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RESEARCH FRONTIERS IN FERTILITY REGULATION

THE ETIOLOGY OF PELVIC INFLAMMATORY DISEASE

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Pelvic inflammatory disease (PID) is a major medical problem worldwide Its treatment and sequelae absorb a significant part of the health resources of many countries (33) The term PID refers either to an acute or a chronic inflammatory response involving the upper female genital tract (endomyometrium, tubes, ovaries, and supporting structures) The term is nonspecific and the condition may result from a variety of infections in a variety of organs, e g gonococcal salpingitis, chlamydial parametritis, and bacteroides tubo-ovarian or pelvic abscesses Unless otherwise indicated, pelvic infections related to pregnancy or gynecologic surgery, or secondary to other gynecologic conditions such as cancer, and their treatment have not been considered in this report because they are traditionally given diagnostic terms other than PID

Although inflammation of the upper genital tract can result from diverse causes such as endometriosis or extension of an intra-abdominal infection, in most cases, infectious PID is considered a sexually derived disease (SDD) resulting from sexually transmitted pathogens (STP) (46) PID is rarely diagnosed in women who do not engage in heterosexual vaginal intercourse The pathogenic micro-organisms responsible for PID are not always transmitted from the women's partner, however, they may be part of the resident flora of her lower genital tract, and they may not become pathogenic until they gain access to her upper genital tract (41)

There are no uniformly accepted criteria for the diagnosis of PID and there is a significant degree of imprecision in this diagnosis As early as 1928, Farr and Findlay reported that salpingitis was correctly diagnosed preoperatively in only 70% of 545 women admitted to a hospital and operated upon with this diagnosis (17) Forty-one years later, Jacobsen and Westrom reported a similar rate of correct diagnosis (65%) of acute salpingitis, compared with the findings at laparoscopy (34)

INCIDENCE

According to Westrom, the annual incidence of PID in modern Western society is estimated to be 1% among women aged 15 to 34 (88) In the high risk age group, 15 to 24, the annual incidence is 2% In the United States, the annual incidence of PID is at least 2% among fecund, sexually active women aged 13 to 44

There is no national reporting system for PID in the United States From a sample of discharge records at 7900 short-stay civilian hospitals, there were an estimated average of 213,000 hospitalizations for PID per year from 1970 to 1975 (36) PID was the primary diagnosis in 65% and the secondary diagnosis in the other 35% of cases Hysterectomies were performed in 23% of the women admitted in whom PID was the principal diagnosis Admissions for PID accounted for about 897,000 hospital bed days each year from 1970 to 1975 (74) Among non-pregnant women over 14 years of age, about 1 in every 100 hospital admissions was for PID (74)

Based on the National Ambulatory Medical Care Survey for the period 1973 to 1977, there were about 1 89 million patient visits for PID per year to office-based private practicing physicians (73) An estimated one-half million physician-hours were required to see and treat these patients One of every 17 of these office visits resulted in hospital admission, accounting for about 55% of all hospital admissions for PID during 1973 to 1977 In addition,

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there were an estimated 680,000 patient visits to clinics and hospital emergency rooms for PID each year (10)

Including only those cases in which the principal diagnosis was PID, there are at least three-quarters of a million cases of PID each year in the United States, with a resulting hospitalization rate of nearly 20% Curran estimated the direct costs of PID and associated ectopic pregnancy in 1979 to be $700 million (10) This figure does not include the costs of treating infertility problems resulting from PID There is no accurate way to measure the total economic consequences of PID, either in the United States or worldwide

MEDICAL CONSEQUENCES OF PID

The medical consequences of PID are well known and include repeated episodes of PID, ectopic pregnancy, infertility, and chronic pelvic pain Westrom reported a greater than 6-fold higher rate of ectopic pregnancy and nearly a 4-fold higher rate of chronic pelvic pain among women with laparoscopically confirmed PID who were followed up for 6 to 14 years, compared to a group of women who did not have PID (87) In the same study, the proportions of women who were unable to become pregnant were 13%, 36% and 75% after one, two and three or more episodes of acute PID, respectively Excluding women who elected not to become pregnant, only 69% of the women who had acute PID became pregnant, compared to 96% of the control group In a later study by the same group of investigators (75), infertility rates among women with laparoscopically verified PID were 22% after the first episode of PID and 46% after subsequent episodes of PID The data from both of these studies probably underestimate the true risk of infertility following acute PID, since the studies included only women with clinically evident disease who were treated medically, it is probable that many cases of PID are subclinical

CAUSATIVE AGENTS

The microbiology of the female genital tract is extraordinarily complex and knowledge of it is limited by the microbiological methods available at present This subject has aptly been described as a microbiologist's nightmare (13) Despite the significant advances in microbiological techniques that have been made during the past 10 to 15 years, an accurate understanding of the microbiology of PID is only beginning to emerge

It is now recognized that *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma hominis* and a variety of other aerobic and anaerobic micro-organisms are causal in most cases of upper genital tract infection in the female (46, 77) The relative contribution of each of these organisms to the development of PID depends upon a variety of factors, including the prevalence of each organism in a given population, the state of health of each woman, and her prior disease history Until recently, it was thought that *N gonorrhoeae* initiated infection in nearly all cases of PID Studies performed over the past 15 years have dispelled this notion, however, and the importance of other micro-organisms in the etiology of PID is now commonly acknowledged (9, 16)

In the United States, gonorrheal infections are associated with 30% to 80% of the PID cases, and 10% to 17% of the women with gonorrhea subsequently develop clinically diagnosed PID (31) Epidemiologic studies from other countries do not always agree on the relative importance of gonorrhea and other sexually transmitted pathogenic organisms in the development of PID Reports from the United States as well as England and Wales have found PID rates to parallel gonorrhea rates (1, 36, 64) A decline in the prevalence of gonorrhea in Sweden was accompanied by a decline in the prevalence of gonococcal PID, at the same time, however, a concomitant increase in the prevalence of all cases of PID was noted (88) These data imply that control of gonorrheal infections alone cannot be relied upon to materially decrease the prevalence of PID

Our understanding of the bacterial pathogenesis of PID has improved with the advent of reliable methods for the growth and identification of anaerobic bacteria (41) Some of the major microbiological advances of the past decade have included a rapid tissue culture method for the diagnosis of *Chlamydia trachomatis* (26) and a microimmunofluorescent technique to identify *C trachomatis* antibodies in sera (85) Unfortunately, these techniques are not uniformly available to clinicians, and the importance of *C trachomatis* in the etiology of PID is only recently becoming recognized among clinicians (42, 46, 77) According to Treharne and co-workers, over 50% of the PID in women under 25 years of age in one city in Sweden was caused by infection with *C trachomatis* (83) In contrast, investigators in the United States generally have reported lower isolation rates (from 5% to 20% using endocervical cultures) of *C trachomatis* from women with PID (35) Perhaps of greater importance is the finding of Jones and co-workers that antichlamydial antibodies were present in the serum of 35% of a group of 172 infertile women (35), these findings are indicative of prior chlamydial infections No doubt, the clinical relevance of chlamydia and other organisms, such as ureaplasma, will become clearer as additional improvements are made in the methods of bacterial isolation Of course, it is important to keep in mind that even with rapid methods of specimen transport and accurate methods of bacterial culture, results obtained from endocervical cultures do not always reflect the microbial picture present at a higher level in the genital tract, even when a lower genital infection accompanies an upper genital tract infection

As noted above, clinical opinion until recent years was that gonococcal infection paved the way for invasion by secondary pathogens at a later date In this regard, studies in which laparoscopy was used to obtain tubal culture and biopsies from women with acute salpingitis have demonstrated two important points The first is that even at the outset, tubal infections are polymicrobial and often include *N gonorrhoeae*, as well as anaerobes, aerobes, and *chlamydia* (14) The second is that fewer organisms are recovered in patients with longer durations of symptoms, even when meticulous attention is paid to the culture methods (14)

EPIDEMIOLOGICAL STUDIES

Over the past decade, numerous epidemiological studies have identified risk factors associated with PID Although many such factors have been identified, it is crucial to remember that these associations do not necessarily imply a cause-and-effect relationship between the presence of the risk factor(s) and the occurrence of PID

Case Control Studies. Prior to 1974, clinical impressions and the medical literature strongly supported an association between sexual activity and the occurrence of PID (50) Since 1974, the association between contraceptive usage, in particular the use of IUDs, and PID has become a focus of attention and has been studied extensively Table 1 lists 18 case control studies (or studies that can be analyzed as case control studies) published since 1973 that have identified factors associated with either an increased or decreased risk of PID The list is not all-inclusive, but represents major contributions to the identification of risk factors for PID For each factor identified, an estimate of the relative risk (RR) of PID was computed for women with the risk factor compared to women without the risk factor An RR value greater than 1 indicates the factor is associated with a significantly greater ($p < 0.05$) risk of PID, and a value less than 1 indicates the factor is associated with a significantly lower ($p < 0.05$) risk of PID If estimates of the relative risks were not provided by the authors, they were derived using standard methods for the calculation of the crude odds ratios If necessary, the original estimates were recalculated, depending upon the methods used by the authors *

Reference	*Risk Factor*	*Est RR*
Noonan & Adams, 1974 (51)	Current use of IUD	7 8
	Current OC use	0 4
Targum & Wright, 1974 (78)	Current use of IUD	9 3
	Prior induced abortion	2 3
Phaosavasdi and co-workers, 1975 (58)	Past current use of IUD	12 2
Brown & Cruickshank, 1976 (5)	Married	0 5
	> 1 sex partner in previous 6 months	3 2
	Age at first intercourse	*
	Previous PID	5 3
Faulkner & Ory, 1976 (18)	Current use of IUD	
	Febrile cases	5 0
	Afebrile cases	2 6
	Prior PID, afebrile cases	3 9
Westrom and co-workers, 1976 (90)	Current use of IUD	3 0
	Age < 26, nulligravid, IUD user	7 6
Eschenbach and co-workers, 1977 (15)	Current use of IUD	2 4
	Intercourse > 2 week	1 4
	Prior pregnancy	1 5
	Intercourses avoided with menses	2 1
	Race non white	1 6
	Previous gonorrhea	1 7
	> 2 sexual partners in past 6 months	1 6
	Current OC use	0 5
	Sterilization	0 3
Thaler and co workers, 1978 (80)	Prior abdominal pelvic operations	11 0
	Prior abortion	2 7
Flesh and co workers, 1979 (19)	Race black	4 0
	Previous PID	3 1
	> 1 sexual partner	5 2
	Current use of IUD	2 2
Osser and co workers 1980 (54)	Current use of IUD	2 4
	Current OC use	0 3
	Prior pregnancy	2 2
Paavonen & Vesterinen, 1980 (56)	Current use of IUD	3 0
	Current OC use	0 3
Potterat and co workers, 1980 (61)	Married	0 5
	Current OC use	0 4
	Race black, Hispanic	2 5
Burkman 1981 (6)	Age < 25 years	1 8
	Education < high school	1 7
	> 1 sexual partner in last 6 months	1 6
	Prior PID	3 2
	Prior gonorrhea	2 5
	Current use of IUD	1 6
	Current contraceptive use other than IUD	0 3
	Race non white	1 3
	Single	1 3
Kelaghan and co workers, 1982 (39)	Current use of barrier contraceptives	0 6
Rosenfelc and co-workers, 1983 (65)	Prior use of IUD	7 2
	Prior OC use	0 4
	Prior use of mechanical contraceptive method	0 3
	History of acute PID	54 6
Wolner Hanssen and co workers, 1983 (92)	Current use of IUD	3 3

*Higher RR for earlier age at first intercourse RR cannot be estimated from these data

Table 1 Estimated relative risks (RR) for factors associated with either an increased or decreased risk of PID.

*The statistical computations in the present report were performed by David A Edelman, Ph D, Medical Research Consultants, Inc, Chapel Hill, North Carolina

Table 1 lists only those factors associated with either an increased or decreased risk of PID In some studies (5, 54, 78) various factors (e g , age, marital status, parity) could not be evaluated, since cases (women with PID) and controls (women without PID) were matched on these factors Also, factors identified in one study as being associated with either a higher or lower risk of PID were not necessarily identified in other studies For example, frequency of intercourse was identified as a risk factor by Eschenbach and co workers (15) but not by others (5, 6) Table 1 does not include those factors not found to be associated with a higher or lower risk of PID There are undoubtedly many more factors than those listed in Table 1 that might have a direct and possibly causal association with the development of PID, e g , the bacterial flora in the patients' vaginas or the presence of urethritis prostatitis in the patients' sexual partners

Other Studies Studies that have evaluated women with PID without any reference to a control group have also identified factors associated with increased risks of PID A wide variety of findings have been reported

Wright and Laemmle found that PID rates in an indigent population were increased in women aged 20 to 24 who were neither currently married nor ever married, and among IUD users (93) Jones and co-workers reported higher PID rates for divorced or separated women, non-white women, and women aged 15 to 30 (36) Potterat and associates implied that the health-seeking behavior patterns of women with gonococcal PID and their male contacts differed from those of women with uncomplicated gonorrhea and their contacts (61) Qvigstad and associates found an association between the risk of PID post-abortion and prior positive cervical culture for *C trachomatis* (63) Gump and co-workers investigated the relationship between serum antibodies to *C tracho matis*, prior to confirmed diagnosis of PID and prior IUD use (28) These investigators found that the proportion of women with prior PID was higher among women who had previously used the IUDs compared to those who had not (28), it was also higher among women with higher antibody titers to *C trachomatis*, regardless of IUD usage

The four major categories of risk factors for PID noted in Table 1 are sexual activity, sociodemographic characteristics, contraceptive method used, and other medical conditions The role of each of these factors is discussed in the following sections

RISK FACTORS

SEXUAL ACTIVITY

PID is predominately a sexually derived disease (SDD) Exceptions include those cases resulting from tubercule infections, extension of appendiceal or other inflammations of the bowel, pelvic surgery, and gynecologic operations such as dilatation and curettage, abortion, insertion of an IUD, and uterine biopsy, to mention a few Unfortunately, the literature on PID contains few studies that specifically attempt to relate sexual activity, PID, and specific bacteriologic agents Of the 16 studies listed in Table 1, only 4 (5, 6, 15, 19) identified some aspect of sexual activity as a risk factor for PID Nevertheless, all studies found factors in which sexual activity was implicated, e g , use of a contraceptive method or prior pregnancy

Only three aspects of sexual activity have been studied and identified as risk factors for the development of PID They are frequency of intercourse, number of sex partners, and age at first intercourse Even so, all studies listed in Table 1 that evaluated these factors (5, 18, 61, 65, 78) were not in agreement as to their importance This is not surprising for two reasons First, in the reported studies, the information on sexual activity obtained by having the patients complete questionnaires or by direct questioning has generally not been validated by any other source to assess its reliability Second, and perhaps more important, only superficial information on sexual activity has been obtained in studies reported to date Specific aspects of sexual activity that have not been evaluated among women with PID include the following frequency of homosexual relations or heterosexual contact with bisexual partners, practice of oral and anal sex, and the sexual exposure and habits of the women's partner(s) Although a complete evaluation of sexual variables would be a most difficult task, a true understanding of the relationship between sexual activity and the development of PID requires that this be done In the past, analyses have generally evaluated only one aspect of sexual activity at a time and have not considered the importance of their interrelationships For example, coital frequency and the number of partners have been evaluated separately in most studies In terms of the risks of acquiring a sexually transmitted disease (STD), a high coital frequency with one partner clearly is not the same as either a high or low coital frequency with many partners Moreover, the number of partners a woman has might be associated with different risks of PID, depending on her marital status, age, and other factors, regardless of coital frequency

The role of the complex variables included in the term "sexual activity" and the interrelationship with sociodemographic factors, contraceptive choices, and other medical conditions is just now beginning to gain the attention of investigators The prevailing view is that coital frequency and number of sexual partners provide measures of the frequency of exposure to sexually transmitted pathogens (STP) The third variable dealing with sexual activity, namely, age at first intercourse, may be only an indirect measure of later sexual behavior with

respect to the exposure to a variety of sexual partners (88)

SOCIODEMOGRAPHIC CHARACTERISTICS

Sociodemographic factors, such as marital status and age, probably are indicators of the sexual behavior that determine the risks of a woman acquiring PID For example, sexually active separated or divorced women have been shown to be at a higher risk of acquiring PID than married women of the same age (36) This association may reflect a higher number of sexual partners among nonmarried women and/or the multiplicity of sexual contacts among the women's partners and a concomitantly higher risk of being exposed to an STP In addition, it seems reasonable to hypothesize that other sociodemographic characteristics might reflect different sexual preferences and lifestyles To our knowledge, these interrelationships have not been specifically investigated

Variables such as race that have been associated with an increased risk of PID may only reflect different prevalence rates of STDs, such as *N gonorrhoeae* or *Chlamydia trachomatis* in various racial-ethnic groups The possibility also exists that there are differences in the host response to sexually transmitted infections that either increase or decrease the risks of developing PID It is also necessary to consider that the health-seeking behaviors of different population groups may differ in such a way as to place them at differential risks of PID From a public health point of view, appropriate educational programs may address behavioral issues such as the number of sexual partners and type of contraceptive used They can do nothing, however, to alter demographic factors such as a woman's age, race, and prior pregnancy history

CONTRACEPTIVE METHODS

Barrier Methods. Both clinical and epidemiological studies have demonstrated that use of barrier (both physical and chemical) methods of contraception reduces a woman's risk of PID (8, 67, 68, 69) Since the bacteriocidal properties of spermicidal preparations protect women from many lower genital tract infections and presumably their subsequent ascent into the upper genital tract, the reduced risk of PID most likely is due to use of the barrier contraceptives per se However, it may also be due to other factors, since the choice of a contraceptive method may be different for different groups of women and be associated with sexual activity factors

Women who use sterilization as their method of contraception are also at a reduced risk of PID (32) It is commonly thought that contraceptive sterilization protects against PID by preventing pathogens from gaining access to large portions of the tubes, as well as the ovaries and the peritoneal cavity This condition of limited access may be especially important during ovulation and menstruation, when a greater susceptibility to PID exists (15) An additional explanation for the lower risk of PID observed among sterilized women is that these women are usually in the demographic subgroups known to have lower risks of PID, e g , married women over 30 years of age These women may live in stable relationships, and their sexual lifestyles and preferences may place them at a lower risk of exposure to externally acquired pathogens that may initiate an infectious process

Oral Contraceptives (OCs). Two reasons have been suggested for the reduced risk of PID among women using oral contraceptives (OCs) (15, 66) that has been found in case control studies (see *Table 1)* The first is that OC use tends to reduce menstrual flow This not only reduces the time during which there is a more favorable environment for bacterial growth, but also reduces the need for the prolonged use of tampons or sanitary napkins The second is that OCs change the characteristics of the cervical mucus Since OC use inhibits the preovulatory estrogen surge there is an absence of receptive mid-cycle cervical mucus that promotes sperm access to the upper female genital tract

Intrauterine Devices. The IUD has been the most extensively studied contraceptive method in terms of the risks of PID, and it has been identified as a risk factor for PID in many studies *(Table 1)* published in the late 1960s and 1970s (11, 12) In 1968, Wright and Laemmle first reported that IUD users had a higher risk of PID compared to users of oral contraceptives (relative risk, 4 9) and compared to women using contraceptive foam (relative risk, 2 6) (93)

The principal difficulty in trying to assess whether or not the use of IUDs increases the risk of PID relates to the selection of an appropriate comparison group Although non-contraceptors appear to constitute an appropriate comparison group, this group in reality includes several subgroups with different risks of PID and therefore may be an inadequate comparison group

The question of whether the higher reported risk of PID among IUD users represents higher risk due to the use of IUDs per se or represents a higher risk as a result of the choice of comparison groups against which IUD users are evaluated has not yet been clearly answered Since the use of barrier or oral contraceptives as well as sterilization protects women against PID, the relative risk of PID to IUD users compared to users of these methods might only reflect the protective effect of the latter methods, rather than a truly enhanced risk of PID among IUD users As previously stated, it is inappropriate to compare the risks of PID to IUD users with those of sterilized women who are inherently at a lower risk of

PID It is equally inappropriate to select non-contraceptors (including women using rhythm, coitus interruptus and any of the so called natural family planning methods) as a comparison group, since the group of non-contraceptors includes the following subgroups, who probably have different risks of acquiring PID

1 Sexually inactive women
2 Sexually active women desiring pregnancy (These women may be at a minimal risk of PID since they generally have stable monogamous sexual relationships
3 Sexually active women not desiring pregnancy but who elect not to use contraceptive methods (This group includes women who have occasional coitus at times when they do not think they can become pregnant These women, as well as others in the non-contraceptor group, may terminate their pregnancies by abortion)

Sexually active women who do not wish to become pregnant but who are not using any contraceptive method represent a unique group If these women are as sexually active as women who use contraceptive methods, they will be repeatedly exposed to the risks of pregnancy as well as PID These women have a far greater risk of becoming pregnant before contracting PID, since the monthly probability of pregnancy is about 5% to 6% and that of contracting PID is about 0 1% to 0 2%

Sexually active non-contracepting women may also be an inappropriate comparison group, because women who become pregnant unintentionally are also exposed to the risk of upper genital tract infections associated with the termination of pregnancy, either at term or by abortion This group of women may include subgroups who are at a high risk of PID For example, Qvigstad and co-workers found that 1 6% of the women with negative serologic cultures for *C trachomatis* developed PID within 1 month of abortion by dilatation and vacuum aspiration (63) In sharp contrast, the rate among women with positive cultures was 20% A similar finding has been reported by others (55) Women who have incomplete septic abortions or upper genital tract infections following induced abortion are not usually classified as having PID, even though the effects of these infections in terms of future fertility may be similar to those of non-pregnant women who acquire any form of PID

It is possible that sexually active, non-contracepting women who do not wish to become pregnant are probably at a different risk of PID compared to sexually active, non-contracepting women who wish to become pregnant To our knowledge, studies that have evaluated the relative risks of PID associated with contraception have not distinguished between these two sexually active non-contracepting groups of women The two groups may also differ with respect to numerous other important factors, including their health attitudes and habits In comparing the relative risks of PID among women using different contraceptive methods, several important factors need to be considered, including the sexual activity of the women and their frequency of exposure to STDs These factors may be quite different for women in the different contraceptive groups For example, Valenti recently reported that the desire for more frequent coitus was greater among IUD users compared to OC users in the first 3 months after initiation of the method (84)

There are two temporal aspects to the risk of PID among IUD users The first is the risk of PID resulting from the insertion procedure per se, and the second is the risk of PID due to factors associated with the IUD and the user The higher risk of PID during the initial month of IUD use (Table 4 3 of 11, 12, 43) is thought to result from the transmission of bacteria from the endocervix to the uterus at the time of the insertion procedure This observation follows from the study of Mishell and co-workers showing that bacterial contamination of the uterus followed IUD insertions, the bacteria were eliminated over a period of weeks (48) Although the prevailing clinical opinion is that the uterine cavity is normally able to maintain sterility (70), several studies have shown bacteria to be present in the uterine cavities of a significant proportion of asymptomatic women who were not using IUDs (71) Some of these studies, however, may include false positive culture results due to the sampling methods used (71)

In a recent case control study reported by Lee and associates in which women were excluded if they were sexually inactive, amenorrheic, sterile, or had recently been pregnant (all factors known to influence a woman's risk of PID), the estimated relative risk of PID to IUD users compared to women using no contraceptive methods was 3 1 for women who used their IUDs for less than 5 months, whereas it was only 1 1 (a value not significantly different from 1, $p > 0\ 05$) for women who used their IUDs for over 4 months (43) These data imply an elevated risk of PID following the IUD insertion procedure itself, but no increased risk due to continuing IUD use This finding is contrary to the commonly accepted opinion that IUD use is associated with an increased risk of PID at any time following insertion Since this study did not ascertain whether the IUD users and users of no contraceptive methods were different with respect to other known risk factors for PID, the reported estimated relative risk of 1 1 may represent either an under or overestimate of the actual risk (43) Moreover, since the nonuser group included women who were at a reduced risk of PID for various reasons, the estimated relative risk value may represent an overestimate of the true value A similar point of view has been expressed by Luukkainen and associates, who suggested that the higher rate of PID for IUD users found in case control studies might only

reflect the risk of PID attributable to the insertion procedure (44)

A major area of concern regarding IUD use has been the presence of tail strings In the early 1960s, many clinicians suggested that the use of strings might put women at an increased risk of infection by providing a pathway for the ascent of bacteria from the vagina to the uterus At the same time, however, it was recognized that the tail facilitated removal of the IUD and avoided complications that might occur at the time of removal if the IUDs were tailless The consensus of experts was that the benefits of adding a tail string outweighed the risks

Two decades later, the question of whether or not the IUD tail per se places women at a higher risk of PID remains unanswered Adequate clinical studies to evaluate the role of the IUD tail in the etiology of PID have not been conducted Four of five studies that have compared tailed and tailless IUDs found no increased risk of PID to users of tailed IUDs (11) These studies, however, were not designed specifically to evaluate the role of the IUD tail In one on-going study in which 1100 women were randomly assigned to use a Copper T IUD either with or without a tail, no statistically significant differences in the PID rates have been found between the two groups of women who have been followed for up to 1 year (7)

Since 1975, the question of whether the risk of acquiring PID is different for IUDs using different types of tail strings also has been debated widely Today, most, if not all, IUD tail strings consist of one or two strands of nylon In the past, some IUDs have used tails made from a bundle of fine filaments (multifilamental tails) e g , Antigon F, Birnberg bow, Dalkon Shield, Latex Leaf, Majzlin spring) None of these IUDs is used in clinical practice in the United States today Comprehensive evaluations of the PID rates for different types of IUDs, regardless of the type of tail string used, have not found any one type of IUD to be consistently associated with higher rates of PID (11, 12)

The controversy over the type of IUD tail was widely published by Tatum and co-workers, who demonstrated in a series of *in vitro* experiments that live *E coli* could move up the Dalkon Shield tail along with fluid under certain laboratory conditions, near the upper knot at the base of the IUD (79) These investigators also reported the presence of anaerobic and aerobic bacteria obtained from Dalkon Shield tail strings removed from asymptomatic users (79) The authors suggested that a multifilament tail could provide a means for bacteria to enter the uterus However, this hypothesis does not explain the clinical observations of the occurrence of PID in women wearing IUDs with monofilament tail strings or IUDs with solid plastic tail configurations (Margulies spiral)

In one *in vitro* investigation (62), bacteria appeared to migrate through a layer of cervical mucous coating the outside of multifilamental and monofilamental IUD tails In a later study by the same group of investigators (72), the apparent migration of bacteria along the IUD tail was confirmed indirectly in IUD users undergoing hysterectomies Aerobic and anaerobic cultures of the uterine cavities demonstrated bacteria in the uteri of 12 of 14 women using IUDs with monofilamental tails and in the uteri of 3 women using IUDs with multifilamental tails Most bacteria cultured in this study were commensals, such as *Gardnerella vaginalis* and *Corynebacterium* sp , but potentially pathogenic organisms such as *Escherichia coli* and *Streptococcus faecalis* were also found The types of bacteria cultured from the extirpated uteri were similar to the types of bacteria present in the vaginas of these same women These authors concluded that the IUD tail in some way interfered with the protective mechanisms of the cervix, thus permitting bacteria to enter the uterine cavity These two studies (62, 72) suggest that there is no significant difference between monofilamental and multifilamental tails with respect to their ability to facilitate the transfer of bacteria from the cervix and vagina to the uterine cavity However, one of the studies (72) included only a few subjects, and all of them had gynecologic conditions requiring surgical treatment For this reason, they may not be representative of all asymptomatic IUD users

The purported ability of bacteria to migrate along the outside or the inside of IUD tails has not been related to the risk of PID for IUD users compared to the risk for nonusers Clearly, the presence of bacteria on either the inside or the outside of the IUD string does not indicate the presence of a pelvic infection Bank and associates performed scanning electron microscopy studies of Dalkon Shield tails removed from asymptomatic women who had used their IUDs for 2 or more years (2) These IUDs had intact tail strings that were found to contain bacteria, both above and below the double knot at the base of the IUD The study did not determine how long the bacteria had been present within the strings The Dalkon Shield tails evaluated by Tatum and associates came from IUDs that were removed electively from women who did not have any symptoms of PID at the time their IUDs were removed (79)

With prolonged use, almost all IUDs undergo physical changes, including distortion of their shape, discoloration, and deposition of mineral salts (especially calcium) on their surface The deposits on IUDs appear to build up over time (27) The significance of these changes, if any, in terms of increasing the user's risk of acquiring PID or developing other significant complications is not known Recent research efforts have been directed at further evaluation of the surface deposits found on IUDs (Lippes Loop, Saf-T-Coil, Dalkon Shield, Copper 7,

