SURVEY OF STUDIES RELATING TO VULNERABLE POINTS IN THE REPRODUCTIVE PROCESSES

By

Warren O. Nelson

In the search for practical, acceptable, and economical methods for controlling the mechanisms of reproduction, it is logical that a route be sought in the application of knowledge of the physiology of the reproductive processes. For many years effective methods of contraception have been available, but because of religious, cultural, or economic objections, these methods have had limited acceptance only. Thus it has become evident that the existing means of limiting conception, be they mechanical, chemical, or dependant upon procedures that limit the coital act to non-fertile periods of the menstrual cycle, are not destined to be the answer to the rapidly growing need for reduction of population increments.

As yet the physiologic control of conception has remained almost totally unexplored. In part this has been due to lack of interest, but of much more importance have been the taboos imposed by religious and cultural groups. Yet, even a casual knowledge of the physiology of reproduction is sufficient to suggest that in this area almost certainly lies the eventual answer to the most important problem that confronts mankind.

The conception and development of a mammalian organism is not a simple process. It is fraught with many complicated and delicate mechanisms, the breakdown of any one of which will result in failure of the organism's development. These range from intricate neuro-endocrine relationships and hormonal balances through the responsiveness of a chain of target organs to the precise timing and sequence of a complicated series of developmental events. Included are such delicately balanced processes as the production and properly integrated release of a battery of hormones, the capacity of many tissues to carry out complicated enzymic phenomena, and the fantastically intricate and balanced events of fertilization and embryonic development. The student of embryo-
logy cannot but marvel that these complex mechanisms do not break down with greater frequency than they do.

Let us examine the points of the reproductive processes which may be regarded as vulnerable, and consequently, susceptible to designed interference. In the following outline, an asterisk marks those points which appear to be particularly vulnerable:

I. Formation and transport of male germ cells and seminal fluid.
   A. Formation of germ cells
      1. Multiplication of spermatogonia
      2. Reduction division of primary spermatocytes*
      3. Formation of sperm from spermatids*
      4. Release of sperm
   B. Transport of sperm
      1. Transport of sperm from testis to urethra
      2. Physiological maturation of sperm in epididymis*
   C. Formation of seminal fluid*
      1. Seminal vesicle secretions
      2. Prostatic secretions

II. Formation and transport of female germ cells and preparation of uterus for implantation.
   A. Formation of germ cells
      1. Growth of ovarian follicles
      2. Maturation of ova*
      3. Ovulation*
   B. Transport of ova
      1. Pick-up of ova by tubes
      2. Transport of ova to uterus
   C. Preparation of endometrium
      1. Secretion of ovarian hormones (estrogen, progesterone)
      2. Action of hormones on uterus*
         a. Proliferation of endometrium
         b. Secretory activity of endometrium

III. Fertilization process.
   A. Transport of sperm in female tract
      1. Movement of sperm from vagina to upper end of tube*
      2. Maturation of sperm within female tract (capacitation)*
      3. Entry of sperm into ovum and union of two pronuclei*

IV. Early embryonic development and implantation.
   A. Embryonic development and implantation
      1. Cleavage of ovum
2. Blastocyst formation
3. Germ layer formation

B. Implantation of ovum
1. Entry of ovum into endometrium
2. Organization of early placenta

This paper cannot pretend to discuss completely the methods of attack which might be levied against these numerous points of vulnerability. We must be content to consider only those that offer the optimum in terms of our present knowledge and chances of success, and further, to limit ourselves primarily to those points which are presently under investigation. In this consideration, the sequence of discussion has no significance of priority but, rather, simply follows the outline as given above.

Spermatogenesis. The production of sperm is an exceedingly complex and highly integrated process. It involves seven to nine cellular divisions and at least two very complex cellular transformations. The phenomena are controlled to varying degrees by a series of hormonal factors and enzymic mechanisms. Experience to date has suggested that the most vulnerable point in spermatogenesis is the meiotic division, which is undertaken by the primary spermatocyte in its process of chromosomal reduction. This phenomenon is unique in that the homologous chromosomes separate after undergoing a complex prophase and, furthermore, apparently is particularly dependent upon unusual metabolic circumstances. Certain drugs, the nitrofurans and related compounds, which interfere with important phases of carbohydrate metabolism, very effectively inhibit in a completely reversible fashion the meiotic division without in any way appearing to influence other testicular mechanisms. When administered in doses adequate for inhibition of spermatogenesis, these compounds also appear to have no discernible actions upon other physiologic processes; but at higher doses they do have certain objectionable effects and consequently are not entirely suitable for use in human subjects.

