Recent Progress in

HORMONE RESEARCH

The Proceedings of the Laurentian Hormone Conference

VOLUME 29

RECENT PROGRESS IN HORMONE RESEARCH

Proceedings of the 1972 Laurentian Hormone Conference

Edited by

ROY O. GREEP

VOLUME 29

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1973

ACADEMIC PRESS, New York and London
A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: Med. 47-38

PRINTED IN THE UNITED STATES OF AMERICA

PREFACE

The 1972 Laurentian Hormone Conference was held at Mont Tremblant, Province of Quebec, Canada, August 27 to September 1 amidst the lovely setting of the Mont Tremblant Lodge. Never has the Conference been favored with more delightful weather. The warming sun, clear mountain air, perfusion of fall flowers, and fine food, all combined with incomparable scientific fare to make this a memorable gathering.

The program lived up to the highest expectations of this traditionally superlative meeting. In keeping with the usual practice, the program had variety and abundant substance. Masterly presentations were followed by vigorous open and wide-ranging discussions. The Conference members were pleased that Mrs. Gregory Pincus could be present to attend the Pincus Memorial Lecture given by Professor Alfred Jost. This elegant and scholarly discussion of hormones in embryogenesis was a fitting tribute to the memory of Dr. Pincus, who was the moving spirit in the founding and establishment of this annual, high-level, endocrine meeting. The program also included the fascinating discovery and hormonal metabolism of pseudohermaphroditism in rats, the mechanism of induction of parturition, hypothalamic regulation of the prehypophysis, the role of microtubules in cells of the thyroid and pancreas, mechanism of steroid hormone action, plus new developments on the structure and function of growth hormone, lactogenic hormones, and gonadotropins.

After a season of listening to endless rounds of 10-minute papers with time for only a question or two, it was very satisfying to hear these extremely carefully prepared summarizations of years of in-depth probings and thoughtful analysis. Add to this ample time for critical discussion by the leading experts in the field and you have science at its best.

The Committee on Arrangements has lost one of its esteemed members in the passing of Dr. Gordon A. Grant. Dr. Grant had been a member of the Committee from 1952 to 1971 and a regular attendant of the Conference. During his early years on the Committee, Dr. Grant was responsible for the local arrangements in Canada and for the securing of public address, projecting, recording, and transcribing equipment. Dr. Grant had long held important posts as biochemist and administrator in the Canadian Division of Ayerst, McKenna and Harrison now known as the Ayerst Laboratories. The Committee on Arrangements wishes to record its appreciation of Dr. Grant's devoted service to the Laurentian Hormone Conference and extends its condolences to his wife, Ruth.

It is my pleasure to thank Drs. James H. Leathem, Neena B. Schwartz, John C. Beck, Vincent P. Hollander, Edwin D. Bransome, Jr., Robert W. Bates, Edwin B. Astwood, and Alfred E. Wilhelmi for chairing the sessions and aiding in preparing the typescripts of the discussions for publication. Personally and on behalf of the Committee on Arrangements, I wish to acknowledge our great debt to our Executive Secretary, Miss Joanne Sanford for her unstinting labors in arranging the Conference and to her associates, Mrs. Mina Rano and Miss Martha MacRae for their heroic efforts in transcribing the lengthy discussions immediately after each session. The helpful cooperation of Academic Press in producing this fine volume merits our grateful appreciation.

Cambridge, Massachusetts
May 7, 1973

ROY O. GREEP

CONTENTS

Studies on Sex Differentiation in Mammals[1]

ALFRED JOST, BERNARD VIGIER, JACQUES PRÉPIN, AND JEAN PIERRE PERCHELLET

Laboratory of Comparative Physiology, University of Paris VI, Paris, France

In opening this lecture dedicated to the memory of Gregory Pincus, I wish first to give recognition to the founder of the Laurentian Hormone Conference and to express the gratitude of all endocrinologists throughout the world to a scientist who so greatly contributed to the knowledge of this branch of science. I wish also to thank the Committee on Arrangements for inviting me to deliver this lecture and for giving me the opportunity to pay a personal tribute of admiration and affection to Gregory Pincus. I knew him for 20 years, first as an outstanding expert in reproductive biology, and later when he developed "The Pill," and thus had so great an impact on mankind. Throughout all those years he remained a warm friend, one of those few personalities who really count in one's life. This friendship started in 1949 during a chat on the grass in front of the main building at the Worcester Foundation for Experimental Biology. His clear-sighted and generous mind was immediately obvious, and his friendly confidence in me was all the more valuable. In 1952 and again in 1965, I had the privilege to participate in the Laurentian Hormone Conference on invitation from Gregory Pincus. During these years, my wife and I met Elizabeth and Gregory Pincus frequently. We benefited from his open and frank manner of looking at life. Today, in honoring his memory, I wish to honor not only an exceptionally gifted and generous man, but also a great man in science.