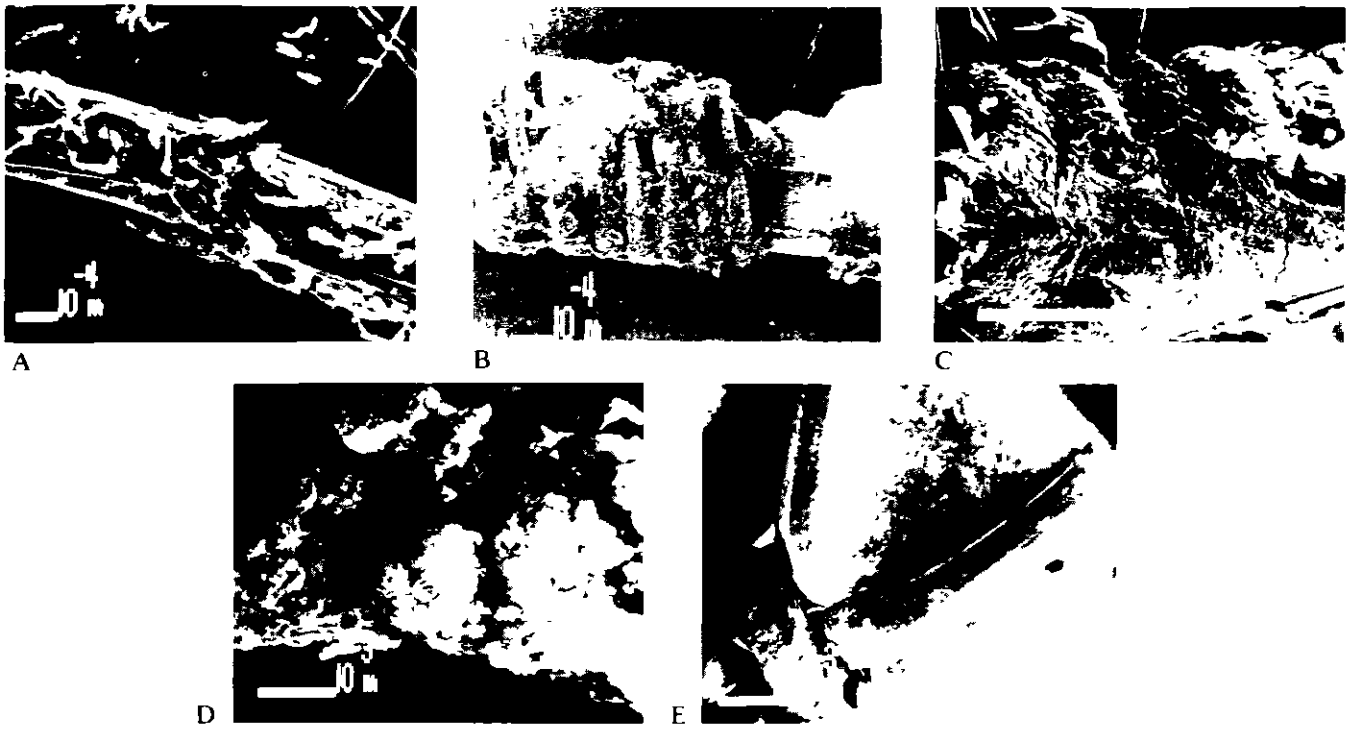

Figure 1 Scanning electron microscopy of various IUDs after removal A Lippes Loop, 100x Flaking encrustations on tail string B Copper-7, 10x, lower margin of copper wire and IUD body C Copper T, 40x, side view of copper wire covered with hard surface encrustation with crevices and irregular topography D Saf-T-Coil, 20x, irregular granular surface encrustation and bulbous nodular formation E Dalkon Shield, 20x, area of lower portion of IUD body and string interconnection, with plaque-like encrustation on topographical areas of the string

Copper T) that have been used for prolonged periods (4 to 15 years) (38) Figure 1 shows scanning electron micrographs (SEMs) of the surfaces of sections of some of these IUDs and their tails The deposits on the surfaces of these IUDs were further evaluated by energy dispersive spectrometry and x-ray diffraction methods These studies identified several minerals and salts on the surfaces and tails of the IUDs *(Figures 2* and *3)* Of particular interest is the identification of calcium apatite crystals on the surface of a Lippes Loop This salt is a common component of dental calculus and results from the mineralization of plaque Similarities between the calculus found on teeth and deposits found on IUDs may be due to a similarity between the bacterial inhabitants of the mouth and vagina, such as anaerobic streptococcus, viellonella and other organisms involved in plaque formation It should be recognized, however, that calcium may precipitate on the surface of IUDs in the absence of bacteria on the IUD surface No evidence has been presented to indicate that the formation of deposits on IUDs varies with the specific type of IUD, it appears to be generic to all types of IUDs (38)

Marrie and Costerton have described transmission electron microscopic studies of IUDs (Copper T, Copper 7, Saf-T-Coil) electively removed, they found microcolonies of bacteria adherent to specific areas of the surfaces of

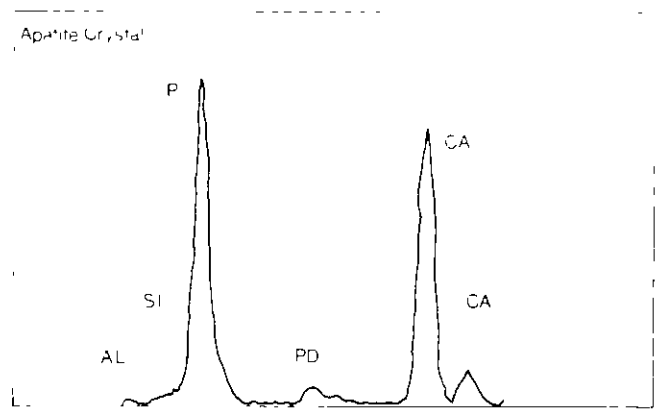

Figure 2 Artist rendition of energy-dispersive spectrometry of calcium apatite crystal from Lippes Loop IUD

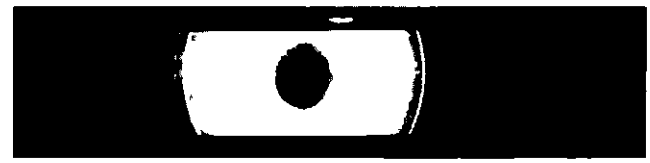

Figure 3 X-ray diffraction pattern of calcium apatite crystal shown in Figure 2

these IUDs (47) These investigators thought the bacteria were not contaminants from the cervix and vagina at the time of IUD removal, but had been on the surface of

the IUDs for a long time From this study and other similar types of studies it cannot be determined whether the bacteria adherent to the IUD surfaces were deposited on the IUDs during the insertion procedure, or "migrated" to the uterus along the IUD string, or came to be there by passive transport, such as by attachment to sperm or other motile organisms

Pelvic actinomycosis is an uncommon form of pelvic infection Until the mid-1970s only about 300 cases had been reported in the literature (12) Beginning in the early 1970s, reports began to appear that associated IUD usage with pelvic actinomycosis Since this time, about 100 such cases have been documented (12, 29) The risk of actinomycosis appears to be generic to all types of IUDs and appears to increase with increasing duration of IUD use (12) In 1976, Gupta and co-workers first documented the recognition of *Actinomyces* in cervical Fast smears stained with Papanicolaou stain (30) This observation has been confirmed by several other investigators (12) Some reports have indicated the presence of *Actinomyces*-like organisms only in cervicovaginal smears In some studies, the prevalence among IUD users was over 40% Gupta noted that in cervicovaginal smears, *Actinomyces* needs to be distinguished from a number of biologically active and inert substances (29) O'Brien and associates have suggested that the prolonged exposure of IUDs to body fluids may result in dissociation of material from the surface of IUDs that is identified as a sulfur granule, which in turn is associated with the presence of *Actinomyces* (52) Gupta concluded that less than 10% of IUD users may have *Actinomyces* organisms detected on Pap smears (29)

In summary, the data suggest that IUD use increases a woman's risk of acquiring PID during the first weeks or months following the IUD insertion This higher risk is probably attributable to the insertion procedure rather than to the IUD use per se The higher risk of PID among IUD users subsequent to the first month of use that has been reported in the literature appears to be based largely on the lower risk of PID among women included in the group(s) against which the risks of PID among IUD users have been compared

OTHER MEDICAL FACTORS

Since about the beginning of the 20th century, the vagina has been known to contain a variety of bacterial species During the past decade, however, with the development of improved culture techniques for anaerobic bacteria, investigators have reported on the extensive aerobic and anaerobic flora of the lower female genital tract in healthy premenopausal women (41) A summary of the more common aerobic and anaerobic species found in studies published since 1970 are listed in Table 2 Although a wide range in the prevalence of the various organisms has been reported, Larsen noted that about ten aerobic and anaerobic species are frequently cultured simultaneously from the vagina and cervix, and that the average number of species per culture is 5 (41) The "normal" flora of the vagina and cervix includes bacteria that may be pathogenic when they gain access to the upper genital tract These bacteria are frequently implicated in serious pelvic infections

In addition to the bacterial species listed in Table 2, *Trichomonas vaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Candida albicans* and other yeasts and *Chlamydia trachomatis* are frequently isolated from the lower genital tracts of symptomatic and asymptomatic women In a group of healthy women attending a health department, Persson and co-workers reported the following rates of positive cultures (57) *M hominis*, 19%, *U urealyticum*, 60%, *C trachomatis*, 5%, yeasts, 22% The precise role of these organisms in the causation of PID is not clear since they exist so frequently in the presence of other pathogens Persson and co-workers suggested that since the above organisms frequently occurred with *N gonorrhoeae*, they might have epidemiologic characteristics similar to that of *N gonorrhoeae* (57)

It is generally thought that lower genital tract infection (LGTI) precedes upper genital tract infection or PID This may or may not always be the case, however, depending on the bacterial etiology of the PID and the mechanisms responsible for the transmission of the bacteria to the upper genital tract Mardh and associates noted that among women with PID not attributable to either a gynecologic procedure or infection in an adjacent organ, a LGTI probably acquired by means of sexual

Aerobes	*Anaerobes*
Lactobacillus	Lactobacillus
Diphtheroids	Propionibacterium
Staphylococcus	Eubacterium
aureus epidermidis	Bifidobacterium
Streptococcus	Clostridium
A,B,C,D + enterococcus	perfringens, other
E coli	Peptococcus
Klebsiella + enterobacter	prevotii, asacharolyticus,
Proteus	magnus, other
Pseudomonas	Peptostreptococcus
	intermedius, productus
	micros, anaerobius
	Gaffkya
	Fusobacterium
	Bacteroides
	fragilis, melaninogenicus,
	bivius other
	Viellonella

Source Larsen B Normal Genital Microflora In Keith L, Berger GS, Edelman DA (eds) Infections in Reproductive Health Lancaster, England, MTP Press (In press)

Table 2 Aerobic and anaerobic bacteria isolated from asymptomatic, premenopausal women

intercourse was nearly always present (46) Obviously, the presence of potentially pathogenic bacteria in the lower genital tract does not necessarily constitute an infection, either in the lower or upper genital tract A major difficulty in assessing the importance of LGTIs in the etiology of PID is that they are not uniformly defined, just as there is no uniformly accepted definition for PID

Even though there is an extensive literature on PID as well as on LGTIs, it is unfortunate that with the exception of *N gonorrhoeae*, studies do not relate the two in a meaningful way Various investigators report that approximately 10% to 20% of women with cervical gonorrhea go on to develop an upper genital tract infection If most LGTIs result from sexually transmitted pathogens, then the risks of these infections also need to be studied in relation to their propensity to cause upper genital tract infections

The interrelationships between the use of various contraceptive methods and the presence of various microorganisms in the vagina, as well as LGTIs, need to be evaluated The literature on this subject is difficult to interpret for a number of reasons Many studies have evaluated women attending venereal disease clinics, some had symptoms of an LGTI, others did not Some women had positive cultures for pathogenic organisms, others did not In some studies, control groups were included that provided a basis for comparison, other studies did not include control or comparison groups In the following paragraphs some of the conflicting results obtained from recent studies are summarized

In a study of women attending a family planning clinic, diaphragm users had a significantly higher isolation rate of fungi from the cervical os or vaginal fornix compared to users of other contraceptive methods (24) In another study by the same group (86), vaginal cultures indicated that the prevalence of *Bacteroides* spp was significantly higher among IUD users, compared to women using other contraceptive methods, IUD users compared to diaphragm users had a higher prevalence of anaerobic cocci, the prevalence of coliforms was higher among diaphragm users, and finally, the prevalence of lactobacilli was increased in the OC group The prevalence rate of each bacterial group was unrelated to age, parity, or social class of the women

According to Osborne and co-workers, the relative risk (about 0 5 to 0 6) of symptomatic vulvovaginitis was similar for IUD, barrier, and OC users, compared to women using other contraceptive methods or no method (53) In contrast, Piot found the risk of "nonspecific" vaginitis to be four times higher for IUD users compared to OC users (59)

Among women attending a sexually transmitted disease clinic, OC users compared to non-users of OCs had higher isolation rates of chlamydia, but lower isolation rates of trichomonads (40) In the same clinic OC users compared to non users had higher yeast infection rates (40) Svensson and associates found the relative risk of cervical infection with chlamydia for OC and IUD users compared to users of other or no contraceptive methods was 2 4 and 0 8, respectively (76) The same study indicated an elevated risk of infection for women who had been pregnant and who had prior PID

In a gonorrhea screening program of women attending a family planning clinic, rates of positive cultures were associated with the contraceptive methods used (3) The prevalence rates were similar for OC users (11 5%) and IUD users (9 9%) and were significantly higher compared to women who were sterilized (3 3%) or women who used barrier contraceptive methods (4 2%)

The question of whether users of different methods of contraception have different susceptibilities to LGTIs has not yet been adequately answered There is some evidence that yeast infections may be more prevalent among OC users (4), and possibly among users of barrier contraceptives Spermicides provide women with some protection against some micro-organisms as a result of their bacteriocidal effects (68, 69)

Prior PID and gonorrhea have both proven to be risk factors for PID Two explanations for this seem plausible First, the tubal epithelium damaged by an infection may lose some of its ability to maintain host resistance and thus become more susceptible to subsequent infections Second, the lifestyles of women who have had a prior episode of PID probably place them at an increased risk of repeated episodes of PID

Prior abortion or pregnancy, as a risk factor for PID, may simply reflect the increased risk of clinical and subclinical infections due to the abortion procedure or delivery and/or sexual exposure without the protective effects of certain contraceptive methods

The well-known clinical observation of the relation between menstruation and the onset of clinical symptomatology of PID has been subject to little study Menstruation usually precedes the onset of PID in about 50% of the cases This observation is consistent with

1 The presence of pathogenic organisms in the cervix and/or vagina,
2 The decline in the relative proportion of aerobic organisms in the cervix in the week immediately preceding menses,
3 The lack of cervical mucus protection during menses,
4 The physiological widening of the cervical and endocervical canal at the time of menses

All these factors and possibly others could explain the clinical picture of post-menstruation PID

CLINICAL CONSIDERATIONS

The classical concept of the pathogenesis of PID (not related to pregnancy, surgery, gynecologic procedures or extensions of a gastrointestinal tract infection) is that lower genital tract infections ascend directly to the upper genital tract along the endocervical mucosa (37, 46) Lymphatic drainage provides an alternative pathway (37, 46) The spread of the infectious process then may pave the way for secondary invasion of the upper genital tract by bacteria normally found in the vagina (46) This concept of PID has been described as "woefully inadequate" (89) A principal omission of this concept is any direct reference to sexual activity preceding the lower genital tract infection

Little doubt exists that genital tract infections can spread through the lymphatics (46) The pelvic lymphatic system is extensive (60), surrounding the entire uterus and providing interconnecting bridges between the cervix and corpus The lymphatic network also spreads laterally to the tubes and ovaries Figure 4 illustrates the lymphatic drainage from the posterior cervix laterally to the pelvic side walls through the broad ligament, inferiorly to the rectum and superiorly along the entire surface of the uterus to the level of the fallopian tubes and ovaries

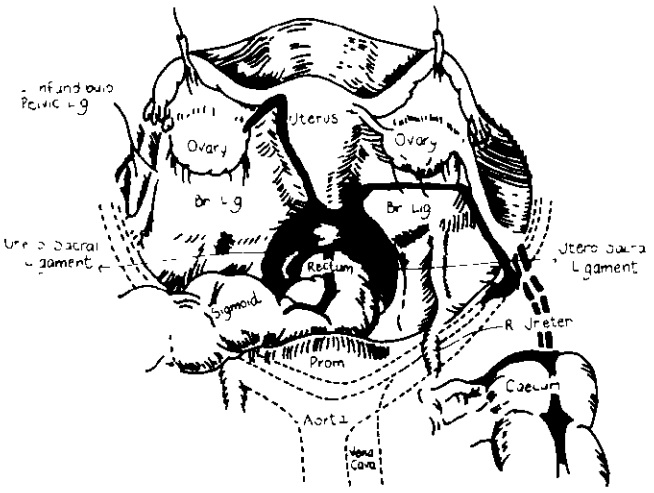

Figure 4. Schematic illustration of lymphatic pathways alongside uterus to tube and ovary (left) and to broad ligament (right). Redrawn with permission after original experiments by Dr. Edward Eichner

Recently, researchers have investigated the mechanisms of the spread of infection from the lower to the upper genital tract in laboratory animals Moller and associates innoculated monkeys with *C trachomatis* or *M hominis* (49) The route of spread of these two bacteria apparently differed somewhat The lymphatics appeared to be more involved in the spread of *M hominis* and less with *C trachomatis* Mardh and associates have reported that in the human, gonococcal infections spread from the lower genital tract canalicularly over the uterine mucosa to the tubes, whereas chlamydial salpingitis derives from infections of the lymphatics and blood vessels of the parametria and broad ligaments where parametritis is first produced (46) This observation is compatible with the recent findings of Gibson and associates that chlamydial infection in the human is more likely to result in parametrial disease including severe adhesions and distal hydrosalpinx formation (22)

The classical theory of the pathogenesis of PID does not consider other possible mechanisms that might promote the ascension of pathogenic bacteria into the upper genital tract These include

1 The male factor, in terms of the bacterial flora of the seminal fluid,
2 Passive transport of bacteria from the lower female genital tract to the upper genital tract,
3 The transport of bacteria into the upper female genital tract by their attachment to spermatozoa or trichomonads

Some aspects of these mechanisms in the pathogenesis of PID are discussed in the following sections

THE MALE FACTOR

Although there have been numerous publications on the bacteriology of the seminal fluid, the role of seminal fluid in the etiology of PID generally has been considered only recently Toth evaluated the bacterial flora of the seminal fluid from groups of fertile men, infertile men without a history of genital tract infection, and men with a history of genital tract infections (81) Isolates of the bacterial flora (aerobic and facultative, as well as anaerobic) were similar for fertile and infertile men without a history of infection A significantly higher number of bacterial isolates were obtained from the semen of males with prior genital infections compared to either fertile or infertile men without a history of infection Four observations of Toth are noteworthy (81) First, most men with bacteriospermia were without symptoms Second, the severity of the bacteriospermia was related to the number of prior sexual partners Third, women married to men with high bacterial counts in their seminal fluid had a greater chance of developing a pelvic infection Fourth, wives of azoospermic men rarely developed PID

The incidence of bacteriospermia in men reflects, among other things, their sexual lifestyles, prior genital tract infections and the treatment of these infections, as well as their use of systemic antibiotics for other conditions During intercourse any bacterial component of the seminal fluid is added to that component otherwise present in the vagina The extent to which the addition of seminal bacteria disrupts the normal bacteriologic state of the

vagina is not known It is likely that the greater the frequency of intercourse with bacteriospermic males, the greater the risk of an LGTI to the female Also, vaginal and cervical bacteria may become adherent to sperm following their ejaculation These events might increase the risk of acquiring an upper genital tract infection Clearly, further research is required to evaluate possible interactions between the bacteriology of the lower genital tract of the female and that of the seminal fluid, and the presence of lower and upper genital tract disease

PASSIVE TRANSPORT OF BACTERIA

Since the early 1930s it has been known that particulate matter can be rapidly transported from the vagina to the uterus and into the fallopian tubes (37) In several animal species, experiments have shown that dead sperm can be transported to the fallopian tubes following artificial insemination (37) The exact mechanisms for the passive transport of sperm and particulate matter from the lower to the upper genital tract are not known, either in humans or in lower animals One possible explanation is that pressure differentials between the peritoneal cavity and the vagina are created by normal respiratory movements of the diaphragm and or uterine contractions Whether the same mechanisms that transport particulate matter and sperm can also transport bacteria to the upper genital tract remains to be studied

BACTERIAL ATTACHMENT

The attachment of bacteria to spermatozoa has been described by several investigators Specifically, the attachment of *N gonorrhoeae*, *U urealyticum*, *E coli* and *C trachomatis* elementary bodies has been demonstrated (20, 23, 25, 91) Figure 5 shows an example of the

Figure 5 Transmission electron microscopy of sperm with *E coli* attached to tail Original magnification 5400x Magnification of inset, 23,000x

in vitro attachment of *E coli* to sperm Probably, there are variations in different strains of bacteria of the same species in their ability to attach to sperm For example, it has been found that certain isolates of *E coli* have only a slight capability to bind to sperm, while others have marked binding abilities (21)

The *in vivo* (20) and *in vitro* (37) studies that show bacterial attachment to sperm have not yet clarified the role of sperm as a mechanism for the transport of bacteria from the seminal fluid or the lower female genital tract to the upper genital tract This can only be established by further studies designed to investigate that specific question Evidence that sperm can provide a mechanism for the transport of bacteria comes from the *in vitro* studies of Toth and co-workers (82) These investigators demonstrated that, in the presence of sperm, aerobic and anaerobic bacteria migrated through a column of ovulatory-phase cervical mucus (82) In the absence of sperm, however, bacteria were not observed to move through the cervical mucus These investigators also noted that no sperm penetration or bacterial migration occurred when cervical mucus was used from either pregnant women or from the luteal phase of the menstrual cycle Toth and co-workers did not determine whether bacterial migration was by the attachment of the bacteria to the sperm, they only observed bacterial migration in the presence of sperm (82) Also, they noted that PID was rare among the wives of azoospermic men Indirectly, this finding implicates spermatozoa in the etiology of PID However, other factors also need to be considered, such as the sexual lifestyles of couples and their exposure to pathogens that might place them a higher risk of acquiring PID

The experiments of Toth and co-workers unify much of the information currently available concerning the pathogenesis of PID (82) In our opinion, the presence of pathogens in the seminal fluid, cervix, or vagina act to increase the likelihood of a woman acquiring PID A high frequency of sexual intercourse may only increase the risk of acquiring PID if there is a significant bacterial contamination of the seminal fluid, sperm, or vagina The lower risk of PID to OC users may be due in part to the absence of ovulatory-phase cervical mucus favorable to sperm penetration and possibly bacterial migration The higher risk of PID to IUD users compared to OC users may reflect the lack of any inhibitory effect of IUDs on ovulatory (estrogenic) cervical mucus

Trichomonads also have been found in the upper genital tract (including the fallopian tubes) of women with serious pelvic infections (45) Trichomonads may thus be causative agents for PID if they act as carriers of other micro-organisms A recent *in vitro* experiment has demonstrated that *E coli* may attach to trichomonads (37)

COMMENT

Improved microbiological methods of the past decade have led to significant progress in our understanding of the bacteriologic etiology of PID In contrast, there has been little significant advance in the understanding of the epidemiology of PID, although some progress has been made toward understanding the mechanism through which women acquire PID The following summarizes our thinking on the development of PID

In general, PID is a sexually derived disease, but those specific aspects of sexual activity that place a woman at an increased risk of acquiring PID have not been adequately studied Micro organisms are transmitted to the lower genital tract during sexual intercourse by direct contact and, or the seminal fluid sperm These micro-organisms are added to those already present in the vagina and cervix

Some LGTIs progress to an upper genital tract infection The risk of this occurring is dependent on numerous factors, including the particular micro-organisms responsible for the LGTI However, the conditions under which a LGTI progresses to PID are not well understood It is questionable whether the risks of LGTIs are different for different methods of contraception, except for a reduced risk to women using spermicides that have bacteriocidal effects (68, 69)

Micro-organisms may be transmitted from the lower genital tract to the upper genital tract through the cervix, lymphatics, by passive transport, by attachment to sperm, in the case of IUD users by migration along the IUD tail string, or by direct extension following any gynecologic procedure in which an instrument is passed through the cervical canal and into the uterus The relative importance of each of these different routes of transmission is unknown and more than one route may be operative concomitantly in the same woman

There are physiologic reasons why the use of oral contraceptives, barrier contraceptives, spermicidal preparations, and contraceptive sterilization protect women against PID For other reasons, women who use natural family planning methods of contraception may also be at a reduced risk of PID On the other hand, the use of IUDs does not protect women from PID Compared to a group of non contraceptors with a similar risk of exposure to STPs, IUD users probably do not have a significantly higher risk of acquiring PID, except for the risk of PID resulting from the insertion procedure

Much of the epidemiologic work to evaluate and identify risk factors associated with PID has been superficial and provides only minimally useful information for either clinical or public health decision makers For the most part epidemiological studies have identified only broad categories of individuals who are at an increased risk of acquiring PID, e g , women who are divorced separated, black, or who have multiple sex partners This knowledge is of limited clinical value since modifying these factors is difficult, if not impossible

With the increasing spread of sexually transmitted diseases worldwide, it would be a mistake to erroneously attribute PID to the use of contraceptive methods that provide no protection against infection to the upper genital tract The widespread use of methods that protect against sexually acquired pelvic infection will have a significant impact on the prevalence of PID, especially if these methods are used by women who are at a high risk of acquiring sexually transmitted pelvic infections

CONCLUSIONS

Future research on the pathogenesis of PID should include the following

1 Evaluation of the interrelationships between bacteriosperma and lower and upper genital tract infections,
2 Investigation of the mechanisms responsible for the presence of infections in the fallopian tubes, adnexa, and ovaries but not in the uterus,
3 Evaluation of the relationships between the use of different contraceptive methods and the risks of lower and upper genital tract infections,
4 Determination of the importance of bacterial transmission by sperm and trichomonads from the lower to the upper genital tract in the etiology of PID,
5 Determination of those specific aspects of sexual activity that are related to increased risks of acquiring lower or upper genital tract infections,
6 Epidemiologic investigations to identify the interrelationships of risk factors for acquiring PID,
7 Evaluation of the physiological and bacteriological events occurring at menstruation that predispose to the onset of PID,
8 Clarification of the difference between so-called primary and secondary bacteriologic pathogens as they relate to the female genital tract,
9 Evaluation of the effect of different contraceptive methods on host resistence as it relates to the onset of PID

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REFERENCES

1 Adler MW Trends for gonorrhea and pelvic inflammatory disease in England and Wales and for gonorrhea in a defined population Am J Obstet Gynecol 138 901 1980

2 Bank HL, Williamson HO Scanning electron microscopy of Dalkon Shield tails Fertil Steril 40 334, 1983

3 Berger GS, Keith L Moss W Prevalence of gonorrhea among women using various methods of contraception Br J Ven Dis 51 307, 1975

4 Bramley M Kinghorn G Do oral contraceptives inhibit Trichomonas vaginalis? Am Ven Dis Assn 6 261, 1979

5 Brown IMcL Cruickshank JG Aetiological factors in pelvic inflammatory disease in urban blacks in Rhodesia SA Med J 50 1342 1976

6 Burkman RT The Women's Health Study Association between intrauterine device and pelvic inflammatory disease Obstet Gynecol 57 269, 1981

7 Cole L Family Health International Research Triangle Park, North Carolina Unpublished data, 1984

8 Cole CH, Lacher TG Bailey JC, Fairclough DL Vaginal chemoprophylaxis in the reduction of reinfection in women with gonorrhea Clinical evaluation of the effectiveness of a vaginal contraceptive Br J Vener Dis 58 314 1980

9 Cunningham FG The etiology and pathogenesis of pelvic inflammatory disease Am Ven Dis Assn 6 221 1979

10 Curran JW Economic consequences of pelvic inflammatory disease in the United States Am J Obstet Gynecol 138 848 1980

11 Edelman DA, Berger GS Keith L Intrauterine Devices and Their Complications Boston, GK Hall & Co , 1979

12 Edelman DA, Berger GS, Keith L The use of IUDs and their relationship to pelvic inflammatory disease a review of epidemiologic and clinical studies Curr Prob Obstet Gynecol V 6 No 3, November, 1982

13 Editorial Pathogenesis of pelvic inflammatory disease Br Med J 16 June, 1979, p 1588

14 Eschenbach DA Buchanan TM Pollock HM Forsyth PS Alexander ER, Lin J S Wang S P, Wentworth BB McCormack WM Holmes KK Polymicrobial etiology of acute pelvic inflammatory disease N Engl J Med 293 166 1975