One of these compounds, furadantin, which is an effective agent in the treatment of urinary tract infections has been studied for its antispermatic effect in men. When used at therapeutic (for urinary tract infections) doses it has a slight, but scarcely significant, inhibitory action (Nelson and Bunge, 1956).

Although the present status of these compounds as practical antifertility agents is no more than mildly encouraging, it may be anticipated that further investigation will provide basically similar agents which can be used effectively and safely in human subjects.

A safe, but not entirely practicable, method of interfering with spermatogenesis in man is available in the use of steroidal hormones which inhibit the
secretion of gonadotrophic hormones. Although the most effective of these are the estrogens, their use is contraindicated due to interference with the endocrine as well as the spermatogenic function of the testes. Effective an antifertility procedure as their application might be, the ensuing reduction of androgen production would make them quite unacceptable. Less objectionable are the androgens, since they replace the endogenous male sex hormone. Indeed, the androgens have been employed successfully in inhibiting spermatogenesis, although in the majority of instances such treatment has been instituted in an attempt to secure a subsequent improvement of spermatogenesis – the so-called »rebound« procedure (Heckel, 1951).

While the administration of androgen would be an effective means of fertility control, it has the disadvantage of requiring constant maintenance of high levels of the hormone, a treatment which would be expensive and not entirely without danger. It is quite possible, however, that some compounds now under investigation will inhibit gonadotrophic stimulation and at the same time provide sufficient replacement of androgen to compensate for the lack of Leydig cell function without provoking excessive effects.

Although no means of preventing the metamorphosis undergone by spermatids in the process of sperm formation is known to be effective in the case of the human being, it should be noted that this complex phenomenon is a very vulnerable point. It is now known that the spermatid-spermatozoon transformation involves important metabolic phenomena (Featherstone et al., 1955; Steinberger and Nelson, 1955), and it is reasonable to suppose that interference with such complex mechanism should not be too difficult to achieve.

**Transport of sperm.** The passage of sperm from testis to urethra involves a series of mechanisms which well may be vulnerable to interference when our knowledge of the constituent processes is more secure. Thus the »flushing« of sperm from the seminiferous tubules through the rete would seem to be particularly susceptible. An even more likely focus of attack on spermatozoa would seem to reside in the so-called »physiological maturation« which they undergo in the epididymis. During the ten days or so that is required for their passage through the epididymal coils, spermatozoa acquire an enhanced capacity for motility and fertility (Young, 1931). The exact nature of this maturation process remains to be explored, but it represents an exceedingly important and promising area of study.

**Formation of seminal fluid.** Too little is known of the relationship of the seminal fluid, beyond its capacity as a vehicle, to the process of fertilization. Evidence has mounted that the fluid contains, among other things, fructose, several amino acids, and a number of reducing substances (Mann, 1954; Gassner, 1952, 1955), but the actual importance of these in so far as sperm function is
concerned remains relatively unknown, and intensive investigation in this area would be of utmost significance. Thus it may be shown that reduction, or increase, in the seminal level of one or more of the constituents will have marked deleterious effects upon the capacity of sperm to effect entry to the ovum. For example it has been suggested by the recent interesting, but unconfirmed, observations of Haag, Goldzieher and Werthessen (1956) on stallions that an excess of certain substances (silver-binding compounds) are related to a significantly reduced fertility.

Since the seminal fluid constituents are provided from a variety of sources – testis, epididymis, prostate, seminal vesicle, and bulbourethral glands – it is evident that the potentially vulnerable foci are numerous. By the same token, however, the problem is complex and must be concerned with a thorough consideration of the entire reproductive tract.