I. Introduction

Twenty years ago, in a contribution to the Laurentian Hormone Conference devoted to "Problems of Fetal Endocrinology: the Gonadal and Hypophyseal Hormones" data resulting from intrauterine surgery on rabbit fetuses, especially castration and decapitation (for hypophysectomy), were presented (Jost, 1953). Since that time the field of fetal endocrinology has grown and a large body of information has been gathered throughout the world and in many animal species including man. However, the solution of problems concerning sex differentiation and fetal testicular hormones has probably not progressed as much. In our laboratory, we resumed more intensive research in the field some years ago. In this paper we discuss freely some of the problems in which we are presently involved, especially those concerning the differentiation of the gonads, without making any effort to give a comprehensive review of the literature (see review in Jost, 1971).

[1] The Gregory Pincus Memorial Lecture.

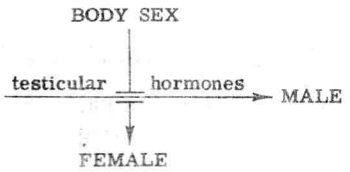

FIG. 1. Scheme to show that every structure would become feminine if not prevented by testicular hormones.

One point discussed in detail at the 1952 Laurentian Hormone Conference dealt with the role of the fetal testis in the development of the male genital tract. It was shown that the masculine characteristics of the body have to be imposed in males by the fetal testicular hormones against a basic feminine trend of the mammalian body. Female organogenesis results from the mere absence of testes, the presence or absence of developing ovaries being unimportant (Jost, 1947, 1953). This observation demonstrated that the fetal testis is a remarkably important endocrine organ and that there is no symmetry in male or in female development. Femaleness corresponds to an intrinsic program of the primordia; it is obtained *in vitro* in the absence of hormones (Jost and Bergerard, 1949; Jost and Bozic, 1951; Picon, 1969). Masculine differentiation is actively imposed on the system at an early stage, no possibility being left for further feminine differentiation. This can be conveniently summarized in a simple scheme showing that every structure would become feminine if not prevented from doing so by testicular hormones (Fig. 1) (Jost, 1970b, 1972a).

The same scheme has been shown by others to be valid in rats and in some other animals for the differentiation of the hypothalamic centers controlling the release of gonadostimulating hormones and of the neural structures which mediate sex behavior in adulthood. A few years ago, after a reconsideration of the available evidence, it was wondered (Jost, 1965, 1970a,b, 1972a,b) whether in mammals the development of the gonads does not obey a similar scheme, where testicular differentiation is imposed at an early stage and under genetic control, by a masculine triggering mechanism, on a primordium which in the absence of this trigger would later become an ovary.[2] Such a working hypothesis is not only verbal, it should be an incitement to experimental studies of gonadal sex differentiation. In the scheme, the horizontal interrupting arrow which schematizes the system imposing maleness actually may correspond to a complex mechanism. This was suggested when the fetal

[2] In birds the situation is probably reversed, and femaleness has to be imposed against a basic masculine trend (Jost, 1965).