15 Eschenbach DA Harnish JP, Holmes KK Pathogenesis of acute pelvic inflammatory disease role of contraception and other risk factors Am J Obstet Gynecol 128 838, 1977

16 Eschenbach DA Holmes KK The etiology of acute pelvic inflammatory disease Am Ven Dis Assn 6 224, 1979

17 Farr CE, Findlay RT Salpingitis A detailed analysis based on the study of five hundred forty five cases — January 1914 to December 1927 inclusive Surg Gynecol Obstet 49 647 1928

18 Faulkner WL Ory HW Intrauterine devices and acute pelvic inflammatory disease JAMA 235 1851 1976

19 Flesh G Weiner JM, Corlett RC, Boice C Mishell DR Wolf RM The intrauterine contraceptive device and acute salpingitis Am J Obstet Gynecol 135 402 1979

20 Friberg J, Fullan N Attachment of Escherichia coli to human spermatozoa Am J Obstet Gynecol 146 465, 1983

21 Friberg J, Fullan N, Keith L Berger GS Edelman DA, Bailey R Paper presented at the Annual District VI Meeting of the American College of Obstetricians and Gynecologists Rapid City, South Dakota Aug 18, 1983

22 Gibson M Gump D, Ashikaga T, Hall B Patterns of adnexal inflammatory damage Chlamydia the intrauterine device and pelvic inflammatory disease Fertil Steril 41 47, 1984

23 Gnarpe H, Friberg J T mycoplasmas on spermatozoa and infertility Nature 245 97 1973

24 Goldacre MJ Milne LJR, Watt B, Loudon N Vessey MP Prevalence of yeasts and fungi other than Candida albicans in the vagina of normal young women Br J Obstet Gynaecol 88 596 1981

25 Gomez CI Stenbach WA James AN Criswell BS, Williams RP Attachment of Neisseria gonorrhoeae to human sperm Microscopical study of effect of trypsin and iron Br J Ven Dis 55 245 1979

26 Gordon FB, Quan AL Isolation of the trachoma agent in cell culture Proc Soc Exp Bio Med 118 354 1965

27 Gosden C, Ross A Louden NB Intrauterine deposition of calcium on copper bearing intrauterine contraceptive devices Br Med J 1 202 1977

28 Gump DW, Gibson M, Ashikaga T Evidence of prior pelvic inflammatory disease and its relationship to Chlamydia trachomatis antibody and intrauterine contraceptive device use in infertile women Am J Obstet Gynecol 146 153 1983

29 Gupta PK Intrauterine contraceptive devices Vaginal cytology, pathologic changes and clinical implications Acta Cytol 26 571 1982

30 Gupta PK Hollander DH Frost JK Actinomycetes in cervico vaginal smears An association with IUD usage Acta Cytol 20 295 1976

31 Hager WD Wiesner PJ Selected epidemiologic aspects of acute salpingitis a review J Reprod Med 19 47 1977

32 Haji SN Does sterilization prevent pelvic infection? J Reprod Med 20 289 1978

33 Infertility and Sexually Transmitted Disease A Public Health Challenge Population Reports, Series L, No 4 Baltimore, The Johns Hopkins University, July 1983

34 Jacobensen L Westrom L Objectivized diagnosis of acute pelvic inflammatory disease Am J Obstet Gynecol 105 1088, 1969

35 Jones RB, Ardery BR Hui SL, Cleary RE Correlation between serum antichlamydial antibodies and tubal factor as a cause of infertility Fertil Steril 38 553, 1982

36 Jones OG, Zaidi AA St John RK Frequency and distribution of salpingitis and pelvic inflammatory disease in short stay hospitals in the United States Am J Obstet Gynecol 138 905 1980

37 Keith LG Berger GS, Edelman DA, Newton W Fullan N Bailey R Friberg J On the causation of pelvic inflammatory disease Am J Obstet Gynecol (In press)

38 Keith L, Bailey R Berger GS, Method M Surface changes in IUDs after long term use Paper presented at the Chicago Gynecological Society, March 16 1984

39 Kelaghan J, Rubin GL Ory HW Layde PM Barrier method contraceptives and pelvic inflammatory disease JAMA 248 184, 1982

40 Kinghorn GR, Waugh MA Oral contraceptive use and prevalence of infection with Chlamydia trachomatis in women Br J Ven Dis 57 187 1981

41 Larsen B Normal genital microflora In Keith L, Berger GS, Edelman DA (eds) Infections In Reproductive Health Lancaster, England MTP Press (In press)

42 Ledger W New concepts in the management of pelvic inflammatory disease J Reprod Med 28 697, 1983 (supplement)

43 Lee NC, Rubin GL, Ory HW Burkman RT Type of intrauterine device and the risk of pelvic inflammatory disease Obstet Gynecol 62 1, 1983

44 Luukkainen T Nielsen N C, Nygren K C Pyorala T Nulliparous women, IUD and pelvic infection Ann Clin Res 11 121, 1979

45 Mardh P A Westrom L Tubal and cervical cultures in acute salpingitis with special reference to Mycoplasma hominis and T strain mycoplasmas Br J Ven Dis 46 179 1970

46 Mardh P A Westrom L, Ripa KT, Moller BR Pelvic inflammatory disease clinical etiologic and pathophysiologic studies In Holmes KK, Mardh P A (eds) International Perspectives on Neglected Sexually Transmitted Diseases Washington, Hemisphere Publishing Co , 1983

47 Marrie TJ, Costerton JW A scanning transmission electron microscopic study of the surfaces of intrauterine contraceptive devices Am J Obstet Gynecol 146 384, 1983

48 Mishell DR Bell JH Good RG, Moyer DL The intrauterine device a bacteriologic study of the endometrial cavity Am J Obstet Gynecol 96 119, 1966

49 Moller BR, Freundt EA, Mardh P A Experimental pelvic inflammatory disease provoked by Chlamydia trachomatis and Mycoplasma hominis in grivet monkeys Am J Obstet Gynecol 138 990, 1980

50 Newton W Keith L The role of sexual behavior in the development of PID J Reprod Med (In press)

51 Noonan A Adams JB Gonorrhea screening in an urban hospital family planning program Am J Pub Hlth 64 700 1974

52 O'Brien PK, Roth Moyo LA, Davis BA Pseudo sulfur granules associated with intrauterine contraceptive devices Am J Clin Path 75 822, 1981

53 Osborne NG, Grubin L, Pratson L Vaginitis in sexually active women Relationship to nine sexually transmitted organisms Am J Obstet Gynecol 142 962, 1982

54 Osser S Gullberg B, Liedholm P, Sjoberg N-O Risk of pelvic inflammatory disease among intrauterine device users irrespective of previous pregnancy Lancet 2 386, 1980

55 Osser S, Persson K Postabortal pelvic infection associated with C trachomatis and the influence of humoral immunity Paper presented at the 5th International Meeting of the International Society for Sexually Transmitted Diseases Seattle, Washington, 1 3, August 1983

56 Paavonen J, Vesterinen E Intrauterine contraceptive device use in patients with acute salpingitis Contraception 22 107 1980

57 Persson K Persson K Hansson H, Bjerre B, Svanberg L, Johnsson T, Forsgren A Prevalence of nine different micro organisms in the female genital tract Br J Ven Dis 55 429, 1979

58 Phaosavasdi S, Vivanichakul B, Rienprayura D, Chutivongse S, Virutamasen P, Snidvongs W Pelvic inflammatory disease in contraceptive acceptors disclosed at transvaginal tubal sterilization In Hefnawi F, Siegel SJ (eds) Analysis of Intrauterine Devices New York, American Elsevier Publishing Co , 1975

59 Piot P Paper presented at the Fifth International Meeting of the International Society for Sexually Transmitted Diseases Seattle, Washington, 1 3 August, 1983

60 Plentl AA, Friedman EA Lymphatic System of the Female Genitalia Philadelphia, WB Saunders Co , 1971

61 Potterat JJ, Phillips L, Rothenberg RB, Darrow WW Gonococcal pelvic inflammatory disease case-finding observations Am J Obstet Gynecol 138 1011, 1980

62 Purrier BGA, Sparks RA, Watt PJ, Elstein M *In vitro* study of the possible role of the intrauterine contraceptive device tail in ascending infection of the genital tract Br J Obstet Gynecol 86 374, 1979

63 Qvigstad E, Skaug K, Jerve F, Fylling P, Ulstrup JC Pelvic inflammatory disease associated with Chlamydia trachomatis infection after therapeutic abortion Br J Ven Dis 59 189, 1983

64 Rendtorff RC, Packer H, Glassco S, Levy J The impact of urban community hospital surveillance for gonorrhoea on the infection rate and complications in the female A progress report Br J Ven Dis 53 364, 1977

65 Rosenfeld DL, Seidman SM, Bronson RA, Scholl GM Unsuspected chronic pelvic inflammatory disease in the infertile female Fertil Steril 39 44, 1983

66 Rubin GL, Ory HW, Layde PM Oral contraceptives and pelvic inflammatory disease Am J Obstet Gynecol 144 630, 1982

67 Siboulet A Maladies sexuelles transmissibles interet des traitments prophylactiques Prophylaxie Sanitaire Morale 44 155, 1972

68 Singh B, Cutler JC, Utidjian HMD *In vivo* effect of contraceptives and non contraceptive preparations on Candida albicans and Trichomonas vaginalis Contraception 5 402, 1972

69 Singh B, Cutler JC, Utidjian HMD Studies on development of a vaginal preparation providing both prophylaxis against venereal disease, other genital infections and contraception II Effect *in vivo* of vaginal contraceptive and non-contraceptive preparations on Treponema pallidum and Neisseria gonorrhoeae Br J Ven Dis 48 57, 1972

70 Sparks RA, Purrier BGA, Watt PJ, Elstein M The bacteriology of the cervix and uterus Br J Obstet Gynaecol 84 701, 1977

71 Sparks RA, Purrier BGA, Watt PJ, Elstein M Bacteriology of the uterus in relation to IUDs and their appendages In Hafez ESE, van Os WAA (eds) IUD Pathology and Management Lancaster, England, MTP Press Ltd , 1980

72 Sparks RA, Purrier BGA, Watt PJ, Elstein M Bacteriological colonisation of uterine cavity role of tailed intrauterine contraceptive device Br Med J 282 1189, 1981

73 St John RK, Blount J, Jones O Pelvic inflammatory disease in the United States Incidence and trends in private practice Am Ven Dis Assn 8 56 1981

74 St John RK, Jones OG, Blount JH, Zaidi AA Pelvic inflammatory disease in the United States Epidemiology and trends among hospitalized women Am Ven Dis Assn 8 62, 1981

75 Svensson L, Mardh P A, Westrom L Infertility after acute salpingitis with special reference to Chlamydia trachomatis Fertil Steril 40 322, 1983

76 Svensson L, Westrom L, March P A Chlamydia rachomatis in women attending a gynaecological outpatient clinic with lower genital tract infection Br J Ven Dis 57 259, 1981

77 Sweet RL Chlamydial salpingitis and infertility Fertil Steril 38 530, 1982

78 Targum SD, Wright NH Association of the intrauterine device and pelvic inflammatory disease a retrospective pilot study Am J Epidemiol 100 262, 1974

79 Tatum HJ, Schmidt FH, Phillips D, McCarty M, O'Leary WM The Dalkon Shield controversy structural and bacteriologic studies of IUD tails JAMA 231 711, 1975

80 Thaler I, Paldi E, Steiner D Intrauterine devices and pelvic inflammatory disease Int J Fertil 23 69, 1978

81 Toth A Alternative causes of pelvic inflammatory disease J Reprod Med 28 699, 1983 (Supplement)

82 Toth A O'Leary WM, Ledger W Evidence for microbial transfer by spermatozoa Obstet Gynecol 59 556, 1982

83 Treharne JD, Ripa KT, Mardh P A, Svensson L, Westrom L, Darougar S Antibodies to Chlamydia trachomatis in acute salpingitis Br J Vener Dis 55 26, 1979

84 Valenti A Paper presented at 6th World Congress of Sexology New York, New York, 1983

85 Wang S P, Grayston JT Immunologic relationship between genital TRIC, lymphogranuloma venereum, and related organisms in a new microtiter indirect immunofluorescence test Am J Ophthalmol 70 367, 1970

86 Watt B, Goldacre MJ, Loudon N, Annat DJ, Harris RI, Vessey MP Prevalence of bacteria in the vagina of normal young women Br J Obstet Gynaecol 88 588, 1981

87 Westrom L Effect of acute pelvic inflammatory disease on fertility Am J Obstet Gynecol 121 707, 1975

88 Westrom L Incidence, prevalence, and trends of acute pelvic inflammatory disease and its consequences in industrialized countries Am J Obstet Gynecol 138 880, 1980

89 Westrom L Clinical manifestations and diagnosis of pelvic inflammatory disease J Reprod Med 28 703 1983 (Supplement)

90 Westrom L Bengtsson LP Mardh P A The risk of pelvic inflammatory disease in women using intrauterine contraceptive devices as compared to non users Lancet 2 221 1976

91 Wolner Hanssen P Mardh P A *In vitro* tests of the adherence of Chlamydia trachomatis elementary bodies to human spermatozoa Paper presented at Fifth International Meeting of The Society For Sexually Transmitted Diseases Seattle Washington 1 3 August 1983

92 Wolner Hanssen P Maren P A Svensson L Westrom L Laparoscopy in women with chlamydial infection and pelvic pain a comparison of patients with and without salpingitis Obstet Gynecol 61 229 1983

93 Wright NH Laemmle P Acute pelvic inflammatory disease in an indigent population Am J Obstet Gynecol 101 979 1968

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RESEARCH FRONTIERS IN FERTILITY REGULATION

LONG-ACTING INJECTABLE NORETHISTERONE CONTRACEPTIVE SYSTEM: REVIEW OF CLINICAL STUDIES

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Synthetic steroid hormones are generally accepted as a safe and efficacious means of fertility control However, the formulations currently available fall short of ideal with regard to ease of administration and lack of side effects There are questions regarding safety following long-term use of formulations containing estrogen, and efficacy may be compromised due to failure of user compliance

To overcome these disadvantages, delivery systems are being developed to improve the benefit-to-risk ratio by providing continuous administration of low levels of contraceptive steroids for up to several years following a single administration

The rationale and the advantages and disadvantages of long-acting controlled release steroidal contraceptive systems have been previously reviewed (1, 6, 11, 12, 14) The most advanced systems include depot steroid formulations, biodegradable and non-biodegradable implants, medicated intrauterine and intravaginal devices, and injectable small particulate systems

This report provides an up-to-date summary of the research supported by the Program for Applied Research on Fertility Regulation (PARFR) to develop a long-acting, injectable, biodegradable microsphere delivery system for the contraceptive steroid, norethisterone (NET) We will trace the development of the microsphere delivery system from the initial prototype formulations to the present, describing the capabilities of the delivery system for controlling both the rate and duration of NET release, in light of the results obtained from the clinical testing program, including both pharmacokinetic and pharmacodynamic studies

NET MICROSPHERE DELIVERY SYSTEM

The NET microsphere delivery system consists of microspheres composed of a biodegradable polymer and NET The microspheres are prepared using a solvent evaporation process that has undergone substantial modification and improvement during the course of this program Microspheres with diameters ranging from 10 μm to 240 μm have been used in studies of rate and duration of NET release, injection efficiency, and biodegradation.

Initially, formulations capable of releasing NET at a near zero order rate were developed and tested in baboons This prototype system was evaluated in clinical trials, and the results gave rise to an improved second generation formulation, which has been tested in multicenter clinical trials Based on the results of these studies, further improvements in the delivery system have been made, and the final version is currently being evaluated in a Phase II clinical study

The microsphere formulation has been varied during the development phase of the research to optimize the NET release rates, biodegradation kinetics, and ease of administration Details regarding the manufacture of the NET microspheres and the improvements made in the microencapsulation process have been published (7, 9, 10) and will not be reviewed here, except to emphasize where appropriate the biological considerations that necessitated improvements in the delivery system design

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PROTOTYPE SYSTEM

The polymer first evaluated as excipient for making NET microspheres was dl-polylactic acid (PLA) PLA had previously been proven safe for human use in absorbable suture material, and long-term toxicity had been thoroughly evaluated Early results showed that microspheres made from PLA using a solvent evaporation process had no adverse effects in rats, thus confirming the histocompatibility of the polymer and removal of potentially irritating solvents and dispersing agents used in the manufacturing process

Prior to animal testing, microspheres made of PLA containing NET were evaluated *in vitro*, in an attempt to define parameters that might be predictive of their performance *in vivo* Scanning electron microscopy was used to monitor the spherical integrity and surface characteristics of the microspheres The rate of NET release from each batch of microspheres was tested *in vitro* These quality control procedures have been repeated with each formulation and batch of the microspheres that has been subjected to *in vivo* evaluation Additionally, following the *in vitro* quality control analysis, each new formulation has been tested in baboons to evaluate safety and pharmacokinetics Figure 1 shows the *in vitro* NET release profile from a representative microsphere formulation and Figure 2 shows a typical scanning electron micrograph of the microspheres

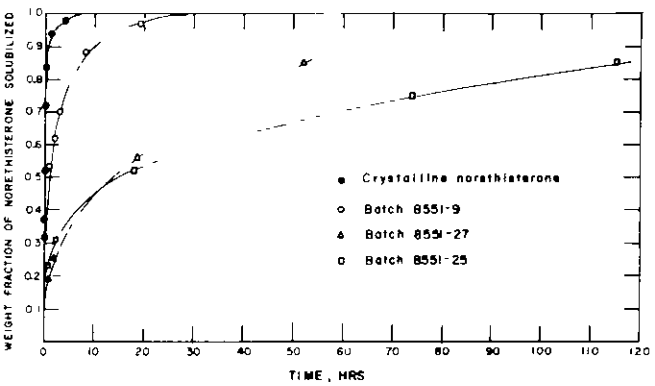

Figure 1 Norethisterone release *in vitro* from microspheres of varying diameter ranges (Source Beck LR, Tice TR Poly (lactic acid) and poly (lactic acid co-glycolic acid) contraceptive delivery systems. In Mishell DR Jr (ed) Long-Acting Steroid Contraception, pp175-199 New York, Raven Press, 1983

Pharmacokinetic studies in baboons were used to demonstrate the dose response capabilities of the prototype system (2, 3) Microspheres 10 μm to 240 μm in diameter containing 25% by weight NET were injected into baboons and serum estradiol (E2), progesterone (P4), and NET concentations were determined by radioimmunoassay (RIA) The slopes of the NET release curves

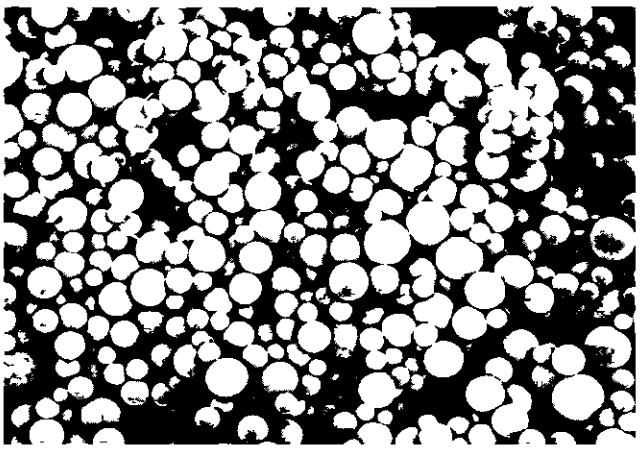

Figure 2 Scanning electron micrograph of norethisterone microspheres (x 450)

using different doses of the same batch were not significantly different from each other, and there was no difference in the duration of NET release The differentiating feature among the different doses was the higher serum NET levels achieved with the higher doses Two of the doses tested, 75 mg and 50 mg NET, were sufficient to inhibit ovarian cyclicity and ovulation in the baboons over a 6-month interval Parallel studies in rats demonstrated that release was occurring primarily by diffusion of the hormone from the microspheres On the basis of these preclinical animal studies, this prototype formulation was judged safe for use in Phase I clinical trials

PHASE I CLINICAL TRIALS

Sixty-three women at three centers were treated with the prototype NET-PLA microsphere formulation (*Table 1*) In the first study (*Group A, Table 1*) 24 women were treated with 50 mg to 100 mg of microencapsulated NET using PLA microspheres that ranged in diameter from 60 μm to 240 μm (8) Poor injection efficiencies experienced by untrained practitioners with saline-suspended microspheres resulted in actual doses ranging from 7 mg to 95 mg of NET, expanding the dose-range of the study Doses of microencapsulated NET ranging from 0 132 mg to 0 267 mg per kilogram body weight had no effect on ovarian function over the 6-month treatment interval, whereas higher doses caused complete or partial suppression of ovarian function for varying periods after treatment

Figure 3 shows the mean serum levels of NET for seven subjects who received doses of microencapsulated NET ranging from 1 22 mg to 2 30 mg NET per kilogram body weight Ovulation was inhibited for 3 to 6 months in this group of subjects

Group	Months*	No	Dose (mg)	P C†	% Load	μm Diameter
A	6	14	100	P	25	60-240
		10	50	P	25	60 240
B	6	19	200	P	24	90 212
C	6	20	150	P	24	90 212
D	3	5	75	C	20 21	63 90
		5	100	C	20-21	
E	3	5	75	C	20-21	63 90
		3	100	C	20 21	
F	3	5	75	C	20 21	63 90
		5	100	C	20 21	
G	3	5	75	C	20 21	63 90
		5	100	C	20 21	
H	3	5	75	C	20 21	90 106
		5	100	C	20 21	
I	6	5	75	C	20 21	125 212
		5	150	C	20 21	
J	6	5	75	C	20 21	125 212
		5	150	C	20 21	
K	3	14	75	C	23 6	45 90

* Expected release duration
† P = PLA, C = PLGA (copolymer) formulation

Table 1 Clinical Trials with Norethisterone Microspheres

Menstrual abnormalities are the most common side effect of long-acting steroidal contraceptives Although this sample size was too small to establish a meaningful correlation between microsphere dose and the amount of menstrual bleeding, the higher doses caused a reduction in the quantity of blood lost in each episode and an increase in the interval between episodes in some subjects

This preliminary trial reinforced the results of the animal pharmacodynamic and pharmacokinetic studies and demonstrated that higher doses would be necessary to achieve ovulation suppression in women for a 6-month period using this formulation

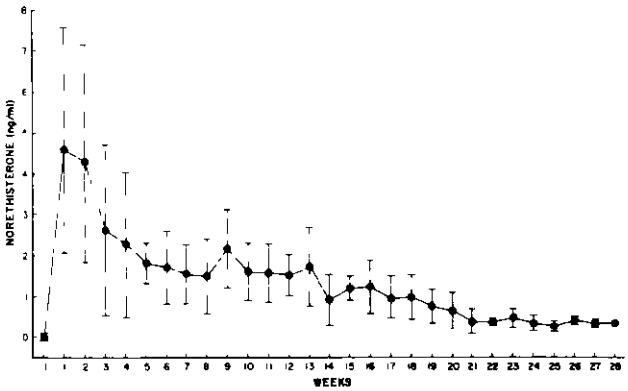

Figure 3 Mean serum levels of immune-reactive NET in 7 human subjects following IM injection of 1 50 ± 0 37 mg NET/kg body weight (Source Beck LR, Ramos RA, Flowers CE Jr, Lopez GZ, Lewis DH, Cowsar DR Clinical evaluation of injectable biodegradable contraceptive system Am J Obstet Gynecol 140 799, 1981

The next studies, therefore, used theoretical doses of 150 mg and 200 mg NET in 25 6% loaded microspheres with diameters ranging from 90 μm to 212 μm (*Groups B* and *C, Table 1*) This size range was prepared as a composite by mixing equal masses of the following specific size fractions 90 to 106, 106 to 125, 125 to 150, 150 to 180, and 180 to 212 μm This ensured even distribution of microsphere diameters over the final size range The vehicle was changed in an attempt to improve injection efficiency to 2% carboxymethylcellulose, 1% tween-20, and 93% water

Twenty patients were treated with microspheres containing 150 mg of NET (*Group C, Table 1*) The actual doses ranged from 22 mg to 144 mg NET (104 ± 37) Twelve subjects received doses greater than 138 mg The serum NET profile was characterized by an initial slight burst (less than 4 0 ng NET/ml) followed by a gradual decline (*Figure 4*) The duration of NET release was approximately 6 months The serum NET profiles in the subjects in this study and those in the former group who received a wider size range of microspheres (60-240 μm) differed in that the smaller microspheres produced higher serum NET levels during the first week and lower levels during the last month post-treatment These differences can be explained on the basis that the smaller microspheres used in the first study had faster release rates

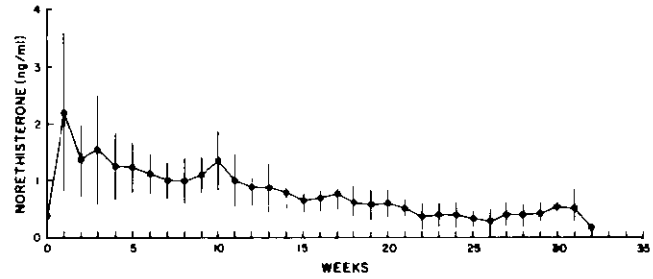

Figure 4 Mean serum levels of immune-reactive NET in 13 women following IM injection of 2 45 ± 0.44 mg NET/kg body weight (Source Beck LR, Flowers CE Jr, Pope VZ, Tice TR, Dunn RL, Gilley RM· Poly (d,l-lactide-co-glycolide)/norethisterone microcapsules clinical study In Zatuchni GI, Goldsmith A, Shelton JD, Sciarra JJ (eds): Long-Acting Contraceptive Delivery Systems, pp 407-417. Philadelphia, Harper & Row, 1984.