Turning to the most likely points of vulnerability in the female reproductive processes, the ovary deserves initial consideration.

**Maturation of ova and ovulation.** It will be recalled that meiotic division in spermatogenesis is particularly susceptible to interference. There is little reason to doubt that the ovarian counterpart of this process is equally vulnerable. While the nitrofurans do not disturb ovocytic division, it is possible, as in the case of spermatogenesis, to prevent ovum maturation and ovulation by the administration of substances which inhibit secretion of the gonadotrophic hormones. Thus it has been known for many years that androgens and estrogens would inhibit ovum and follicular maturation in lower animals and women. More recently it has been shown that large doses of progesterone have a similar effect (Pincus, 1956). While these better-known steroidal hormones are not likely, for a variety of reasons, to be practical antifertility agents, it is almost certain that related compounds will be evolved for this purpose. Indeed, several compounds, including 17-ethynal-19-nortestosterone and two substances having the structure of 17-ethyl-19-nortestosterone, the difference being in the placement of the double bond in ring A, have shown significant promise in the inhibition of ovulation in lower animals and human beings, and undoubtedly, other highly effective compounds will become available.

While this consideration of steroidal substances appears to emphasize their effect upon ovarian function, consequent to the inhibition of gonadotrophin secretion, other avenues of interference are possible. Of particular significance in this regard are agents which may exert an inhibitory effect at the level of the ovary, in some way negating the action of the gonadotrophins. Two or three kinds of procedures seem to be possible, although their practicability remains undemonstrated.

Certain plants, notably those of the genus Lithospermum, are said to inhibit the action of gonadotrophic hormones (Noble et al., 1950), and in at least two
laboratories, the fractionation of Lithospermum extracts has provided compounds which may have antifertility activity (Gassner; Ginsberg, personal communications). If chemical synthesis of these compounds can be secured, it will be possible to study their effectiveness and mode of action on a large and conclusive scale.

Another means of interfering with the action of gonadotrophins is available through the use of antihormones (Maddock et al., 1953), although the practicability of these as an antifertility measure remains questionable. In the same category would be the relatively unexplored field of organ-specific antibodies (Gorbman; Bishop; Segal, personal communications). Theoretically it should be possible to secure by suitable immunologic procedures antibodies against the ovary or even against some specific elements of the ovary, and thus prevent their normal activity.

The objection inherent to all these procedures as antifertility measures lies in the fact that the endocrine function, as well as the gametogenic activity of the ovary, is subject to inhibition. However, continued investigation and refinement of techniques may eventually provide methods of selective interference in such a manner as to affect the ovum only.

*Action of hormones on uterus.* While it is possible to inhibit the secretion of the ovarian hormones by measures that interfere with ovarian activity, there are very obvious objections to such procedures. More practical would be procedures that would inhibit the action of estrogen and/or progesterone on the endometrium in a direct fashion, thus preventing nidation of the ovum. Such interference with the proliferative response or, even more important, the secretory response of the endometrium would provide an effective barrier to continued ovum development. Several lines of attack seem reasonable.

Alteration of the normal estrogen-progesterone balance is a method that would be effective, but its acceptability is open to question since it probably would be difficult to control. More logical would be the introduction, at appropriate times in the menstrual cycle, of a substance which would compete with or negate the action of progesterone. Thus a physiologically inactive analogue of progesterone may have the capacity to compete with or replace progesterone to a greater or lesser degree in the sequence of events which occur in the endometrium. Thus it might be possible to prevent the development and/or maintenance of a secretory state and so interfere with nidation of the embryo. The recent studies of Velardo et al. (1956) in which the administration of pregnandione and pregnanediol inhibited the normal response of the endometrium to progesterone bear directly upon this means of interfering with conception.

A related approach to this target is being undertaken by Lieberman and his colleagues (personal communication), who are endeavoring to secure progesterone-protein complexes which will have antibody activities. Success in this
project might provide substances which, when administered at suitable times in
the cycle, would negate the effect of progesterone on the endometrium. A varia-
tion of this avenue of study might be attempted by the use of procedures de-
dsigned to stimulate the production of an organ-specific antibody, in this case
one directed toward the endometrium.

