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PERSPECTIVES IN REPRODUCTIVE BIOLOGY

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Probably no feature of a living organism qualifies more clearly as the essence of life than its ability to reproduce and perpetuate the species. Even viruses which have few, if any, of the other characteristics of living things are able to reproduce or to be reproduced by their host cells. For the survival of the species, each generation must produce new individuals to replace the ones killed by predators, parasites or the ageing process.

Reproduction is not simply conception, but a cyclic process (Fig. 1) that involves the formation of eggs or sperm in one individual, their union in fertilization, and the development of the fertilized egg to a mature individual which, in turn, forms sperm or eggs. In considering reproduction we can begin at any point in this cyclic series and proceed until the cycle has been completed. Reproduction at the molecular level is a function of the unique capacity of nucleic acids to undergo replication, transcription and translation. These processes depend, in turn, on the specificity of the relatively weak hydrogen bonds between specific base pairs. Reproduction at the biological level of the whole organism ranges from simple fission in bacteria and other unicellular organisms, a process which usually does not involve sex, to the incredibly complex structural, functional and behavioral processes of reproduction in the higher animals and man. Many facets of the transfer of biological information from one generation to the next in man are under endocrine control: the development of the genital tracts in the male and the female, the processes of spermatogenesis oogenesis, and ovulation, and the intricate patterns of sex behavior that ensure that male and female gametes will be released at the same time and at the same place so that they can unite to form a zygote. Fertilization is followed by the complex sequence of developmental processes and cellular differentiation by which a zygote becomes an adult organism ready, in turn, to reproduce the next generation.

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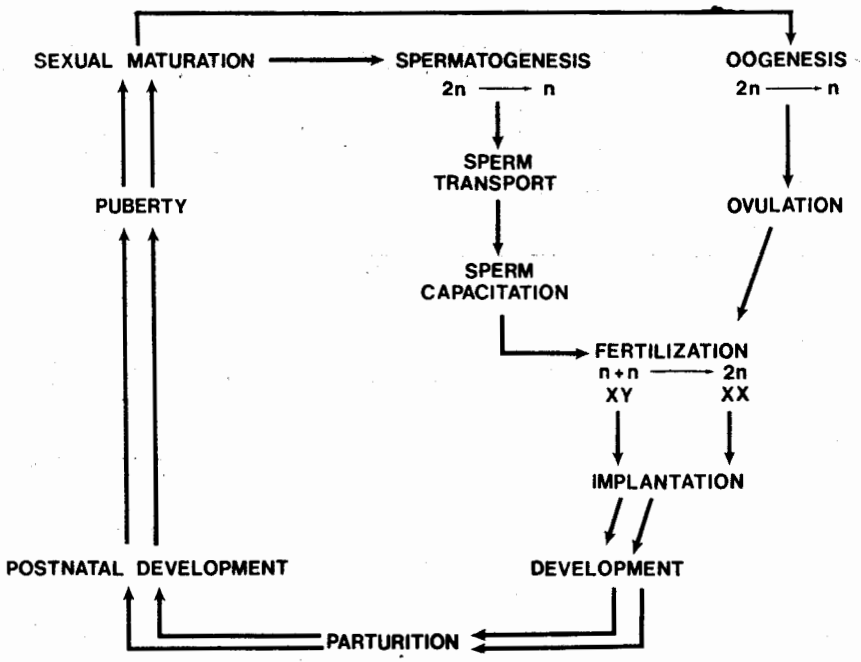

Figure 1. A diagram emphasizing the cyclic nature of the reproductive process.