Nineteen patients were treated at a second center (13) with the same formulation and size range using an even higher dose, 200 mg NET (*Group B, Table 1*) Injection efficiencies were excellent in this study, averaging 92 ± 3% Ovulation was inhibited for at least 24 weeks in 16 of 18 subjects The serum NET levels were higher and serum NET curves parallel to those in subjects who

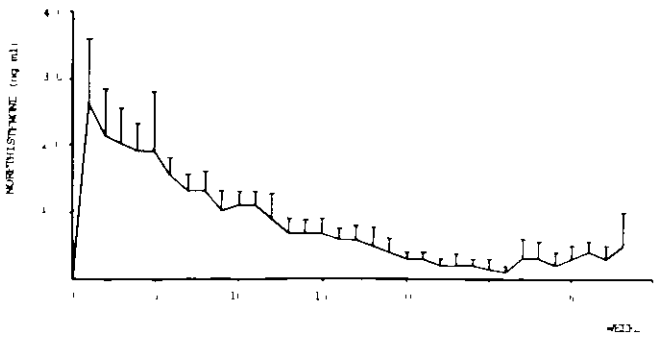

Figure 5 Mean serum levels of immune-reactive NET in women following IM injection of PLA microspheres containing 200 mg NET (Source: Rivera R, Flores C, Aldaba S, Hernandez A Norethisterone Microspheres 6-month System Clinical Results In Zatuchni GI, Goldsmith A, Shelton JD, Sciarra JJ (eds) Long-Acting Contraceptive Delivery Systems, pp 418-424 Philadelphia, Harper & Row, 1984

received the lower dose (*Figure* 5) One subject resumed ovulatory cycles 16 weeks post-treatment, after NET levels had dropped below 2 ng ml Two subjects had single progesterone values suggestive of ovulation during the first 7 weeks post-treatment These elevated progesterone values, however, were found to be inconsistent with the other weekly samples, preventing definitive diagnosis of ovulation In most subjects, serum NET levels fell below 1 0 ng ml between the 10th and 22nd weeks post-treatment, and were below 0 5 ng ml usually by week 25

Bleeding patterns were altered in these subjects, with a range of 1 to 10 bleeding episodes per subject over the 239-day reference period Total spotting days per subject ranged from 1 to 85, and total bleeding days from 3 to 58 Bleeding episodes were an average of 6 6 days, ranging from 3 to 16 days in length The longest nonbleeding interval was 166 days In general, the average number of bleeding and spotting days per 30-day reference period decreased with increasing time from injection, with a maximum of 9 68 in the second 30 days to a low of 3 21 per subject 241 to 270 days post-treatment

We concluded from these dose response clinical studies that PLA microspheres are capable of delivering NET continuously for approximately 6 months, and that the duration of ovarian suppression is dose-dependent within a given microsphere formulation and size limit The shape of the particular release curve within a given formulation is dependent upon microsphere size distribution and dose of NET contained in the microspheres

Comparison of the serum NET profiles attained using different doses at three centers shows the influence of microsphere size on NET release rates The size distribution of microspheres in the first study was 60 μm to 240 μm, whereas the size distribution used at the other two centers was 90 μm to 212 μm Smaller microspheres provided more rapid release and higher initial serum NET levels, with a resulting steeper slope of the curve and shorter duration of action A narrower size range results in flatter release curves more nearly approaching zero order release (*Figures* 4 and 5) These curves, however, are characterized by a gradual decline in serum NET levels over the treatment period

The early animal and human studies demonstrated the good potential of the microsphere system, however, it was determined from biodegradation studies that up to 12 months were required for the PLA microspheres to completely biodegrade Altering the molecular weight of the polymer and reducing the diameter of the microspheres did not significantly change the biodegradation interval

We became concerned, further, that repeated injections of PLA microspheres at 6-month intervals might allow an undesirable build-up of PLA in the muscle tissues at the injection site Additionally, small amounts of hormone associated with this residual polymer might be released at a later time We concluded that a PLA excipient that has a 12-month biodegradation time is not optimal for systems designed to provide steroid treatment for 6 months or less We therefore centered our research efforts on developing a polymer formulation that provides better synchronization between duration of NET release and biodegradation of the polymer

SECOND GENERATION NET MICROSPHERE FORMULATION

To achieve faster biodegradation, polyglycolic acid (PGA) was incorporated into the polymer We found that when molar ratios of lactide glycolide were decreased in stepwise fashion, biodegradation time was reduced proportionately (7, 9) NET microspheres made with the more rapidly biodegrading copolymers were evaluated in baboons to determine the pharmacokinetics of specific copolymer formulations (7) From these studies we selected a copolymer with a lactide glycolide molar ratio (85 15) that biodegrades in 6 months

Pharmacokinetic studies in baboons using NET microspheres made from the copolymer poly lactide-co-glycolide (PLGA) resulted in release profiles uniquely characteristic of this formulation The copolymer formulation produces a biphasic release curve (*Figure* 6) A diffusional release pattern characterized by decreasing NET levels with time, similar to those previously described for the PLA systems, occurs during the first 10 weeks post-treatment The duration of this diffusional release phase is variable, depending on formulation A secondary rise in the serum NET level occurs later during the

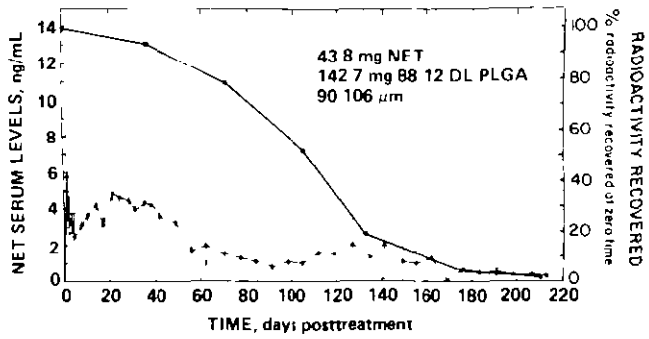

Figure 6. Biodegradation curve of copolymer superimposed on mean serum levels of NET in baboons treated with the copolymer formulation (Source: Beck LR, Pope VZ, Flowers CE, Cowsar DR, Tice TR, Lewis DH, Dunn RL, Moore AB, Gilley RM. Poly (d,l-lactide-co-glycolide)/norethisterone microcapsules an injectable biodegradable contraceptive. Biol Reprod 28 186, 1983)

treatment interval This secondary phase corresponds with the period of rapid polymer biodegradation (*Figure 6*)

We concluded, from the pharmacokinetic studies in baboons, that NET release from copolymer-fabricated microspheres occurs via both diffusion and bioerosion We anticipated that this dual release mechanism could be exploited to develop formulations having more discrete release intervals, uniform steroid levels, and less "tailing off" of NET release than occur with the PLA formulation

Following further baboon studies, a master batch of microspheres comprising 22% NET in a copolymer excipient containing 86% to 88% PLA and 14% to 12% PGA respectively was made for use in clinical trials Five centers participated in a study to evaluate this PLGA NET microsphere formulation with appropriate characteristics to provide 3 months of continuous NET release Two microsphere diameter ranges, 63 μm to 90 μm and 90 μm to 106 μm, were evaluated at doses of either 75 mg or 100 mg NET

The smaller microspheres (63-90 μm) released NET for approximately 20 weeks in ten women treated with two different doses, 75 mg and 100 mg (*Group D, Table 1*) The release profiles in both groups of women were biphasic (*Figure 7*) After an initial post-injection peak, serum NET levels gradually declined until about 8 weeks after treatment, when a secondary rise and fall in NET levels occurred between 8 and 20 weeks post-treatment Ovarian function was suppressed for 3 to 4 months in all subjects who received the full dose of either 75 mg or 100 mg of NET (4, 5)

The serum NET curves of the subjects receiving the two doses were parallel during the initial diffusional release phase, with the 100 mg dose producing proportionately higher serum NET levels During the biodegradation

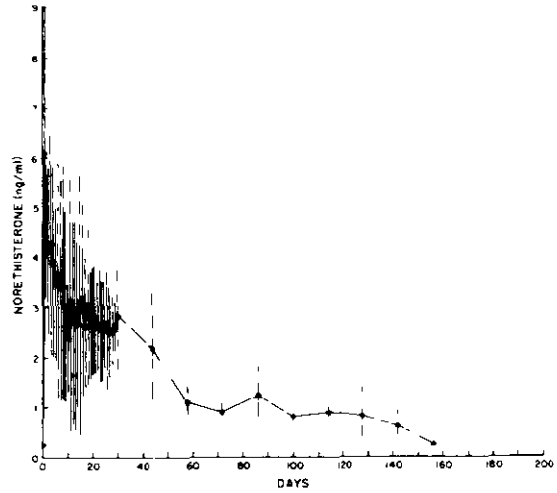

Figure 7 Serum levels of immune-reactive NET in Group D women following IM injection with 75 or 100 mg NET in PLGA microspheres. (Source Beck LR, Flowers CE Jr, Pope VZ, Tice TR, Dunn RL, Gilley RM Poly (d,l-lactide-co-glycolide)/norethisterone microcapsules clinical study In Zatuchni GI, Goldsmith A, Shelton JD, Sciarra JJ (eds) Long-Acting Contraceptive Delivery Systems, pp 407-417 Philadelphia, Harper & Row, 1983

phase, the curves remained parallel, although the serum NET levels in the subjects receiving the higher dose were only slightly greater (*Figure 7*)

There were no significant dose-related effects on bleeding patterns in these two groups of women, although the low dose group appeared to have less spotting during the 120- to 140-day interval post-treatment

Two post-treatment uterine biopsy specimens were obtained from each of these subjects and were evaluated for progestin effects by light microscopy Small glands, slightly hypertrophic stromal cells, and limited predecidual reaction about the arterioles, characteristic progestin responses were evident in these biopsies Glands with classical secretory exhaustion, a characteristic response to oral contraceptives, were uncommon Breakthrough bleeding was attributed to disruption of the integrity of the surface epithelium, which allowed blood to escape into the uterine cavity Tissue degeneration with leukocytic infiltration was also present These endometrial changes are typical responses to norethisterone

The injection efficiencies in this study averaged 90%, reflecting the benefit of careful instruction of the practitioners by technicians experienced in the procedure for injecting the microsphere suspensions, and use of the improved injection vehicle

Five subjects received the 75 mg NET dose and three received 100 mg NET at a second center participating in

the 3-month Phase I clinical trial of the copolymer formulation (*Group E, Table 1*) Three subjects were dropped from this study because of difficulty with the injection

The 100 mg group had only slightly higher serum NET levels than the 75 mg group (*Figure 8*) No ovulatory progesterone levels (≥ 3 ng/ml) were reported in the 74 to 108 days post-treatment during which blood samples were obtained

A third center (*Group H, Table 1*) tested equivalent doses, but used microspheres ranging in diameter from 90 μm to 106 μm This center had participated in one of the earlier studies, and the experience that had been acquired with the injection protocol was reflected by injection efficiencies ranging from 90% to 99% in all subjects Ovarian cyclicity was suppressed for an average of 22 weeks following treatment, with the earliest occurrence of ovulatory progesterone levels at 14 weeks post-treatment, and the longest suppression exceeding 26 weeks

Menstrual alterations were the only side effects reported in this study Six subjects experienced non-bleeding intervals of greater than 90 days' duration, four of these had over 150 days between bleeding episodes Three subjects had over 50 days of spotting and/or bleeding in the 189 to 210 day reference period, with a mean of 36 for all subjects Although bleeding episodes were 5 days or less in half the subjects, four subjects had bleeding episodes exceeding 10 days in length There appeared to be no relationship between dose and bleeding patterns in these two groups

Serum NET levels were consistently higher in the subjects who received 100 mg NET (*Figure 9*) during both release phases, similar to release profiles in subjects at the first two centers Increasing the diameter of the microspheres

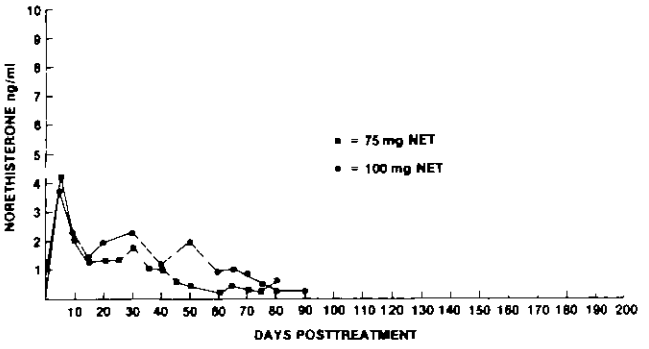

Figure 8 Serum levels of immune-reactive NET in Group E women following IM injection with 75 or 100 mg NET in PLGA microspheres

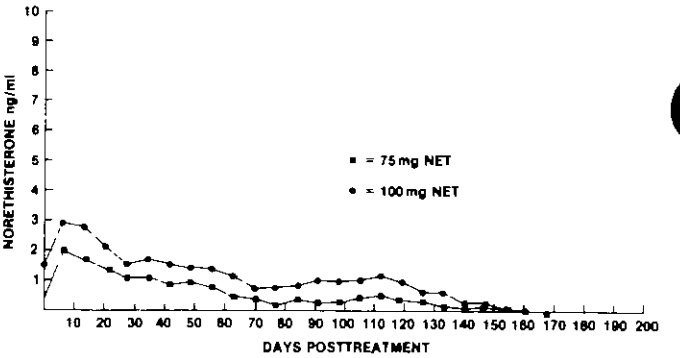

Figure 9 Serum levels of immune-reactive NET in Group H women following IM injection with 75 or 100 mg NET in PLGA microspheres

did not significantly affect the release profiles, when compared to the subjects who received the 63 μm to 90 μm microspheres

Composite NET release curves for each dose from the subjects at these three centers (75 mg n = 14, 100 mg n = 13) are similar to those found at the individual centers, with the increased number of subjects appearing to minimize peaks and valleys (*Figure 10*) These release profiles demonstrate the capability of the copolymer system reliably to provide continuous controlled release of contraceptive quantities of NET This particular formulation consistently released NET for approximately 4 months in women subjects

The fourth center (*Group F, Table 1*) experienced poor injection efficiencies resulting from the use of needles having improper bore size for microsphere injection This

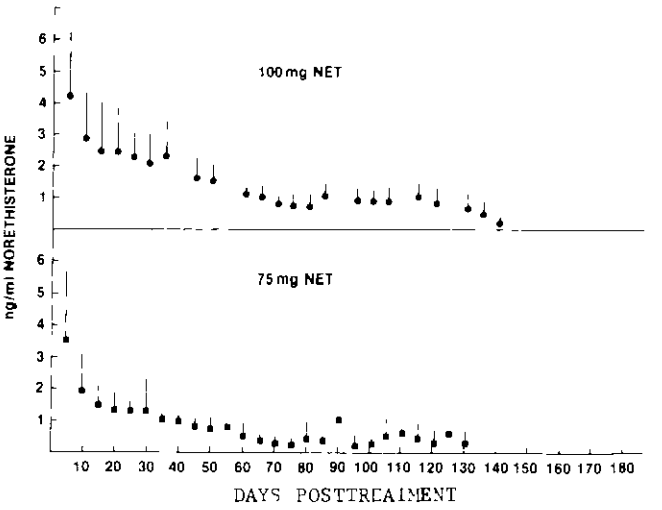

Figure 10 Serum levels of immune-reactive NET in Group D, E and H women following IM injection with 75 or 100 mg NET in PLGA microspheres

center tested 63 μm to 90 μm diameter microspheres at 75 mg and 100 mg doses Seven injections were attempted using needles with a bore size smaller than 18-gauge Three subjects had normal ovulatory cycles following treatment, and two had prolonged first cycles before resuming normal cyclicity One subject had ovulatory progesterone levels throughout, but lower than normal estradiol peaks and scanty blood flow, and another resumed ovulation the second month post treatment Injection efficiencies ranged from 5 7% to 16 5% in these subjects We concluded from this that minimum needle bore size needed for this formulation was 18-gauge The remaining three subjects were treated using an 18-gauge needle for injection, and ovulatory progesterone levels were not encountered until 93, 109 and 112 days post-treatment These three subjects, therefore, had treatment effects similar to those seen following successful injections at the other three centers

Problems with injection also occurred at the fifth center (*Group G, Table 1*) The practitioners subjectively estimated that injection efficiency, based on material remaining in the syringes, was between 90% and 100%, but actual doses, determined by measuring residual NET in the syringes, ranged from 31 8% to 95 7% (n = 8) of intended doses Because of the multiple doses resulting from variable injection efficiencies, no attempt was made to group these 10 subjects according to dose Nine of 10 subjects had ovulatory serum progesterone levels within 102 ± 5 days post-treatment, regardless of dose

Serum HDL and cholesterol were quantitated before and at the end of the treatment interval in the subjects at four centers (n = 40) No significant effects on serum lipid levels were observed following continuous administration of NET for 3 months

A second clinical study at two centers (*Groups I and J, Table 1*) was undertaken to further evaluate the effect of microsphere size on pharmacokinetics For this study, the size range of the microspheres was increased to 125 μm to 212 μm Two doses were evaluated in 10 subjects at each center Five subjects at each center received a 75 mg NET dose and five received a 150 mg dose Nine subjects in Group I were anovulatory for the first 5 months post-treatment Three subjects who received the 75 mg dose and one who received the 150 mg dose ovulated between the 5th and 6th month post-treatment

Serum NET profiles in these subjects were biphasic (*Figure 11*) Increasing the diameter range from 63 μm to 90 μm up to 125 μm to 212 μm shifted the biodegradation phase to the right, effectively extending the duration of action Serum NET levels gradually declined during the first 10 weeks post-treatment, with the biodegradation phase providing increased NET levels during the 13th through 20th weeks post-treatment

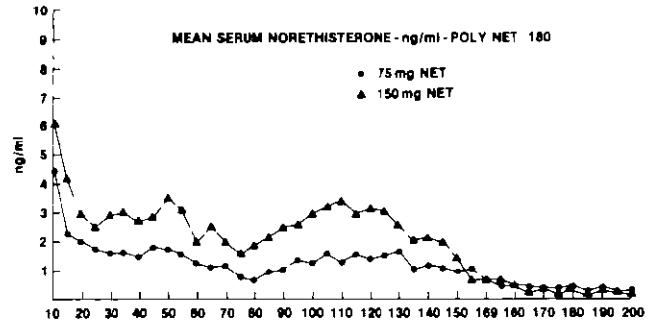

Figure 11 Serum levels of immune-reactive NET in Group I women following IM injection with 75 or 150 mg NET in PLGA microspheres 125-212 μm in diameter

The total spotting and bleeding days per 30-day period post-treatment ranged from 0 to 29 per patient Fifteen of 71 periods were amenorrheic, and 19 30-day intervals contained greater than 7 days of spotting and bleeding Eleven 30-day periods with greater than 7 bleeding or spotting days occurred in the first 3 months post treatment, three of which were single periods in the 2nd or 3rd months in three patients receiving the 150 mg dose Eight periods with greater than 7 bleeding or spotting days occurred in the second 90 days post-treatment, three in the low dose group and five in the high dose group The amenorrheic periods were distributed fairly evenly between the two groups, with one patient in each dose group experiencing 120 days without bleeding, and the other amenorrheic periods randomly distributed among the other subjects

At the second center (*Group J, Table 1*), one subject in each group had ovulatory progesterone levels 160 days post-treatment, and the other eight subjects had no progesterone value over 3 ng/ml during the 160- to 175-day sampling interval Serum NET levels were generally lower than those in subjects at the first center, and a dose-related difference in release profiles was not evident (*Figure 12*) The reason for this discrepancy between the two centers is most likely due to different injection

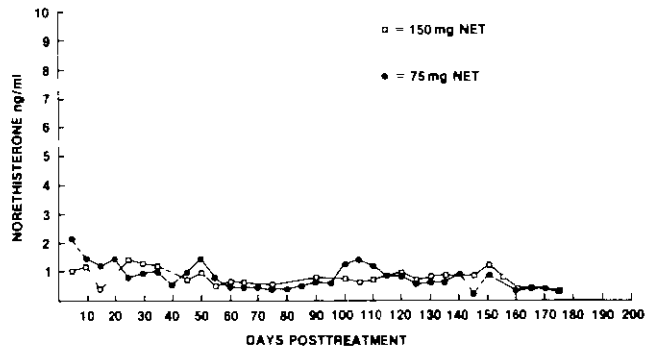

Figure 12 Serum levels of immune-reactive NET in Group J women following IM injection with 75 or 150 mg NET in PLGA microspheres 125-212 μm in diameter

efficiencies This has not been confirmed, however, because injection efficiencies were not established at the second center

As with the 3-month study, serum lipoproteins were unaffected in the women receiving the 6-month NET doses at both centers

PHASE II STUDIES

We decided, on the basis of the results of the multicenter Phase I clinical study, to initiate a Phase II study (*Group K, Table 1*) to evaluate the contraceptive efficacy of the copolymer microsphere formulation using a 75 mg dose of microencapsulated NET Based on past experience, we thought microspheres made of 85% polylactide and 15% polyglycolide, ranging in diameter from 45 μm to 90 μm and containing 20% by weight norethisterone, should provide 3 months of contraceptive protection in women

A batch of microspheres was prepared according to these specifications using an improved process that results in high quality microspheres A comparison of the quality of microspheres produced by this process to the earlier process has been previously published (10) Although the microspheres produced by the new process contained the identical polymer and norethisterone concentration, the *in vitro* rate of norethisterone was slower

Parallel *in vivo* studies in baboons and women demonstrate that the quality of the microspheres significantly affects pharmacokinetics The serum norethisterone profiles in 14 human subjects treated with microspheres produced by the improved process are shown in Figure 13 Unlike earlier formulations, the serum levels of norethisterone gradually increased for 30 days to 40 days post-treatment Early fast release, or burst effect, as it is commonly termed, does not occur One of the subjects in this study became pregnant during the first month

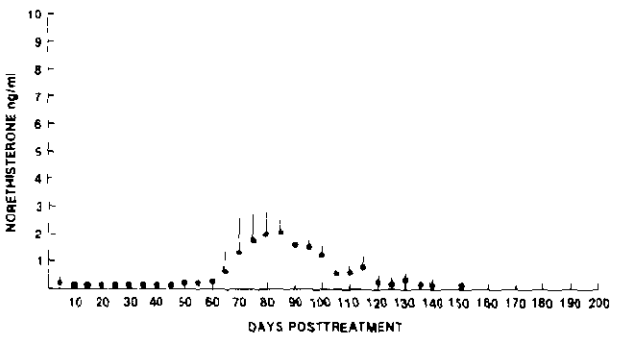

Figure 13 Serum levels of immune-reactive NET in Group K women following IM injection with 75 mg NET

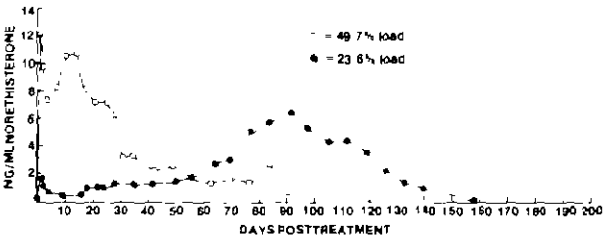

Figure 14 Serum levels of immune-reactive NET in baboons following IM injection with 75 mg NET in 24% loaded or 50% loaded PLGA microspheres

post-treatment because of the lower than expected serum norethisterone levels We decided, therefore, to discontinue further studies using this dose and batch of *microspheres*

Based on the pharmacokinetic studies in women and baboons, and the results from additional animal experiments, we concluded that the change in the serum norethisterone release was due to better encapsulation of norethisterone in the polymer matrix

Improvement in the microencapsulation process has reduced the amount of free or unencapsulated norethisterone in a formulation, which in turn reduces the early burst effect characteristic of all the earlier formulations Better-quality microspheres produced by the improved process provide more precise control of norethisterone release Although the higher quality microspheres are superior from a controlled release standpoint, they were not suitable for use in the Phase II clinical trial because the initial release rates were too low

Additional experiments were performed to determine the best way to achieve faster release rates without compromising the improvement that had been gained in the quality of the microspheres We found that the norethisterone release rate during the first 30 days post-treatment could be significantly accelerated by increasing the concentration of norethisterone in the microspheres

Figure 14 compares serum norethisterone profiles in baboons treated with the same dose of microencapsulated NET, 75 mg Formulation A contains 24% by weight norethisterone and formulation B 50% Both microsphere formulations were prepared by the improved process, yields high quality microspheres

This experiment clearly demonstrates that the higher norethisterone-loaded microspheres produced substantially faster release rates during the first 30 days post treatment Comparative scanning electron micrographic studies show that the two formulations are of equal quality

We also learned from these experiments that increasing the concentration of norethisterone in the microspheres to 50% substantially improves the injectability of the formulation This improvement results from a reduction in total mass of the particles to be injected For example, the weight of microspheres necessary to achieve a 75 mg dose of norethisterone from formulation A is 341 mg, whereas with formulation B only 150 mg is required We know from the results of the multicenter Phase I study that injectability is a serious problem The use of more heavily loaded microspheres should solve the injection problem In addition, we have initiated studies at two different centers to develop an improved injection vehicle and to determine the optimum needle size and syringe design

Although these studies are still in progress, substantial improvements have been reported, and we feel confident that the injectability problems will be solved prior to initiation of the Phase III studies

CONCLUSIONS

Evaluation of the course of this research program reveals a number of problems and the importance of well-coordinated pharmacokinetic studies in both animals and human volunteers The latest norethisterone microsphere formulation being used in expanded Phase II efficacy studies differs substantially from the original prototype system Improvements have been made in the manufacturing process, pharmacokinetics of the norethisterone microspheres, biodegradation kinetics of the polymer, and the injectability of the formulation

We have attempted to make improvements in response to the problems as they surfaced during the animal or human studies This continuum of improvement over a number of years demonstrates the inherent flexibility of this delivery system The ability to make changes in the performance of the delivery system in response to safety and physiological considerations is highly advantageous

The applicability of this delivery system for human use was realized by undertaking a number of Phase I studies in which minor changes were made in the dose and composition of the microsphere formulations Our purpose is to perfect a delivery system for application in women Although we found the baboon to be an extremely useful and predictive animal model, final evaluation and fine-tuning of the delivery system has been based on the results of the actual clinical trials As a result, we now have an improved process for the formulation of norethisterone microspheres that provides a more precise control of release, we have improved the pharmacokinetics of the delivery system by optimizing the drug loading and size distribution of the microspheres, and we have optimized the biodegradation of the delivery system by changing the molar ratios of polylactic and polyglycolic acid

Finally, we have substantially improved the injectability by improving the vehicle for preparing microsphere suspensions and by increasing the concentration of norethisterone in the microspheres, thereby minimizing the mass of particles to be injected These improvements bring us to the present state of the art and a formulation that we feel is suitable for use in expanded Phase II studies

This research program is still in progress, and although we feel we have reached a new point on the continuum of improvements, need for further modifications and improvements may become apparent during the course of the Phase II and III clinical trials

Looking backward, we see a foundation of polymer and microencapsulation technology developed during this research that supports not only this contraceptive application but a number of other controlled release drug formulations

Looking forward, we see few remaining obstacles to the eventual approval and wide-scale use of this injectable contraceptive system in both developing and developed countries We anticipate that additional improvements will come through the use of alternative steroids and or combinations of different steroids This will provide better bleeding control, which remains the single most objectionable feature of this long-acting injectable contraceptive Studies are currently underway to improve the bleeding control by the use of alternative progestogens and/or combinations of estrogens and progestogens

In presenting this review, we have emphasized the problems we have encountered during the course of this research, because these problems established the need for improvement and the justification for the research In continuing this research, we expect additional problems and anticipate further improvements

ACKNOWLEDGEMENTS

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REFERENCES

1 Beck LR, Cowsar DR, Pope VZ Long acting steroidal contraceptive systems Zatuchni GI (ed) Research Frontiers in Fertility Regulation, Vol 1 No 1 Chicago PARFR Northwestern University, July 1980

2 Beck LR, Cowsar DR Lewis DH Cosgrove RJ Jr Riddle CT, Lowry SL, Epperly T A new long acting injectable microcapsule system for the administration of progesterone Fertil Steril 31 545, 1979

3 Beck LR, Cowsar DR, Lewis DH Gibson JW, Flowers CE Jr New long acting injectable microcapsule contraceptive system Am J Obstet Gynecol 135 419 1979

4 Beck LR, Flowers CE Jr, Pope VZ, Tice TR, Dunn RL, Gilley RM Poly (d,l lactide co glycolide) norethisterone microcapsules clinical study In Zatuchni GI, Goldsmith A Shelton JD, Sciarra JJ (eds) Long Acting Contraceptive Delivery Systems pp407 417 Philadelphia, Harper & Row, 1983

5 Beck LR Flowers CE Jr Pope VZ Tice TR Clinical evaluation of an improved injectable microcapsule contraceptive system Am J Obstet Gynecol 147 815 1983

6 Beck LR Pope VZ Controlled release delivery systems for hormones a review of their properties and current therapeutic use Drugs 27 528, 1984

7 Beck LR, Pope VZ, Flowers CE, Cowser DR, Tice TR, Lewis DH Dunn RL Moore AB, Gilley RM Poly (d,l lactide co glycolide) norethisterone microcapsules an injectable biodegradable contraceptive Biol Reprod 28 186, 1983

8 Beck LR Ramos RA Flowers CE Jr Lopez GZ, Lewis DH Cowsar DR Clinical evaluation of injectable biodegradable contraceptive system Am J Obstet Gynecol 140 799, 1981

9 Beck LR Tice TR Poly (lactic acid) and poly (lactic acid co glycolic acid) contraceptive delivery system In Mishell DR Jr (ed) Long Acting Steroid Contraception Vol II, pp 175 199 New York Raven Press, 1983

10 Lewis DH Tice TR Polymeric consideration in the design of microencapsulation of contraceptive steroids In Zatuchni GI, Goldsmith A Shelton JD, Sciarra JJ (eds) Long Acting Contraceptive Systems pp 77 95 Philadelphia Harper & Row 1983

11 Goldzieher JW Benagiano G Long acting injectable steroid contraceptives In Mishell DR Jr (ed) Advances in Fertility Research Vol 1 pp 75 115 New York Raven Press, 1982

12 Mishell DR (ed) Long Acting Steroid Contraception New York Raven Press, 1983

13 Rivera R Flores C Aldaba S Hernandez A Norethisterone microspheres 6 month system clinical results In Zatuchni GI Goldsmith A Shelton JD Sciarra JJ (eds) Long Acting Contraceptive Delivery Systems, pp418 424 Philadelphia Harper & Row 1984

14 Zatuchni GI Goldsmith A Shelton JD Sciarra JJ (eds) Long Acting Contraceptive Systems Philadelphia Harper & Row 1984

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RESEARCH FRONTIERS IN FERTILITY REGULATION

IMMUNOLOGIC METHODS OF FERTILITY REGULATION: REPORT OF A WORKSHOP*

Reproductive immunology has been identified as a topic of interest by several national and international agencies supporting contraceptive development The National Institute of Child Health and Human Development (NICHD) has a vested interest in both the basic and applied aspects of research on immunocontraception Both contract and grant support mechanisms of NICHD are used to provide direct financial assistance for research in this field The Office of Population, Agency for International Development (AID), is currently interested in identifying and defining an increased role it could play in enhancing developments in this field Limited support for some applied aspects of immunocontraception has been provided to investigators through programs such as the Program for Applied Research on Fertility Regulation (PARFR) and the Population Council The World Health Organization (WHO) Special Programme of Research in Human Reproduction began the Task Force on Immunological Methods of Fertility Regulation (Birth Control Vaccines) in 1973 and intends to initiate clinical trials in 1985 The Government of India accords high priority to immunocontraception

Owing to these interests, a joint National Institutes of Health (NIH) AID PARFR Workshop on Research and Development of Immunologic Methods of Fertility Regulation was convened at NIH on April 16-18, 1984

