chapter 3

Principles of Reproductive Biology and Development

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Chapter 3

Principles of Reproductive Biology and Development

INTRODUCTION

Normal reproductive function comes about only as a consequence of interactions among multiple physiological systems. In the narrowest sense, reproduction is the union of sperm and ovum to form a new biological entity. Yet the union of gametes is merely a signal event in the continuum of physiological processes comprising normal reproductive function, Prior to fertilization, for example, the maturation of sperm and egg depends on the coordinated secretion of multiple hormones. At coitus, synchronized neural reflexes and appropriate reproductive behaviors are required to bring gametes together. After conception, embryonic growth depends on the integrity of the zygote and a remodeling of the maternal circulatory system. The later growth and development of the offspring are a function of both prenatal and postnatal nutrition.

For purposes of this report, reproductive function is used in the broadest sense possible. It encompasses:

- the functional and structural integrity of the sperm and ova;
- differentiation and development of the internal and external reproductive organs and endocrine glands;
- activation of the adult reproductive system at puberty;
- senescence of the adult reproductive system (e.g., menopause);
- behaviors associated with or subserving reproduction (e.g., libido);
- maternal and paternal prenatal events;
- embryonic and fetal events (e.g., organogenesis);
- maternal postnatal events (e.g., lactation); and
- child health and development.

The significance of some aspects of reproductive function not overtly related to fertility is often underestimated; because they are held to be strictly private matters, many of these subjects tend to go undiscussed. In fact, an individual's reproductive function and, should it occur, reproductive dysfunction, can be of extraordinary personal importance. Impotence, menstrual pain, and loss of libido exemplify instances of reproductive dysfunction that can have substantial impact on individual well-being and human relationships.

Concern about reproductive processes is not limited to the brief periods in an individual's lifetime during which reproduction may actually occur. Reproductive function is an integral part of everyday human health and well-being. Before, during, and after the childbearing years, reproductive hormones may act, for example, on such variables as resistance to heart disease and cancer, immune function, complexion, bone mineral content, and feeling and mood. Threats to reproductive function can take place at nearly any point during an individual's lifespan. In fact, the most insidious hazards to reproductive function may be those whose immediate effects are apparently benign, but whose ill effects surface at a later date.

Viewed from this perspective, the bounds of typical reproductive function and the task of defining atypical reproductive function seem impossibly broad in scope. Yet, by using an array of well-defined endpoints, it is possible to assess human reproductive function in both a qualitative and a quantitative manner.

MEASUREMENT OF REPRODUCTIVE FUNCTION: RELATION TO WORKPLACE HAZARDS

For the couple desiring to reproduce, it may be argued that the only meaningful index of reproductive function is the ability to produce a healthy baby when they wish to do so. At any given time, the couple either can, or cannot, procreate. But, for the purposes of this report, this final common denominator of successful procreation must be dissected into numerous constituent factors in order to: 1) examine the nature of reproductive function and dysfunction, and 2) relate reproductive dysfunction to a potential workplace hazard. Multiple endpoints of reproductive function further serve to define the reproductive status and physiological well-being of the majority of the population who are, at any given time, not procreating,

Endpoints used for measuring reproductive function may be divided into two groups: 1) those serving as indices of reproductive function independent of fertilization, and 2) those serving as such indices after fertilization. There are close parallels between male and female reproductive processes up to the point at which sperm and egg mature. Thereafter, most of the reproductive processes related to procreation occur in the female, as the fetal-placental-maternal system exhibits many stages without counterpart in the male.

Table 3-1 lists measures by which reproductive function may be assessed in adult men and women. The measures listed are limited to those that are readily observable in a relatively noninvasive fashion. In order to have broad applicability in a workplace or outpatient setting, such measures are obtainable by one or more of the following means:

- a detailed patient history,
- a physical examination,
- blood samples,
- semen samples, or
- urine samples.

Table 3-1 illustrates the disparity between the ease with which male and female reproductive parameters can be assessed. That is, sperm are readily accessible, while eggs are not Table 3-2 lists measures by which reproductive function may be assessed in the adult woman and her offspring during pregnancy and after birth. Again, the measures listed are limited to those readily obtainable in the relatively noninvasive fashion just described. A comprehensive discussion of the methods used to assess reproductive function, including more sophisticated methods than those listed in these tables, appears in chapter 5.

NORMAL REPRODUCTIVE BIOLOGY AND DEVELOPMENT

Hormonal Control Mechanisms

In both men and women, the hypothalamus, an area at the base of the brain, serves as a fundamental neural regulator of the body's reproductive function. It receives neural and hormonal input from the brain and endocrine glands and responds to these stimuli by secreting luteinizing hormone-releasing hormone (LHRH) and other hormones. The hypothalamus releases LHRH into tiny blood vessels which surround the pituitary gland, With a target so nearby, LHRH is released in minute amounts and breaks down quickly. As a consequence, this vital reproductive hormone—a telling indicator of reproductive function—is possible but difficult to detect in peripheral blood circulation.

LHRH acts on cells of the anterior pituitary gland to promote secretion of two hormones, lu - teinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH, known as gonadotropin, direct hormone and gamete production by the testes and ovaries. As the gonads release hormones in response to stimulation by LH and FSH, these gonadal hormones act at the hypothal-

Table 3-1.—Measures of Reproductive Function Readily Obtainable Prior to Fertilization

	Affected individual		
Endpoint	Male	(Both)	Female
Sexual function:	Erection Ejaculation	Libido Behavior	
Endocrine system:		Luteinizing hormone Follicle-stimulating hormone Steroid hormones (androgens, estrogens, and progestins)	Cervical mucus quality
Germ cells:	Sperm number Sperm motility Sperm shape (morphology) Chromosomal integrity Fertilizing ability		
Fecundity:	Testicular integrity Semen quality	Integrity of external genitalia	Ovarian integrity Blockage of oviduct Menstrual regularity Amenorrhea Anovulatory cycles
Secondary sexual characteristics:		Breast development Facial and axillary hair growth Sebaceous glands	
Reproductive lifespan:		Age at puberty	Age at menopause

SOURCE: Office of Technology Assessment.

Table 3.2.—Measures of Reproductive Function Readily Obtainable After Fertilization

	Affected individual		
Endpoint	Female	(Both)	Offspring
Endocrine system:	Human chorionic gonadotropin Steroid hormones, especially progesterone		
Health during pregnancy:	Hemorrhage Toxemia	Fetal death Spontaneous abortion	Morphology Chromosomal aberrations
Perinatal period:		Premature birth Postmature birth:	Death Chromosomal aberrations Birth defects Birth weight Apgar score
Postnatal period:	Lactation		Infant death Childhood morbidity Childhood malignancies Development Behavior
Reproductive lifespan:	Age at menopause		Age at puberty

SOURCE: Office of Technology Assessment.

amus and pituitary gland to reduce the secretion of LH and FSH. In this way, a feedback loop operates, involving the hypothalamus, pituitary gland, and gonads. A defect at any point in the hypothalamic-pituitary -gonadal axis or in the metabolism of their modulator hormones will interrupt the normal pattern of reciprocal hormone secretion among these organs.

The moment-to-moment secretion of LH and FSH is best described as episodic, or pulsatile, with a frequency of 1 to 2 hours under normal conditions. The pattern of episodic gonadotropin secretion represents endocrine signaling from the hypothalamic-pituitary unit to the gonads, thus directing normal ovarian and testicular activity (6,14,58). In addition, larger alterations in the pattern of gonadotropin pulses are correlated with dramatic changes in reproductive function, as in the peripubertal period, at menopause, and in certain pathological conditions. The pattern of hormone secretion is difficult to detect when the plasma concentration of gonadotropins is low, as in prepubertal individuals.

Normal, premenopausal, adult women (but not men) exhibit a second cyclic mode of hormone secretion. This cyclic secretion is marked by a periodic, synchronous burst of LH and FSH release, known as the preovulatory LH surge. Estrogens secreted by the cells in the ovaries act upon the brain to trigger the preovulatory surge. Thus, coordination of both neural and ovarian signals is required for normal ovulation to occur.

In order to map the pattern of LH and FSH secretion—and thus judge hypothalamic-pituitary function-it is necessary to draw serial blood samples at frequent intervals. A single blood sample yields no information about the pattern of gonadotropin secretion, although it can some times identify gross abnormalities in hormone levels

The episodic nature of LH and FSH secretion is a consequence of episodic release of LHRH from the hypothalamus. In this way, intrinsic properties of the central nervous system mediate gonadotropin secretion and, ultimately, gonadal function. It is through the central nervous system that psychological, emotional, sensory, and environmental stimuli can profoundly influence reproductive function.