Even our definition of sex has been refined in recent years since we can now distinguish the genetic sex of an individual, determined at the time of fertilization by the X–X, X–Y mechanism, from his gonadal or phenotypic sex. In the normal course of development, the Leydig cells of the fetal testes produce testosterone which leads to the development of the vas deferens, prostate, seminal vesicle and other structures of the male reproductive tract. The testes produce a second hormone, the mullerian duct inhibitor, which leads to the regression of the mullerian ducts that normally form the oviducts, uterus and other structures of the female reproductive tract. Female embryonic devment is produced not by the presence of estrogen, but by the lack of androgens and by the lack of mullerian duct inhibitor. And X–O individual with Turner's syndrome or gonadal agenesis has vestigial gonads, which produce little or no estradiol, and yet the reproductive tract is like that of an immature female. The human with testicular feminization syndrome is X–Y and hence a genetic male. The gonads present are testes and the patient, a genetic and gonadal male, has a normal male concentration of testosterone in serum and urine. The cells of the body, however, do not respond to androgens and the external genitalia and general body form are those of a female; however, the mullerian duct inhibitor produced by the testes has resulted in the lack of a uterus and oviducts while the vagina is very short. Thus we can speak of the endocrine sex of an individual and finally we can distinguish the psychological sex of an individual which may differ from his genetic or phenotypic sex.

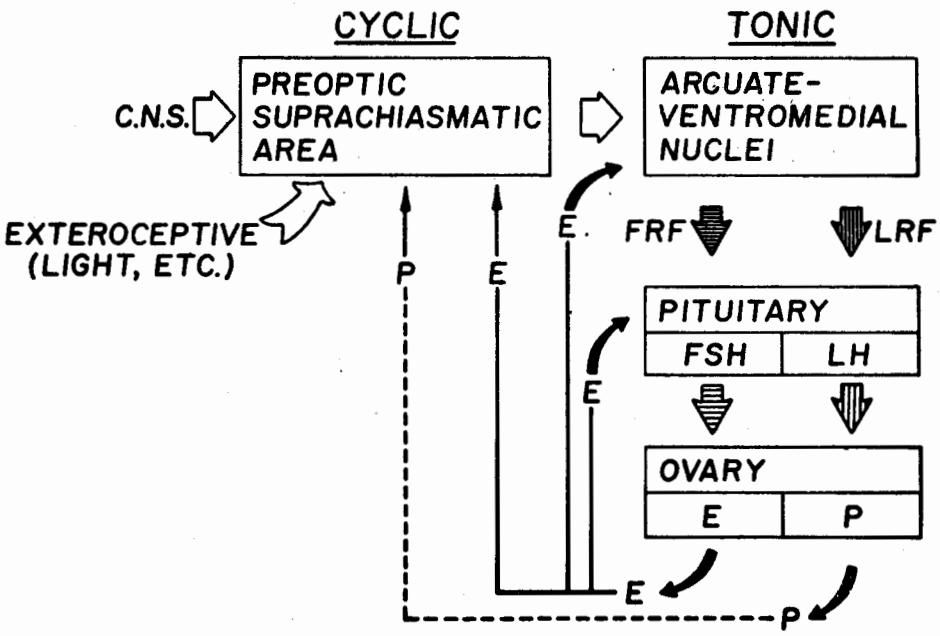

Figure 2. A diagram illustrating the several levels of hormonal control of gonadal function and the feedback control of pituitary and hypothalamic function by ovarian hormones[1].

Research in recent years has shown that the reproductive process in man and other higher animals is under several levels of endocrine control (Fig. 2). At the first level are the steroid sex hormones produced by the gonads: testosterone by the Leydig cells of the testes and estradiol and progesterone by the follicular and luteal cells of the ovary. The structure and function of the gonads are, in turn, under the control of gonadotropins secreted by the anterior pituitary. FSH stimulates the development of the testes and its seminiferous tubules in the male, and the development of the ovary and its follicles in the female. LH stimulates the development of Leydig cells of the testes and their production of androgens, and ovulation and the production of progesterone in the ovary. The production and release of gonadotropins is regulated by a specific releasing factor secreted

Pyro Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

Figure 3. The amino acid sequence of the decapeptide gonadotropin relaasing hormone[2].

by the hypothalamus. This relasing factor, a decapeptide (Fig. 3), is secreted under the control of a number of neural factors such as stimuli from the amygdala, and the system is modulated by the concentrations of estradiol and progesterone which can exercise both positive and negative feed-back control of the secretion of the releasing factor. A small rising concentration of estradiol may trigger the release of the releasing factor but a continuous high concentration will inhibit the secretion of gonadotropin relasing factor (Fig. 4). This latter effect is the basis for the use of estrogens in oral contraceptives.