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The organizers of the workshop wish to acknowledge the enthusiasm and active participation of the speakers and the observers This report was compiled by Jeff Spieler, Agency for International Development and is the result of a collaborative effort on the part of all those involved in the workshop Special thanks for its preparation must be extended to Nancy Alexander Gabe Bialy Laneta Dorflinger Mike Harper Mike McClure Jeff Spieler and Vern Stevens

The purpose of the workshop was to review current research on reproductive immunology, with specific reference to research on sources of antigens that have the potential for eliciting an immune response and can interfere with reproductive processes in both the male and the female Participants were experts in reproductive biology, endocrinology, biochemistry, immunology, and pathology (see page 11) It was the intention that the workshop result in recommendations to investigators and funding agencies interested in conducting and supporting research and development activities in this field

One important issue considered was whether efficacious, safe, and reversible vaccines for fertility regulation can be developed The current research in the area and related topics were reviewed and discussed (see page 10), with primary emphasis on research to develop vaccines based on 1) gonadotropins or their subunits and fragments, 2) sperm antigens, and 3) antigens derived from the zona pellucida Prerequisites for an acceptable vaccine were discussed in detail The workshop participants identified research needs and stressed the importance of research on both basic and applied aspects of reproductive biology and immunology The overall complexity of developing contraceptive vaccines indicates the need for interdisciplinary research

It was the consensus that immunologic approaches offer some unique advantages in fertility regulation Considerable research effort, however, must be mounted to recognize their full potential Although some approaches have advanced to the stage where clinical testing is imminent, these as well as other approaches require additional preclinical research

The proceedings and the recommendations of the participants (indicated by italics) are summarized in the sections that follow

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ANTI-HORMONE VACCINES

Research efforts are being directed toward the development and evaluation of efficacy and safety of antifertility vaccines using the gonadotropins and their subunits and various peptide fragments For example prototype vaccines are being developed using the beta subunit of human chorionic gonadotropin (hCG beta), the beta subunit of ovine luteinizing hormone (oLH beta) and the carboxy terminal peptides of hCG beta In extensive safety tests in animals, including non human primates, hCG beta, oLH beta and the hCG beta carboxy terminal peptide vaccines showed no evidence of acute or chronic side effects (29)

In rhesus monkeys, immunization with oLH beta caused a shortened luteal phase with reduced progesterone levels In this study, monkeys were observed for more than 5 years, and detailed histopathological studies were conducted at the end of that period (30) No damage to the pituitaries of the animals was observed, despite the continued existence of circulating antibodies reactive to LH Although the mechanism of action of anti-hormone antibodies is not completely defined, it was demonstrated that animals with high levels of circulating antibodies do not become pregnant after multiple matings (36)

The observation of impaired or reduced ovarian function in animals immunized with oLH beta (30) raised a discussion of the possibility that such induced endocrine imbalances could lead to breast tumors or other malignant endocrine diseases, as has been seen in relation to an insufficient luteal phase Obviously, subjects participating in clinical trials with oLH beta would need to be monitored for several years at least In human trials conducted some years ago in India, Finland, Chile, and Brazil, immunization with an hCG beta subunit linked to tetanus toxoid did not produce any detectable side effects (6)

Further safety testing of hCG beta or oLH beta was not recommended by the workshop participants, since the number of animals that could be used for such studies would not likely reveal a potential, low-frequency problem resulting from immunization However, it was agreed that sufficient safety testing should be conducted on new vaccine formulations to permit approval of clinical trials by appropriate drug regulatory authorities

The major limitation to the anti-hormone vaccine (as well as other potential vaccines) is likely to be the genetically determined variability among individual recipients in response to immunization It was speculated that such variation in both magnitude and duration of vaccine induced immune response may be reduced by the use of potent adjuvants, however, the number of non responders could still be significant *Simple, non invasive methods to identify such non responders were believed to be an important area for research* Despite this concern, it was the consensus that Phase I clinical trials to test the immunogenicity of at least two or three of the best characterized anti-hormone vaccines could be initiated as soon as approved by appropriate regulatory authorities

Early clinical trials will answer many important questions, such as the variation in the level and duration of antibody production among women Since several different vaccines are likely to be in clinical trials within 1 to 2 years, *it was recommended that the exchange of sera among investigators be encouraged and that a reference "standard" serum bank for comparing antibody levels attained from different vaccines be established* The availability of standard reagents and standard methodology will be required to permit comparisons between laboratories

Several areas of research for the development of other anti-hormone vaccines were discussed, including anti-FSH and anti-LHRH Questions were raised as to the advantages of an anti-LHRH vaccine over a long-acting, injectable LHRH antagonist The most advanced primate study for male immunocontraception (22) used FSH as the antigen, however early promising results were followed by an unexplained recovery of spermatogenesis in spite of high antibody titers

While there was no immediate role envisioned for anti-steroid hormone vaccines in human fertility regulation, it was thought that research studies employing them are likely to be of immense value in animal husbandry and veterinary medicine, and will provide much needed data on basic immunology and reproductive biology *There was a consensus that more research is needed in the area of antigen carriers, new adjuvants, and chemical modification of antigens*

When a method is ready for clinical testing, it was thought that studies of the mechanism(s) of action should be initiated, if they are not already underway These data will reveal how a given vaccine is affecting fertility, and may provide insight for developing new vaccines

While the participants encouraged clinical studies of anti hormone vaccines at an early date, they did not endorse any specific method and could not vouch for the purity or lack of toxicity of individual vaccines at this time

ANTIGENS IN THE MALE

Development of a vaccine based on sperm represents a promising approach to contraception Interference may be feasible at several sites, during sperm production in the testes during sperm maturation in the epididymis, or during sperm interaction with the egg in the female

reproductive tract The number of known sperm antigens that could be used as a vaccine is limited In fact, it was postulated that only 1% of the proteins in sperm have been identified to date (3) This situation is changing rapidly, however, due to the advent of monoclonal antibody (Mab) technology *It is important that basic studies of spermatogenesis, sperm maturation, sperm function, and fertilization be continued* Only with such information will we know which antigens will prove most useful for vaccine development

Lactate Dehydrogenase (LDH-C_4) Some known antigens of sperm include LDH-C_4, protamine, acrosin, hyaluronidase, plasma membrane antigens, and other differentiation products The most completely characterized antigen is LDH-C_4, which is synthesized during spermatogenesis and becomes localized to the mid piece and tail of spermatozoa This sperm specific isoenzyme has been crystallized from mouse testis Although there are some differences in the amino acid sequences of this tetrameric protein from various species, there is considerable cross-reactivity of antibodies raised against the mouse LDH C_4 with the comparable enzyme of other mammals, including rabbits, baboons, and humans

Although LDH-C_4 is considered to be an internal antigen, a significant amount of the antigen is also on the surface of sperm Systemic immunization of females with LDH-C_4 resulted in a reduction of fertility in mice, rabbits, and baboons (12) Infertility was correlated with high antibody titers, and in baboons the ovulatory cycles were not affected (12) When antibody titers fell, normal pregnancies occurred Samples of oviductal fluid from systemically immunized rabbits have been shown to contain antibodies to LDH-C_4, and IgA has been detected in uterine washings of immunized mice (12) These data suggest that immunoglobulins produced following immunization with LDH-C_4 enter the female reproductive tract Antibodies probably prevent fertilization by causing spermatozoa to become agglutinated or immobilized Studies have recently been initiated that attempt, by immunizing with LDH-C_4, to stimulate IgA secretory systems and promote antibody secretion into cervical mucus and oviductal fluids in rhesus monkeys

Antibodies to LDH C_4 cause a marked reduction in fertility (approximately 80%) in female baboons While there is some concern that the contraceptive effect is not complete, increased fertility suppression may be achieved by altering the protocol to enhance antibody procuction These changes would involve the route of administration (local versus systemic), dosage and schedule, adjuvant, and antigen (primate versus murine LDH-C_4)

Until these parameters are tested, LDH-C_4 remains one of the most promising target antigens for an antifertility vaccine Nevertheless, *participants agreed that an in vitro test to measure antibody effectiveness would circumvent problems of multiple fertility trials*

One advantage of LDH-C_4 is that its structure is well defined, thus, the development of a synthetic vaccine is facilitated Since production of a vaccine will depend on the capacity to synthesize large amounts of antigen, current studies are focusing on evaluating selected peptide sequences that can be synthesized Fertility evaluation of baboons immunized with one such peptide, consisting of amino acid residues 5 to 15 of LDH C_4, conjugated to diphtheria toxoid, is currently underway (11) After the first mating, only one of eleven baboons conceived There are at least five other peptide sequences that could be tested and that may be even more effective in preventing conception Although LDH-C_4 and its fragments have some attractive properties, questions of efficacy still require resolution in order to develop a vaccine based on this antigen However, as long as antibodies to LDH-C_4, or to any other sperm antigen, for that matter, will agglutinate spermatozoa, the potential for a contraceptive effect exists

Other Internal Sperm Antigens. The protamines, a family of nuclear proteins, may not be as amenable to antibody attack as other sperm constituents because they are not readily accessible to the antibody Nevertheless, these antigens, as well as other internal antigens, may be vaccine candidates at some future date Perhaps it will be possible to induce the Sertoli cells to transport substances into the maturing spermatogenic cells prior to their release from the seminiferous epithelium, thus rendering them incapable of subsequent fertilization (3) *With a better understanding of sperm production, new immunological methods of suppressing spermatogenesis without altering steroid hormone production may be possible*

Use of Monoclonal Antibodies. Identifying sperm-specific antigens has been facilitated by monoclonal antibody (Mab) techniques Such antibodies provide a powerful tool to deduce the role of sperm antigens in germ cell differentiation, sperm maturation, capacitation, and fertilization Since studies in many species will provide important basic information for vaccine development, a library of anti-human sperm Mabs is required Some Mabs to specific regions of sperm have already been described Several Mabs to the acrosome, equatorial region, post acrosomal region, and tail of human sperm have been shown to impede sperm penetration of hamster eggs Some Mabs react only with capacitated human sperm One Mab directed against the surface of guinea pig sperm induces an acrosome reaction *More studies on sperm function and fertilization will allow a better definition of possible sites for intervention* Those Mabs that are directed against phylogenetically conserved antigenic determinants will be useful in

permitting functional evaluation in laboratory species For example, an anti human sperm Mab that reacts with mouse sperm will allow studies of its antifertility action in mice Recent advances in hybridoma technology make it feasible to utilize human Mabs for antifertility vaccines through passive immunization

Application of Mabs as immunoaffinity ligands has permitted the isolation of new antigens Immunization of animals with these antigens may provide important information as to whether high titers of these antibodies will prevent fertility Mabs as reagents will greatly facilitate studies on the ontogeny of surface antigens and their organization into functional topographic domains

Another approach to the identification of antigens has been the evaluation of serum samples from individuals considered to have immunologically mediated infertility Western blot techniques have been used to evaluate such serum samples Further assessment of these antigens excised from gels may provide useful data concerning naturally occurring antibodies

In this context participants discussed the usefulness of supporting the WHO serum bank (Task Force on Birth Control Vaccines, Special Programme of Research in Human Reproduction) Concern was expressed that some of the serum samples were from patients who had not been adequately clinically characterized Although the WHO serum bank has been useful in allowing a comparison of tests for assessing immunologic infertility, its importance for advances in vaccine development has not yet been demonstrated

Further basic efforts should be directed toward studies of immunosuppressive and anti-complement factors in seminal plasma These substances should be characterized and defined since their presence may affect the antigenicity of sperm antigens, regulate whether intercourse can provide a booster effect, and affect other immunological events at a local level

Differences in the immune response to sperm antigens should be studied Only through studies in genetically defined or inbred strains of animals can a better understanding of the basis for optimal immunization regimens be determined and vaccines developed capable of overcoming poor immune responsiveness

Whether a vaccine involving sperm antigens could be used more appropriately in females than males remains open to discussion Two feasible approaches in females involve active immunization or a passive local delivery, such as vaginal administration of the anti sperm antibody Possibly, a vaccine that stimulates production of antibodies to several different sperm antigens will be most efficacious, although no one has yet reported 100% fertility suppression even with crude extracts that presumably contain all sperm and or testis antigens

THE OVUM AND ITS INVESTMENTS

Several unique antigenic substances are associated with oocyte development and may provide suitable targets for immunologic contraception Although there may be specific antigens associated with the oocyte itself such antigens have not been purified In contrast, antigens specific to the oocyte investment — the zona pellucida — have been identified Antigens from this noncellular layer may prove to be an excellent target for immunologic inactivation Most recent research has concentrated on studying the antigens of the zona pellucida

It remains to be seen whether other differentiation antigens can be found that appear or are expressed in the ovum only after fertilization (perhaps associated with the fertilizing spermatozoon) The availability of such post fertilization antigens would reduce the possibility of cross reactions with oocytes in the ovary A particular class of structural nuclear proteins, the lamina A and C, has been detected in the cytoplasm of the ovum before fertilization (7) Remarkably, these two polypeptides suddenly become non-immunoreactive 2 or 3 minutes after fertilization in a wave-like manner that is dependent on the release of intracellular calcium, and then gradually reappear again a few hours later The significance of this observation and its possible utility for immunocontraception remains to be determined

It is clear that if antigens that are associated only with later stages of oocyte development in the preantral or antral follicles, or with fertilization, can be defined, they would have great advantages since the risk of endocrine disturbance or, more serious, permanent damage to the germ cells and primordial follicles and associated sterility would be avoided Although it will be ideal if a contraceptive vaccine based on ovum antigens is reversible, this cannot be guaranteed For some individuals who have completed their family, irreversible methods of contraception are both attractive and acceptable, as is evidenced by the popularity of sterilization by surgical methods Furthermore, irreversible methods of immunocontraception may offer non-surgical approaches to sterilization that would be of value in developing and developed countries

Zona Pellucida Antigens It was strongly emphasized that significant progress in the isolation and characterization of zona antigens has only been made through the use of standardized separation and purification techniques These techniques include two-dimensional high resolution

gels and the most sensitive protein detection methods available, e g silver staining *It was recommended that these techniques be used routinely* Application of such techniques has facilitated the identification and isolation of several zona pellucida antigens discussed below

The zona pellucida is a complex structure composed of three major and several minor glycoprotein families with multiple antigenic determinants The molecular weights of these glycoproteins are difficult to determine, because of heterogeneity due to extensive glycosylation However the apparent molecular weights of the most abundant antigens from the major family range from 55K to 80K, and it is the 55K antigen that is being investigated most extensively

For the present, the zona pellucida of the pig provides the best immunogen, because of its availability, and because antibodies to the zona pellucida of this species cross react with the zona antigens of several other species, including rabbits, nonhuman primates, and humans Current information indicates that zona antigens derived from rodent species will not be suitable as the basis for a contraceptive vaccine for humans, owing to the lack of cross-reactivity with the human zona (10, 18)

Efficacy of Immunization with Zona Antigens. Active immunization with whole zona pellucida or passive immunization procedures with antibodies to zona pellucida can have dramatic effects on fertility in a variety of species Studies involving active immunization of several species with zona pellucida antigens have also demonstrated strong antifertility effects (10, 16, 21) In addition, passive immunization with monoclonal antibodies to purified zona pellucida antigens has been shown to be effective in preventing fertilization in mice

The immunologic response to zona antigens is remarkably consistent among individual animals and between species, and is also long-lasting (at least 1 year) This has been demonstrated in both rabbits and squirrel monkeys (9, 17)

Potential Problems and Drawbacks. There have been indications in several species that antibodies to some of these complex immunogens do alter ovarian function and ovum development In rabbits, ovulation ceased within 3 months after immunization with zona pellucida, and histologic examination revealed that the ovaries lacked oocytes (21) Whether this effect will occur in higher primates is not yet known Initial immunization in primates was conducted in squirrel monkeys, which do not exhibit an overt menstrual cycle (17) Unfortunately, histologic examination of the ovaries was not conducted in that study Future research in other species may prove enlightening

For reversible contraception the most desirable interference with zona function would be one preventing fertilization Inhibition of ovulation without concomitant change in endocrine function would also be desirable Elimination of all oocytes from the ovary would be irreversible and, therefore, may be less desirable in women It could be of significant benefit, however, in agricultural practice and for domestic pets

More detailed studies are needed to determine the mechanisms involved in alterations of fertility following immunization with different zona antigens When this information is available, selection can be made of the most appropriate antigens for further development

Aside from discerning the mode of action, there is also the larger issue of provision of an adequate supply of the chosen antigen Large-scale collection of zonae pellucidae from slaughter houses for processing is obviously not a practical long term solution to this problem It seems appropriate to start using modern techniques of molecular biology to help with the preparative scale purification of these antigens

Quantitation of the Immune Response Use of standardized and quantitative methods, as outlined above, will be of great benefit in achieving progress with regard to characterization of the immune response *For quantitation, it is recommended that investigators avoid the use of assays involving the measurement of fluorescence in intact zonae Suitable tests for quantitation would include radioimmunoassay and non isotopic assay detection systems such as ELISA or biotin avidin*

Collaboration Among Investigators *All investigators, both in the USA and abroad, currently working on zona pellucida and oocyte antigens should be identified and a workshop convened to discuss the status of ongoing research and to outline the standardization of techniques and approaches being used When standardized methodology has been adopted, exchange of antigens and antisera can be initiated At that time a bank of reference preparations should be established*

New Information Obtainable from Further Research. Remarkably little is known about the biochemical and molecular events associated with the early stages of oocyte growth and follicular development Since the zona pellucida proteins are synthesized and secreted during these early stages, they provide unique stage-specific markers for development of the follicle *The studies described above also should allow generation of specific molecular probes and antibodies that can be used to elucidate the mechanisms and control systems of oogenesis, and more precisely define the process of fertilization*

ACCEPTABILITY AND EFFECTIVENESS OF CONTRACEPTIVE VACCINES

In view of the wide scale acceptance of vaccination and parenteral administration of drugs, fertility regulating vaccines should prove to be an acceptable, and therefore popular approach to family planning. Furthermore, it would seem likely that immunologic methods can be selected that do not disrupt the menstrual cycle or cause the metabolic disturbance and concomitant side effects associated with some of the currently used hormonal contraceptives.

Because vaccines will provide long acting fertility regulation and could be administered by trained paramedical and non-physician personnel, they are likely to be enthusiastically received by administrators of family planning programs. Furthermore, antifertility vaccine programs could be included within the scope of the MCH services and integrated not only with family planning but also with exclusive immunization programs (e.g., WHO Expanded Programme of Immunization), thus further improving on the availability of family planning.

There was considerable discussion among participants on how effective any birth control vaccines must be to warrant consideration. Views ranged from the opinion that even a 30% effectiveness would have demographic impact (if it were the only available or acceptable method) to the *general consensus that less than 90% effectiveness would not be acceptable.*

There are two distinct considerations, the percent of nonresponders in a population and the contraceptive effectiveness in responders. It has been demonstrated in animals that there are genetically controlled species and strain differences to immunogens. In addition, there are inter-animal differences in immune response within the same strain. *Therefore, techniques to predict or immediately identify poor responders or non-responders in a human population would be useful.*

Reversibility is an important issue. Although it is preferable to develop reversible methods, potentially irreversible methods may be acceptable, particularly since sterilization is currently the most widely used method of fertility regulation. *There was a consensus that irreversible approaches should have a greater method effectiveness than reversible ones.*

The discussion on this topic terminated with the following points:

1. A vaccine that is at least 90% effective should be sought.
2. In the absence of the desired level of effectiveness, a given approach should not be abandoned immediately. Instead, techniques for improving the response by modifying the immunogen, carrier, adjuvant, or delivery schedule should be investigated.
3. The actual or percent reversibility of any given approach will have to be determined empirically. At this time, the major consideration should be to obtain and maintain an appropriate antibody response over a desired period of time.
4. Depending on the nature of the antigen, it may be preferable to utilize a homologous immunogen animal model system than a heterologous one in order to obtain better information on duration of action, reversibility, and safety.

SAFETY CONSIDERATIONS

Safety is an important consideration in the development of a contraceptive vaccine. Aspects of the comprehensive document (33) developed by the WHO Task Force on Birth Control Vaccines for testing the efficacy and safety of birth control vaccines were discussed. *In view of new knowledge obtained during the past several years, this document, which was formulated in 1977 and published in 1978, should be updated.* Some issues of safety and immunopathology were discussed with regard to these original guidelines. While immune complex deposition could be a worrisome complication, the effect of circulating immune complexes is an open question.

Four major potential complications were proposed for consideration:

1. Induction of organ specific autoimmune disease (e.g., of the pituitary, ovary, or testes).
2. Induction of antibodies to hormone receptors that may follow an immune response to peptide hormones.
3. Enhancement of the risk of neoplasia to organs that carry antigens involved in a vaccine (e.g., hCG and immunization of men with sperm antigens), as well as an indirect biological effect resulting from, for example, the effect of unopposed estrogens on the uterus.
4. Genetically controlled variation in the immune response (i.e., some individuals may not develop a sufficient immune response) and long term biological effects of immunization. It was stressed that the genetic control of the immune response must be dissociated from the biologic consequences of the immune response.

There is evidence to indicate that each of these possibilities is a valid concern. It was thought that for testing purposes in a responding species, autoimmune pathology, should it occur, would be observed histologically within 6 months after high titers of antibody are obtained.

CARRIERS, CONJUGATION METHODS, ADJUVANTS, AND VEHICLES

Methods used in development of certain vaccines were reviewed Examples of antigen carriers were described and requirements for appropriate chemical coupling of antigens to carriers were outlined The participants stressed the importance of the capability of preparing an immunogen with batch-to batch reproducibility with regard to its chemical and immunologic properties Chemical methodology suitable to industrial scale production will have to be developed

Several experimental synthetic adjuvants with potent antibody stimulating properties have been developed Some of these compounds may be useful in overcoming genetically-related unresponsiveness to reproductive antigens Vehicles or delivery systems for vaccine components are not in an advanced stage of development Most experimental vaccines have employed oil-in-water emulsions or alum precipitates The safety of these products is important and must be considered *Some scientists believe that polymer based delivery systems can be designed to release an appropriate amount of immunogen and or adjuvants from the injection site and research in this area of vaccine development is encouraged* Advances in the synthesis of lymphokines and immunoregulatory products of bacteria are also of importance when considering methods of enhancing an immune response Methods resulting from research are likely to be applicable to the development of a wide variety of antifertility vaccines

USE OF RECOMBINANT DNA TECHNOLOGY IN DEVELOPMENT OF CONTRACEPTIVE VACCINES

An important issue discussed was the use of molecular biology and recombinant DNA technology to produce large quantities of purified antigen Such large-scale production of pure antigen will be a critical and perhaps limiting factor in ultimately providing a contraceptive vaccine for family planning programs

Technology is rapidly advancing in the area of genetic engineering It is now possible to produce large proteins and even glycoproteins using this technology Even rare messenger RNAs have been used to establish clones It was pointed out that once a cDNA clone is available to produce a specific antigen, it might be possible to make small changes in the DNA of that clone to enhance immunogenicity of the antigen Recombinant DNA technology can also be used to produce smaller peptide fragments of a protein, which could possibly confer immunity to the full protein

There was discussion of the use of genetic engineering technology to produce large quantities of antibodies for passive immunization programs For example, a DNA library can be made from a clone producing a desired monoclonal antibody and those genes inserted into a high producing cell line Large quantities of the original specific monoclonal antibodies would then be produced

Recombinant DNA vaccine viruses may be produced that would express specific antigens useful for a contraceptive development program The approach would entail the molecular cloning and characterization of cDNA, encoding the antigen of interest, and incorporation of the cDNA into a common vaccine virus such as vaccinia In this way, purified antigen would not be necessary and the immunization program would follow well-established routes Such an approach would be favored where large numbers of doses would be necessary for large target populations

Finally, it was thought that recombinant DNA technology will be increasingly important in the identification of new antigens specific to the ovary or testis These new antigens might prove superior to currently known antigens as a basis for a contraceptive vaccine By a technique such as subtractive hybridization, new differentiation-specific antigens can be identified *This area of research, while very basic, is essential for future evolution of contraceptive vaccine technology*

DELIVERY SYSTEMS

A concerned research effort is necessary to develop new methods for vaccine administration Current techniques require booster injections to maintain effective antibody titers Development of new delivery systems that could provide for timed release of the vaccine would obviate the need for booster injections Technology of this type has already been successfully applied to the release of contraceptive drugs For the release of steroids and LHRH analogs, constant release has been desired In order to achieve appropriate release rates for peptides, blends of microcapsules with a range of lag periods have been used *Therefore, it should be possible to produce a microcapsule delivery system for vaccines that will incorporate a series of suitable delays in antigen release, resulting in effective immunity with a single-shot vaccine* This is especially important in developing countries, where more follow up difficulties may be encountered if booster shots are necessary

CONCLUSIONS

Throughout the workshop, the need to provide both stimulation and continuing support of basic and applied research was reiterated. There was consensus that the basic-applied viewpoint of research in reproductive immunology represented a graded continuum not amenable to discrete partitioning. Both aspects warrant urgent initiatives in support of immunocontraceptive research and development. *Nevertheless, it was recommended that financial support for the field be increased by donor agencies, that programs supporting basic research via grant mechanisms issue new Requests for Applications (RFAs), and that those agencies funding applied research issue new Request for Proposals (RFPs) addressing several of the areas emphasized during this workshop.* Multidisciplinary research and collaboration between interested investigators are essential if the formidable challenge of developing immunocontraceptives is to be met successfully.

REFERENCES AND SELECTED READINGS*

1. Alexander NJ: Antibodies to human spermatozoa impede sperm penetration of cervical mucus or hamster eggs. Fertil Steril 41:433-439, 1984.

2. Anderson DJ, Alexander NJ: A new look at antifertility vaccines. Fertil Steril 40:557-571, 1983.

3. Bellve AR: Personal communication, 1984.

4. Bellve AR: The molecular biology of mammalian spermatogenesis. In Finn CA (ed): Oxford Reviews of Reproductive Biology. Oxford, Oxford University Clarendon Press, Vol. 1, pp 159-261, 1979.

5. Bellve AR, O'Brien DA: The mammalian spermatozoon: structure and temporal assembly. In Hartman JF (ed): Mechanisms and Control of Fertilization. New York, Academic Press, Inc., 1983, pp 55-137.

6. Contraception 13(2): [entire issue] 1976.

7. Donovan MJ, Mayhew PL, Bellve AR: Dynamics of the lamin proteins during fertilization and early embryogenesis in the mouse. Reflection of the calcium transient. Cell (submitted for publication, 1985).

8. Drell DW, Dunbar BS: Monoclonal antibodies to rabbit and pig zonae pellucidae distinguish species-specific and shared antigenic determinants. Biol Reprod 30:445-457, 1984.

9. Dunbar BS: Personal communication, 1984.

10. Dunbar BS, Wolgemuth DJ: Structure and function of the mammalian zona pellucida, a unique extracellular matrix. In Satir B (ed): Modern Cell Biology, Vol 3, pp 77-111. New York, Alan R. Liss, Inc., 1984.

11. Goldberg E: Personal communication, 1984.

12. Goldberg E: Current status of research on sperm antigens: Potential applications as contraceptive vaccines. In Zatuchni GI (ed): Research Frontiers in Fertility Regulation, Vol 2, No 6. Chicago, PARFR 1983, pp 1-11.

13. Isahakia M, Alexander NJ: Interspecies cross-reactivity of monoclonal antibodies directed against human sperm antigens. Biol Reprod 30:1015-1026, 1984.

14. Miller AB, Barclay THC, Choi NW, Grace MG, Wall C, Plante M, Howe M, Cinader B, Davis FG: A study of cancer, parity and age at first pregnancy. J Chron Dis 33:595-605, 1980.

*Not all references are cited in the text

15. Naz RK, Alexander NJ, Isahakia M, Hamilton MS: Monoclonal antibody to a human germ cell membrane glycoprotein that inhibits fertilization. Science 225:342-344, 1984.

16. Sacco AG: Immunocontraception: Considerations of the zona pellucida as a target antigen. In Wynn RM (ed): Obstetrics and Gynecology Annual, Vol 10, pp 1-26. New York, Appleton-Century Crofts, 1981.

17. Sacco AG, Subramanian MG, Yurewicz EC, DeMayo FJ, Dukelow WR: Heteroimmunization of squirrel monkeys *(Saimiri sciureus)* with a purified porcine zona antigen (PPZA): Immune response and biologic activity of antisera. Fertil Steril 39:350-358, 1983.

18. Sacco AG, Yurewicz EC, Subramanian MG, DeMayo FJ: Zona pellucida composition, specie cross-reactivity and contraceptive potential of antiserum to a purified pig zona antigen (PPZA). Biol Reprod 25:997-1008, 1981.

19. Saling PM, Raines LM, O'Rand MG: Monoclonal antibody against mouse sperm blocks a specific event in the fertilization process. J Exp Zool 227:481-486, 1983.