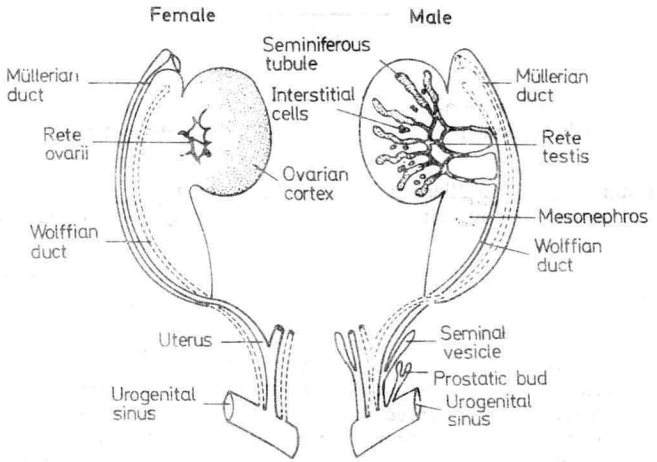

FIG. 2. Composite scheme showing some homologies in the development of male and female organs from the undifferentiated condition characterized by the presence on the mesonephros of a double set of ducts (Wolffian and Müllerian ducts) and of a gonadal primordium. In the female or in the castrated male rabbit fetuses only the Müllerian ducts persist, and a female system develops. In males the testis is responsible for the disappearance of the Müllerian ducts and for the development of the male characters. The differentiation of the urogenital sinus and external genitalia is not shown. Chronological differences in male and female development are emphasized in the text (see Fig. 6). From Jost (1970b).

testicular hormones controlling the development of the genital tract were studied and when a dual testicular control was suspected, without mentioning the hypophysial control of the fetal testis itself, in some species (see Jost, 1966).

Figure 2, which summarizes some homologies in the development of male and of female organs, may help to clarify the following discussion.

II. The Fetal Testicular Hormones Controlling Differentiation of the Body Sex

From the very beginning of our experiments it was realized that testosterone or methyltestosterone given to castrated rabbit fetuses replaced the fetal testis in masculinizing most of the fetal structures, but that it could not duplicate the fetal testis in inducing the retrogression of the Müllerian or female ducts (see Fig. 2) (Jost, 1947, 1953, 1965). The concept was introduced that the fetal testis might produce at least two, or two kinds of, morphogenetic secretions one (or more) masculinizing hormone(s), and a Müllerian inhibitor, the action of which is not

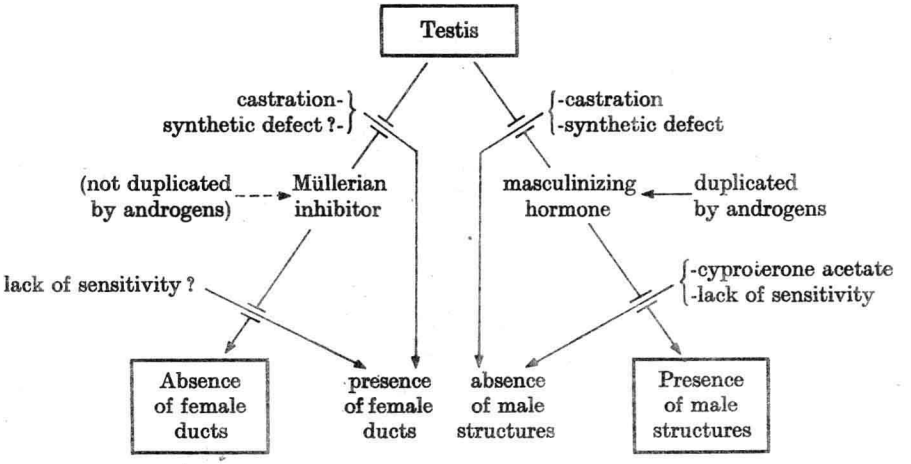

FIG. 3. Scheme summarizing the testicular control of the differentiation of the sex ducts and sex characters. Some conditions capable of interfering with the normal testicular activity are indicated (crossing arrows). The data concerning cyproterone acetate refer to experiments made on rabbit fetuses (in rats the effect is not the same). From Jost (1970b).

duplicated by steroidal androgens (Fig. 3). This gives a simple explanation for the condition of the genital tract in humans afflicted with so-called "testicular feminization," or in pseudohermaphroditic rats described by Stanley and Gumbreck (1964) or in Tfm mice (Lyon and Hawkes, 1970): in these cases the fetal testes induced the disappearance of the Müllerian ducts but failed to masculinize the genital tract, probably owing to androgen insensitivity of the tissues. Testicular 17α-hydroxylase deficiency resulting in impairment of testosterone synthesis had similar effects (New, 1970).

Another very interesting piece of evidence was obtained when Elger (1966) (confirmed by Jost, 1967) treated pregnant rabbits with the antiandrogen cyproterone acetate; in male fetuses all male characters were prevented from developing, but the Müllerian ducts were absent. This could be understood if cyproterone acetate opposed the masculinizing androgen but did not oppose the Müllerian inhibitor.