Although we are assured that the normal endometrial cycle depends upon
both estrogen and progesterone, we are almost totally ignorant of the manner
in which these hormones achieve their effects. Of interest have been the studies
of Shelesnyak (1956) on the relationship of histamine to the decidual response
of the endometrium. This lead indates that histamine may be an intermediary
between progesterone and the responsive tissues and that an interference may
be imposed by antihistaminic substances as, indeed, Shelesnyak’s further studies
have shown. Suggestions such as these point clearly to the importance of serious
study of the enzymic mechanisms of the endometrium and their relationships
with hormonal levels and tissue responses. Almost certainly an understanding
of these will reveal methods of interfering with fundamental mechanisms of
the endometrial cycle.

Although the antifertility substance reported by Sanyal (1952) as occurring
in the pea, Pisum Sativum, and identified by him as 2,6-dimethyl hydroquinone,
is supposed to exert its effect by inhibiting progesterone activity, at least three
groups of investigators have not been able to confirm this finding. There is
some evidence, however, that the compound may interfere with fertility by
inhibiting gonadotrophin activity (Pincus, 1956) and Thiersch (personal com-
munication) is exploring its effect on very young embryos.

Probably the most acceptable method of effecting control of fertility would
be a physiologic means of preventing penetration of the ovum by spermatozoa.
since interference at this stage might be achieved with less likelihood of dis-
turbing other reproductive processes. Interruption of fertilization might be
achieved at any one of several points.

Transport of sperm to upper end of tube. At the present time no agreement
exists as to the manner in which sperms ejaculated into the vagina reaches the
upper end of the Fallopian tube where fertilization is supposed usually to occur.
In all probability the most important factor is the contraction of the uterine
and tubal musculature. Although many investigations have been made on the
relationship between hormonal levels and uterine contractility, we as yet know
relatively little about the mechanisms controlling this musculature. Such studies
as those of Csabo (1956) appear to offer important clues as to possible methods
of interference with sperm transport at critical points. Perhaps we may learn
to control the little-understood uterotubal junction so as to obstruct sperm entry
to the tubes, or to modify tubal motility in such a way as to prevent sperm
ascent to the ampullary and fimbriated segments of the tube.
It is possible, too, that a better understanding of the part played by the cervical mucus in sperm passage may yield rewarding information. Studies on this material have suggested that the change in amount and viscosity during the cycle are related to sperm passage at the time of ovulation, but too little precise information is available on the chemical and physical characteristics of cervical mucus to provide for logically conceived attempts to alter the material so as to interfere with sperm passage. Such studies are presently being made by Scott-Blair and Folley (personal communication) and others.

Under normal circumstances the seminal fluid liquefies soon after ejaculation, and the individual sperm are freed to become actively motile. However, in some infertile men liquefaction is much delayed or may occur to a very limited degree. The reasons for failure of normal liquefaction are not understood, nor is motility by any means completely inhibited in its absence, but it is probable that should we be able to impose circumstances inimical to liquefaction, we might have at hand a means of inhibiting activity of the sperm.

Normally, sperm show little or no tendency to clump or aggregate in a liquefied seminal fluid, but in some men, usually infertile, sperm agglutination is a common phenomenon. A recent observation in this regard has been made by Wilson (1956). It may be supposed that this clumping is due to an excess of agglutinogens, or to the absence of antiagglutinating factors. If, as Lindahl (1954) has suggested, these factors are produced in the reproductive tract, it may be possible to interfere with their normal production, or to tip the balance in favor of agglutination in such a way as to promote wholesale clumping and consequent inactivation of the sperm.

Maturation of sperm in the female reproductive tract. Evidence has been submitted by workers in at least three laboratories which indicates that sperm undergo a form of physiological maturation in the female reproductive tract. Thus it would appear that only after a sojourn of 4 to 6 hours in the uterus and tubes do sperm have the capacity to effect fertilization. The nature of this maturation phenomenon is not understood, but it is clearly evident that once it is known, we may be able to contrive some means of interfering with the process and thus render sperm less able to accomplish fertilization. At the present time, projects relating to this study are underway in at least two laboratories (Chang, 1955; Noyes, 1953).