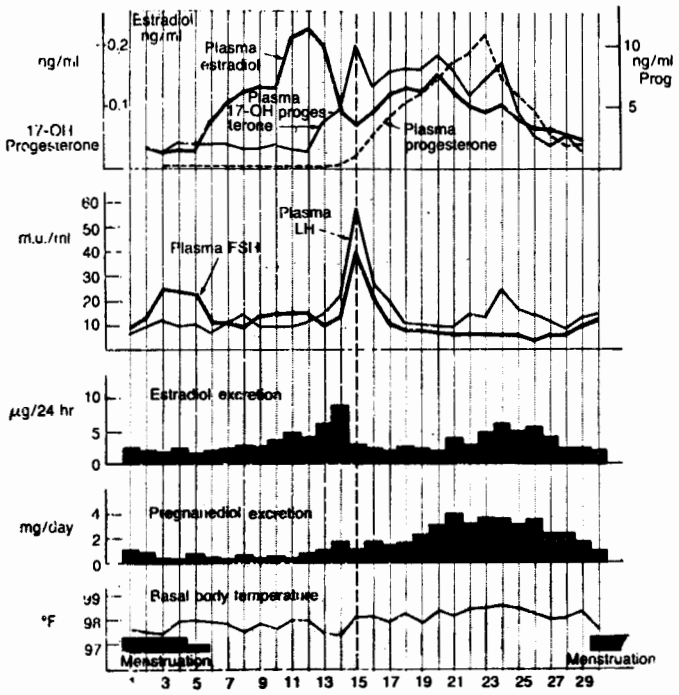

Figure 4. Diagram illustrating the concentrations of gonadotropins, estrogens and progestins in the plasma during a single human menstrual cycle. The urinary excretion of estradiol and pregananediol and the changes in basal body temperature are also shown[3].

Women and female primates have menstrual cycles and undergo spontaneous ovulation. The rhythm of the human menstrual cycle and probably ovulation itself can be influenced by a number of environmental and nutritional factors. Spontaneous ovulators have some sort of light dependent hypothalamic clock which provides the neural stimulation for the release of a hypothalamic releasing factor in response to the estrogen surge (Fig. 5). The releasing factors are produced by neurons that end in the median eminence

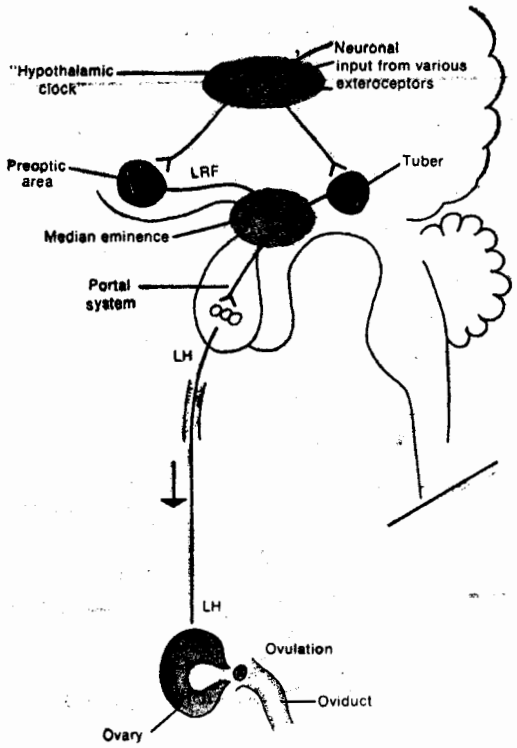

Figure 5. Spontaneous ovulation in man and other primates. The secretion of releasing factor from the hypothalamus is stimulated by impulses coming from various exteroceptors which are modulated by some sort of biological clock in the hypothalamus[3].

