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Rodrigo Guerrero

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The Aging of Gametes: The Known and the Unknown

Rodrigo Guerrero, M.D.

Dr. Guerrero has a Ph.D. in epidemiology and population science from Harvard University where he has recently been a visiting professor and lecturer in the medical school. Formerly general director of the department of health at the Universidad del Valle in Cali, Colombia, he now serves as Minister of Health in Cali.



I. Introduction

The effects of aging gametes, both spermatozoon and ovum, have received considerable attention in the last few years. The role of aged gametes in chromosomal malformations has been studied extensively in different animal species and it has been postulated that the "rhythm" method of family planning may lead to a variety of chromosomal and developmental malformations by increasing the probabilities that aged spermatozoa or ova be involved in fertilization.^{1, 2, 3} The morality of the "rhythm" method has been challenged⁴ on the basis that it may lead to pathological conceptions. It is the purpose of this paper to review the concepts and evidence as they exist today, relating to the effects of the aging of gametes and other similar physiological phenomena, in particular as they relate to the use of natural family planning methods (NFP). More detailed reviews of the general problem of aging gametes may be found elsewhere.^{5, 6, 7}

II. Aging of Spermatozoa

The effects of the aging of spermatozoa in the male tract (before ejaculation) and in the female tract (after ejaculation) are conveniently separated. It is generally agreed that survival in the male tract is longer, in the order of several weeks, while survival in the female tract is in the order of several days. Typically, aging in the male tract is

accompanied by a gradual decline in fertility without resulting defects in the offspring.^{8,9} However, pre- and post-implantation losses¹⁰ and chromosome abnormalities¹¹ have been reported for aged rabbit spermatozoa. The effects of aging in the male tract of human spermatozoa have been difficult to study for several reasons. Spermatozoa are continuously produced after adolescence and thus, since mixing of old and fresh spermatozoa is known to occur in the epididymis,¹² prolonged sexual abstinence is not a synonym of old spermatozoa. Furthermore, leakage of spermatozoa into the urethra occurs¹³ and there seems to be a selective advantage when young sperm compete with old at the time of fertilization.¹⁴

Aging of sperm in the female tract is accompanied by a decrease in the fertilizing capacity in the studied animals^{15,16} pre- and post-implantation losses in the rabbit,¹⁷ gross abnormalities in the hen,¹⁸ and chromosome abnormalities in the rabbit.^{19,20} The effects of aging of human spermatozoa in the female tract have been difficult to study. Marshall²¹ however, explored the teratogenic effect of aged human sperm in 81 pregnancies with negative results. By using basal body temperature (BBT) as an indicator of ovulation and coital records from natural family planning clinics with a similar methodology to the one used by Marshall, Guerrero and Rojas²² reported an increased probability of abortion as the responsible insemination took place at increasing days before the shift in BBT in a total of 965 pregnancies. Since ovulation may fluctuate several days before or after the thermal shift, Hilgers²³ and Roetzer²⁴ have considered the results invalid. Basically what Guerrero and Rojas reported was that the day of insemination for 75 spontaneous abortions occurred significantly before the thermal shift when compared with 890 term deliveries. The objections mentioned before are untenable unless the fluctuation of ovulation around the shift is different for abortions and term deliveries. An insemination six or seven days before the shift in BBT should reflect the same degree of aging of the spermatozoa both for an abortion case or a term delivery unless ovulation selectively tends to occur several days before the shift in abortions, but not in term deliveries. There is no reason to believe that this may be the case.

An unpublished analysis of the pregnancies observed in the Fairfield International Collaborative Study has shown that conceptions resulting from inseminations at increasing periods before the shift in BBT have an increased probability of spontaneous abortion.²⁵ This new set of data reaffirms the original claim by Guerrero and Rojas.

III. Aging of Ova

In contrast with the spermatozoa which are continuously produced during reproductive life, human oocytes are already formed and arrested at one of the meiotic stages of development at the time of

birth. During reproductive life, some of these oocytes, generally one per menstrual cycle, mature beyond the arrested stage and are released at ovulation. As a consequence, oocytes released later in reproductive life are, of necessity, older than those released at earlier stages. This type of aging sometimes called "natural" or "physiological," is probably responsible for the well demonstrated excess of mongoloid children (Down's syndrome or trisomy 21) born to older women reported by most authors.^{26, 27} Other types of aging of the ovum are separated into intrafollicular aging (sometimes also called preovulatory overripeness), when the ovum is retained in the follicle beyond normal time, and intratubal aging (also called postovulatory overripeness) when the ovum is shed at a normal time but fertilization occurs later.

