present. Under estrogenic stimulation, during the latter part of the follicular phase, a preponderance of large cornified cells with pyknotic nuclei are evident. These stain pink with eosin. Clinically, a common index of estrogen influence is the cornification index, which

represents the percentage of cells in a vaginal smear which have acidophilic cytoplasm.

During the luteal phase, the vaginal smear changes gradually to contain epithelial cells with degenerating nuclei and cytoplasm rich in granules. Mucus, bacteria, and cornified cells are also present.

On occasion, vaginal cytology may be misleading in assessing the occurence and time of ovulation.

4. *Changes in the Cervix*

The cervix has an epithelium histologically distinct from that of endometrium. Under cyclic hormonal production, characteristic changes occur. During the follicular phase, the glandular elements increase in number while the epithelial cells become taller. Their secretion, the cervical mucus, is an alkaline gel, rich in protein and fructose which have a nutritive function for spermatozoa. At the approximate time of ovulation, in response to increased estrogen production, the mucus becomes more copious, clear, thin, acellular, and somewhat elastic. This property permits the mucus to stretch into threads from 5 to 15 cm in length. The "thread test" (spinnbarkeit) is used clinically as an approximate expression of estrogen influence.

Because of the presence of abundant sodium and potassium chloride, the mucus assumes a characteristic fern-like pattern when dried. At this stage the mucus is easily penetrable by spermatozoa, and sperm survival is favored.

Following ovulation, muscle tone rises under the influence of progesterone, and the cervical os becomes tightly closed. The cervical glands become branched, and their secretion changes in chemical and physical properties. The mucus becomes viscous, forming a secure cervical plug. Because of changes in electrolyte composition, ferning disappears. In this phase, the mucus cannot be stretched without breaking. The secretion decreases and becomes opalescent and viscid. During menstruation, desquamation and some loss of blood may occur.

5. *Changes in the Fallopian Tubes*

During the follicular phase, the tubal mucosal cells actively proliferate, while in the luteal phase secretion is increased. At the time of ovulation, muscular and ciliary activity increases considerably.

6. *Other Signs Related to Ovulation*

a. Temperature Changes: Progesterone is thermogenic, and there-