Male Reproductive Function

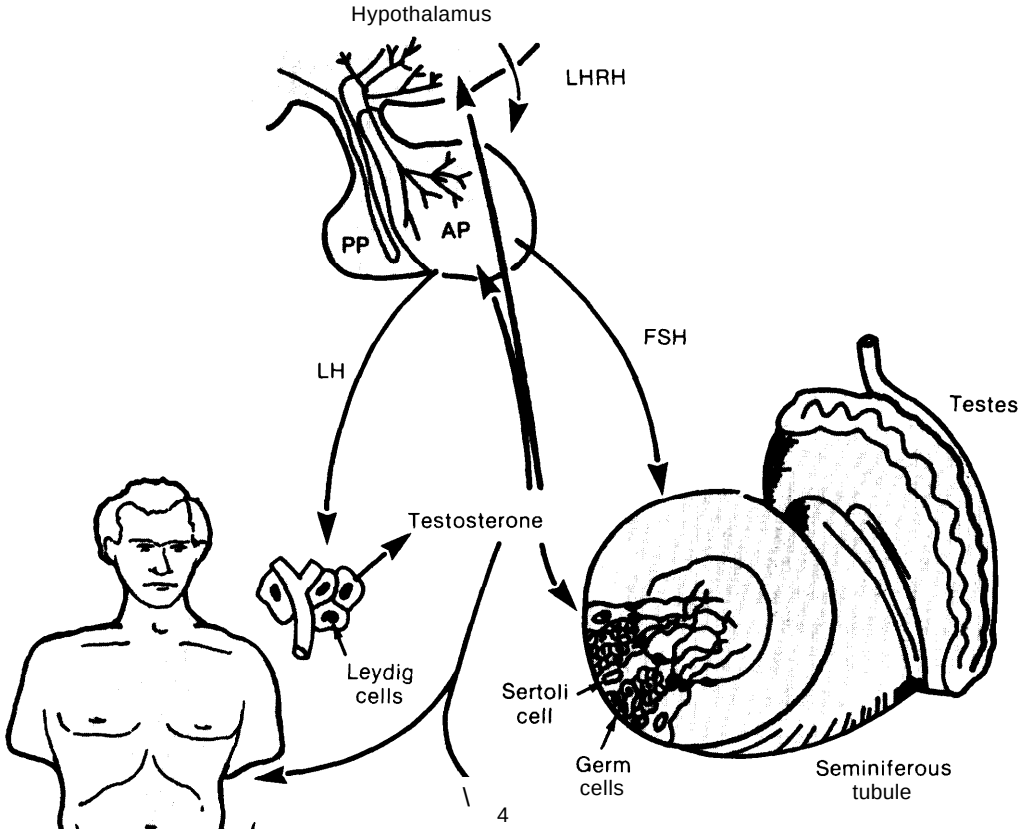
In the male, the testes are the target of the LH and FSH released by the pituitary gland (figure 3-l). The testes serve two functions, producing both gametes (sperm) and hormones, notably testosterone. Sperm develop in the loops of seminiferous tubules within the testes; these tubules make up the bulk of the testes. Testosterone is produced by the Leydig cells, which are scattered throughout the testes and lie outside the seminiferous tubules. Damage to the sperm-producing tubules does not necessarily affect testosterone production by the Leydig cells. However, a deficit in testosterone production by the Leydig cells is likely to be accompanied by impaired sperm production because of feedback to the pituitary and hypothalamus.

Sperm are produced continuously in the testes beginning at puberty and continuing throughout life. A decline in sperm production may occur as men age, becoming apparent in the sixth decade and beyond (22,41). Such an age-related decline in sperm production is not observed in all study populations (44), and the response of the testes to aging is variable (41).

Sperm production begins with division of sperm precursor cells, the spermatogonia, within the seminiferous tubules. Spermatogonia are generally thought of as falling into two broad categories—those in a self-renewing pool and those in a proliferating pool of cells. Most spermatogonia are in the latter. These spermatogonia divide to produce two daughter cells that are destined to become spermatozoa. A few more spermatogonia exist in a pool of cells that renew themselves. These spermatogonia produce two daughter cells that can either remain in the population or commit to the proliferating pool of cells.

When spermatogonia are damaged or killed by a toxic agent (e.g., ionizing radiation) reproductive function in the male maybe greatly impaired. There is some evidence that a third type of spermatogonium that rarely divides under normal cir-

Figure 3-1 .—The Male Reproductive System



Key:

PP: posterior pituitary
AP: anterior pituitary
LH: luteinizing hormone
LHRH: luteinizing hormone-releasing hormone
FSH: follicle-stimulating hormone

SOURCE: Adapted from S M Harman, "Cltnlcal Aspects of Aging of the Male Reproductive System, " *The Agirrg Reproductive System (Aging, Volume* 4), E. L. Schneider (ed) (New York: Raven Press, 1978), pp. 29-58.

cumstances may begin to actively divide to replenish the population of spermatogonial cells, and in this way, the testes may regain sperm-producing capacity. Although it may be temporary, interruption of fertility can have lifelong consequences in that timing of procreation can be crucial. The gonad itself may be the target of toxic agents (e.g., DBCP). In such cases, depending upon the extent of exposure, gonadal damage can be irreversible.

The final stages of sperm maturation take place during passage of the sperm from the testes through the long, coiled epididymis. Maturation involves changes in motility, metabolism, and morphology. Sperm then leave the body in the semen, a fluid comprised of secretions of the seminal vesicles, prostate, and glands adjacent to the urethra. Ejaculation is a two-part spinal reflex that involves: 1) emission, the movement of the semen into the urethra; and 2) ejaculation proper, the propulsion of the semen out of the urethra at the time of orgasm.

The process of forming sperm from primitive stem cells in the seminiferous tubules consumes an estimated 64 to 74 days; the sperm take an additional 9 to 12 days to pass through the epididymis. For this reason, changes in the sperm-producing activities of seminiferous tubules are generally not immediately reflected in ejaculated semen.

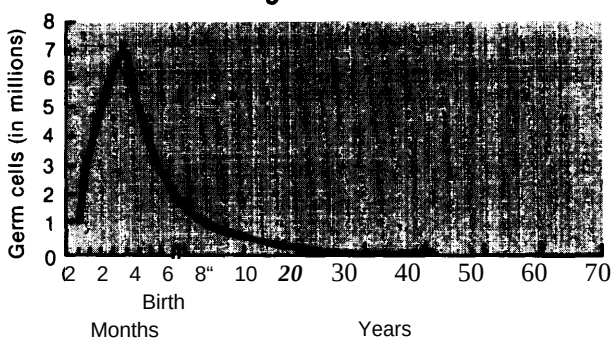
Testosterone has a number of actions. It diffuses into the seminiferous tubules to promote sperm development. Testosterone is also secreted into the general circulation, where it acts at the hypothalamic-pituitary unit to modulate the release of LH. (FSH release by the pituitary gland is modulated by a protein factor called inhibin, which is secreted from the seminiferous tubules.) Testosterone acts to promote growth and development of male sexual organs, causing an increase in size of the penis, prostate, Cowper's gland, and seminal vesicles, and promoting secretory activity of the latter three glands. Male secondary sex characteristics (e.g., increased muscle mass, beard growth, deep voice, and underarm and pubic hair) are all developed and maintained by testosterone. Sex drive in men increases in puberty as testosterone rises, usually decreases in the event of castration, and is restored by exogenous testosterone in 'men with dysfunctional testes.

Female Reproductive Function

In the female, the target organs of LH and FSH are the ovaries. Within each ovary are primitive germ cells, called oocytes. The number of oocytes in the ovaries is fixed prenatally and is greatest during the fetal stage of development, when it reaches several million. After peaking in the seventh month of gestation, the number of oocytes decreases to fewer than 1 million at birth, and continues to decline markedly throughout life (figure 3-2). Only about 400 oocytes are actually ovulated during the period of female fertility. In contrast to the continuing renewal of germ cells throughout an adult male's life, no new oocytes are formed after the fetal stage in the female.

The female menstrual cycle averages 28 to 29 days, but may range from 21 to 50 days (13). Each month, LH and FSH stimulate growth of a selected group of ovarian follicles–small spheres of cells that surround a developing egg. Concomitant with the growth in size and number of follicular cells is the production of estrogenic hormones by these ovarian cells. Estrogens are responsible for the thickening of the uterine lining, or endometrium. Estrogens also stimulate and maintain secondary sex characteristics (e.g., growth of breasts, development of a flared pelvis, and distribution of body

Figure 3.2.-Relation Between Oocyte Number and Age n Women



SOURCE: Adapted from D. R. Mattison, M. S. Nightingale, and K. Shiromizu, "Effects of Toxic Substances on Female Reproduction," Environ, *Hea/th Perspect. 48:43-52, 1983.*

fat to hips and thighs), and induce cyclical alterations in cervical mucus.