Intrafollicular (Preovulatory) Overripeness

Intrafollicular overripeness in laboratory-bred xenopus²⁸ and artificially induced in the rat^{29, 30} and in the rabbit has been shown to produce chromosomal aberrations (trisomies, monosomies, tri- and tetraploidies) and an increased frequency of monozygotic twinning in the rabbit.³¹ Also an increased frequency of abnormalities has been reported in long cycles spontaneously occurring in aged rats³² which has been interpreted as indicative of preovulatory overripeness. Typically, preovulatory overripeness has been reported to be associated with an increased proportion of sterile inseminations, and an increase in abortions and chromosomal malformations. The similarities of anatomic and chromosomal anomalies found in human spontaneous abortions led Mikamo³³ to postulate that overripe ova (probably intrafollicular) may be a cause of spontaneous abortions.

There seem to be some indications that intrafollicular overripeness, as indicated by ovulations late in the menstrual cycle, occur in the human. Hertig,³⁴ Boue,³⁵ and Cohen³⁶ have reported several cases that suggest that the day of ovulation, as judged by different criteria, in spontaneous abortions is delayed. However, no significant difference in the mean day of ovulation for spontaneous abortions and term deliveries was found by Guerrero and Lanctot.³⁷ The fact that irregular cycles are more frequent very early and late in reproductive life, a time where reproductive losses are more frequent, has also been interpreted as indicative of the preovulatory overripeness effect.³⁸

Intratubal (Postovulatory) Overripeness

Intratubal overripeness has been typically described in association with a decreased fertilization rate and an increase in abnormal embryonic development as indicated by an augmented frequency of spontaneous abortions and chromosomal abnormalities in rats,^{39, 40} mice,^{41, 42, 43} guinea pigs,⁴⁴ rabbits,⁴⁵ hamsters,⁴⁶ and swine.⁴⁷ Although many chromosomal anomalies have been reported, the most

consistent finding has been that of polyploidy probably as a result of dispermy (fertilization by two spermatozoa) or digyny (fertilization without the expulsion of the second polar body). Other less extensive anomalies such as monosomies and trisomies have also been reported, although with less frequency.

The occurrence of postovulatory overripeness has been presented as a theoretical causal⁴⁸ possibility of mongolism⁴⁹ and of other malformations⁵⁰ in the human. The high incidence of birth defects of the central nervous system in certain parts of the world where the population is predominantly Catholic has been suggested by some as due to post-ovulatory overripeness due to high frequency of rhythm use.⁵¹ Even most enthusiastic supporters of this theory will have to admit that it is no more than a hypothesis and that little, if any, additional evidence exists to support it. Animal research does not substantiate the hypothesis. Furthermore, a recent retrospective study indicates that this is not the case.⁵²

German⁵³ postulated that a decreased coital frequency as it accompanies increased parental age by increasing the chances of fertilization of overripe ova, might lead to mongolism. There are other things that go together with changes in the coital frequency as parents get older, most notably, the physiological aging of the ova of the mother, that may better explain this reported association.

There are two other studies that seem to indicate that intratubal aging occurs in the human. Boue *et al.*,⁵⁴ analyzing the day of insemination in 24 cases of spontaneous abortion, found a 79% incidence of chromosomal abnormalities when the responsible insemination had fallen outside two days before or after the presumed day of ovulation. The numbers are small and the results are not statistically significant. The second study is the already mentioned work by Guerrero and Rojas⁵⁵ that found an increased frequency of abortions when the responsible insemination had taken place after the thermal shift in BBT. This finding is interpreted by the authors as suggesting that aged ova (intratubally) if fertilized, may lead to more spontaneous abortions. Roetzer,⁵⁶ however, considers that given the fluctuations of ovulation around the thermal shift that may occur, this work constitutes no evidence at all. The answer to Roetzer's criticism is the same as mentioned before on the spermatozoa side, that is, unless a differential fluctuation of ovulation around the shift occurs for abortions and term deliveries, the result, although not constituting a proof, suggests that this may be the case in humans.

IV. Other Physiological Considerations

Before discussing some of the practical implications that the previously expressed concepts may have on the application of natural family planning methods, there is some other information that has to be considered.

The correct interpretation of the previously mentioned data requires that the concept of the probability of conception be understood. Animal experiments, both for aging of spermatozoa and aging of the ova, have always reported that aging of gametes is accompanied by a decrease in the fertilization rate. That is as either gamete is aged the chances that it fertilizes or be fertilized gradually decrease to a point that conception is no longer possible.