In some other animal species the effects of cyproterone acetate on sex differentiation are more complex (review in Jost, 1972c). In male rat fetuses cyproterone acetate opposes the masculinization of those parts of the genital tract which become masculinized on or after day 18 (e.g., urogenital sinus), but it does not prevent the action of the fetal testis on the Wolffian ducts and on the development of seminal vesicles

before day 18 (Jost, 1968). This and other data would suggest a shift in the nature of the masculinizing substance produced by the rat fetal testis on day 18 (Jost, 1970c, 1972c; Elger *et al.*, 1970). Although it has been verified that at an age corresponding to sexual differentiation the rat fetal testis produces biologically active androgens (Jost, 1948) and is capable of converting labeled progesterone to testosterone *in vitro* (Noumura *et al.*, 1966), the exact nature of the fetal testicular masculinizing hormone at different developmental stages should be studied further. The physiology of the fetal receptors is another aspect of this study: Wilson and Lasnitzki (1971) observed that the urogenital tubercle, when incubated *in vitro*, has the capacity to convert testosterone into dihydrotestosterone before its sexual differentiation, while the duct system acquires this capacity only after gender identification.

The Müllerian inhibitor still has to be isolated. It can be assayed on fetal ducts cultivated *in vitro*. In a few trial experiments made a long time ago testosterone did not induce the retrogression of the Müllerian ducts of male rat fetuses *in vitro* (Jost and Bergerard, 1949). More recently in our laboratory, Drs. N. Josso and R. Picon took over that work. The inhibitory effect of fetal testes on Müllerian ducts was analyzed (Picon, 1969, 1970), and it was observed that rabbit (Picon, 1971), human (Josso, 1971a), or calf (Jost *et al.*, 1972; Jost, 1972b) fetal testes inhibit rat Müllerian ducts. Testosterone, however, does not (Josso, 1971b).

Dr. Josso (1972), now in her own laboratory, recently isolated seminiferous tubules and interstitial cells of calf testis; only the former have the Müllerian inhibiting capacity *in vitro*, a result which agrees with the situation prevailing in the rat fetus, whose Müllerian ducts are inhibited by the testis before the interstitial cells differentiate morphologically.

Experimentation on the Müllerian duct inhibitor is pursued by the senior author of this paper with the help of Mrs. O. Valentino. Müllerian ducts of 14.5-day-old fetuses of either sex or of 15.5-day-old male fetuses are used. The effects of fetal testes, steroids, and other substances or extracts were compared. For instance, prostaglandins E_1, E_2, and $F_{2\alpha}$ at concentrations of 10^{-6} *M* to 10^{-4} *M* did not induce Müllerian regression (Fig. 4). Müllerian regression has been obtained inconstantly with some water-soluble fractions isolated from the testes of 17- to 19-day-old rat fetuses, but these results have to be repeated and confirmed before being reported in more detail.

When the Müllerian inhibitor will be available, its role in the sex differentiation not only of the genital tract, but also of the gonads will become accessible to experimental inquiry.