Follicular growth continues throughout the follicular phase of the menstrual cycle. One dominant follicle then prevails, while the 20 or more other follicles at the same stage of development begin to degenerate. At ovulation, the dominant follicle ruptures in response to a surge of LH and FSH, and the ovum travels down the oviduct to the uterus. Fertilization of the ovum by a sperm usually takes place in the oviduct, within 24 to 36 hours after ovulation. The follicular cells of the dominant follicle remaining in the ovary form a temporary endocrine organ called the corpus luteum.

During the second half of the menstrual cycle, the luteal phase, the corpus luteum produces high levels of progesterone in addition to estrogens. These hormonal changes prepare the uterus for a possible pregnancy. If a fertilized egg does not reach the uterus and begin to implant, the corpus luteum regresses, the uterine lining is discharged, and menstruation occurs. (Figure 3-3 summarizes the female reproductive cycle.) The luteal phase usually consumes about 14 days. Variability in the length of the overall menstrual cycle, from 21 to 50 days, typically results from varying duration of the follicular phase, rarely from variations in the luteal phase, although shortening of the luteal phase may profoundly affect the ability to support implantation of the fertilized egg (see chapter 5).

Menopause, the cessation of menstrual cyclicity, occurs when the ovary is virtually depleted of oocytes, and is marked by diminished production of ovarian estrogens, bursts of LHRH release, sudden body-temperature fluctuations, and other changes of a longer term. It occurs, on average, at about age 50 (figure 3-4). The destruction of oocytes at any time from the fetal period through adulthood may lead to premature ovarian failure, and premature menopause. As oocytes age, the chances of developmental abnormalities in offspring increase.

Embryogenesis and Fetal Growth

If fertilization of the ovum occurs (24 to 36 hours after ovulation), cell division is initiated and continues during the next 3 to 4 days as the early embryo, called a blastocyst, passes down the oviduct. The blastocyst implants in the lining of the uterus 6 to 7 days after ovulation. During the second and third weeks following conception, extraembryonic membranes are laid down and the development of the three layers of cells (endoderm, mesoderm, and ectoderm) occurs. Thus, by the time the first menstrual period is missed, the embryo is in the primitive "streak" stage,

The embryonic period takes place between weeks 3 and 8 to 9 of pregnancy [1] This is a critical phase of development, during which cell differentiation proceeds at an accelerated pace. During this period, the brain, eyes, heart, upper and lower limbs, and other organs are formed.

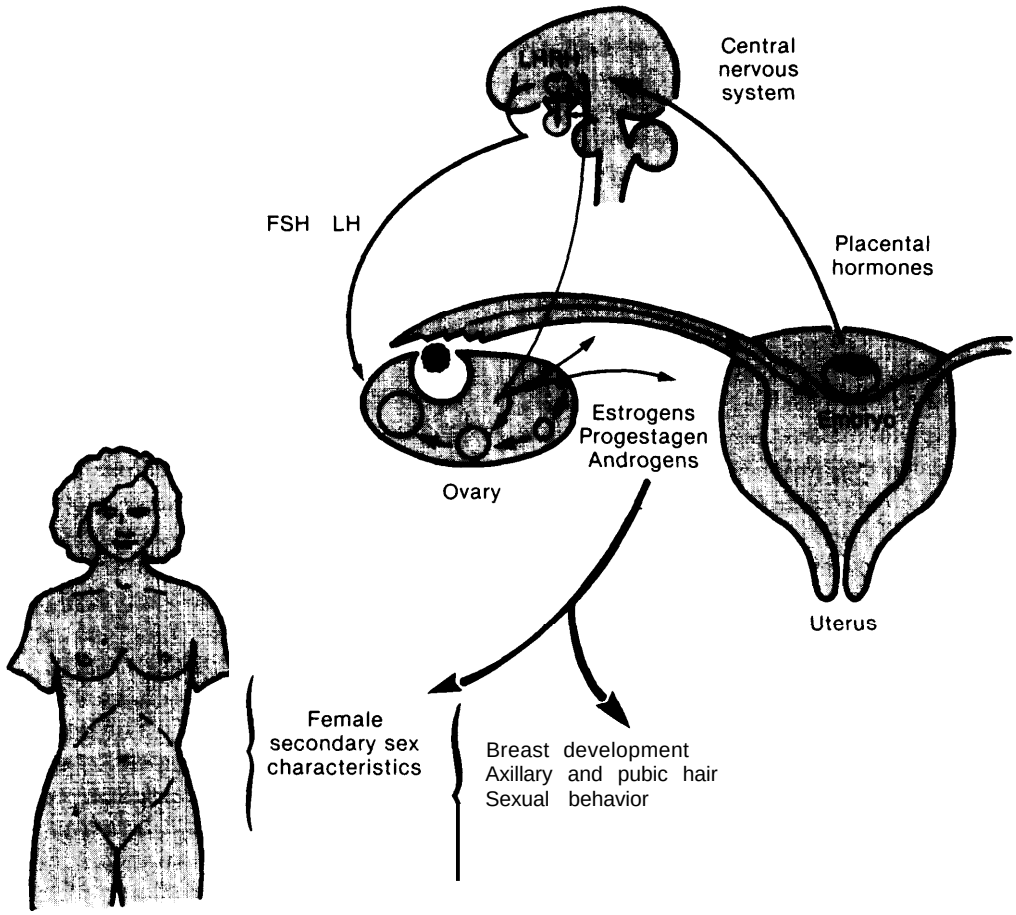
The fetal period is considered to have begun after the major organs have developed. It extends from approximately 8 or 9 weeks of gestational age until birth. This period is both a time of fetal growth and continued biochemical and physiological maturation of tissues and organs. Early in the fetal period, during weeks 9 to 11, the external genitalia differentiate, The growth and development of the nervous system occurs largely in the later fetal stages, during the second and third trimesters of pregnancy. It is important to note that the growth of nerve cells, or neurons, and the formation of connections between neurons, called synapses, continue in humans even after birth. Table 3-3 summarizes the timing of embry - onic and fetal development, and figure 3-5 places the periods of embryogenesis, organ-system development, and fetal growth in the perspective of a full-term pregnancy.

The Pregnant Woman

If a fertilized egg reaches the uterus and begins to implant, the nascent placenta produces the hormone hCG, human chorionic gonadotropin. This hormone signals the corpus luteum to continue producing progesterone and estrogens in order to maintain the uterine endometrial lining.

[1] References to time during pregnancy are often made in two ways. If the time from conception, or time of gestation, is enumerated (as in this text), a full term pregnancy spans about 38 weeks. If pregnancy is timed from the last menstrual period, about 2 weeks are added, making a term pregnancy equal to about 40 weeks.

Figure 3-3.—The Female Reproductive System



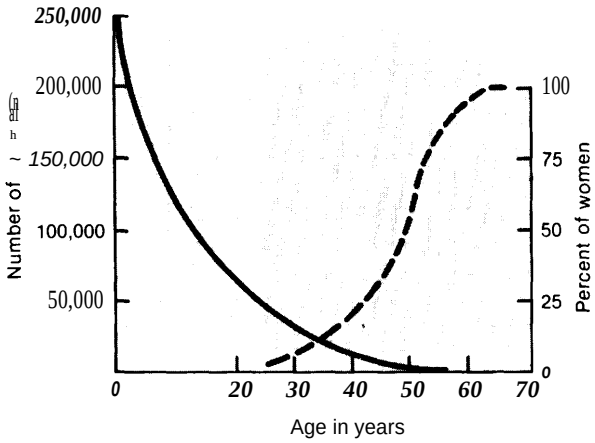
Key:
LH: luteinizing hormone
FSH: follicle-stimulating hormone
LHRH: luteinizing horm~ne-releasing hormone

SOURCE: Adapted from E. K. Sllbergeld and D. Mattison, personal communication, 19S4.

Secretion of hCG is the earliest biochemical change indicative of pregnancy. Chorionic gonadotropin has been detected in plasma and urine as early as 6 to 9 days after conception; that is, very soon after implantation of the primitive embryo into the uterine endometrium, Under in vitro conditions, hCG secretion has been detected at 7 days after fertilization, in the absence of implantation (15), suggesting that hCG release by the developing embryo occurs even prior to implantation. In a spectacular demonstration of the diagnostic value of hCG measurement, doubly elevated hCG levels in blood have been used to diagnose the occurrence of twins, just 2 to 3 weeks after conception (23).