If this is also true in the human, one would expect a gradual increase in the probability of conception (fertilization rate) as ovulation is approached, and a decrease afterwards. Barrett and Marshall⁵⁷ found such an increase by analyzing a series of Basal Body Temperature records and using a mathematical model to be able to analyze cases in which more than one insemination had taken place in the fertile period. Results are graphically presented in Figure 1. Observe that there is a gradual increase until one day before the shift in BBT, and a more rapid decline after it.

A pioneering work done in the field by Vollman⁵⁸ in which he reported the number of sterile and fertile cohabitations by given days in the menstrual cycle, has allowed us to compute the same type of information and is also presented in Figure 1. Note that a conception took place on Vollman's day -8 (in fact, according to his data, that day would have a 2.8% probability of conception). Analyzing 22,896 charts from the Fairfield International Collaborative Study, Guerrero and Rice⁵⁹ have done a similar analysis. Results are presented in Figure 2. Since our numbers were larger than the previous studies, they permitted us to study the influence of the age of the woman on the probability of conception. As can be seen, the age group less than 28 years shows an almost identical distribution to the one presented by Barrett and Marshall, but women 29 to 33 and 34 to 38 appeared to have a much lower rate of conception. In women older than 39 years, despite a small number of exposures in the fertile period, there were no conceptions.

It has been known that fecundity (that is the potential for having children as opposed to fertility which is the concrete expression of that potential in a given number of children), decreases with age, but it has been generally interpreted as an indication of anovulatory cycles. Further analyses are being done to ascertain whether this decrease in fecundity is a real biological phenomenon and not an artifact.

In general, it can be said that the fertile period begins several days before the temperature shift and shows a very low increase until six days before the shift where the risk of conception begins to increase sharply, reaching a maximum two days before the shift. Afterwards, there is a sharp decrease and already the first day after the thermal shift, the risk of conception is very low and remains so until the

following cycle. It can be thought that the shape and actual values for the probability of conception diminish with increasing age of the woman. It would be extremely interesting to study the probabilities of conception in relation to the hormonal levels of the woman since animal experiments indicate that gamete survival may be enhanced by higher hormonal levels.⁶⁰

An attempt to summarize the main events of the menstrual cycle in relation to the probability of conception is presented in Figure 3. Note that the day of the luteinizing hormone (LH) peak antecedes by two days the thermal shift. It is generally accepted that LH peak coincides with ovulation. Day -2 also coincides with the peak of the mucus symptoms.

V. Practical Considerations as They Apply To the Use of NFP Methods

The first consideration which is necessary to make is that NFP methods are a global category which encompasses several methods of different effectiveness and with different advantages or disadvantages. A distinction has to be made between the calendar methods (that are based on the Ogino-Knaus discoveries), the mucus method (which is based on the recording of mucus symptoms, sometimes called ovulation or Billings method), and the symptothermic method (which uses a combination of symptoms and signs to detect ovulation). Calendar rhythm is different according to which numbers are used to determine the fertile period. The symptothermic method may be used restricting sexual activity to the postovulatory period or using also the preovulatory period following either a calendar schedule or mucus symptoms. The symptothermic method restricting sexual activity to the postovulatory period may also be different according to which method is used to determine the shift and whether the shift and other symptoms are used to discern ovulation. Therefore, extreme caution must be exercised when comparing the results of the different methods. In the same manner, if there are any risks involved in the use of NFP, they have to be analyzed individually for each specific method.

VI. Possible Harmful Effects

As a general principle, it can be said that those NFP methods which are based on the diagnoses of the occurrence of ovulation, such as the symptothermic or the mucus method, offer the distinct advantage of preventing the possible harmful effects of preovulatory overripeness, if it occurs, in abnormally delayed ovulations. It must be remembered that this type of overripeness which may occur late in the repro-

ductive life of women, has been reported in animals associated with chromosomal anomalies of the trisomy type (mongolism or Down's syndrome is a trisomy). Calendar rhythm offers no protection for this type of problem. In relation to the possible effects of postovulatory overripeness, it can also be said that both the symptothermic and mucus methods offer good protection since the time period allowed for the resumption of sexual activity is generally three days, more than enough for the ovum to have reached an infertilizable stage (the time limit for most animal ova doesn't go beyond 8 to 12 hours. There is no reason to suspect that the human ova are different).