20. Saling PM, Waibel R, Lakoski KA: Immunological identification of sperm antigens that participate in fertilization. In Hedrick JL (ed): The Molecular and Cellular Biology of Fertilization. New York, Plenum Press (in press).

21. Skinner SM, Mills T, Kirchick HJ, Dunbar BS: Immunization with zona pellucida proteins results in abnormal ovarian follicular differentiation and inhibition of gonadotropin-induced steroid secretion. Endocrinol 115:2418-2432, 1984.

22. Srinath BR, Wickings EJ, Witting C, Nieschlag E: Active immunization with follicle-stimulating hormone for fertility control: a four-and-a-half year study in male rhesus monkeys. Fertil Steril 40:110-117, 1983.

23. Stevens VC: Potential methods for immunological fertility control in the female. In Benagiano G, Diczfalusy E (eds): Endocrine Mechanisms in Fertility Regulation, pp 141-161. New York, Raven Press, 1983.

24. Stevens VC, Cinader B, Powell JE, Lee AC, Koh SW: Preparation and formulation of a human chorionic gonadotropin antifertility vaccine. I. Selection of a peptide immunogen. Am J Reprod Immunol 1:307-314, 1981.

25. Stevens VC, Cinader B, Powell JE, Lee AC, Koh SW: Preparation and formulation of a human chorionic gonadotropin antifertility vaccine. II. Selection of adjuvant and vehicle. Am J Reprod Immunol 1:315-321, 1981.

26. Stewart TA, Bellve AR, Leder P: Transcription and promotor usage of the *myc* gene in normal somatic and spermatogenic cells. Science 226:707-710, 1984.

27. Talwar GP: Structured vaccines for control of fertility and communicable diseases. In Chandara RK (ed): Critical Reviews of Tropical Medicine, pp 245-269. New York, Plenum Press, 1984.

28. Talwar GP, Gupta SK, Gaur A, Singh G, Kaur IP, Das C, Singh V, Singh O, Sadal D, Iyer KSN: Anti-hCG and anti-LHRH monoclonals and their applications. In Isojima S, Billington WD (eds): Reproductive Immunology, pp 69-80. Amsterdam, Elsevier Science Publishers, 1984.

29. Thau RB, Witkin SS, Bond MG, Sawyer JK, Yamamoto Y: Effects of long-term immunization against the β-subunit of ovine luteinizing hormone on circulating immune complex formation and on arterial changes in Rhesus monkeys. Am J Reprod Immunol 3:83, 1983.

30. Thau RB, Yamamoto Y, Sundaram K, Spinola PG: Human chorionic gonadotropin stimulates luteal function in Rhesus monkeys immunized against the β-subunit of ovine luteinizing hormone. Endocrinology 112:277, 1983.

31. Tung KSK: Antifertility vaccine: Considerations of their potential immunopathologic complications. Int J Fertil 121:197-206, 1976.

32. Wheat TE, Goldberg E: Sperm-specific lactate dehydrogenase C_4. Antigenic structure and immunosuppression of fertility. In Rattazzi MC, Scandalios JG, Whitt GS (eds): Isozymes: Curr Top Biol Med Res 7:113-130, 1983.

33. WHO Task Force on Immunological Methods for Fertility Regulation. Evaluating the safety and efficacy of placental antigen vaccines for fertility regulation. Clin Exp Immunol 33:360-375, 1978.

34. Wolgemuth DJ, Celenza J, Bundman DS, Dunbar BS: Formation of the rabbit zona pellucida and its relationship to ovarian follicular development. Develop Biol 106:1-14, 1984.

35. Yamamoto Y, Gunsalus GL, Sundaram K, Thau RB: Antibodies against the β-subunit of ovine luteinizing hormone can decrease the clearance of human chorionic gonadotropin in Rhesus monkeys. Am J Reprod Immunol 5:164, 1984.

36. Yamamoto Y, Gunsalus GL, Sundaram K, Thau RB: Characterization and anti-oLHB antibodies acting as contraceptives in Rhesus monkeys. I. *In vitro* binding properties. J Reprod Immunol 4:295, 1982.

37. Yamamoto Y, Thau RB: Characterization of anti-oLHB antibodies acting as contraceptives in Rhesus monkeys. II. *In vivo* neutralizing ability for gonadotropic hormones. J Reprod Immunol 5:195, 1983.

AGENDA

Joint NIH/AID/PARFR Workshop on Research and Development of Immunologic Methods of Fertility Regulation.

April 16-18, 1984, Bethesda, Maryland

1. *Introduction:* Dr. G. Bialy, Mr. J. Spieler
2. *Areas for Possible Disruption of the Reproductive Process Through Immunologic Methods:* Dr. N. Alexander
3. *Current Status of Research and Development on Specific Approaches and Prospects for the Future*
   Anti-hormones: Dr. R. Thau
   Anti-sperm: Dr. E. Goldberg, Dr. P. Saling
   Anti-egg: Dr. B. Dunbar, Dr. A. Sacco
4. *The Indian Experience — Priorities, Lessons to be Learned, Current Status, and Prospects for the Future:* Dr. G. P. Talwar
5. *International Approaches to Birth Control Vaccine Development:* Group
6. *Identification of New Antigens*
   Investigation of Immunologically Conditioned Infertility: Dr. D. Anderson
   Systematic Dissection of Membrane Antigens (Sperm and Ovum): Dr. A. Bellve
7. *Vaccine Development*
   Overview of Vaccine Development: Dr. J. Robbins
   Production of Vaccine Antigens — Natural Versus Synthetic Antigens, Including Use of Recombinant DNA and Solid Phase Synthesis Techniques: Dr. B. Chin
   Carrier Proteins, Adjuvants, Conjugation Procedures, Delivery Systems/Formulation: Dr. V. Stevens
8. *Immunopathology and Other Safety Considerations:* Dr. K. Tung
9. *Strategy Formulation*
   Brainstorming: Group
   Recommendations: Group

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TRANSCUTANEOUS MALE STERILIZATION

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Vasectomy is one of the safest, simplest, and most effective methods of fertility regulation Compared to female sterilization, it is safer and cheaper and has a similar rate of effectiveness In spite of these advantages, in most countries the number of contraceptive female sterilizations performed each year continues to exceed the number of male sterilizations performed (30) In Latin America, the Caribbean, the Middle East, and Africa, fewer than one-half million couples rely on vasectomy for contraception, compared to about 5 million couples in the United States and about 12 million couples in both India and China (30) The reasons cited (30) for the low prevalence of vasectomy in some countries include

1 Lack of vasectomy services
2 Emphasis on providing female and not male sterilization services
3 Negative attitudes of physicians toward vasectomy

Even if there were a greater emphasis on the provision of male sterilization services there might not be a greatly increased demand for these services, especially in cultures where men have a fear of surgery in the scrotal area and where vasectomy is equated with castration However, many experts argue that a simple nonsurgical method of male sterilization would have the potential to overcome the fears associated with the standard methods of vasectomy requiring a scrotal incision

Advantages of Transcutaneous Procedures For over 20 years, research has been conducted to develop a transcutaneous method of male sterilization in which the scrotum is punctured by a needle or needle-like instrument By means of this instrument, chemical agents or electro-coagulation can be used to block the vas lumen The ideal has been to develop a procedure that is inexpensive, can be administered by trained paramedical personnel, and can be performed with simple instrumentation The potential advantages of transcutaneous vas occlusion compared to standard surgical vasectomies include the following (12)

1 The risk of postsurgical hemorrhage is eliminated At present, about 1 6% of surgical vasectomy patients develop postoperative hematomas (31)
2 The risk of postoperative infections should be greatly diminished Presently, about 1 5% of men undergoing surgical vasectomy procedures develop infections (31)
3 The procedure should be more acceptable to men who have a fear of genital operations
4 Surgery and surgical equipment are not required
5 The procedure can be taught to paramedical personnel Once learned the procedure can be done rapidly at low cost

Despite the many advantages of transcutaneous male sterilization, only a minimal amount of research has been conducted to develop this method for widespread use in sterilization programs

In spite of the apparent simplicity and advantages of a transcutaneous sterilization procedure, outside of the People's Republic of China, very little work has been done to evaluate the safety and effectiveness of these

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procedures in men Most of the work has been limited to sporadic efforts to investigate the effects of various sclerosing agents using animal models Some efforts have been made to improve the technical feasibility of the procedures through the development of improved equipment

The purpose of this report is to review and summarize these research efforts and to provide an update on current research In the following sections current research and the results from animal and human studies are reviewed for each of the listed approaches to transcutaneous sterilization

METHODS OF TRANSCUTANEOUS STERILIZATION

The following methods of transcutaneous sterilization have been evaluated in animals and or man

1 Intratesticular injection of chemical agents to affect spermatogenesis
2 Intraepididymal injection of chemical agents to affect sperm transport
3 Obstruction of the vas lumen by the intravasal injection of chemical (sclerosing) agents or by electrocoagulation of the vas lumen

Intratesticular Methods. For over 25 years, research has been underway to develop a male contraceptive pill that would interfere with spermatogenesis, either by direct action on the pituitary by suppressing the production of gonadotropins or by direct interference with spermatogenesis in the testes Numerous steroidal and non-steroidal agents have been evaluated, including luteinizing hormone releasing hormone (LHRH) agonists and antagonists, progestogens, and androgens, either in combination or alone, and other drugs such as gossypol, a phenolic compound isolated from the cotton plant (15, 22, 29) These drugs are usually administered orally or by systemic injection, and their use has been associated with undesirable side effects, including loss of libido and various effects on accessory sex glands Although many of these compounds can produce oligospermia, they do not consistently produce azoospermia Also, the effects of most of these compounds are temporary, and repeated administrations are required to maintain oligospermia azoospermia As an alternative to the interference of drugs, the direct injection of compounds into the testes has been investigated (32, 33)

Wiebe and Barr evaluated the effects of the direct injection of aqueous 1, 2, 3-trihydroxypropane (THP, glycerol), a normal component of living cells, into the testes of Sprague-Dawley rats (32, 33) Spermatogenesis was inhibited by a direct and local action of THP on the seminiferous tubles The THP injections had no apparent effects on mature sperm stored in the epididymides The first mating of treated rats resulted in normal offspring After the fourth postinjection week no matings resulted in pregnancy The THP injections produced long-term infertility (up to 21 weeks) without producing any significant effects on Leydig cell steroidogenesis, on testosterone and LH and FSH serum levels, or on secondary sexual characteristics and mating behavior No undesirable side effects were noted The investigators found that by 14 days after injection of THP, there was a 50% reduction in the weight of the testes Although this is not considered an undesirable side effect in animals, in man a reduction in testicular size might limit the acceptability of the procedure Additional studies are currently being undertaken to determine the mechanism of action of THP on the testes

The only other intratesticular method that has been investigated is the use of ultrasonic energy In one study of the effects of different ultrasound intensities on the testes of mature rabbits, ultrasound at 1 5 W per sq cm for 15 minutes produced degeneration of the seminiferous tubules (23) Whether the use of ultrasound can be developed into a practical method of nonsurgical sterilization will require further evaluation One obvious limitation of the method, especially in developing countries, is the cost of the equipment and the difficulty in obtaining routine maintenance

Intraepididymal Injection. Although the injection of sclerosing agents directly into the epididymis is technically simpler than injection into the vas lumen, the intraepididymal approach to nonsurgical sterilization is known to have been evaluated in only three studies The advantages of intraepididymal over intravasal injections are that the cauda epididymis is easily palpated and intraluminal placement of the needle in the epididymis is not necessary

Bowman and coworkers evaluated the effects of injections of anhydrous calcium chloride dissolved in sterile saline directly into the cauda epididymis of mature rams on ejaculate volume, sperm concentration, and mounting time (4) Five rams received injections of calcium chloride and five received injections of saline By 2 weeks after the injections, the calcium chloride-treated rams had a significant reduction in ejaculate volume and sperm concentration None of the animals became azoospermic, but semen analysis was characterized by the absence of sperm motility and by head-tail fragmentation Mounting times were not affected The sterilizing effects of the calcium chloride most probably were due to its necrosing effects on the epididymis No histopathology studies were performed

Lewis and Garcia evaluated the effects of three sclerosing agents (formaldehyde, methylcyanoacrylate [MCA] and quinacrine hydrochloride) in mature *Macaca fascicularis*

monkeys (18) These agents were injected directly into the cauda epididymis either transcutaneously or after exposure of the epididymis through a scrotal incision All animals were sedated with ketamine hydrochloride intramuscular injection (0 1 ml kg of 100 mg ml solution) The results of the study are summarized in Table 1 and show that none of the agents evaluated was effective Histologic evaluations of testicular biopsies performed after 6 months showed normal testicles in all monkeys but one That MCA-treated monkey had testicular atophy Other complications included abscess formation at the site of injection in two MCA treated animals and two quinacrine-treated animals In the formaldehyde-treated group, a moderate to marked inflammatory cell infiltrate and fibrosis were noted in most testicles evaluated Four animals (1 MCA-treated, 3 quinacrine-treated) died after the intraepididymal injections Three deaths occurred on the day of the procedure and one (in a quinacrine-treated animal) occurred 1 week after the procedure Autopsies did not reveal the cause of death in any of the animals In the quinacrine-treated group, death may have resulted from the quinacrine or from the combined toxicity of quinacrine and ketamine Based on the unsatisfactory results obtained with all of the agents evaluated, no additional studies are being undertaken with intraepididymal injections of sclerosing agents

Experimental Group	*No Initially Azoospermic*	*Recanalization*	*No Azoospermic After 6 Months*
Formaldehyde (4%) in glycerate (n=5)	4	2	2
MCA (0 5 cc) (n=5)	5	2	3
Quinacrine hydrochloride (100 mg) in water (n=5)	4	3	1

Source Lewis and Garcia (18)

Table 1 Results of a study of intraepididymal injections of various agents

Davis evaluated the intraepididymal injection of formaldehyde in alcohol and MCA in adult mongrel dogs (8) In the first two animals evaluated (one treated with formaldehyde in alcohol and one with MCA) severe toxicity and death occurred within 1 week of the injection The cause of death of these animals was not stated, but may have resulted from the relatively large doses of the drugs administered Gross examination indicated bilateral acute necrosis of the epididymides and testes in both animals In other animals, in which lower doses of these chemicals were injected directly into the epididymis, extensive necrosis of the epididymides and testes was observed

The appeal of the intraepididymal approach to transcutaneous sterilization is that it is easier to inject a chemical into the epididymis than into the vas lumen To date, the limited evaluations of the intraepididymal injection of chemical agents has shown this to be an unsatisfactory approach to male sterilization Whether improved results can be obtained with other chemical agents remains to be evaluated

Intravasal Methods. The concept of transcutaneous vas occlusion with either chemical agents or electrocoagulation is not a recent one Over 20 years ago Lee, in a series of experimental studies in dogs, evaluated the effects of electrocoagulation of the vas through a transcutaneously inserted electrode, or the transcutaneous injection of different concentrations of phenol, glycerophenol, or a combination or quinine and urethane (17) He also investigated the effects of the transcutaneous injection of liquid Biowax, a wax used in cosmetic surgery (17) Shortly after injection, the liquid Biowax solidifies, causing obstruction of the vas Lee noted that if a radiopaque material were mixed with the Biowax, its placement could be checked using x-rays Although none of the transcutaneous methods tested by Lee resulted in vas occlusion in all animals injected, the studies did demonstrate that the transcutaneous approach was a feasible method of vas occlusion

Chemical Agents. Numerous chemical agents have been injected into the vasa of rats, dogs, and rabbits to evaluate their effects in producing vas occlusion The agents evaluated are listed in Table 2 Most of the studies have been experimental, in that they have evaluated relatively few animals and have been conducted to screen chemicals that might be worthy of either more extensive trials in animals or preliminary trials in man

Of the many chemical agents that have been evaluated in animals, only two are known to have been tested in man 3 6% formaldehyde in 90% ethanol (5, 11) and 4% formaldehyde in 90% ethanol (6, 7) and a carbolic acid, n-butyl alpha cyanoacrylate mixture (21) The mode of action of all of the sclerosing agents tested is thought to be similar, they produce local necrosis and fibrosis and vasal closure through scarring

One of the principal objectives in choosing a chemical agent for use in human sterilization procedures is to select one that has minimal toxic effects and will produce a minimal amount of damage if injected into structures other than the vas Ideally, damage caused by the chemical should be limited to the basal epithelium of the vas, without damage to the muscularis Although it is not the intent of this review to summarize the effects of each of the chemical agents used for vasal occlusion in various animals, it is noteworthy that in most of these procedures the epididymides and testes were not affected by the intravasal injections Also, toxicity appeared to be minimal None of the investigators reported any animal deaths or severe adverse reactions that could be attributed to the intravasal injections

Chemical Agent & Reference	*Animals Evaluated*
Sodium chloride (5, 12)	Rat
Ethanol (5, 12, 13)	Rat, dog
Formaldehyde (5, 12)	Rat, dog
Formaldehyde with ethanol (5, 12)	Rat, dog
Silver nitrate (5, 12)	Rat, dog
Acetic acid (5, 12)	Rat
Sodium tetradecyl sulfate (5, 12)	Rat
Potassium permanganate (5, 12)	Rat
Sodium morrhuate (5, 12)	Rat
Camphor (28)	Rat
Potash (28)	Rat
Quinacrine dihydrochloride (28)	Rat
Quinacrine, urethane (17)	Dog
Phenol (17)	Dog
Glycerophenol (17)	Dog
Biowax (17)	Dog
Phenol, cyanoacrylate (25)	Rabbit
Methyl cyanoacrylate (8, 25)	Rabbit, dog
Tincture of iodine, potassium iodide, sinograffin, carboxymethyl cellulose (25)	Rabbit, dog
Phenol, glycerine, sinograffin, gum tragacanth (25)	Rabbit
Silver acetate alginate (8)	Dog
Calcium chloride, gum tragacanth, glycerine (21)	ns
Sodium, cod liver oil acid, gum tragacanth, glycerine (21)	ns
Carbolic acid, gum tragacanth, glycerine (21)	ns
Carbolic acid, n butyl alpha cyanoacrylate (21)	ns
Ethyl alpha cyanoacrylate (21)	ns
n Butyl alpha cyanoacrylate (21)	ns

ns = not stated

Table 2 Chemical agents evaluated to produce occlusion of the vas in animals

Based on their studies of the intravasal injection of various sclerosing agents in rats and dogs (whose vasa are similar to those of man), Coffey and Freeman elected to evaluate a combination of 3.6% formaldehyde in 90% ethanol in human trials (5, 11). One advantage to the use of this combination is that both chemicals are easily metabolized and leave no residuals to produce adverse effects. The method used by these investigators was simple and did not require the use of any elaborate surgical equipment. One disadvantage of the procedure is that the investigators found it necessary to inject 1 ml of 1% lidocaine alongside each vas before injection of 0.25 ml of the formaldehyde and ethanol mixture. After each vas was located and stabilized between the thumb and forefinger, the mixture was injected through a 25 gauge needle. By the 24th post-procedure week, 7 of the 8 men injected were azoospermic. The other man had a sperm count of 67 million sperm per ml at the 14th post-procedure week. Once azoospermia was established, none of the men followed-up for the 14-40 weeks was found to again have sperm in his semen.

Additional trials of the intravasal injection of formaldehyde in ethanol have been conducted by Davis (6, 7). The procedure used by Davis was essentially the same as the one used by Coffey and Freeman. Davis injected 0.5 ml 4% formaldehyde in 90% ethanol into each vas. In the first 27 procedures, an Allis clamp was used to stabilize the vas. In the next group of 27 men, a specially designed clamp was used to stabilize the vas. A 25-gauge needle was passed through holes in the jaws of the clamp to inject the formaldehyde in ethanol mixture into the vas. The results of the studies by Davis are summarized in Table 3.

Series	*No. Subjects*	*Percent*
First		
Azoospermic within 16 weeks	13	48.2
Failures	11	40.7
Decease in sperm count further follow-up required	3	11.1
Second		
Azoospermic within 10 weeks*	17	65.4
Failures	4	15.4
Decrease in sperm count further follow-up required	5	19.2
Failed injection (retractile testes)	1**	

* Includes two men who had repeat injections.
**Not included in calculation of percentages.
Source: Davis (6, 7).

Table 3 Results of studies of the transcutaneous injection of 4% formaldehyde in 90% ethanol

In the second series of subjects there was a significant increase in the incidence of azoospermia. Whether this was due to the use of the specially designed clamp or was due to the increased experience of the operator cannot be determined from the study data. For six men, the duration of follow-up was insufficient to determine success of the procedure. Davis noted that once a man became azoospermic, he remained that way (7). Some of the men, for whom the procedures had been classified as a failure, had a transient drop in their sperm counts that later returned to near baseline levels. This drop was most likely due to an initial inflammatory reaction in the vas following the injection, causing temporary obstruction of the vas.

The combined data on the intravasal injection of formaldehyde in ethanol into men show that the time to achieve azoospermia is highly variable and may take up to several months. The differences in the failure rates reported in the series by Freeman (11) and Davis (6, 7) may be due to the different lengths of follow-up rather than to factors related to the way in which the procedures were performed.

Using dogs, Davis compared the effects of direct injections of MCA or silver acetate alginate into the vas lumen with effects of perivasal injections of these agents (7). In most cases, the injections produced vasal occlusion, whereas perivasal injections did not. These data indicate that for transcutaneous injections of

sclerosing agents to be effective in occluding the vas, they must be made directly into the vas lumen Although this is a technically more difficult procedure, the requirement should not limit its widespread use

In sharp contrast to the few studies on transcutaneous sterilization procedures with sclerosing agents in the United States, reports from the Peoples' Republic of China indicate that since 1972, this procedure has been used in over 500,000 men, with satisfactory results (2, 20, 21) The sclerosing agent used is carbolic acid and n-butyl alpha cyanoacrylate In spite of the very extensive experience with intravasal injections of chemical agents, very little information is available on the procedure either in Chinese or Western medical journals Precise data are not available on the effectiveness of the procedure or on the incidence and types of complications occurring either at the time of or after the procedure In the most recent report from the Peoples' Republic of China, long term follow-up data were given for two series of men (21) The first series included 919 men who had been followed up for up to 10 years The second series included 640 men who had been followed for up to 8 years The only stated difference between the two series was that in the second series 0 02 ml compared to 0 01 ml of the sclerosing agent was used Of the 1,345 men who were followed up and examined, small nodules could be palpated at the site of intravasal injection In 99 4% of the vasa examined, the diameter of the nodules was estimated to be less than 0 5 cm Only one man reported that the nodules were painful Follow-up data relating to the effectiveness of the procedures are summarized in Table 4 For both series, azoospermia was achieved in 95 9% of the men In the second series, the pregnancy rate among spouses was reduced from 11 5% to 2 6% Of the 109 pregnancies recorded in both series, 56% occurred to the spouses of men who had been shown to be azoospermic Since no information was given on the time between vas injection and the time the pregnancies occurred, it cannot be determined if the pregnancies occurred before azoospermia had been confirmed

The transcutaneous intravasal sterilization procedure developed by the Chinese is simple and requires the use of minimal surgical equipment (19, 20, 21) The following briefly describes the principal aspects of this procedure, which has been widely and successfully used in China since 1972

The vas is stabilized by use of a vas deferens fixing clamp This is a straight hemostat with flattened tips that permits the vas to be grasped without injuring the scrotum With the patient under local anesthesia, the operator clamps the vas and grasps it between the thumb and index finger A sharp needle is inserted into the vas perpendicularly The needle is withdrawn and a blunt needle is placed through the puncture hole. The operator can usually feel when the needle has entered the vas lumen Two simple tests can be performed to determine whether the needle is in the vas lumen

1 A syringe containing 4 ml of air is attached to the needle With the vas firmly compressed by an assistant at the site of puncture and at the distal end, 2 ml of air is injected into the vas The syringe plunger is released If the needle has been placed in the vas lumen, the plunger should return to its original position within a few seconds
2 A small amount of saline is injected through the blunt needle If the needle is in the vas lumen, the injection should proceed easily and should be made without undue pressure Examination of the scrotal skin and subcutaneous tissues should indicate no local edema

If the second test is used, all of the saline is aspirated from the vas before injection of the sclerosing agent The vas sclerosing agent (0 045 ml of carbolic acid, n-butyl alpha cyanoacrylate) is then injected through the blunt needle into the vas The drug polymerizes after about 20 seconds The needle is then withdrawn Only about 0 02 ml of the drug is actually injected into the vas The procedure is then repeated for the other vas and both puncture sites are covered with sterile gauze The procedure takes about 10 minutes to perform

Electrocoagulation The use of transcutaneous sterilization with electrocoagulation was first reported by Lee in 1964 (17) In that study, bilateral closure of the vas was obtained in four dogs and unilateral closure on two others In one dog, testicular atrophy was noted This atrophy was attributed to extensive burns to spermatic vessels other than the vasa In the Peoples' Republic of

First Series 919 men followed up to 10 years	
Semen analysis performed 456 men	
Azoospermic (%) 95 6	
Couples evaluated 829	
Pregnancies (%) 11 5	
Semen analysis for the 95 men whose spouses became pregnant	
Azoospermic — 54 7%	
Sperm in semen — 42 1%	
No semen analysis — 3 2%	
Second Series 640 men followed for up to 8 years	
Semen analysis performed 404 men	
Azoospermic (%) 96 3	
Couples evaluated 577	
Pregnancies (%) 2 6	
Semen analysis for the 14 men whose spouses became pregnant	
Azoospermic — 64 3%	
Sperm in semen — 21 4%	
No semen analysis — 14 3%	

Source Li S Zhu J (21)

Table 4 Summary of long-term follow-up data from The Peoples' Republic of China on the effectiveness of the transcutaneous injection of a sclerosing agent

China the use of transcutaneous electrocoagulation of the vas has been investigated in animals, but no information on the procedures was given (21)

In 1966, Schmidt first reported on a vasectomy procedure he had used in 144 men A bilateral incision was made in the scrotum, the vasa were divided, and the vas ends were electrocoagulated with unipolar electrodes (27) Schmidt found that the incidence of sperm granulomas could be minimized if the vas ends were electrocoagulated rather than ligated In a later series of 1,000 vasectomies in which electrocoagulation of the cut vas ends was used, Schmidt reported no failures, and fewer than 1 % of the men had clinically significant sperm granulomas or other complications (26) In the procedure used by Schmidt, the vasa were electrocoagulated in such a way that the lesion was confined to the vasal epithelium, lamina propria, and part of the muscle wall

To further advance the electrocoagulation procedure developed by Schmidt, bipolar electrodes have been developed The use of these electrodes can eliminate the danger of damage to vessels other than the vas resulting from stray currents, and at the same time confine the lesion to the vasal epithelium and lamina propria Preservation of most of the vasal muscles is thought to be important, because the muscle is the source of the fibrous tissue that ultimately results in occlusion of the vas

Adair developed a transcutaneous electrocoagulation procedure that used bipolar electrodes (1) In an initial series performed by the investigator, there were no failures and no clinically significant complications attributable to the procedures Subsequently, an independent, multiclinic evaluation of the procedure developed by Adair that included 33 men was curtailed due to the high failure rate of the procedure to attain azoospermia (24) In the second series, the only complication was a scrotal hematoma It has been suggested that the failure rate of the procedure was high because placement of the tip of the bipolar needle into the vas lumen was extremely difficult, due to the relative diameters of the bipolar needle and vas lumen (10) The bipolar needle has a diameter of 1 6 mm, the average diameter of the human vas lumen is 0 55 mm, but it may be distended to 1 2 mm (16)

Black, at the Marie Stopes Clinic in the United Kingdom, is currently investigating a transcutaneous electrocoagulation procedure (3) The procedure gradually evolved through several steps designed to provide a simpler, quicker, and less traumatic method of sterilization Initially, the vasa were electrocoagulated after they were pulled out through a small scrotal incision Electrocoagulation was then performed through a small scrotal incision, but with the vas in the scrotum The final step in the development process was to perform the electrocoagulations transcutaneously Black has performed about 80 of these procedures using prototype bipolar electrodes (3) Although the success rate of his transcutaneous procedure does not yet approach that of the standard vasectomy procedure used at the Marie Stopes Clinic, Black is of the opinion that improvement of the electrodes and some changes in the technique of performing the electrocoagulation will result in an effective procedure

In the United States, Denniston will evaluate whether electrocoagulation of the vas by insertion of the electrode into the vas lumen under direct vision, and without division of the vas, will result in high rates of vasal closure (9) The effectiveness of this procedure will be compared to the standard electrocoagulation procedure If electrocoagulation of the vas under direct vision, and without division of the vas, results in high rates of vasal closure, additional trials will be undertaken in which electrocoagulation will be performed transcutaneously

COMMENT

Over the past 20 years, a transcutaneous method of male sterilization has been sought in a haphazard manner and the limited developmental efforts have been sporadic In spite of the apparent simplicity of the procedure and its potential for widespread use and acceptance no systematic efforts have been undertaken in the United States to develop a transcutaneous procedure, except for the research supported by the Program for Applied Research on Fertility Regulation (PARFR) over the past 7 years

Compared to standard surgical vasectomy procedures, there are many advantages to the transcutaneous procedures that are applicable to both developed and developing countries, including a possible reduction in the incidence of certain complications commonly associated with vasectomy, such as scrotal hematomas and infections

In the United States, fewer than 100 men have had transcutaneous sterilization procedures either by injection of formaldehyde in alcohol or electrocoagulation In sharp contrast to this, reports from the Peoples' Republic of China refer to over 500,000 transcutaneous sterilization procedures using a cyanoacrylate mixture Unfortunately, the reports from China provide few data on the effectiveness of the procedure or on its short-term or long-term complications Also, information on the development of the procedures and any associated toxicology is not available in the literature

In view of the many advantages of the transcutaneous sterilization procedure, the importance of an organized research program is obvious if such a procedure is to become widely available in the United States and elsewhere Electrocoagulation of the vas and occlusion of the vas with sclerosing agents are the two best candidates for further development Regardless of the methods of transcutaneous sterilization used, criteria should be developed for determining acceptable failure rates of the procedures Failure can be defined in terms of either the pregnancy rates of the partners of the sterilized men or the proportion of men who achieve azoospermia within a specified time period Consideration should also be given to defining an acceptable proportion of men who do not achieve azoospermia but who become severely oligospermic

The scanty literature on transcutaneous male sterilization procedures indicates that the research efforts have been directed to developing a procedure that will result in azoospermia in a very high proportion of men after a single application of the agent This may not be feasible Therefore, research efforts might concentrate on a two application procedure Such a procedure could be highly effective with minimal rates of complications or side effects If, for example, a single application procedure produces bilateral vasal occlusion and azoospermia in only 90% of the men, then a two-application procedure can be expected to produce azoospermia in 99%.