at the base of the brain where they are released and pass into the hypophyseal portal vessels through which they reach the pituitary (Fig. 6). The releasing hormone stimulates the release of a surge of LH which initiates ovulation. The pituitary of the male also produces and secretes gonadotropins. Indeed, the pituitary of a male transplanted into a hypophysectomized female rat will support a normal estrous cycle. However, although an ovary transplanted into a castrate male rat will be able to develop ripe follicles, it will not undergo ovulation since there is no cyclic release of an LH surge from the male pituitary. The difference between the sexes, the development of the hypothalamic clock, appears at a critical stage early in the development of the central nervous system in the rat. In the male, testosterone inhibits the development of the hypothalamic cyclic center. A young female injected with testosterone will have the activity of the cyclic center in her hypothalamus permanently abolished. This apparently does not occur in the human female fetus or infant suffering from a deficiency of 11-β-hydroxylase or 21-hydroxylase in the adrenal and, therefore, exposed to very large amounts of androgens during development. This female will become virilized but will retain the ability to give off LH surges as an adult. A female infant suffering from adrenal cortical virilism can be treated with cortisol to suppress the flood of ACTH which is leading to the overproduction of androgens. If she is maintained

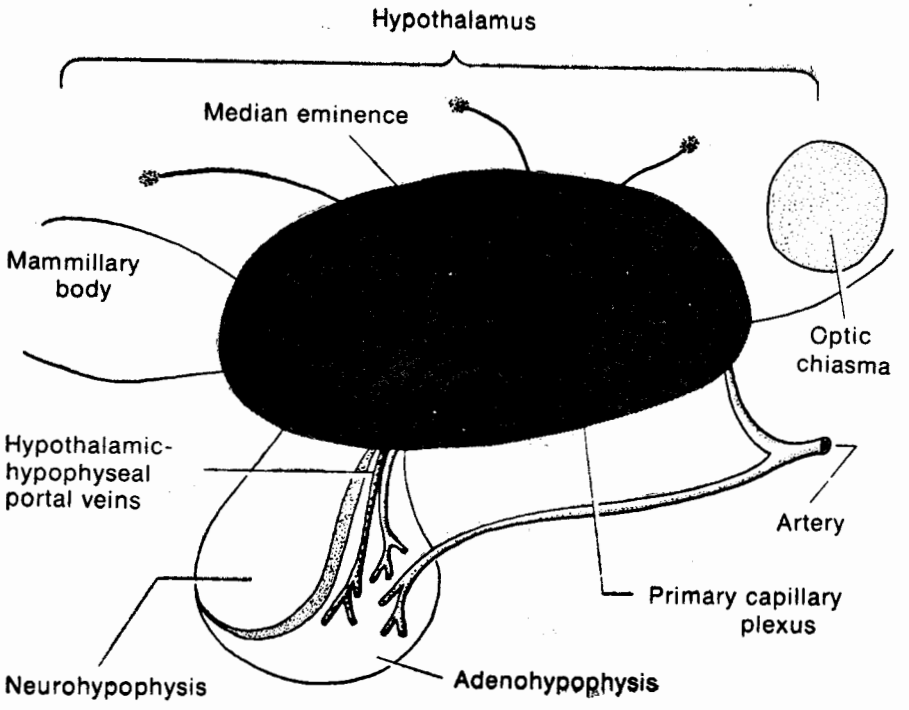

Figure 6. Diagram of the relations of the blood vessels supplying the pituitary and hypothalamus indicating the hypothalamic-hypophyseal portal veins by which hormones are transferred from the hypothalamus to the pituitary[3].

on therapy she can develop into a normal adult female with regular menstrual cycles and ovulatory surges of LH and will retain the capacity to become pregnant.

In most animals the periods of reproductive activity recur in a cyclic fashion regulated by the season of the year, by the photoperiod, and by other environmental factors. The increased photoperiod in the spring is associated with increased pituitary activity and the increased gonadotropin secretion affects sexual behavior and sexual receptivity. In some mammals ovulation is stimulated by copulation. The coital reflex is mediated through the hypothalamus leading to a release of releasing hormone and of LH (Fig. 7). The effective stimulus in the rabbit may be a single copulation, but in the female short-tailed shrew at least 19 copulations per day are required to induce ovulation. The term estrus or "heat" is applied to the period of sexual receptivity in the female and the term "rut" is applied to the period of male sexual activity. Human females are sexually receptive at anytime in the ovarian cycle and at any season of the year. Human males after puberty are in constant rut.