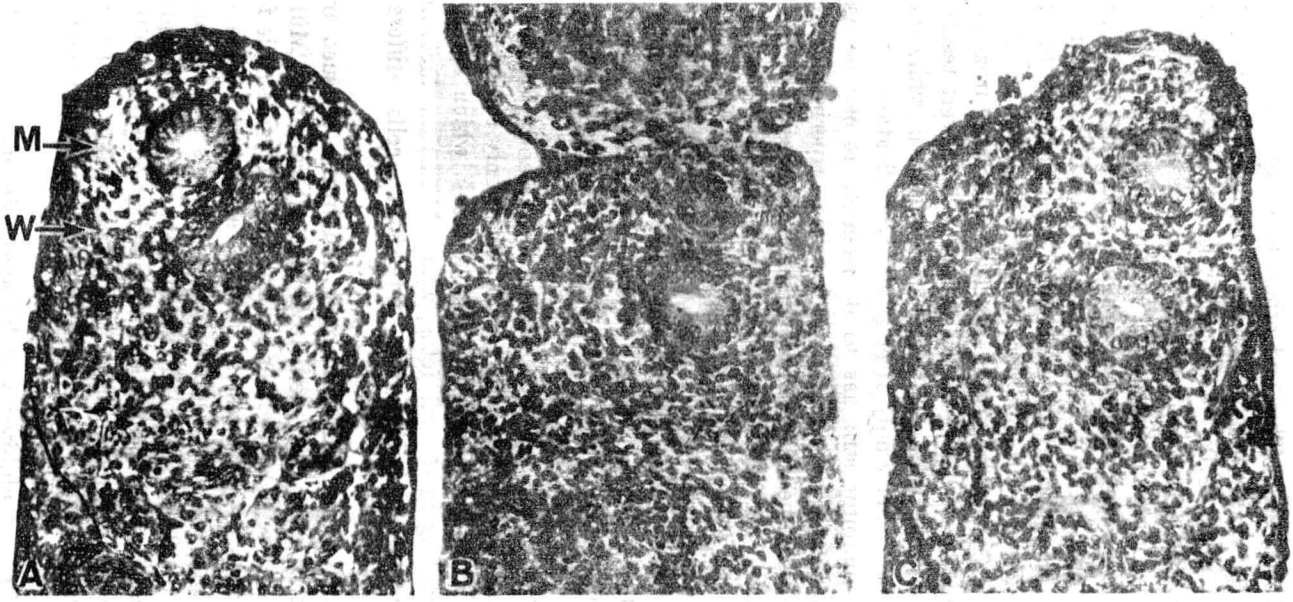

FIG. 4. Histological sections through the genital ducts (M = Müllerian; W = Wolffian) taken from 14.5-day-old female rat fetuses and cultivated *in vitro* for 3 days (medium 1066). (A) Control. (B) Ducts cultivated in contact with a rat fetal testis (top); the Müllerian ducts has almost completely regressed. In the section illustrated, it still can be seen. (C) Ducts cultivated in the presence of 2.5×10^{-5} *M* prostaglandin. $F_{2\alpha}$. The Wolffian ducts normally regress at a later developmental phase. ×240.

III. Sexual Differentiation of the Gonads

Before discussing gonadal differentiation, it seems fitting to recall one basic structural characteristic of adult testes and ovaries (Fig. 5). In both gonads the germ cells are stored in contact with a single type of cells (Sertoli cells or granulosa cells) in structures which are isolated from the other tissues or from blood by a more or less complex basement membrane or wall. The functional significance of this condition still has to be explored further, but it seems necessary for the survival of the germ cells. It is noteworthy, and it will be emphasized again later, that during development the male germ cells are enclosed in the seminiferous cords much earlier than are female germ cells in their follicles.

The question of how a testis or an ovary develops from its early embryonic primordium is given a rather simple answer in most elementary textbooks. The situation is not as clear if one looks at original papers. For more than a century many very good biologists have discussed the question and debated theoretical interpretations without reaching a general agreement.

Each gonad develops as a tiny swelling of the inner aspect of the mesonephros, usually near a glomerulus, or at least near mesonephric tubules as is the case in the rat (Fig. 7). At the surface of this swelling the cells are arranged more or less regularly, in a so-called epithelium, although many of these cells are not very different or distinct from those lying beneath, which are often referred to as mesenchymal cells. Some cells produce connective fibrils at early stages and can be considered cells of the connective tissue; others look undifferentiated, and

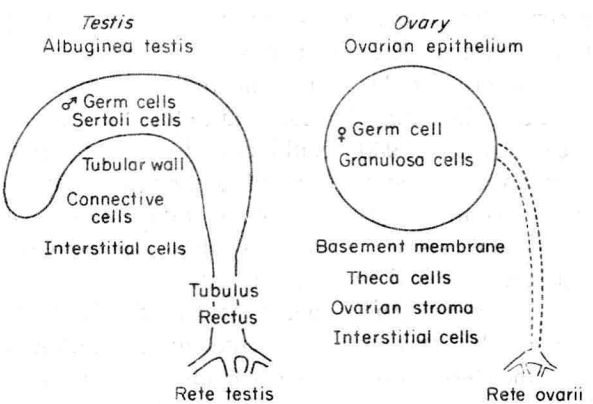

FIG. 5. Schematic representation of the main cellular components of a testis and of an ovary.

their derivation or lineage is difficult to trace. Among these somatic cells, the usually larger primordial germ cells can be distinguished. Their extragonadal origin is now generally accepted (see Pinkerton *et al.*, 1961).

A. Some Classical Theories

Preconceived theoretical ideas such as the necessary derivation of an epithelioid cell from a preexisting epithelium (i.e., from the "germinative epithelium" or from a preexisting mesonephric structure) have often influenced interpretations in the past. Cellular movements (e.g., ingrowth of cell cords from the superficial epithelium) were also advocated, but were impossible to prove in fixed tissues. Moreover, the watery cells of young fetuses are difficult to preserve satisfactorily with conventional fixative fluids (this probably explains why many papers contain mainly drawings or low power illustrations).