During the first 60 days of gestation, the secretion of hCG doubles approximately every 2 days (5). This leads to an exponential rise in maternal plasma hCG concentration with very little individual variation. Maternal plasma hCG levels during the first 60 days of pregnancy can thus be

Figure 3"4.—Reiation Between Age, Oocyte Number, and Menopause



SOURCE: Adapted from D. R. Mattison, M. S. Nightingale, and K. Shiromizu, "Effects of Toxic Substances on Female Reproduction," *.Envirorr. Health Perspect. 48:43-52, 1983*

used to accurately predict gestational age. After 60 days' gestation, hCG levels vary widely and are of little value for predicting gestational age (28,29).

A high rate of embryonic loss occurs during the early phase of the normal reproductive process. It was suggested more than 60 years ago that embryonic death is so widespread in mammals, including humans, that it should be accepted as a normal phenomenon (47). For example, the conception rate per menstrual cycle for a normal couple of reproductive age having unprotected intercourse is nearly 50 percent, whereas the viable pregnancy rate is approximately 25 percent (52) (see figure 3-6). This loss of embryos is particularly high in the very early stages of pregnancy, 1 to 2 weeks after conception. Estimates of embryonic and fetal wastage in women are depicted in figure 3-7. These data have been used to estimate the probabilities of conception, recognizable pregnancy, and live birth in women who are attempting to reproduce. Upon exposure to spermatozoa, the probability of fertilization of an ovum is estimated to be 84 out of 100. By the time pregnancy is recognizable, half of all embryos have been lost. During the remainder of pregnancy, another 25 percent perish and are spontaneously aborted. The entire process—from exposure of an ovum to a spermatozoan through parturition —results in an estimated probability of a live birth of only 31 out of 100 (3). Employing a different frame of reference, the success rate of pregnancies following implantation of the conceptus is estimated to be 57 percent, with 43 percent ending in spontaneous abortion (32).

Pregnancy generates changes in the physiology of the pregnant woman (reviewed in (10)). Her blood volume increases to 150 percent of its nonpregnant volume. The resulting moderate dilution of red cells in the plasma is the anemia of pregnancy and is normal. However, the pregnant woman may be particularly vulnerable to other factors that induce further anemia, including poor nutrition and iron deficiency. Because of the increase in blood volume, her heart works harder, and more blood goes to all her organs.

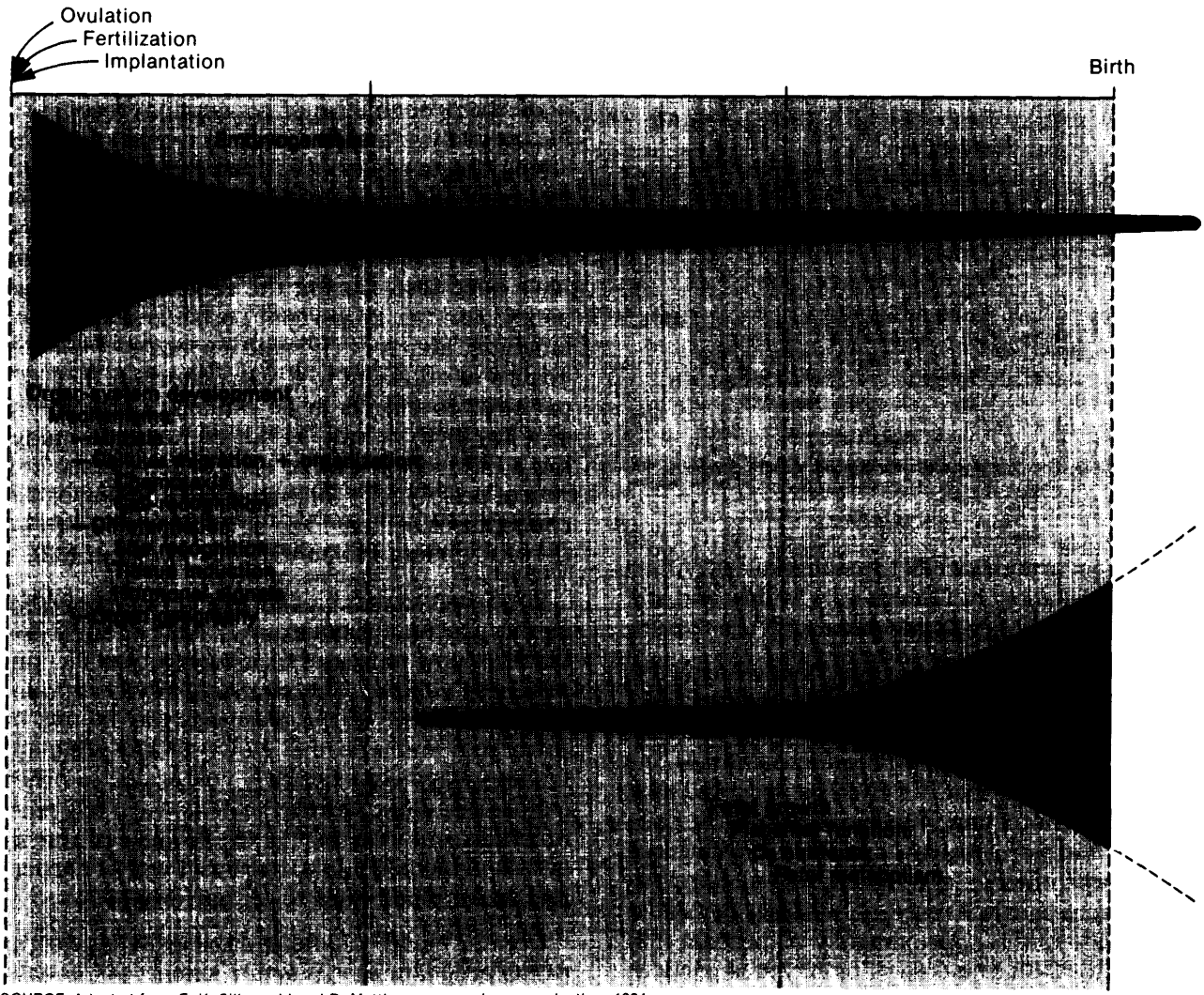
Greater blood volume and the growing weight of the pregnant uterus act in concert to increase

Table 3-3.—Stages of Embryonic and Fetal Development

Period	Time after conception	Stage	Time after conception
Fertilized ovum	First week	Cleavage	1-3 days
		Blastocyst.	4-5 days
		Implantation	7 days
Embryonic streak . . .	2-3 weeks	Gastrula	7-8 days
		Neurula	20 days
Embryo	3-8 weeks	Tail-bud embryo.	29 days
		Complete embryo	35-37 days
		Metamorphosing embryo . .	38-56 days
Fetus	9-40 weeks	First fetal	56-70 days
		Second fetal	70-140 days
		Third fetal	140-280 days

SOURCE: Adapted from R H. Blank, *Redefining Human Life: Reproductive Technologies and Social Policy* (Boulder, CO: Westview Press, 1984).

Figure 3-5.—Embryogenesis and Fetal Growth: Three Trimesters of Gestation



SOURCE: Adapted from E. K. Silbergeld and D. Mattison, personal communication, 1984.

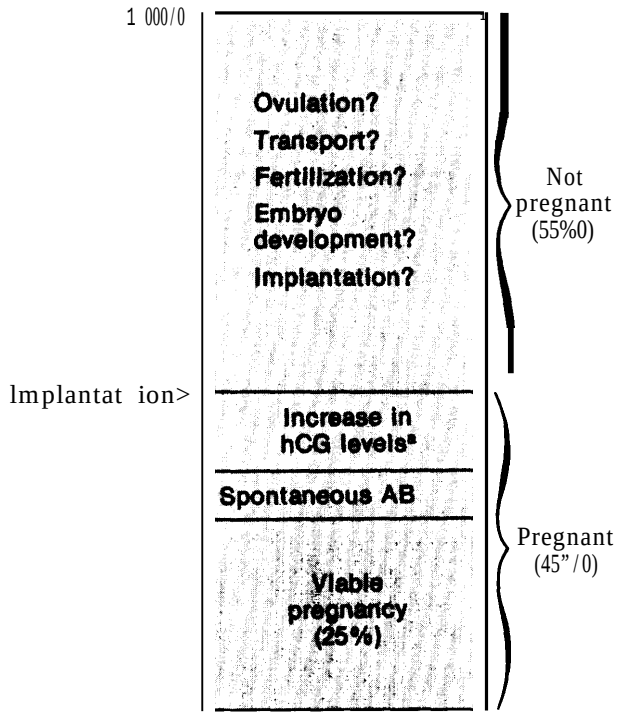
pressure on the leg veins during pregnancy. Sitting or standing in one position may become uncomfortable, and the risk of developing varicose veins in the legs is increased. The weight of the enlarging uterus also increases strain on the lower back. The pregnant woman's kidneys serve to fil ter wastes from both her blood and that of the fetus. The increased blood flow to the kidneys and pressure on the bladder can cause the pregnant woman to urinate more frequently, particularly as pregnancy progresses.