The nature of the reproductive process plays a considerable role in determining the organization of animal societies, their migrations and their behavior. The reproductive

process also has effects on the organization of human society. It is interesting, for example, to contemplate what kind of family units and human society might have evolved if women were sexually receptive only in April and October or to speculate as to how the patterns of human behavior might be altered if women emitted some visual or olfactory stimulus at the fertile period or if they ovulated reflexly after each coitus.

Although the morphologic features of the reproductive tract have been known for a long time, there have been major increases in our understanding of the fine structure of the constituent cells of the reproductive tract. Probably no single cell has been studied quite so intensively by electron microscopy as the sperm[4]. This incredibly complex cell

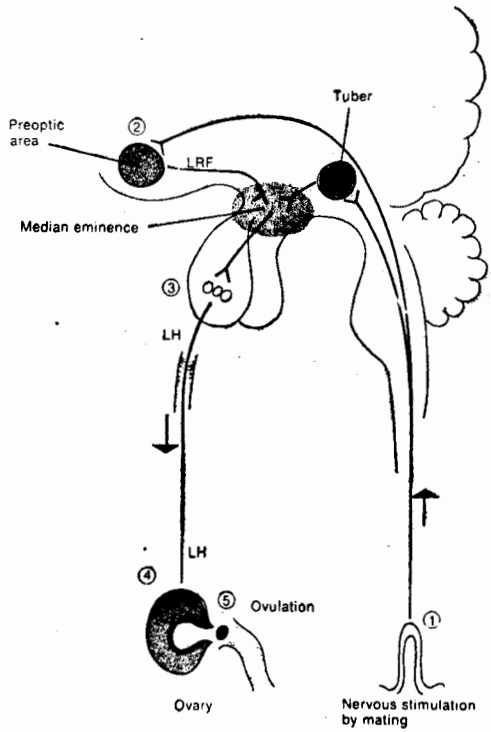

Figure 7. Diagram of the nervous pathways involved in reflex ovulation in animals such as the rabbit, cat and mink. The stimulation of receptors in the vagina by the mating act generates impulses which pass to the hypothalamus and bring about the secretion of relasing factor. This passes to the pituitary and causes the release of luteinizing hormone which goes via the blood stream to the ovary and initiates ovulation, the release of the egg[3].

is of great interest, not only because of its role in reproduction, but also because it provides a striking model system of the changes that can occur in cell differentiation.

There have been tremendous advances in our understanding of reproductive physiology in the past ten years. Clearly if man wants to tamper with the reproductive