Sperm entry into ovum. The process of union between the two germ cells, effected when the ovum is penetrated by the sperm and the two pronuclei merge, may ultimately prove to be one of the prime targets for antifertility measures. Only recently, studies on hyaluronidase and hyaluronidase inhibitors suggested that the answer may have been found. However, these observations have not been confirmed (Millman and Hartman, 1956) and at the present time
a great deal of uncertainty exists both with regard to the role of hyaluronidase in the fertilization process and the feasibility of interfering with fertilization by oral use of such hyaluronidase inhibitors as phosphorylated hesperidin. Although the current situation has thus been rendered less encouraging than it was a few years ago, one cannot doubt that further studies on the intimate mechanisms involved in fertilization will reveal facts important to the problem of fertility control. Thus studies relating to the nature and possible modification of the follicular ground substance and of the vitelline membrane and to the nature of the sperm-ovum contact (a form of agglutination) are likely to be particularly rewarding. Workers actively engaged in these studies include G. Asboe-Hansen and his colleagues, A. Tyler and his students, D. Bishop, and P. Lindahl (personal communications).

**Embryonic development.** The final area of study which I shall attempt to summarize at this time relates to interference with early embryonic development. Needless to say, antifertility measures which choose this as the point of attack are less likely than most other methods to receive widespread acceptance. Nevertheless, there can be little doubt that the early embryo is highly vulnerable to deleterious influences.

This discussion has already centered upon the mechanisms involved in preparation of the endometrium for nidation. Successful prevention of this preparation would result in failure of the blastocyst to implant and be followed quickly by its death. Probably this set of circumstances is a common spontaneous occurrence, and when it does occur the female is unaware of the transient presence of a fertilized ovum since her menstrual period appears on schedule.

The process of implantation and establishment of the placental attachment occupies the last week of the usual menstrual cycle and is presumed to be carried out largely through the medium of enzymic digestion, by the trophoblast, of the maternal tissues. We know little or nothing of the details of this mechanism and cannot now determine how effectively the process may be prevented. However, recent studies with certain antimetabolites have shown that the recently implanted embryo is highly vulnerable to the effects of substances that interfere with fundamental anabolic mechanisms. Although several workers have noted this phenomenon, the most extensive studies have been those of Thiersch (1956) who has examined the effects of a large series of antimetabolites. Particularly effective have been azaserine, 6-mercaptopurine, and aminopterin. The mechanism whereby these substances destroy the young embryo appears to be one in which the compound competes with naturally occurring materials that are important to the embryo.

While these compounds are highly effective, their use is fraught with some danger if great care is not exercised, since other physiologic processes in the mother may be influenced. Furthermore, in case of aminopterin, at least, failure
of the drug to kill the embryo may be followed by serious anomalies in the fetus.

The nitrofurans and related substances also destroy the young embryo although the mechanisms whereby they act is somewhat different. It is possible that they inhibit, in the embryo, as they do in the testis, important phases of the carbohydrate cycle. Anomalous fetal developments have not been observed in animals receiving the nitrofurans, but side effects attendant on the dose that would be required (see discussion on testis) are likely to interfere with their use for this purpose in human beings.

It may be anticipated that other even more effective antimetabolites will be found, some are already under study, and it is likely that among these will be some which can be used more safely than those presently available and which may be acceptable among some peoples.

SUMMARY

An attempt has been made to outline the points in the reproductive processes which are vulnerable to interference and to point out some of the significant studies which have been done or are in progress. To date so little effort has been made to focus specifically on the problem of interfering with the physiologic mechanisms of reproduction that most of the information at hand is a by-product of studies concerned with enhancing fertility, or concerned only with some more or less isolated aspects of reproductive physiology. However, these observations have provided us with a substantial basis for designing future studies, and it may be confidently anticipated that the awakening interest in the subject and awareness of the need for more concerned efforts in the study of antifertility measures, we shall have a rapidly mounting fund of information on which to draw in formulating effective, acceptable, and safe methods for controlling fertility.

REFERENCES

Lieberman, S.: Personal communication.