Coping With Pregnancy Loss

Embryonic or fetal loss causes maternal and paternal grief reactions. The grief pattern seen parallels that which has been described in facing death in adulthood (25), namely:

- shock,
- disorganization,
- volatile emotions,
- guilt,
- loss,

Figure 3-6.—The Percentage of Normal Women Who Conceive per Menstrual Cycle and the Outcome of These Pregnancies



[a]In some pregnancies clinical diagnosis is not made but the woman does have a transient increase in serum human chorionic gonadotropin (hCG) levels

SOURCE: Adapted from M. R Soules, "The In Vitro Fertilization Pregnancy Rate: Let's Be Honest With One Another, " *fertility& Sterility43(4)'511* -513, 1985

- relief, and
- reestablishment of an emotional balance.

A 1984 study found that the strongest stage of grief in pregnancy loss was guilt. This stage took the longest time to begin to resolve, and was the one in which the couples needed the most support and assistance. Women stated that if only they had not jogged, or had sexual intercourse, or fallen, or if they had eaten better, the spontaneous abortion might not have happened. Others had to deal with previous events that represented higher risks, such as medical illnesses or heavy cigarette smoking (30).

Although society is sensitive toward the couple who experiences pregnancy loss, there is a tendency not to express this sympathy. There are, for example, no accepted rituals for mourning an early pregnancy loss. Wakes and funerals are uncommon for a nonviable fetus. In-depth, emotionally supportive counseling sessions are considered an essential part of care for couples who experience a pregnancy loss (30).

Lactation

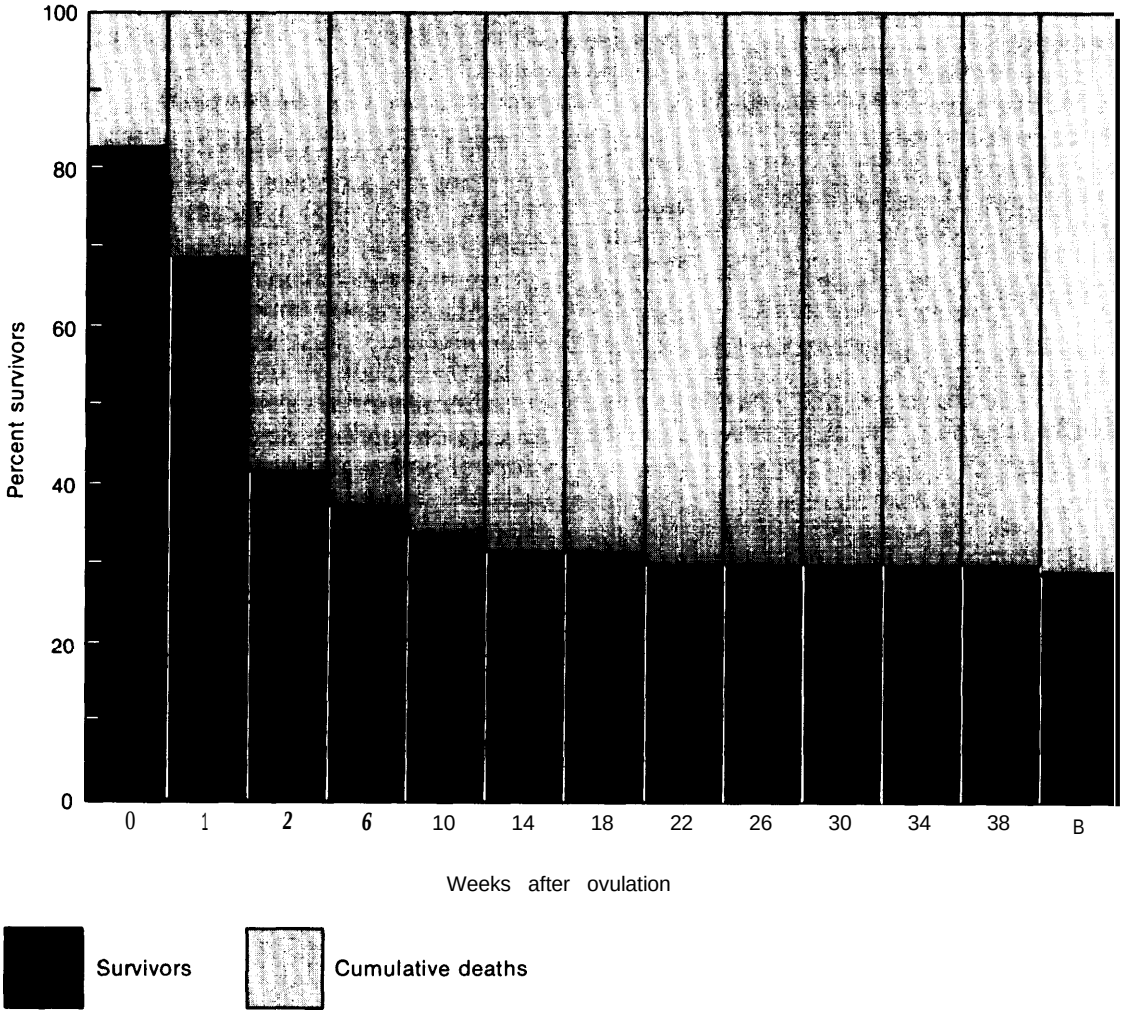
The breast is a complex organ that both synthesizes and excretes. When feeding a growing infant, the mother typically produces a liter of milk per day, containing protein, fat, carbohydrate, minerals, vitamins, hormones, and antibodies. All nutrient components are fully digestible. The product is delivered sterile, on demand, and with the carbohydrate and protein suspended in a mineral/aqueous system. The fat is excreted as a milk-fat globule, Because breast milk is a mixture of both water and fat, it can serve as a vehicle for a wide variety of substances present in maternal tissue or blood. Many constituents present in maternal blood plasma may be present in breast milk. Chemical or drug excretion into breast milk may be accomplished by binding to milk protein or to the surface of milk fat globules. It is also possible that fat-soluble chemicals (e.g., DDT, PCB, most insecticides) maybe trapped entirely within the milk-fat globule (2,19,61).

Sexual Development: puberty

Puberty is the period of transition between the juvenile state and adulthood. During this stage of development, secondary sex characteristics appear and mature, the adolescent growth spurt occurs, profound psychologic effects are observed, and fertility is achieved. These changes are in part a consequence of maturation of the hypothalamic-pituitary-gonadotropin unit, stimulation of the sex organs, and secretion of sex steroid hormones (17). A complex biological and maturational event, puberty actually spans several years, and is not well understood in terms of its onset.

Most American girls (98.8 percent) enter puberty between age 8 and age 13, with a mean age of 11 years (43). They complete their secondary sexual development in an average of 4.2 years, with a range of 1.5 to 6 years (32). Menarche (the first menstrual period) occurs fairly late in the maturational process and is the salient event for the pubertal girl. The first menstrual period appears at an average age of 12.8 years (56).

Figure 3-7.—Percentage of Surviving and Lost Human Embryos and Fetuses at Different Stages of Pregnancy



SOURCE: J. D. Biggers, "In Vitro Fertilization and Embryo Transfer in Human Beings," *New Engl. J. Med.* 304(6):336-342, 1981.

Some sign of puberty is first shown by 98.8 percent of normal American boys between 9 and 14 years, with a mean age of 11.6 years (43). Boys complete secondary sexual development in an average of 3.5 years, with a range of 2 to 4.5 years (33).

ABNORMAL DEVELOPMENT

Historical Perspective

Since the 1950s, tests of the effects of selected chemicals on reproduction have been conducted by the pharmaceutical industry, using animal models. The prospective sires and dams are usually exposed to the test chemical by diet, and measurements are made of reproductive endpoints (e.g., pregnancy rate; successful parturition; number, viability, and growth rate of offspring).

This section reviews abnormal development of the embryo/fetus; a discussion of abnormal reproductive function from puberty through adulthood appears in ch. 5.

As a consequence of the thalidomide tragedy in the early 1960s (see chapter 2), intensive efforts were mounted to detect substances capable of producing structural abnormalities in developing fetuses. The ability to detect skeletal and external malformations was emphasized, because techniques were available to detect those types of effects (60). These efforts were placed in a practical context as awareness grew that nearly all substances or agents are capable of adversely affecting the conceptus, if the dose is sufficiently great (24).