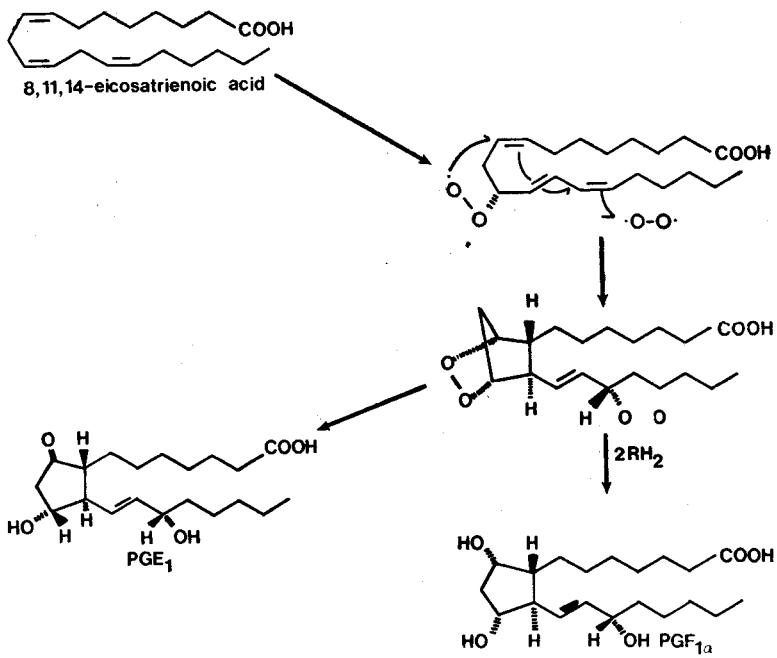

Figure 8. Diagram illustrating the synthesis of prostaglandin E_1 and prostaglandin $F_1 \alpha$ from the long-chain polyunsaturated fatty acid, 8, 11, 14-eicosatrienoic acid.

process to limit human fertility in some acceptable fashion he must first know everything possible about the process itself. The drive in recent years to discover safe, effective and acceptable methods of contraception has been the stimulus for many studies of reproductive physiology and has yielded a satisfying increase in our understanding of these processes. Our knowledge of the molecular events involved in hormonal control of reproductive function has grown and will undoubtedly continue to grow. For example, a whole new class of compounds, the prostaglandins (Fig. 8), has been discovered and much effort has been expended to understand the normal physiologic roles of these fascinating fatty acid derivatives. Their practical importance in facilitating the birth process and in inducing abortion before term is a development of the last half dozen years[5]. All of these studies, are leading us to an appreciation of how complex the reproductive process is and how remarkably the several processes are controlled and interdigitated by hormonal and neural factors. In addition to regulating the secretion of gonadotropins, the hypothalamus controls many aspects of reproductive behavior. The sex behavior that ensures that male and female will meet and bring their respective gametes into juxtaposition at the proper time in the estrous or menstrual cycle is orchestrated by many hormones. Sperm development, maturation, transport and capacitation, oogenesis, ovulation, fertilization, implantation, the maintenance of pregnancy and even parturition are regulated by an incredibly complex series of hormonal and neural controls. We still have much to learn about the nature of

the molecular messages controlling each step. Each of these processes must occur in proper sequence and in a proper temporal relationship to preceding and following steps. Some of the contraceptive measures depend upon upsetting the temporal sequence of the events such as those resulting in implantation of the blastocyst.

What remains to be learned in the future? We must certainly enlarge and broaden our understanding of reproductive biology, especially of human reproductive biology. The details of the reproductive process are different enough in primates, and especially in the human, so that the rat, rabbit, sheep and cow are not necessarily valid models for the human in matters reproductive. An increased understanding of human reproduction is needed so that effective clinical advice can be given not only to solve the global problem of unwanted excess fertility, but also to solve the numerically smaller, but equally disastrous, personal problems of unwanted sterility. The World Population Conference in Bucharest in 1973 recommended that "family planning and related services should aim at prevention of unwanted pregnancies as well as the elimination of involuntary sterility or subfecundity to enable couples to achieve their desired number of children." The successes to date[6,7] in devising safe and effective oral contraceptives, intrauterine devices, and long-term implanted silastic capsules that release estrogens or progesterone must not lead us to believe that the problem has been solved and there is no more to be done. When we understand more fully all of the processes involved in reproduction, we may find that other points in the reproductive process can be attacked more effectively, safely and acceptably with even fewer side effects than the methods in use today. It would be a tremendous breakthrough if we could predict the time of ovulation reliably by some simple means 48 hours in advance of the event. There are changes in the patterns of fatty acids secreted by the vagina during the cycle[8] but these are not consistent enough to be a useful predictor. We have not yet achieved any notable success in finding a contraceptive for the male[9]. The ones tested so far are unaceptable because they are general cell toxins or reduce libido and potency.