In women, nonsurgical methods of sterilization have been evaluated extensively (14) Methods currently under investigation in the United States include one or two applications of MCA and three applications of quinacrine However, in the Peoples' Republic of China, nonsurgical methods of sterilization have been developed and used successfully since the late 1960s (34, 35) All of these methods are more difficult to perform than transcutaneous male sterilization and they have a higher risk of potentially serious complications, such as uterine perforation and intraperitoneal placement of the chemical agents

Also, assessing the effectiveness of the procedure in the female is more difficult If radiopaque MCA is used, flatplate x-rays can be taken to determine if MCA was present in the fallopian tubes Alternatively, some time after the procedure, hysterosalpingograms may be performed to determine if there is bilateral tubal closure

Neither of these two procedures is 100% accurate and neither is appropriate for areas where there are scarce medical resources In countries that have the available medical resources, flatplate x-rays and hysterosalpingograms significantly add to the cost of the procedure Evaluation of sterility in the male is a relatively easy matter Semen analysis may be performed with simple laboratory equipment by laboratory technicians who have minimal training If the two or three application nonsurgical sterilization procedure will be acceptable to women, there is no reason why a two application procedure should not also be acceptable to men

Future research efforts on nonsurgical methods of male sterilization need to focus on the use of sclerosing or occluding agents or electrocoagulation of the vas when delivered as either a one or two application procedure

REFERENCES

1 Adair EL Transcutaneous closure of the vas deferens a new procedure Unpublished 1980

2 Anonymous New method of male sterilization Chin Med J 93 205 1980

3 Black T Population Services Personal communication 1984

4 Bowman TA Senger PL Koger LM Gaskins CT, Hillers JK Blockage of sperm transport using intraepididymal calcium chloride injections in rams J Anim Sci 46 1063, 1978

5 Coffey DS Freeman C Vas injection a new nonsurgical procedure to induce sterility in human males In Sciarra JJ Markland C Speidel JJ (eds) Control of Male Fertility Hagerstown. Harper & Row Publishers 1975

6 Davis JE, Richart RM A new method for obstructing the vas deferens by direct injection of chemical agents a non operative technique of male sterilization Unpublished 1980

7 Davis JE New methods of vas occlusion In Zatuchni GI Labbok MH, Sciarra JJ (eds) Research Frontiers in Fertility Regulation Hagerstown. Harper & Row Publishers 1980

8 Davis JE Study of the vas occlusion in animals using chemical agents Unpublished 1982

9 Denniston G Population Dynamics Personal communication, 1984

10 Free M Program for the Introduction and Adaptation of Contraceptive Technology (PIACT) Personal communication 1984

11 Freeman C Preliminary human trial of a new male sterilization procedure vas sclerosing Fertil Steril 26 162, 1975

12 Freeman C Coffey DS Sterility in male animals induced by injection of chemical agents into the vas deferens Fertil Steril 24 884 1973

13 Freeman C, Coffey DS Male infertility induced by ethanol injection into the vas deferens Int J Fertil 18 129, 1973

14 Goldsmith A Edelman DA Nonsurgical methods of female sterilization In Sciarra JJ (ed) Obstetrics and Gynecology V 6 Philadelphia Harper & Row Publishers (in press)

15 Heber D Swerdloff RS Brain peptides and fertility control in the male In Zatuchni GI Labbok MH Sciarra JJ (eds) Research Frontiers in Fertility Regulation Hagerstown Harper & Row Publishers 1980

16 Huika JF Davis JE Vasectomy and reversible vas occlusion Fertil Steril 23 683 1972

17 Lee HY Studies on vasectomy I Experimental studies on nonoperative blockages of vas deferens and permanent introduction of nonreactive foreign body in the vas New Med J 7 117 1964

18 Lewis RW Garcia RR The results of epididymal ablation by sclerosing agents in the nonhuman primate Fertil Steril 41 465 1984

19 Li S Clinical application of the vas deferens puncture Chin Med J 93 69 1980

20 Li S Non operative sterility research with intravasal injecting drug a clinical report Reprod Contracept 1 24 1981

21 Li S Zhu J Non operative sterility research with intravasal injection drug (clinical report) Unpublished 1984

22 Lobl TJ Bardin CW Chang CC Pharmacologic agents producing infertility by direct action on the male reproductive tract In Zatuchni GI Labbok MH Sciarra JJ (eds) Research Frontiers in Fertility Regulation Hagerstown, Harper & Row Publishers 1980

23 Mahmoud KZ Abdou MS Hemeida NA Safouri LS Mahmoud S Girgis SM, Fahim MS Effect of ultrasound on mature rabbit testes (Abstract) Arch Androl 9 87 1982

24 Program for Applied Research on Fertility Regulation (PARFR) A multisite evaluation in developed and developing countries of a technique and equipment for transcutaneous closure of the vas deferens by electrocoagulation Unpublished 1981

25 Richart RM Li XZ A rapid and effective percutaneous intra vas injection for male sterilization Unpublished 1984

26 Schmidt SS Prevention of failure in vasectomy J Urol 109 296 1973

27 Schmidt SS Technics and complications of electrical vasectomy Fertil Steril 17 467 1966

28 Setty BS Dasgupta PR Kar AB Chemical occlusion of vas in rats Contraception 6 329 1972

29 Smith DK Rodriguez Rigau LJ Steinberger E Hormonal methods for male contraception In Zatuchni GI, Labbok MH Sciarra JJ (eds) Research Frontiers in Fertility Regulation Hagerstown Harper & Row Publishers 1980

30 Vasectomy — safe and simple Population Reports Series D No 4 November December 1983 Population Information Program The Johns Hopkins University

31 Vasectomy What are the problems? Population Reports Series D No 2 January 1975 The George Washington University Medical Center

32 Weibe JP Barr KJ Suppression of spermatogenesis without inhibition of steroidogenesis by a trihydroxypropane (glycerol) solution (Abstract) Biol Reprod 28 (suppl 1) 256 1981

33 Weibe JP Barr KJ The control of male fertility by 1 2 3 trihydroxypropane (THP glycerol) rapid arrest of spermatogenesis without altering libido accessory organs gonadal steroidogenesis and serum testosterone LH and FSH Contraception 29 291 1984

34 Wu Y-H Qian P L Tien S P Nonsurgical procedures for manual instillation of a phenol atabrine paste (PAP) for female tubal occlusion In Zatuchni GI Shelton JD Goldsmith A, Sciarra JJ (eds) Female Transcervical Sterilization Philadelphia Harper & Row Publishers, 1983

35 Zheng H G Chen X H A study of phenol mucilage induced tubal occlusion for sterilization Analysis of the results in 4 784 cases In Zatuchni GI Shelton JD Goldsmith A Sciarra JJ (eds) Female Transcervical Sterilization Philadelphia Harper & Row Publishers 1983

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RESEARCH FRONTIERS IN FERTILITY REGULATION

Methods of Monitoring Ovarian Function and Predicting Ovulation: Summary of a Meeting

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Ovulation itself cannot be observed externally The only definite proof of its occurrence is establishment of pregnancy, which is normally the outcome being regulated We are therefore forced to predict the *timing* of ovulation on the basis of indirect indicators that lie at varying physiological distances from ovulation itself The distances result in uncertainties that can be resolved only with statistical analyses, and then only imperfectly Unfortunately, the ability to predict the timing of ovulation indirectly does not guarantee that the predicted ovulation does, in fact, take place The imprecision and uncertainty of current indirect methods provide the impetus for monitoring ovarian function and predicting ovulation

Other pressures to improve current methods and develop new ones come from various health and research groups Natural family planning (NFP) programs seek to improve the reliability of methods for defining the fertile period of the menstrual cycle and to extend their utility to periods of lactation, onset of menarche, and the approach of menopause Artificial insemination and *in vitro* fertilization programs look for more complete information on the timing of ovulation and the period of optimum fertility to maximize the chances of conception while minimizing the cost and inconvenience of preliminary testing

Excellent reviews exist covering broad aspects of the problem of predicting ovulation (36,47,49,81,82,85,86) or dealing with specific methodologies (7,14,16-18,40,42,50, 53 56,69,73,75,76,79,80,83,84) However, a comprehensive discussion combining the state of the art with practical considerations of method application was needed

To assist groups administering family planning programs and to help investigators not trained in clinical chemistry to choose the best techniques among the wealth of methods presently available, an international meeting on Methods of Monitoring Ovarian Function and Predicting Ovulation was organized and sponsored by Family Health International, with a grant from the United States Agency for International Development (USAID)

The meeting, which was held in Durham, North Carolina in December, 1984, contrasted the most sophisticated methodologies for monitoring ovarian function and predicting ovulation, as used by *in vitro* fertilization clinics, with the limited methods available for use in anthropological field studies and in most NFP programs The goal was to stimulate discussion of available methods in terms of their limitations and potential These discussions provided a basis for deciding which methods could be used most successfully in any program or study Additionally, they helped define what approaches require further development or testing and what that development or testing should entail

Another purpose for the meeting was to stimulate a general exchange of information among the participants to catalyze formation of the collaborations necessary to produce a new generation of techniques These should be less invasive and more rugged, reliable, and precise than the present ones They should also monitor ovarian function and/or predict ovulation in a wide variety of clinical and nonclinical settings

The meeting did not attempt to address all known

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MONITORING FOLLICLE DEVELOPMENT & THE MENSTRUAL CYCLE

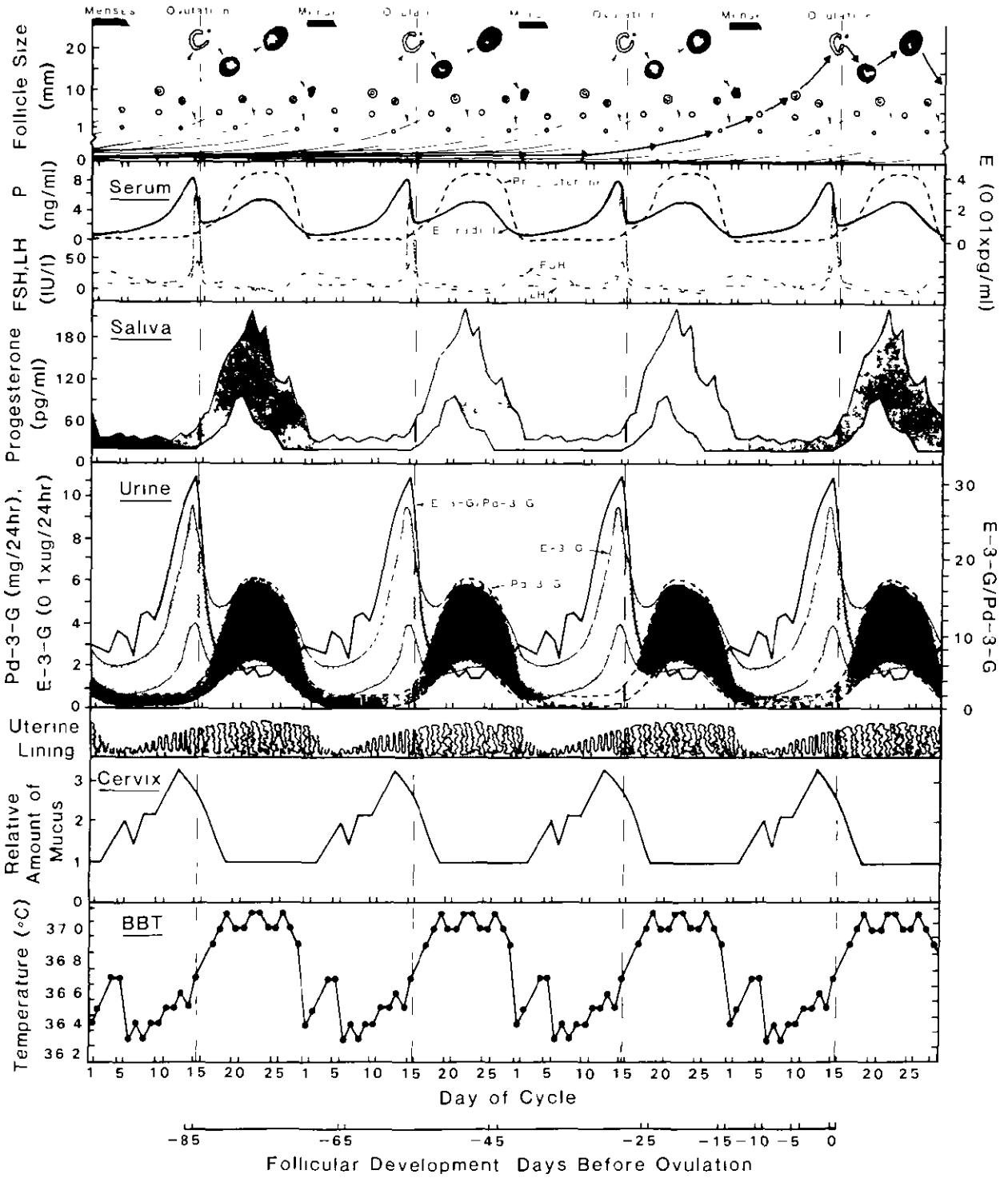

Figure 1 Selected events occurring during development of an ovulatory follicle Note that development occupies three full cycles Parameters include size of developing follicles measured by ultrasound or during laparoscopy, gonadotropin concentrations in serum, estrogen and progestogen concentrations in serum, saliva, and urine, ratio of urinary steroids state of endometrial proliferation, relative cervical mucus volume, and basal body temperature An average 28-29 day cycle was used as a model to which were fitted results presented in several references (4 8 9,49,55 73,75) Confidence intervals for salivary progesterone include ±1 standard deviation of the mean (55,73), for urinary steroids, the 80% confidence interval is shown (9)

methodologies It did, however, touch upon a broad spectrum This report is a summary of the meeting, the complete proceedings will be published at a later date

THE BIOLOGY OF OVULATION

Folliculogenesis and the Menstrual Cycle

Follicular growth and several of its correlates are summarized in Figure 1 Growth, beginning about 85 days before ovulation (26) involves expansion of the ovum from about 20 μm to 80 μm (3), an increase in follicular diameter from about 30 μm to 18-27 mm (42,53), and a spectrum of cellular maturation events in both the ovum and the follicle cells At about days 5 7 of the ovulatory cycle, estradiol-17β, 90% of which is produced by the preovulatory follicle (3,4), begins to rise toward a peak that occurs, on average, 1 day prior to ovulation (86) The exponentially rising estradiol on days 11-13 triggers release of a surge of LH that begins 32±6 hours before ovulation and peaks about 15 hours later (15,68,81,85,86) Rising estradiol also stimulates proliferation of the uterine endometrium and the production of a watery, stretchy form of cervical mucus Metabolites of estradiol produced outside the ovary, including estrone-3-glucuronide (E_1-3-G), begin rising in urine about 1 day after commencement of the increase in follicular production The midcycle LH surge alters metabolism within the follicles so that they begin to produce progesterone in preference to estradiol Within 26 28 hours after the beginning of the LH surge, the oocyte completes the first meiotic division and becomes competent for fertilization (13,15)

After ovulation, the follicle cells that remain in the ovary metamorphose into the luteal cells, forming the corpus luteum As the corpus luteum forms, it produces increasing amounts of progesterone, as well as some estradiol Production of progesterone halts the production of cervical mucus and coincides with a decline in LH It also increases urinary levels of the major progesterone metabolite pregnanediol-3α-glucuronide (Pd-3-G) (62) and causes a rise of 0 2-0 5°C (0 4-1 0°F) in basal body temperature (BBT) (27,47) If fertilization does not occur, the corpus luteum regresses about 9-11 days later (22) The fall in circulating steroids causes the shedding of the endometrium during menstruation, about 10-14 days after ovulation (57,67,71,80)

Gamete Viability and the Fertile Period

Two of the main objectives in monitoring ovarian function or predicting ovulation are to increase or decrease fertility To accomplish either, the life-spans of gametes within the female reproductive tract should be known Estimates based on cervical mucus (CM) evaluations, BBT charts, and reported coitus and pregnancy have been computed (5,8,58,81) Royston (58) estimates ovum

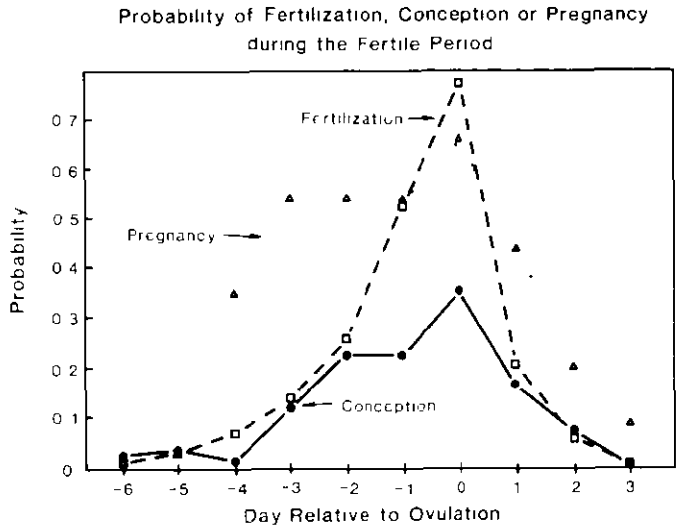

Figure 2 Probabilities of fertilization, conception, and pregnancy during fertile period (8,58) Fertilization and conception were calculated from a mathematical model in conjunction with a large collection of BBT charts and pregnancy records Pregnancy was estimated from data in a multicenter WHO study (79)

life-span after ovulation to be 0 7±0 1 day (± 1 standard error of the mean) with a 99% confidence interval reaching 3 2 days, sperm viability is estimated to be 1 47±0 20 days, with a 99% confidence limit reaching 6 8 days The period of maximal fertility will thus extend from about 1 5-6 8 days before ovulation to 0 7 3 2 days after ovulation This is borne out by the probabilities that single acts of intercourse occurring on individual days near ovulation lead to conception (8,58,79) *(Figure 2)* It is estimated that 90% of all pregnancies occur in an interval extending from 4 days before ovulation to 1-2 days after ovulation or, since ovulation cannot be observed but the timing of the LH peak can, from LH peak -3 to LH peak +2 (13,58)

Given the physiologic ontogeny described, predicting the limits of the fertile period means observing a clear change in one index, such as estradiol, at least 4 days before ovulation and another change, such as an increase in progesterone, 1-2 days after ovulation

Chronology of Indicators

Table 1 provides a chronology of indicators that have been used to predict the limits of the fertile period (FP) and the time of ovulation Most of these indices or end points involve determinations of rises above a baseline or attainment of a peak value Prospective evaluation of such quantitative changes requires serial sampling The necessary sampling frequencies increase as the duration of the changes sought diminishes, e g , detecting the brief peak of the LH surge requires more frequent samples than detecting the broad estradiol peak Algorithms

(problem solving schemes) using these quantitative shifts or changes to predict limits or events must take into account the intervals between samples and are no more accurate than the sampling interval used A rise may be detected as an abrupt increase in variance about a moving average (66,77), e g , BBT based methods (8,47), or as a cumulative sum or assay result exceeding a concensus threshold value, e g , Royston's treatment of BBT and urinary steroids (13,15,37,57,59,81) or the adjustment of the detection limit for Brown's home assay kit for Pd 3-G (9) Detection of a peak necessitates identifying a decline following a series of increasing values or a sustained decline in the rate of rise of an accumulating sum By its nature, a peak can only be identified retrospectively

Endpoint	*Mean*[a]	*SD*	*Range* *Min*	*Max*	*Source*
First day of mucus	-7 3	2 5	-13	-3	81
Rise of E 3 G Pd 3 G	-7 2	2 8	-15	-2	81
Rise of urinary E 3 G	-5 6	1 9	-11	2	81
First day of fertile mucus	-5 1	2 6	12	1	81
Rise of salivary estradiol (E_2)	5 0		-		55
Rise of serum E_2	3 4[b]	0 9[c]	- 7	2	86
Follicle Size 15 ± 2 mm	-2 7	0 5	—	—	42
Peak of E 3 G Pd 3 G	-2 5	2 3	-10	0	81
Peak volume of mucus	-2 0	1 0	- 4 5	-1 5	9
Peak of salivary E	-1 5	—	—	—	55
Peak of urinary E 3 G	-1 3	1 9	- 9	-4	81
Rise of LH	-1 3[b]	0 3[c]	- 2 3	-1	13
Rise of serum E_2	-1 0[b]	0 3[c]	- 2	0	13
Rise of LH	0 7[b]	0 2[c]	1 9	0 3	13
Peak of fertile mucus	0 4	2 2	10	5	81
Rise of serum progesterone (P)	-0 3[b]	0 3[c]	1 3	0	13
Rise of urinary Pd 3 G	0 2	3 0	—	-	57
Rise of BBT	1 1	2 0	- 2	7	57
Rise of salivary P	1 5	—	—	—	55

[a] Values were recomputed from listed sources by adjusting for relative average time of ovulation after the LH peak and by subtraction of any constants added in the original sources

[b] median

[c] 95 confidence limits of the estimated median

Table 1 Chronology of endpoints predictive of limits of fertile period and timing of ovulation in days relative to ovulation (=LH peak + 0 7 days)

The success rates of several methods in predicting the limits of the fertile period or the timing of ovulation are summarized in Table 2 Single endpoints combined with computations achieve 80-90% accuracy in predicting limits of the fertile period that fully encompass the defined interval There is an obvious parallel relationship between predicted length of the fertile period and predictive success The 10-11 days suggested by maximum gamete life spans may be required to achieve success rates in excess of 90% Combinations of two or more methods can prospectively delineate the fertile period in >95% of cycles Such prediction rates are needed for confident use of the methods by individual women monitoring individual cycles Unfortunately, in lactating women the success rates drop dramatically One reviewer found that the symptothermal method (STM) of NFP, which uses multiple indices of fertility, failed to accurately define the fertile period in 4 5-13 9% of cycles monitored (27)

Since predictive errors of a single day can markedly increase the probability of conception (see *Figure 2*), reducing incorrect definition of the fertile period from 10% to 5% could diminish the observed conception rates by nearly half, even if periodic abstinence is the only contraceptive measure being used (13) This would be an important contribution in areas where periodic abstinence methods are frequently used or in those groups that suffer lower success rates normally, e g , lactating or perimenopausal women

Method	*FP Duration*	*SD*	*Range* *Min*	*Max*	*FPs Entirely Covered*	*Source*
Limits						
CM + E 3 G			-	-	100	—[a]
STM (BBT + CM)	13 4	2 9	9	21	98	81
CM	11 9	2 9	5	19	91	81
BBT + calendar	11 8	3 3	6	21	90	81
E 3 G (rise + peak)	9 3	2 2	6	15	83	81
E 3 G Pd 3 G (rise + peak)	10 7	2 3	8	17	84	81
Salivary E	—	—	—		86	13
Salivary E_2 + P	—	-			80[b]	-[a]
Ovulation						
CM		—	—	—	90	84
Ultrasound[c]	—	—	—	—	88	15
Rise of LH	—	—	—	—	83	23

[a] Reported by W P Collins at the FHI Meeting on the basis of a WHO study comparing various immunochemical methods

[b] The small number of cycles evaluated for salivary steroids and the combinations used for predictions probably account for a value for salivary E + P lower than that for salivary E alone

[c] Compared to laparoscopic evidence

Table 2 Accuracy of several methods at prospectively defining Fertile Period (FP) (fully encompassing LH peak -3 days to LH peak +2 days) or predicting ovulation to within ± 12 hours Analyses were accomplished using the CUSUM analysis of Royston (57, 59, 81), the LH peak was located retrospectively by a concensus reference method using assay of LH in serum or urine and or ultrasound

If the exact timing of ovulation itself needs to be predicted, CM or STM, which show considerable variability (see *Table 1*), should be augmented by other indicators such as observations of the increase in follicle size, the rise or peak in LH levels, the attainment of peak estradiol, or the initial rise in progesterone The art of combining indices to predict the timing of ovulation has been highly refined by *in vitro* fertilization clinics It is routine to remove oocytes from follicles after they have completed meiosis I but before they have been ovulated, a window of roughly 2-4 hours (15,21-23,68)

CURRENT NFP INDICES

Cervical Mucus

The cervix produces thick, sticky, opaque mucus an average of 5-10 days prior to ovulation, in response to rising levels of estradiol in the absence of progesterone (81) As estrogen titers rise further, copious amounts of a lubricative, stretchy, transparent mucus, referred to as "fertile-type" mucus, appear on day -8 to -2 (81) As estradiol titers continue to rise, the volume of "fertile" mucus continues to increase until 1-3 days before ovulation (6,7,10,49,69,81) Production of slippery mucus continues until about half a day before ovulation (81) This last day of production of fertile-type mucus is called the "peak" day and is followed by 0-3 days during which sticky, opaque mucus is produced as estrogen levels decline and progesterone levels rise following ovulation (35) After sufficient training (1-3 months) to correctly recognize and chart changes in mucus, a woman can use appropriate rules to delineate the limits of her fertile and infertile periods and the approximate timing of her ovulations

The increase in quantity of cervical/vaginal fluid was investigated by Usala and Schumacher (69) and led to the development of the Ovumeter cervical fluid aspirator The device is a modified syringe that is inserted deep into the vaginal vault, near the cervix, and then filled Prediction of ovulation and the end of the fertile period can be made by charting fluid volume aspirated versus day of the cycle and by applying an appropriate algorithm Reported accuracy is near 75%, and cost is minimal Potential difficulties in properly and reproducibly inserting the device and collecting cervical mucus may diminish its possible impact Additional field trials will be needed before any final conclusions as to its utility can be reached

Interferences with mucus evaluation methods can arise from vaginal infections, seminal drainage after intercourse, individual variation in the detection of various mucus types, or individual variability in estrogen response The degree of interference usually diminishes as the user gains experience

Cervical mucus patterns also show considerable variation during lactation (10,27) In a study of 55 women over 100 cycles, half of those cycles shown to have defective luteal phases showed abnormal mucus patterns, the incidence of normal mucus patterns increased from 58% during the first postpartum cycle to >80% beyond the fifth postpartum cycle (27) Brown (10) reported that 42% of a group of 33 lactating women did not have mucus symptoms that matched the steroid patterns found in urine

CM evaluations and BBT charting are physically noninvasive, but the time and effort involved in collecting and recording these data can be significant, so use of these methods requires strong motivation Even though the intensive training and rules surrounding collection and use of data do alter life-styles, the methods seem well accepted by many women Moreover, the existence of the support network involved in training and the benefits derived by women using these methods often serve as powerful incentives to continue their use

Method costs are negligible, although training and follow-up costs can be substantial, depending on the services provided

Easily accessible indices such as CM and BBT have proven reliable for normal, healthy women as long as the guidelines for data collecting, recording, and interpretation are followed (7,8,47,83,84) However, the range of variability in these endpoints has provided impetus for development of hormone measurement systems Further testing and hormonal verification of strategies using CM, BBT, or other indices such as cervical position are necessary in perimenarchial, perimenopausal, and lactating women — groups that experience the widest variations in hormone and cycle patterns