In relation to the possible abortifacient effect of spermatozoa aged in the female tract, it is clear that the symptothermic or mucus method restricting sexual activity to the postovulatory period offer, by definition, total protection. When these methods are also used with sexual activity in the preovulatory period or in the case of the calendar method, there exists the possibility that an aged spermatozoon fertilizes the ovum and there follows an increased probability of abortion. In estimating the magnitude of risk involved, however, it is of extreme importance that the knowledge about probabilities of conception be incorporated. If we accept the Guerrero and Rojas⁶¹ result, a conception resulting from an insemination six days before the shift has around a 12% chance of being spontaneously aborted. If we want to know what is the risk of an insemination six days before the shift leads to an abortion, then we must first multiply the risk of conception on that day by the risk of abortion given that a conception took place. If this is done we obtain $.05 \times .12 = .6\%$. Less than one percent. The same approach would have to be followed on the ovum side. In effect, to find out the risk of an insemination ending on spontaneous abortion three days after the shift, we have to multiply the probability of abortion given the fact that a conception occurs. If we do that (again accepting the Guerrero and Rojas⁶² and Guerrero and Rice⁶³ data), we obtain $.001 \times .24 = .02\%$, or one-twentieth of one percent. It must be remembered that the risk of a spontaneous abortion in a normal pregnancy is believed to be between 10 to 15%. The graphic representation of the risks of abortion and of conception in relation to the BBT shift are presented in Figure 4.

VII. What Is Not Known

The title of this conference was "Aging of Gametes. What is Known and Unknown." I have attempted to summarize existing knowledge of this field, and as it was mentioned, most of the knowledge comes from animal species whose reproductive mechanisms might be different.

The little evidence which exists in humans has still to go through the usual scientific process of repetition and verification in different sets of data and by different observers.

The existence in humans of preovulatory overripeness is an area in which populations using natural methods can produce valuable information. The possible etiologic factors of Down's syndrome can also be explored. The verification of the probabilities of conception and its possible reduction with age would be extremely useful information not only for NFP users but also for all those concerned. The study of the precise temporal relationship of ovulation and the mucus symptoms or BBT chart would permit a clearer answer to the many questions mentioned.

It is clear that the unknowns grossly outnumber the few aspects that we already know. It is clear that the current trend toward the use of NFP methods offers a unique responsibility and opportunity. We have the responsibility to investigate seriously the different aspects of human physiology of reproduction. We have to find an answer to each of the problems which has been presented in relation to NFP methods. More important than that, we have the opportunity to explore, with respect and honesty, the delicate aspects around the transmission of life as few other groups can.

Figure 1

Probabilities of conception for given days in the menstrual cycle in relation to the shift in basal body temperature.

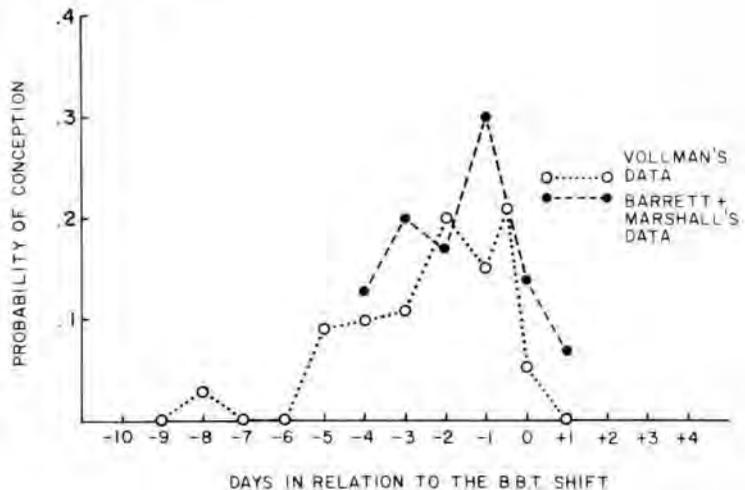


Figure 2

Probabilities of conception for given days in the menstrual cycle in relation to basal body temperature shift, according to age of women. (Guerrero and Rice data)