One of the newer developments in the contraceptive field is the uterine therapeutic system which prevents conception, but confines the drug response largely to the uterus[7]. The T-shaped intrauterine device releases 65 micrograms of progesterone per day for a year, after which it can be removed and replaced with a new one. In a field test of some 2,000 women years[10] the pregnancy rate was 0.8 per 100 women years and the continuation rate was 80%. The contraceptive effectiveness was similar to the most effective hormonal methods and the continuation rate was superior. Other effective devices release synthetic progestins such as medroxyprogesterone acetate. The presence of the progesterone-releasing intrauterine device resulted in a decrease in endometrial enzymes such as alkaline phosphatase, β-glucuridonidase, acid phosphatase and lactic dehydrogenase within three months[11]. The use of depot contraceptives consisting of a polymer-steroid amalgam pellet implanted under the skin appears promising. The polymer gradually undergoes hydrolysis to release the steroid. The steroid polymer combination can be made into a ring and inserted into the vagina so that the steroid is absorbed through the vaginal mucosa[12]. Another method under development is that of inducing menses by an orally active anti-progesterone given on days 24 to 28 of the cycle[13]. Clearly if the effect of progesterone

in maintaining the endometrium can be overcome with an antiprogesterone, menses will ensue whether or not pregnancy has occurred.

In preparing antibodies for the radioimmunoassay of steroids it was noted that animals given injections of testosterone bound to bovine serum albumin had elevated levels of total serum testosterone but decreased amounts of free testosterone[14]. They consequently had high levels of circulating gonadotropins. The elevated levels of FSH and LH resulted in hyperplasia of the testes. Females immunized with estradiol bound to bovine serum albumin had lengthened estrous cycles or persistent estrus[15]. Thus, immunization with steroids bound to proteins leads to neutralization of the biologic effects of the endogenous hormone since the hormone in the serum becomes bound to the circulating antibodies and is unavailable to the receptors in the cytosol of the target tissues. This leads secondarily to increased secretion of tropic hormones due to the lack of feedback on the pituitary and hypothalamus. To test the possible usefulness of this system in contraception rhesus females were given estradiol bound to bovine serum albumin. They subsequently developed antibodies to estradiol and become anovulatory, but the ovaries were enlarged and polycystic due to the increased LH resulting from the decreased free circulating estradiol. Giving antibodies to estradiol or to progesterone to pregnant rats led to the resorption of the fetus[16]. Thus although it seems clear that one could interfere with the reproductive process by antibodies to estradiol or to progesterone, the long-term effects of the method, such as enlarged polycystic ovaries, do not appear very promising as a contraceptive technique in the human.

An antibody has been prepared to the β subunit of human chorionic gonadotropin (anti β-hCG) and is being tested clinically as an antifertility measure[17]. By binding the hCG produced by the trophoblast and preventing its maintenance of the corpus luteum and production of progesterone it prevents the continuation of the implanted blastocyst.

There is a great deal yet to be learned about the hormonal control of reproductive behavior. Which hormones, estradiol, testosterone, dihydrotestosterone or others, are effective in controlling reproductive behavior and in which parts of the central nervous system do the hormones act? Is testosterone converted to estradiol in the central nervous system in significant amounts and does this conversion have significant effects on reproduction? There are receptors for both estradiol and dihydrstestosterone in certain regions of the brain and indeed both males and females have receptors for estrogen in the hypothalamus and pituitary.

We must not infer that the need for further research in both basic and applied reproductive biology has in any way been diminished by the notable achievements of the past decade. We now have several methods of contraception that are effective and safel but each has restrictions and contraindications for individuals with certain medical problems. It is imperative to continue the studies of reproductive biology to determine whether other points of attack in the reproductive process may yield even better contraceptive methods.

Demographers dream of a day when it might be feasible to add an antifertility substance to the drinking water, much as we now add fluorides for the prevention of caries.

When a couple wanted a child, they would then apply to the government to use the appropriate antidote. In light of what we know, this appears likely to remain a dream. It would be totally impractical and unwise to deliver any hormonal agent or any cytotoxic agent in the drinking water or food since the dosage could not be regulated. An amount of estrogen appropriate to inhibit ovulation in an adult woman would cause serious problems in a man or in an immature female or male.

To enable each couple in the world to exercise its privilege of having the number of children they want at the time they want them, much more must be learned about all aspects of the reproductive process to make sure that the wanted pregnancies have a successful outcome and to learn how the process can best be interfered with to prevent unwanted conceptions.

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