Methodologic advances since the 1960s have permitted detection of soft-tissue deficits and some functional deficits. These include alterations in central nervous system function (7), intestinal function (11), and respiratory function (42). As a result, the concept of teratology has evolved into a broad concept that includes structural and functional aspects of reproductive and developmental capability.

Terminology

The field of developmental toxicology is evolving rapidly, and its vocabulary is consequently in a state of flux. In late 1984, the Environmental Protection Agency (57) summarized the relevant terminology as follows:

- Developmental toxicity is the induction of adverse effects on development occurring up to the time of puberty. The four principal manifestations of developmental toxicity are: 1) death of the conceptus, 2) structural abnormality, 3) altered growth, and 4) functional deficiency.
- Embryotoxicity and fetotoxicity refer to any toxic effect on the conceptus occurring as a result of prenatal exposure. The distinguishing feature between the terms is the period during which the insult occurs. These terms include malformation, altered growth, and in-utero death.
  - —Altered growth is a significant alteration in fetal or neonatal organ or body weight. A change in body weight may or may not be accompanied by a change in skeletal maturation. Altered growth can be induced at any stage of development, may be reversible, or may result in a permanent change.
  - —Functional teratogenesis refers to aerations or delays in the postnatal abilities of the individual or organ system, following exposure to an agent during critical periods of prenatal or postnatal development.
  - —A malformation is defined as a permanent structural deviation that is generally incompatible with or severely detrimental to normal postnatal survival or development. These types of defects are also called teratogenic effects. A variation is defined as a divergence beyond the usual range of structural constitution, but which may not have as severe an effect as a malformation on survival or health. Distinguishing between malformations and variations is difficult, since there exists a continuum of responses from the normal to the extreme deviant. Other terminology that is often used, but no better defined, includes anomaly, deformation, and aberration.

Developmental toxicants thus induce functional teratogenesis, structural malformations, altered growth, or variations. Toxicants can act during either the embryonic or fetal periods, and can kill the embryo or fetus. Developmental toxicants may be equally toxic to both the parents and the embryo/fetus. If exposure occurs at, or sufficiently near to, the adult toxic dose, both the embryo/fetus and pregnant woman are likely to be harmed[3] (21,27),

A teratogen can be defined in several ways. As indicated, the EPA defines teratogenic effects as functional alterations or delays in postnatal abilities and structural malformations that are generally incompatible with or severely detrimental to normal postnatal survival or development. A teratogen can also be defined as a substance that adversely affects the embryo at doses below those necessary to produce overt signs of toxicity in the pregnant woman (53). Yet another definition states that a teratogen is an agent that produces a malformation at any dose (2 I).

Thalidomide remains the premier, but not sole, example of a chemical—a pharmacologic in this

[3]Some substances may be equally toxic to woman and embryo. If exposure occurs at, or sufficiently near to, the adult toxic dose, both the embryo and woman will be affected. The woman may recover, but the embryo can be irrevocably damaged (19).

instance—uniquely hazardous to the developing embryo. It has a marked selectivity for a particular target in humans, the limb buds of the conceptus. Thalidomide is able to injure the conceptus at dose levels so small as to be essentially harmless to the pregnant woman. However, most developmental toxicants can affect the woman as well.

The evolution of the concept of developmental toxicity and teratogenicity over the past 20 years has implications for public policy. For example, the Toxic Substances Control Act (TSCA; Public Law 94-469), written in 1976, classifies some chemicals as "teratogens)" thereby implying the exclusion of substances that may cause other developmental effects. Section 4(b) of TSCA states that testing standards may be prescribed for carcinogenesis, mutagenesis, and teratogenesis by the Administrator of the Environmental Protection Agency. Section 4(e) requires the Administrator to develop a list of chemicals for priority attention. The chemicals listed are those known or suspected to cause or contribute to cancer, gene mutation, or birth defects. Section 1O(C) requires coordination between the Administrator and the Secretary of the Department of Health and Human Services for research on rapid screening techniques for carcinogenic, mutagenic, and teratogenic effects of chemicals.

The wording of these sections of TSCA is generally consistent with contemporary understanding of cancer and mutations. However, insertion of the words "developmental toxicants" would clarify the existing statute with regard to contemporary understanding of the word "teratogen."

Mutagens

A mutagen is an agent capable of altering the structure of deoxyribonucleic acid (DNA), the genetic material of a cell, The basic process of mutagenesis may be spontaneous or induced by some agent, and may involve the alteration of a single cell. If the event occurs in a sperm progenitor or egg cell, the cell may die or the mutation may be transmitted to progeny of the affected parent. This kind of mutation, called a germ cell mutation, may be expressed, for example, as fetal wastage, sterility, structural or functional defect, or inherited disease. If the event occurs in a cell other than a sperm or an egg, the result may be cell death or the formation of daughter cells that produce altered gene products or tumors. This type of mutation is called a somatic cell mutation (46). Mutations in somatic cells imply the existence of a germ cell genetic hazard if the inducing agent also reaches the gonads. Mutations may or may not be harmful either to the affected individual or to the progeny.

Impaired Embryogenesis and Fetal Growth

During its earliest phase, prior to implantation and beginning organogenesis, the fertilized ovum (table 3-3) is largely resistant to certain types of toxicants. That is, toxic insults occurring during the preimplantation stages that do not kill the embryo usually do not have an adverse outcome. During this early embryonic period—the first 3 weeks of pregnancy—the most probable effects of toxic influences on the embryo are severe damage and death, followed by spontaneous abortion (16).

After implantation, the organs develop rapidly in a complex series of overlapping and interdependent events. The embryonic period is the primary, although not the sole, period for the induction of congenital malformations. During embryogenesis, the rate of cell division and the timed differentiation of primordial cells into organ systems confer a period of increased vulnerability to toxic effects. This is the period during which most structural teratogens act; functional teratogens may act later on, as well. The expression of teratogenicity varies with dose and with timing of exposure during gestation (51).

During the fetal stages and extending into early postnatal life, major functional and tissue maturation occurs. An agent acting during this period of time can markedly disrupt these processes. Such insults would be expressed not as major gross anatomical abnormalities, but rather as decrements of anticipated function (21). For this reason, most damage occurring in fetal stages is likely to be regarded as a type of functional injury, rather than as the gross malformations or devel-

opmental disruptions that may occur during the earlier embryonic period (16).

The major organs are already formed by the beginning of the fetal stages, after which it is too late to cause gross morphological abnormalities. For example, after the palatine shelves have already fused with one another to form the palate, cleft palate cannot be induced by any agent. Nevertheless, a substantial amount of development continues after the embryonic stages, and in-utero exposure of the fetus has been established as capable of producing altered postnatal functional capabilities. Such alterations have been produced in numerous organ systems (e.g., central nervous system, gastrointestinal tract, and cardiovascular system) (21).

Exposure of the developing nervous system to toxic influences may result in enduring behavioral deficits or abnormalities. Behavioral teratogenesis may thus be induced during organogenesis, in the later fetal stages of pregnancy, and even post-partum. Ingestion of mercury, alcohol, or addicting drugs, for example, can cause behavioral deficits or abnormalities in later fetal stages.

The exact nature and severity of induced impairments to embryogenesis and fetal growth depend on such factors as the time of exposure, the severity of exposure, and the nature of the substance itself (see table 3-4), Although it is generally not possible to examine a defective newborn and determine precisely when, during pregnancy, a malformation occurred, it is often possible to determine a gestational age beyond which it could not have been precipitated (2 I).

Table 3.4.—Principies of Teratogenesis and Timing of Embryonic and Fetai Toxicity

- Teratogens often adversely affect only a portion of exposed individuals; large individual differences in susceptibility exist.
- Susceptibility to embryotoxins depends on the genetic makeup of the embryo and the environmental conditions and lifestyle variables surrounding the parents.
- Toxic agents may be devastating to the embryo but harmless to the parents.
- A toxic agent may produce defects at different levels of biological organization resulting in biochemical, physiological, or behavioral anomalies that may not be apparent at birth.
- A toxic agent may affect the embryo even when given prior to conception either to the mother or to the father.
- The kind of effect a genetic or environmental toxin produces depends on the stage of development during which it acts.
- The same toxic agent may disrupt the developmental program and produce a congenital malformation at one stage, but merely injure an organ or produce no effect at all at another stage.
- The earlier in the formation of a structure a toxic agent acts, the more complete is the damage to that structure.

SOURCE: Adapted from A.S. Goldman, "Critical Periods of Prenatal Toxic Insults," *Drug and Chemical Risks to the Fetus and Newborn,* R. H. Schwartz and S. J. Yaffe (eds.) (New York: Alan R. Liss, Inc., 1980).