Some old theories are worth recalling since they still have consequences. In 1870 Waldeyer developed the concept that every embryo passes through a period of potential hermaphroditism because it has discrete female and male structures: (1) the female structures were the "germinative epithelium" (which was believed to proliferate the egg tubes previously described by Valentin and by Pflüger in developing ovaries) and the Müllerian ducts; (2) male structures were represented by the Wolffian ducts and nephric derivatives producing the seminiferous tubules and the male germ cells. Sex differentiation resulted from the development of one of the two components. These views were rapidly amended, but in essence largely maintained, by those who claimed that both the primary sex cords (potential seminiferous tubules) and later the Pflüger's cords (ovigerous cords) are proliferated from the germinative epithelium. However, this description never obtained a general consensus. The seminiferous cords were repeatedly reported to differentiate inside the gonadal blastema—by local autodifferentiation according to Prenant (1889). Fischel (1930) could not observe any participation of the so-called germinative epithelium in human gonadal organogenesis; nor did Odor and Blandau (1969) in developing mouse ovaries, studied with the electron microscope. Gropp and Ohno (1966) reached a similar conclusion in a histochemical study of the calf gonads.

Witschi (1951) revived the concept of gonadal hermaphroditism in a different way in his theory of corticomedullary antagonism. He assumed that the undifferentiated gonad is made up of two morphologically distinct and physiologically antagonistic components: the cortex, represented by the coelomic epithelium; and the medulla, ingrown from the mesonephric blastema. In mammals the delineation of the two territories

at the time of incipient sex differentiation is not very clear. Both components participate in the differentiation of the gonads. The cortex "furnishes the primitive gonad with follicle cells that become granulosa cells in the ovary, and sustentacular cells (of Sertoli) in the testis." The medulla differentiates the rete tubules, and "in both sexes it furnishes the interstitial cells and, in advanced stages of ovogenesis, contributes to the formation of the follicular thecae" (Witschi, 1962). The inductive capacity was attributed to the follicle cells in females and to the interstitial cells in males (Witschi, 1967).

Theories postulating morphological and functional hermaphroditism or ambisexuality have a serious drawback in that they understate the chronological asymmetry of gonadal differentiation in the two sexes. Males differentiate very early, females very late.

B. Chronology of Sexual Differentiation

Figure 6 summarizes the developmental chronology in the human fetus as compiled from the literature (Jost, 1971, 1972a). A similar table may be made for the calf fetus, which will be discussed later. The table shows that the testes develop long before the ovaries.

The sequence of events occurring in both sexes can be listed chronologically as follows: (1) differentiation of the first seminiferous cords in males accompanied with incipient cellular scarcity at the surface of the primordium (future albuginea testis); (2) approximately a week

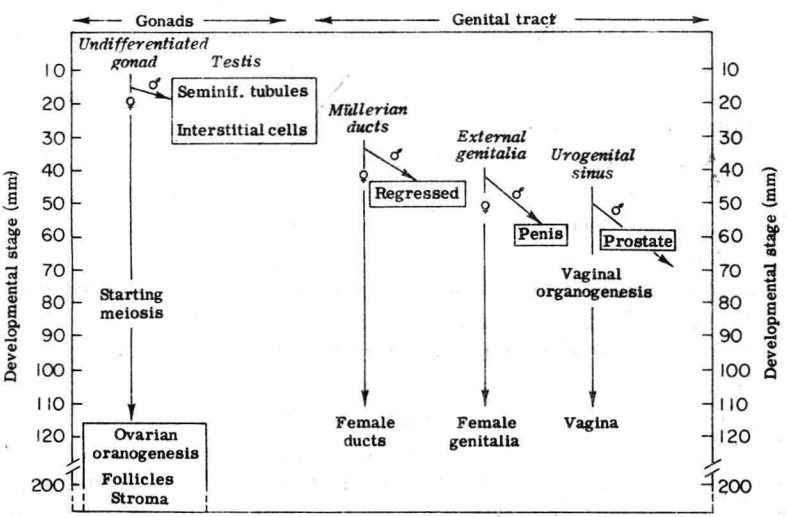

FIG. 6. Summary of the chronology of sexual development in the human fetus according to fetal crown–rump length (ordinates). From Jost (1971).