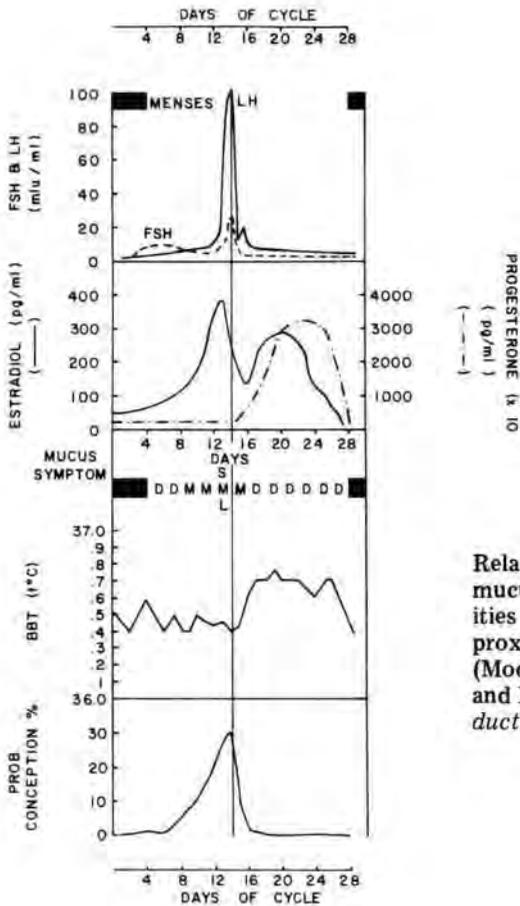
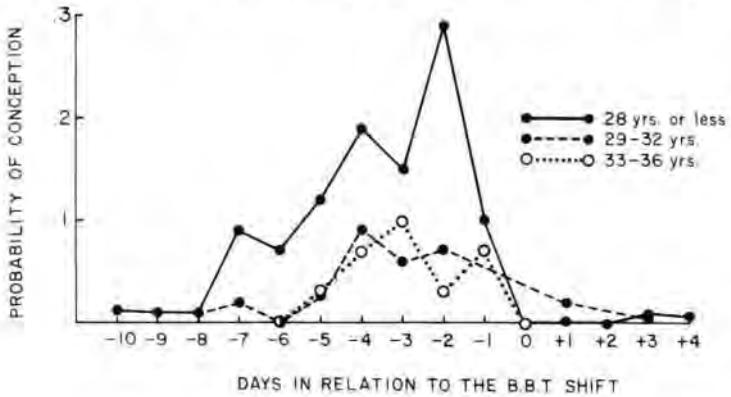
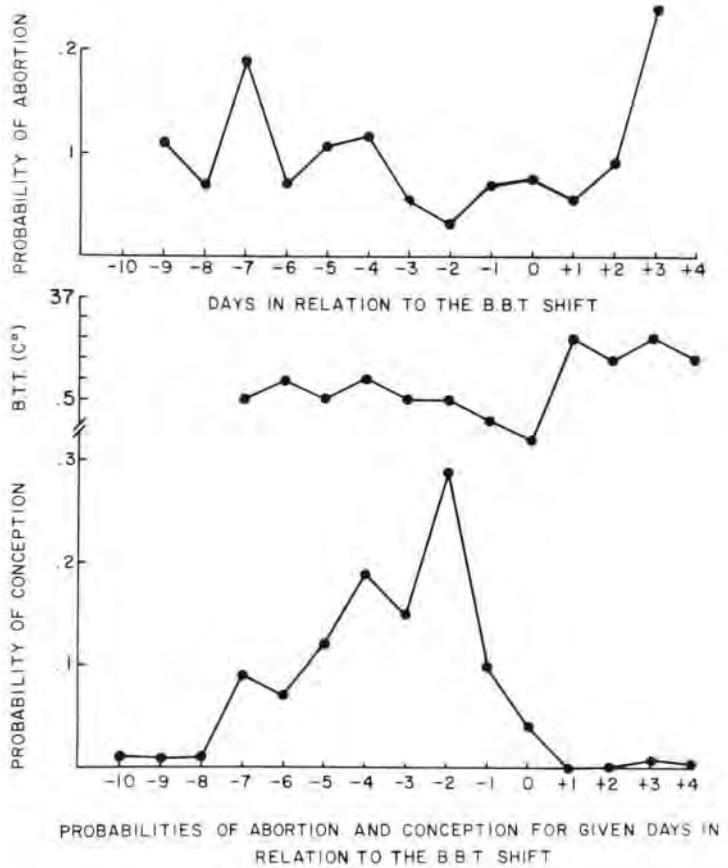


Figure 3

Relationship of hormonal levels with mucus symptoms, B.B.T. and probabilities of conception. Vertical line approximately indicates day of ovulation. (Modified and adapted from Odell, W. and Moyer, D.L., *Physiology of Reproduction*, 1971, C.V. Mosby Co.)

Figure 4

Relationships of the probabilities of abortion and conception during the menstrual cycle to the basal body temperature.



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61. See note 22 above.

62. *Ibid.*

63. See note 25 above.