MECHANISMS OF ACTION OF REPRODUCTIVE AND DEVELOPMENTAL TOXICANTS

The mechanisms of reproductive and developmental toxicity can be reduced ultimately to some effect that interrupts the normal functioning of a cell, tissue, organ, or organism (8), A toxicant, whether a chemical, physical, or biological agent (see chapter 4), acts by interrupting biological processes, including the transfer of energy and information necessary for normal reproductive function and development.

Following exposure, for example, to a toxic chemical, the compound must be distributed to the target organ ie.g., hypothalamus, pituitary gland, gonad, uterus, epididymis, or liver), where it exerts its toxic effect, Within the target organ, the toxin interacts with a critical cell or subcellular component, disrupting an event necessary for normal reproductive function. If this interaction goes unrepaired, the toxic effect—altered reproductive function–will be produced. The toxic effect may be highly specific and affect only a single function of a single cell type. Or it may be broad and nonspecific, with multiple sites of toxicity within the organism. Within each target, this multistep process precedes the ***occurrence*** of reproductive toxicity (34).

Metabolism of the chemical by the liver or kidneys, for example, may result in toxicity that is more or less apparent. In some cases, a compound

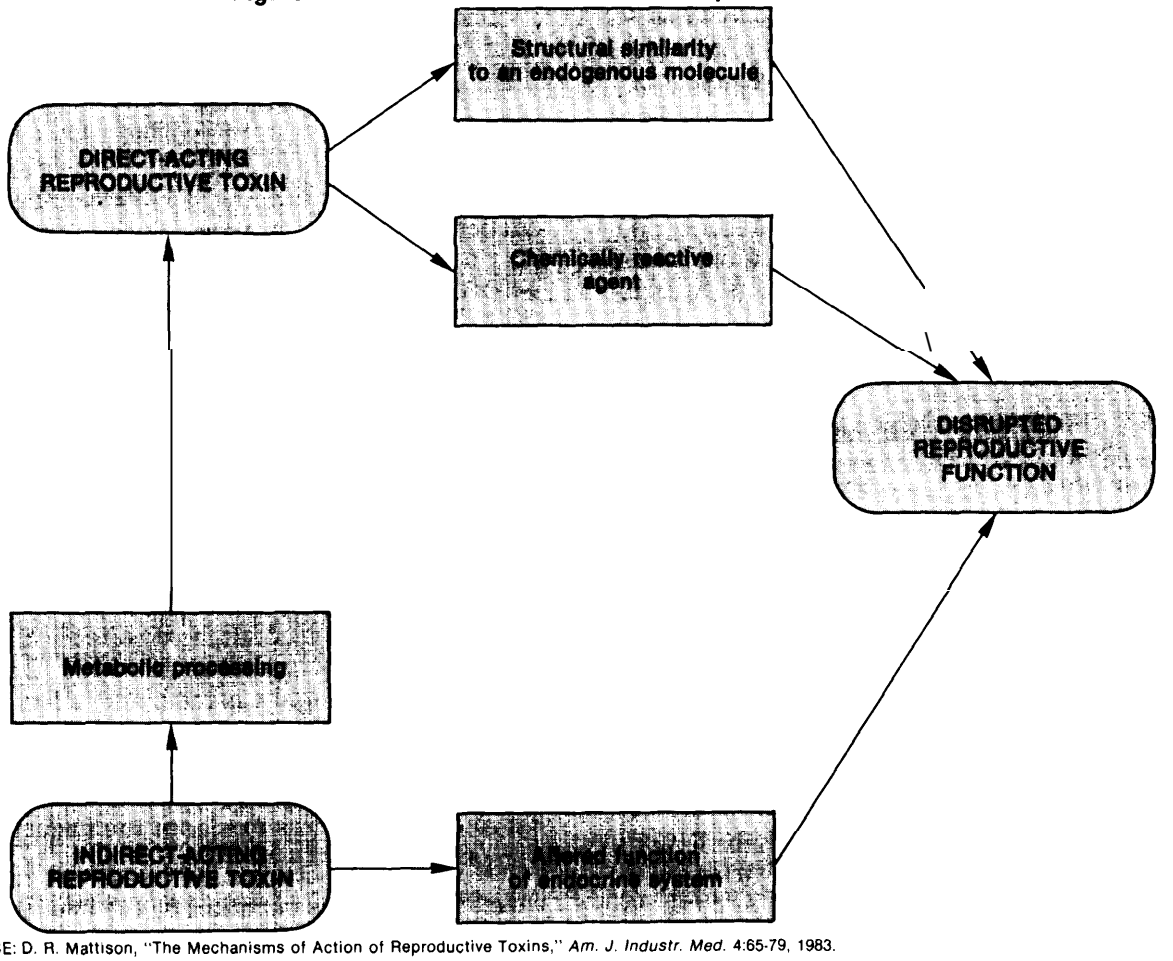
may be metabolized and cleared from the body, and no adverse effect will occur. In other cases, metabolic products may be more toxic or long-lived than the original toxin.

Reproductive toxins may act directly: 1) by virtue of structural similarity to an endogenous compound (e.g., hormone or nutrient); or 2) because of chemical reactivity, such as the ability to alter the structure of, or denature, a protein hormone. Some reproductive toxins may act indirectly, requiring metabolic processing or conversion within the body before exerting a toxic effect. The metabolize formed may then act through one of the direct mechanisms of reproductive toxicity (i.e., structural similarity or chemical reactivity). Other indirect-acting reproductive toxins may exert their effects by producing alterations in the body's physiological control systems (e.g., activation or inhibition of enzymes) (34). Figure 3-8 illustrates these mechanisms of action of reproductive toxins.

It is also possible for reproductive toxins to exert adverse effects through multiple mechanisms. For example, polychlorinated or polybrominated biphenyls (PCBS, PBBs) may act indirectly by activation of subcellular enzymes. These same compounds may also act directly by virtue of their ability to mimic the structure and function of steroid hormone molecules (34).

A great deal of attention is being given to research efforts to discover the mechanisms of action of agents known to disrupt development. Current knowledge, however, falls markedly short of identifying even the developmental se-

Figure 3-8.—Mechanisms of Action of Reproductive Toxins



SOURCE: D. R. Mattison, "The Mechanisms of Action of Reproductive Toxins," *Am. J. Industr. Med.* 4:65-79, 1983.

quences leading to some adverse effects, much less the precise cellular and molecular mechanisms involved in disruptions of normal structure and function of either the reproductive system or in-utero development (21). Nevertheless, it is possible to enumerate general developmental mechanisms that can be disrupted and lead to altered development. These include:

- faulty cell or tissue differentiation;
- excessive, or in some cases inadequate, cell death during development;
- improper cellular migration;
- faulty intercellular communication; and
- disrupted metabolism, manifested as altered respiration, absorption, excretion, or secretion.

Three issues are central to understanding the mechanisms of action of reproductive and developmental toxicants; these issues also illustrate the overall complexity of reproductive toxicology (34). They are:

- Species differences: Differences in reproductive toxicology among species are a reflection of variations among species. In mechanisms of hormonal control, for example, there are differences in anatomy, metabolism, and pharmacokinetics.Q In some instances, these species differences are poorly understood. A reproductive toxin in one species may not be toxic in another (including humans) because of differences in reproductive or toxicological mechanisms. The teratogenicity of thalidomide is an instructive example of species susceptibility in that rat and mouse are relatively insensitive, while rabbit, human, and nonhuman primates are sensitive (49). Another example is the difference exhibited by rats and mice in sensitivity to oocyte destruction by aromatic hydrocarbons (e.g., benzo(a)pyrene) (36).
- Gender differences: This issue is crucial because of the differences in anatomy and biological control mechanisms for reproduction in the male and female. Because of the ease of accessibility of gametes and gonads in the male, more suspect compounds have been screened in animal studies and demonstrated toxic to males than to females. Whether this represents an actual gender difference in ga - metic or gonadal toxicity or is simply an artifact of experimental designs is as yet unknown. More parameters are accessible for evaluating sperm, for example, than more-difficult-to-obtain oocytes (table 3-l).
- Time frame for toxicity: Knowledge of the window of sensitivity during which a structure or function may be affected by reproductive and developmental toxicants is of critical importance. A developing organ such as the ovary (35) may be susceptible to the harmful effects of a reproductive toxin, yet the same agent may have no effect on the developed organ. Little is known, for example, about differences between the immature oocyte and the mature, preovulatory oocyte with respect to susceptibility to reproductive toxins.

[4]Pharmacokinetics refers to the study of the action of a chemical in the body over a period of time. It includes the processes of absorption, distribution, localization in tissues, transformation into other chemicals with biological activity, and excretion.

REPRODUCTIVE DYSFUNCTION IN THE POPULATION AS A WHOLE[5]

In 1982, approximately 2,4 million married American couples, or 8.4 percent of those in which the wives were of childbearing age (I5 to 44) were unintentionally infertile, The epidemiologic profile of infertile couples reveals: 1) a greater proportion of infertile couples among blacks than whites, 2) a tendency to have experienced one or no live births, and 3) a tendency for the woman to be age 30 or over with less than a high school education. Although the overall infertility rate among married couples (excluding those who have been surgically sterilized) has not changed since the 1960s, subgroups of couples in which the wife is age 20 to 24 or black have experienced substantial increases in infertility

[5]This section is a summary of the detailed analysis of reproductive impairment in the general population that appears in app. A,

(39,40). It is important to note that many infertile couples are only temporarily affected and may eventually bear a viable infant irrespective of medical treatment (12).

The causes of infertility are often complex, difficult to pinpoint, and variable among individuals. Infertility is attributed in roughly equal proportions to men and women among married couples (18). The known and suspected causal factors of infertility can be categorized as:

- environmental, including pollutants;
- pathological, including infectious diseases;
- heritable, such as genetic syndromes;
- iatrogenic, or medication-induced, including contraceptive and therapeutic drugs;
- nutritional;
- ascribed, including race, maternal or paternal age; and
- sociobehavioral, including "recreational" drugs, stress, and exercise.

Analysis of these factors reveals large gaps in scientific knowledge of the causes of infertility, and even sparser knowledge about possible synergism with occupational factors.

Infant mortality rates in the United States are higher than those of many developed countries. The proportion of infant deaths due to birth defects has risen to more than 20 percent, because: 1) the rate of birth defects has not fallen as rapidly as the overall infant death rate, and 2) improvements in prenatal and postnatal care have reduced the infant death toll from other causes. The overall infant death rate for blacks is almost twice that for whites, and more than three times higher for infant deaths that are due specifically to low birth weight or prematurity. Although the overall rate of birth defects is lower among blacks than whites, the proportion of black infants of low birth weight is almost twice that of white infants, probably because of: 1) the higher proportion of preterm black infants, and 2) the higher proportion of black mothers possessing risk factors for bearing low birth-weight infants.

Birth defects afflict about 7 percent of live-born infants in the United States (31). About one-half of these birth defects are apparent at birth; the remainder become clinically apparent within 1 year. Some of the most common defects involve the cardiovascular system and the male urogenital system. Many of the more common birth defects, such as Down syndrome or neural tube defects, have a substantial impact on the individual, family, and society because of the severity of their physiological and functional effects. Single neural tube defects (those with no major associated defects) decrease in incidence following a gradient across the United States from East to West and are most common in white and female newborns (26). Several other defects, including Down syndrome and clubfoot, are most common in the Northeast.

The causes of the majority of birth defects are unknown. Individuals may be affected differently by a given causal agent, and some may not be affected at all. Age, health, and personal habits of both male and female, and extent of prenatal care in the female are some of the characteristics that can influence the risk of adverse fetal effects. Attempts to isolate and identify work-related reproductive hazards must take these variables into account (50). The timing and extent of fetal exposure to the agent during gestation may also vary its effect.

Sociobehavioral factors have received much attention in the quest to understand the causes of birth defects. Alcohol is teratogenic when consumed by the mother in large amounts (defined variably) and can result in '(fetal alcohol syndrome)" characterized by central nervous system dysfunction, mental retardation, growth deficiency, and facial deformities (54). Among neonates of alcoholic mothers, 83.3 percent had birth weights under the tenth percentile compared with 2.3 percent in a nonalcoholic sample (55). In a prospective study of the relationship between birth weight and alcohol consumption during the first trimester of pregnancy in 31,604 pregnancies, the authors found that consuming at least one to two drinks daily was associated with a significantly increased risk of producing a growth-retarded infant. Conversely, consuming less than one drink daily had minimal to no effects on intrauterine growth and birth weight. The authors note that "an occasional drink has only a trivial effect on intrauterine growth" (38). Conclusions regarding

the effects of alcohol consumption, although probably valid for heavy drinkers, may be tentative because of the difficulty of assessing all possible impacts on prenatal development. These include factors often associated with excessive alcohol consumption such as smoking, heavy coffee consumption, abuse of drugs, lower socioeconomic status, and poor nutrition. In addition, most studies do not control for the father's consumption of alcohol or other paternal risk factors.

Cigarette smoke and nicotine are also harmful, carrying an increased risk of: 1) prematurity; 2) low birth weight, due partly to fetal malnutrition resulting from depression of placental circulation or maternal appetite; and 3) perinatal death (45,54). A pregnant woman who smokes two packs of cigarettes a day may reduce the oxygen supply to her fetus by 25 percent (l). Effective October 1985, new warning statements were required (Public Law 98-474) on the packages and advertising of all cigarette brands sold in the United States (59). Two of these statements call specific attention to the hazards imposed by maternal smoking upon the offspring, for example:

> SURGEON GENERAL'S WARNING: Smoking by Pregnant Women May Result in Fetal Injury, Premature Birth, and Low Birth Weight.

Data on the effects of passive smoking-inhalation of the spouse's or co-worker's smoke by the pregnant woman-on the fetus are not available.

In sum, more complete knowledge of causal factors for both male and female infertility and birth defects in the population at large is needed to accurately isolate and identify reproductive hazards specific to the workplace. Epidemiological surveillance using incidence data is capable of detecting only unusually high rates of infertility or birth defects in certain worker populations, and only after many people have been affected. Even then, epidemiological data are often not sensitive enough to pick up more subtle changes (see chapter 5), and national prevalence data may not pinpoint locally high rates of infertility and birth defects. Furthermore, many indicators of reproductive impairment, such as early spontaneous abortion, are difficult to detect and are therefore underreported.

SUMMARY AND CONCLUSIONS

The complexity of the continuum called reproductive biology and development is masked by a tendency to focus on discrete components of the process, such as the sperm cell or the egg cell or the embryo. Reproductive function also encompasses pregnancy, lactation, child health and development, puberty, adult behavior, reproductive senescence, and the integration of reproductive physiology with the overall health of the individual. Failure to recognize the integral role of each of these components as part of reproductive function leads to an underestimation of the sensitivity of normal reproductive biology and development to perturbation.

Reproductive function in adult men and women can be assessed by relatively simple means, including a detailed patient history, a physical examination, blood samples, semen samples, and urine samples. When only these means are employed, a disparity exists between the ease with which male and female reproductive parameters can be assessed. Sperm are readily accessible, while eggs are not. However, evaluation of the causes of particular aspects of reproductive dysfunction is difficult. Diagnostic techniques are discussed in chapter 5.

Embryonic loss is a normal part of the reproductive process. Only one-quarter to one-third of all embryos conceived develop to become live-born infants. The remainder are lost at some stage between fertilization and the end of pregnancy. Data such as these are hard to obtain, and estimates vary, because the loss of embryos is particularly high in the early stages, before clinical diagnosis of pregnancy is made.

The terminology of the evolving field ***of develop***mental toxicology is rapidly changing. The four principal manifestations of developmental toxicity are: 1) death of the conceptus, 2) structural

abnormality, 3) altered growth, and 4) functional deficiency. Structural abnormalities and alterations or delays in postnatal abilities are teratogenic effects. Insertion of the term "developmental toxicant" for the term '(teratogen" in the language of TSCA would clarify the existing statute to coincide with contemporary understanding of the word "teratogen. "

The complexity of reproduction and development is mirrored by the complexity of biological mechanisms underlying toxicology, which involve absorption, distribution within the body, metabolism (toxification and/or detoxification), excretion, and repair (34).

Toxicants may produce their adverse reproductive or developmental effects by one of several mechanisms. Some agents may act directly, either by virtue of direct chemical action, or by structural similarity to endogenous molecules (e.g., hormone mimics or antagonists). Other agents interrupt reproductive processes indirectly, either by metabolic processing to a direct-acting toxicant (e.g., metabolic activation to form an active chemical), or by altering the normal endocrine balance (e.g., increased steroid hormone clearance) (34).

The causes of the unintentional infertility being experienced by some 2.4 million U.S. married couples are varied and difficult to pinpoint. Moreover, for some couples, infertility is a temporary phenomenon. The known and suspected causes of infertility can be grouped as environmental, pathological, heritable, iatrogenic (i.e., medication-induced), nutritional, and sociobehavioral. Birth defects afflict about 7 percent of live-born infants. As in the **case** of infertility, the causes of many birth defects are often unknown or speculative. Analysis of reproductive impairment in the population as a whole (see appendix A to this chapter) provides a background against which to identify any increased incidence of reproductive dysfunction that may be workplace-related.

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