This may be best accomplished in two stages First, a limited pilot study of correlations between predictive effectiveness of the physiologic indicators and biochemical indices in lactating women should be done This should be followed by a second, larger study of the efficacy of NFP indicators based on pregnancy rates corrected for fetal losses and the presence of luteal defects Conduct of a study evaluating the extent of subjective biases caused by anticipating "correct" results in the application of these methods among regularly menstruating or lactating women also seems appropriate Development of devices, e g , poetic or musical lists of rules or plastic cumulative sum calculators resembling star guides, which simplify training, data collection, and data recording, should also improve the reliability of these methods

Basal Body Temperature

Basal body temperature rises 0 2-0 5°C (0 4-1 0°F) due to the midcycle increase in progesterone caused by the transformation of the ovarian follicle to the corpus luteum in response to the LH surge Although progesterone begins to rise about 24 hours after the beginning of the LH surge or 8 hours before ovulation, the rise from the hypothermic plane existing during the follicular phase to the hyperthermic plane present during the luteal phase can be observed only an average of 1 day after ovulation Moreover, since temperature shifts also occur in response to other agents, the menstrual cycle shift must be verified by a continuation of the elevated temperatures for at least 3 days (65) Therefore, being strictly retrospective, BBT cannot predict ovulation It can only detect probable ovulation and can only predict the end of the fertile period

The BBT rises, which occur over a period of 2-8 days, assume several characteristic forms including a smooth, rapid rise, a smooth slow rise, a slow, step wise rise, and a slow, saw-tooth rise (47) The uncertainty of the correlations of these multiple forms with the underlying hormonal levels and exact stage of follicular development leads to a conservative definition of the limits of the preovulatory fertile period the entire follicular phase These uncertainties also result in the rule that to avoid pregnancy 3 days of abstinence (5 days for a smooth, slow rise) are required following the initially observed BBT increase, even though this increase usually follows ovulation by a period approximating the life-span of the viable oocyte The rules provide protection from risk of pregnancy but entail extensive abstinence that may result in method failures due to "rule breaking "

The small increment of BBT has spawned a number of technical innovations that make its occurrence more obvious mercury thermometers with expanded scales electronic thermometers, some supplemented with the microcircuitry required for data storage and computation, and recording charts with vertically expanded scales (27,47,51)

This index is susceptible to many sources of error reading and recording temperatures, the presence of infections or emotional upsets, or nonadherence to the rules for reading BBT The rules specify that a woman should take her temperature by mouth, vagina, or rectum Readings may be made 3-5 minutes after inserting a mercury thermometer Vaginal or rectal temperatures may be read 15-90 seconds after inserting an electronic thermometer, but oral readings must allow at least 2 minutes for temperature equilibration The woman should take her temperature at the same time each morning prior to engaging in any type of activity Royston's suggested subtraction of 0 1°C per hour delay in measuring the temperature (60) serves only as a rough correction Alone, it cannot correct for variations in lifestyle that were not envisioned when the rules for using BBT were formulated

Monophasic BBT charts lacking a discernible temperature rise suggest anovulation or luteal defects The incidence of these aberrations is high during lactation, menarche and menopause (27 29,48) Vollman (71) showed that the incidence of monophasic BBT charts is age dependent During the year of menarche 55 7% of menstrual cycles showed monophasic BBT charts By gynecologic age 23 29, the proportion dropped to 1 2% then rose to 34% in the perimenopausal period After resumption of menses during lactation Hatherley found that 34% of the 273 women in his study recorded monophasic BBT charts (34) Short luteal phases may be detected by the presence of short, <8 day, hyperthermic periods on BBT charts (27,28,51) The frequency of monophasic BBT charts is also cycle length dependent 57 1% in cycles of <17 days, 1 8-4 8% in cycles of 25 32 days, and 41 3% in cycles of >60 days (71)

Algorithms

Algorithms for prospectively interpreting BBT begin by establishing a baseline, e g , the average of the temperature from days 4-11 after the beginning of menses A coverline or reference line, is drawn 0 05°C (0 1°F) above this baseline and serves to approximate the upper bound of a confidence interval allowing statistical comparisons between the baseline mean and the subsequent daily temperature measurements A rise above the coverline signals the recent occurrence of ovulation and allows computation of the end of the fertile period

Royston (57,59) also suggests a cumulative sum technique (CUSUM) where differences from a coverline are summed and tested against a "decision interval" that combines the signal to be tested with an adjustment of the baseline for the signal's potential variability The results for BBT are cross-checked by the coverline technique, three consecutive temperatures must equal or exceed the reference level The same approach is being adopted for prospectively testing steroid or gonadotropin measurements In these cases, transformation of the observed signal pattern to its first derivative, as embodied by the CUSUM, improves the sensitivity of the algorithm for detecting rate of change and consequently also for detecting increases in signal level This improvement is most important for signals that change slowly e g , estradiol levels, it has much less impact on those such as LH that change rapidly and are already easily detected by using threshold levels

Introduction of additional algorithms based on mathematical models of the physiological process or on trend

statistics that incorporate information on sampling frequency should improve the predictive capabilities of both the physiologic evaluation methods, e g , CM volume, and the biochemically based methods, e g , serum estradiol levels Effort seems best directed to development of formulae that mathematically combine the results of several endpoints into single values that can be used as predictors The reduction in variability and the increase in predictive reliability resulting from such approaches is exemplified by the use of the E_1-3-G/Pd-3-G ratio in preference to separate measurements of E_1-3-G or Pd-3-G (13,15-18,57,81,82) For the purposes of monitoring ongoing programs, efforts should also be directed toward development of techniques for extracting the maximum amount of information on ovarian function from single, cross-sectional, or infrequent samples

The Symptothermal Method (STM)

BBT and CM are most effective in defining the fertile period when used together in STM *(see Table 2)* In contraceptive practice the users learn rules governing data collection (daily BBT and CM evaluations) and charting, and procedures for interpreting and applying the results The method can also be used to increase the probability of conception by concentrating intercourse near the middle of the fertile period

Like CM, the method requires a lengthy training period, 3-12 months, as well as support and follow-up It also requires a number of signs to be monitored and recorded daily to allow correct application of the various rules and computations Although these requirements may lead to low continuation rates that suggest a need to augment STM with other methods such as urinary steroid home-assay kits (9,10), it defines the fertile period in 98% of cycles studied (81) and is very well received among women who continue to use it (47)

Cervical Changes

Other changes in the cervix can be monitored to identify the phases of the cycle (27,40,41,51) This can be done by self- or partner-assisted palpation of the position, resilience, and openness of the cervix During the fertile phase the cervix is higher, straighter, less apposed to the vaginal wall, softer and more open Although the method is ostensibly simple, definitely inexpensive, and less influenced by the presence of infection or semen than CM, it is subjective and greatly dependent on the woman's motivation It requires considerable training, support, and practice to differentiate the symptoms and to utilize them with confidence (27,47,51) These indices have not yet been evaluated in multicenter clinical trials, nor has synchronization of the symptoms with CM signs or hormone levels been fully tested Still, these are potentially useful indicators of the time of ovulation and the limits of the fertile period

OTHER INDICES

Ultrasound

Follicular size monitored by ultrasound is the most proximal predictor of impending ovulation, it can serve as a reference for other methods Follicles are imaged as areas having ultrasound density identical to the adjacent full bladder but contrasting with surrounding, less dense ovarian tissue Objects of 5-10 mm are clearly discernible to ±1 mm (4,13,15,26,42,50,53) Ultrasound is capable of detecting growing follicles as early as the first 5 days of the menstrual cycle, can register the presence of pre-ovulatory follicles of at least 15 mm in diameter at 2-3 days prior to ovulation, and can sometimes indicate the presence of a detached, ripe ovum cumulus complex within a Graafian follicle approximately 16-24 hours prior to ovulation (W P Collins, FHI meeting) Although ultrasound serves as an excellent index of imminent ovulation, several reports (13,15,61,68) indicate that there is a rather wide range of follicular sizes (17-27 mm) from which mature, fertilizable eggs may be aspirated This variability in attained size and attendant steroid content (11) limits the predictive precision of ultrasound However, since it can detect the collapse of the antrum following ovulation, ultrasound has an internal retrospective check on the accuracy of its own predictions

Ultrasound must be done serially if accurate information is to be obtained Moreover, it depends on modestly sophisticated electronics at present and cannot determine the biological functionality of the follicles examined Until the beginning of the fertile period, even serial ultrasonic scans cannot distinguish which of the remaining 4-6 growing follicles will become atretic and which will eventually ovulate (4,13,15)

The introduction of inexpensive, portable instruments may make this method accessible to many clinics and or women in the near future, at least in developed countries

Gonadotropins

Gonadotropins directly stimulate follicular growth and maturation Although FSH fluctuates during the cycle, the chemical lability of the molecule makes it difficult to assay The similar, but less pronounced, pattern of its changes compared with those of LH make measurement of LH more desirable for predicting ovulation The exact patterns obtained are critically dependent on the frequency of sampling (2,87) They result from the interplay of two factors 1) pulsatile releases of gonadal and pituitary hormones that result ultimately from the pulsatile nature of LHRH release by neuroendocrine cells within specific areas of the hypothalamus, and 2) clearance of the hormones from the bloodstream by catabolic tissues The synthetic and catabolic tissues are susceptible to modulation by internal neuronal or hormonal inputs, they

frequently demonstrate their own unique circadian release patterns, which change with age (21,68,67), and they are influenced by such exogenous factors as diet, stress, and medication

Most assays utilize serum as the source fluid, but changes are also measurable in urine about 3 hours after they occur in serum, e g , Hi-Gonavis (Mochida, Tokyo) or OvuSTICK (Monoclonal Antibodies, Inc , Mountainview, CA) colorimetric dipstick measurement of LH (15,23,37,68) These proteins do not pass into saliva or other secreted fluids The rise and peak in serum or urinary LH levels are tightly clustered at midcycle, with only relatively minor fluctuations outside this period, the surge thus serves as a temporal reference point for establishing the chronology of events during the cycle The rise and peak occur much too late, however, to predict the onset of the fertile period

Serum or Urine? The rapidity of the LH surge requires frequent sampling Unless urine is used, this presently involves repeated venipuncture Although venipuncture is physically invasive, it is widely accepted because of its association with palliative and curative medicine Still, it often cannot be used effectively for frequent serial sampling because of problems with trauma, infection, and red cell depletion Adoption of an alternative sampling protocol using a finger lance (30) may overcome these problems but may not be as well accepted because of a lack of historical ties to medical practice The informational content of serum may still make it the fluid of choice, even if only rather infrequent samples can be collected

Although urine collection might be considered easier than serum collection, it may be socially unacceptable in some cultures The problem can be exacerbated by any requirements to accumulate and store urine specimens either for 24 hour periods or as serial samples Use of short-term, spot, or early morning urines (EMU) will help The best approach may be the use of self-tests that do not require supervision or storage and thereby minimize any embarrassment or inconvenience

Assays. Quantitation of LH can be accomplished by any of a number of immunoassay techniques (14) In contrast to steroids, there are no rapid, high volume physicochemical reference assays, such as gas chromatography or mass spectrometry, currently available Some of the choices available for gonadotropin or steroid immunoassays are summarized in Table 3 Assays now in use for gonadotropins and prolactin in serum or urine perform well with regard to specificity, parallelism, accuracy, bias, precision, and sensitivity Assays for progestogens in serum, saliva, and urine also meet high standards for these criteria, as do assays for estrogens in serum Estrogen immunoassays in other fluids and progestogen assays in milk do, however, need further characterization before they can be applied with equal confidence

Immunoassays rely heavily on the characteristics of the antisera employed for their specificity and sensitivity In turn, the production of the antisera relies on the purity and molecular distinctiveness of the available isolated hormones to assure specificity If one hormone is contaminated by another or strongly resembles it, an immunization will generate a mixture of antibody molecules that bind to both hormones Unless the antisera are extensively treated prior to use, they cannot be expected to demonstrate absolute specificity Despite the fact that isolates of LH, FSH, hCG, and TSH suffer at least minor purity and resemblance problems, many excellent antisera have been produced The most specific ones are directed against the binding sites present on the hormonally unique β subunits of the above two-subunit hormones

Antisera	1	Polyclonal		
	2	Monoclonal		
	3	Chimeric		enzyme is part of antibody molecule
Format	A1	Homogeneous		no separation of bound from free ligand
	A2	Heterogeneous	a	Precipitate bound with 2nd Ab Protein A, 2nd Ab coated magnetic or polymeric beads solvent perturbation by PEG PVA, PVP salts
			b	Precipitate free with dextran coated charcoal
			c	Rinse Ab Ag coated test tubes, microtiter wells, sticks, filters
	B1	Immunoassay		limited Ab (background affects at high values)
	B2	Immunometric assay		excess Ab (background affects at low values)
	C1	Direct assay	a	sample not pretreated
			b	Acid or noncompetitive modifier added
	C2	Extraction assay		Haptens extracted with solvent & purified before assay, tracers added to monitor recovery
Endpoint	1	Label	a	Radioactivity moderate sensitivity, -H high sensitivity, -I, S
			b	Intrinsic labels or conjugated enzymes
			i	Optical signal turbidity, visible chromogen, phosphorescence luminescence, fluorochrome (organic molecule rare earth chelate time resolved signal)
			ii	Electrical signal redox label, biosensor
	2	Site of label	a	Ligand
			b	Primary antisera
			c	Secondary antisera (in "sandwich" assays)

Not all variants are available for all ligands Disposal costs of radiation stimulate development of other forms of assay The least expensive assays will probably be those direct immunoassays using optical signals readable on inexpensive equipment luminometers fluorimeters or colorimeters

Table 3 Some immunoassay variations available

Monoclonal Antibodies. The generation of monoclonal antibodies against the various hormones has gone far to diminish cross-reactions In this approach (43), the spleen of an immunized mouse or rat is isolated and the individual lymphoid cells are fused with myeloma cells The cultured progeny of these fused cells are clones and secrete single types of antibody molecules selective for unique antigenic determinants on the hormone molecules originally injected The same spectrum of antibodies is produced as in the usual *in vivo* polyclonal approach, but here each of the antibody types is produced in only one of several hundred tissue culture wells Specific, single molecular species of antibody that do not demonstrate cross-reactions and that provide the other assay characteristics desired can be chosen and employed in various forms of assay

Genetically Engineered Hormones. Interassay and interlaboratory variability would be drastically reduced by the production of single hormones from genetically engineered mammalian cells Reports now exist of the cloning and successful introduction of the genes for both subunits of human LH into cultured mammalian cells that subsequently secrete hormone into culture media (44) As these materials become available, they should serve as a basis for assay standardization along with the use of monoclonal antibodies Until they are being produced by more than one or two firms or laboratories, it may be advisable to initiate a quality monitoring program to ascertain that properties of the products do not deviate substantively with time from those of the current purest hormones

Reference Reagent Collections Reference reagent distribution programs exist at the National Institutes of Health, the World Health Organization, and the National Institute for Biological Standards These programs address the generally limited availability of hormones purified to homogeneity and help laboratories establish concensus standards for use in testing and monitoring assays Inclusion in these collections of stores of secondary reference preparations, commonly used antisera, newly developed monoclonal antibodies, and genetically engineered protein hormones should improve the current problems with interlaboratory variability and quality control Ready access to secondary standards derived from all of the bodily fluids should improve the database for establishment of concensus values and will go far toward providing benchmarks for use in development of new assays

Costs. In assaying large numbers of samples, the costs of materials, personnel, and equipment are all important factors For the physicochemical techniques, such as mass spectrometry, used for assessment of steroids, the cost per sample may be hundreds to thousands of dollars Immunologic approaches have normally been less expensive The cost of the equipment and personnel required to run a clinical assay laboratory and to develop antisera and reagents for its use results in charges for each sample tested on the order of $3-$50 (U S) Actual reagent and materials costs for most of these assays are less than $5, frequently less than $1 Thus, if a laboratory must be established to develop or run the assays, costs will be substantial until the volume of samples disperses them

Necessary repeated generation and testing of new antisera to the same antigens has in the past inflated the cost of most immunoassays Introduction of monoclonal antibodies and genetically engineered hormones, chimeric antibodies, and enzymes that may be produced in bulk quantities, tested once and distributed for many years, should decrease this aspect of assay cost measurably Costs will also decline as radioisotopes and their attendant disposal costs are superceded by other labels

Steroids and Steroid Metabolites

Serum. In serum, total estradiol is a sensitive index of ovarian function capable of predicting the onset of the fertile period and ovulation, as well as reflecting the initiation and termination of luteal function *(Figure 1)* (2,4,6,8,13,15,16,37,42,49,56,68,77,80,81,85,86) Levels rise during the follicular phase from 1-5 pg ml (4-20 pmole l) to 100-400 pg/ml (400-1600 pmole l) on the day before ovulation They then fall to 20-100 pg ml (80-400 pmole l) on the day of ovulation, before rising again to 100-200 pg/ml (400-800 pmole l) during the midluteal phase Deviations from this characteristic pattern can signal specifically follicular or luteal malfunctions

Changes in total progesterone extractable from serum define not only the period of formation and function of the corpus luteum but the adequacy of its function Levels rise from a baseline of less than 1 ng ml (3 nmole l) during the follicular phase to a plateau of 10-20 ng/ml (30-60 nmole l) 3-9 days after ovulation in the normal cycle Thereafter, they fall back to baseline during luteolysis, on average 12±2 days after ovulation (22,57,67) The initial rise in progesterone signals the beginning of responses to rapidly rising LH levels and precedes the usual time of ovulation slightly, making it a very late predictor of ovulation and an index of the end of the fertile period (9,13,15,16,81,85,86) The rise is strongly suggestive of ovulation but does not prove its existence since normal luteinization can occur in the presence of an entrapped oocyte The level and duration of the progesterone plateau do, however, indicate the sufficiency of luteal function If these levels fail to rise to roughly 5-7 ng ml (15-25 nmole/l) or fall prior to day 8 after ovulation, any fertilizations that occur will fail to implant and die (22)

Thus, although progesterone is a less effective predictor of ovulation than estradiol, LH, or estradiol induced effects, e g , CM production its evaluation provides crucial information on luteal function that is requisite for determination of accurate conception rates Estimates of the success of prediction of ovulation or limits of the fertile period should include some evaluation of luteal function to correct the positive bias introduced by conception losses due to luteal inadequacies Such adjustments are of utmost importance in women returning to cyclic ovarian function postpartum or during peripubertal or perimenopausal ages, since these periods are all known to be associated with a high incidence of anovulatory cycles and or defective luteal phases (27 29,48)

Saliva. Of the estrogens circulating in serum >95% are bound to proteins (24,39) Molecules bound to carrier proteins are effectively sequestered against immediate catabolism by the liver The free, physiologically active steroid that is available diffuses into all intra- and intercellular fluids including those that are secreted A nearly identical situation exists for most steroids, including estradiol 17β progesterone testosterone, estriol, some corticoids, and for many drugs and pesticides (46,54,70)

Relatively small quantities 0 3-11 pg ml (1-50 pmole l), of free estradiol are found by immunoassay of the unchanged steroid in saliva The levels fluctuate during the cycle in a pattern roughly paralleling that of estradiol in serum A peak of 2 or 11 pg ml (8 or 50 pmole l), depending on the assay system used (20,37,55,74), occurs 1 2 days prior to ovulation followed by a nadir of roughly 2 days and a rise back to a plateau of 0 8 or 8 pg ml (3 or 30 pmole l) during the luteal portion of the cycle Good success with predictions of the limits of the fertile period has been achieved across several laboratories that participated in a WHO-sponsored evaluation of the measurement of serum and salivary steroids (W P Collins, FHI meeting) Though the assay requirements were particularly demanding, 80-85% of the cycles studied demonstrated correct delineation of the fertile period *(see Table 2)*

Quantitation of free progesterone in saliva by similar methods indicates a range that is easier to measure 23±2 pg ml (74±6 pmole l) in the follicular phase (74) to 112±40 pg ml (355±126 pmole l) in the midluteal phase (88) This is still only 1 2% of the total steroid measurable in serum The pattern of progesterone in saliva faithfully mimics that seen in serum (12 13,19,20,37,55,73,88) Salivary progesterone also has been used successfully in monitoring luteal dysfunction, establishment of pregnancy and placental steroidogenesis and resumption of ovarian function during lactation (54,55 73 74)

The rate of saliva production seems to decrease at night in most individuals Fortunately, most of the assays for salivary steroids have proven insensitive to salivary flow rate (54 55) This allows application of citric acid to the tongue to increase flow rate and decreases the time required for sample collection Collection of saliva is probably the least invasive fluid sampling method available Collection of whole saliva by expectoration seems to be accepted in many cultures and by most individuals even when serial samples must be obtained on a daily or even hourly basis and then stored prior to mailing or assessment It is desirable to educate subjects prior to sampling to prevent contamination problems caused by eating and drinking or any "helpful" sample substitutions that might otherwise occur

Problems with this approach relate to variations in salivary composition The pronounced variability in the mucus content can be readily handled in most instances by freezing and thawing the samples, sedimenting any residues by centrifugation, and then doing measurements on aliquots of the nonviscous supernatant More problematic is the presence of serum contamination in saliva, which will lead to false high readings unless the steroids normally associated with carrier proteins are eliminated before assay The usual small amounts of serum from eating and tooth brushing can be eliminated by rinsing the mouth before collecting the saliva specimen, more serious bleeding from mouth trauma or periodontal disease will require special treatment or disregard of the affected samples (54)

Progesterone in Milk Collection of breast milk seems well tolerated by most cultures Hartman and Prosser (33) have used it as an accessible fluid in which progesterone may be measured Rather than approximating the levels found in saliva, progesterone concentrations in milk closely mimic total concentrations in serum Since the composition and rate of milk production vary with length and intensity of lactation (32,33,52), measurements of the steroid levels as a function of milk production rate or as a function of total lipid content would be informative, especially since the steroid could be preferentially concentrated in these lipids Judgments on the limitations and or practicality of this approach await further testing

Urinary Steroid Metabolites Estradiol and other steroids, drugs, and pesticides are metabolized to more polar, water soluble compounds such as glucuronides or sulfates that cannot diffuse freely through tissues This metabolism involves initial alteration by the liver, secretion into the bile, further modification by intestinal bacteria, reuptake by the lymph or bloodstream, and final conjugation in the liver or kidney, i e , enterohepatic circulation A major portion of the metabolized compounds is excreted in urine

The metabolic products are accumulated within the bladder between micturitions If the clearance rate is assumed to be uniform, production rates of metabolites can be computed from their concentration, urine volume, and the time between micturitions As a practical matter, this is done by collecting an early morning urine, EMU, which approximates an 8 hour span, or by collecting all the urine voided over a day, 24-hour urine The impact of accumulation of urinary metabolites over these periods is two-fold First, the amounts available for measurement are substantial, μg-mg 24 hours Second, predictions based on urinary metabolites are constrained by the delays in metabolic clearance and collection of urine, an EMU reflects serum conditions averaged over the previous 8 hours, and cannot predict events with better than an accuracy of ±8 hours

The relative levels of steroid conjugates excreted when several are produced from a single compound, e g , estradiol (4,9,13), may vary over time in response to changes in enterohepatic metabolism and those factors that influence it This could cause difficulties in monitoring ovarian function, especially in cross-cultural studies where diet may vary Theoretically, this problem may be overcome by employing assay systems capable of detecting several of the major metabolites simultaneously In practice, E_1-3-G appears to be as good a predictor of ovulation as total urinary estrogens, the main advantage of measuring total estrogens is an increase in assay signal

The initial clearance of estradiol-17β from serum is rapid, but the entire catabolism and excretion process can take up to 2 days (4,37) Catabolism leads to the production of a spectrum of estrone, estradiol and estriol glucuronides, and sulfates The best assay systems have been developed for E_1-3-G, several high affinity, high specificity antisera and monoclonal antibodies are now available (13,17,18, 25,62,63,75,76,82) The initial rise of E_1-3-G levels occurs an average of 5-6 days prior to ovulation It can serve as an excellent marker for the start of the fertile period and as a good predictor of ovulation

The metabolism of progesterone produces several compounds, the most abundant being Pd-3-G Levels begin to rise above baseline on the day of ovulation and achieve a plateau that parallels serum progesterone levels during the mid-luteal phase Alone, Pd-3-G yields an estimate only for the end of the fertile period However, when used as a ratio with E_1-3 G the rapidly changing status of the follicles and the corpus luteum become more obvious, the E_1-3-G Pd-3-G ratio rises with increasing estradiol production during the follicular phase then declines rapidly as the corpus luteum forms, due both to decreasing estradiol levels and to increasing progesterone levels *(Figure 1)* Since both steroids are released by the ovary and both undergo enterohepatic metabolism, progesterone (or Pd-3-G) measurements serve as internal corrections for the variability of estradiol (or E_1-3-G) levels Use of the ratio of steroid concentrations also eliminates the need to standardize the volume of urine evaluated, i e , the ratio is independent of sample volume These corrections stabilize the data obtained and emphasize the follicular phase rise in estradiol As a result, the E_1-3-G Pd-3-G ratio rises fully 7 days before ovulation and encompasses the entire preovulatory fertile period The peak ratio occurs 1-3 days before ovulation (57), prior to the increase in progesterone This is adequate to define the period of highest measured fertility and to provide a secondary prediction of time of ovulation

Home Assay Kits. Brown (9,10) is developing home assay kits for urinary Pd-3-G and for E_1-3-G for use as an adjunct to current NFP methods Diluted urine is added directly to the assay reagents, which include an antisera, directed against E_1-3-G or Pd-3-G, a steroid conjugated to lysozyme, and a bacterial cell wall preparation Binding to antibody decreases the activity of the lysozyme it hydrolyzes the cell walls more slowly In the presence of urinary steroid metabolite, the steroid-lysozyme is free, and the enzyme hydrolyzes the cell walls rapidly The rate of clearing (loss of turbidity) of the assay vial is compared to that of a vial diluted with water containing a "threshold" level of urinary conjugate, 1 2 mg 24 hr for Pd-3-G, 15 μg 24 hr for E_1-3-G, levels chosen on the basis of field trials (9,10) as sufficient to detect or to predict ovulation, respectively The contents of the vial either clear more rapidly than the standard or remain turbid The endpoint has less room for the type of interpretive variation encountered when color changes or graded colors are used as endpoints Rather, it serves as a concrete, dichotomous signal to the user either she has ovulated, or she has not (Pd-3-G), either she is in the fertile period, or she is not (E_1-3 G)

Despite early difficulties with measurements of low levels due to high assay background, successful field tests of self-test kits for Pd-3-G have already been completed (9,10), widespread testing and eventual application of the Pd-3 G kit await completion of bulk preparation and packaging of the reagents

Steroid Assays. Steroids and steroid metabolites are low molecular weight entities with well known, defined structures These properties allow more stringency to be applied to their quantitation than is the case for the proteins They can be prepared in large quantities by means of organic synthetic techniques These well defined materials can be intrinsically labeled with tracer isotopes, chemically linked to colored, fluorescent or phosphorescent labels, or conjugated to enzymes (9,14) They also may be isolated by simple techniques such as solvent extraction or chromatography, e g , high performance,

HPLC, gas liquid, GLC, or thin layer, TLC, chromatography Purified steroids or steroid metabolites can be assayed by direct chemical techniques that rely for their specificity on a reaction at a specific chemically reactive grouping, e g , acid-fluorescence of corticoids, or by physical chemical techniques that rely on detection of specific chemical groupings, e g , mass-spectrometry

These physicochemical assays provide the reference techniques against which all forms of immunoassays of steroids should be compared Results obtained with most established immunoassays for steroids in serum have been compared to these methods The vast quantities of steroids in urine have allowed application of these approaches following hydrolysis of the steroid conjugates (9) The growing importance of the unhydrolyzed steroid conjugates as accessible indicators and the variability of the spectrum of steroids in urine suggests that reference techniques should also be applied directly to the conjugated forms There have been few laboratories and many fewer investigator years applied to examination of steroid levels in saliva or breast milk It is not surprising that verification of the accuracy and specificity of the immunoassays for steroids in these fluids is incomplete (55,72) The job is complicated by the low levels present but such verifications are necessary before strict correlations can be made between steroid quantitations in serum, saliva, milk, and urine Other forms of immunoassays for monitoring steroids in any bodily fluid must also be verified by correlation to reference techniques before they should be applied, especially in health care or self-monitoring programs

The methods for quantifying estradiol or estradiol metabolites stand the best chance of forming the basis of predictive schemes that require only infrequent sampling Improvements in urinary assays that should be encouraged entail production and use of high affinity monoclonal antibodies and production of broader spectrum assays Estradiol measurements in serum serve as a reference method in clinical situations, on-site applications in the home or in field studies have not been developed yet, largely due to the invasiveness of blood sampling and to problems inherent in assaying low levels of steroids The still lower quantities of estradiol in saliva will necessitate production of even higher affinity antisera and or use of more sensitive endpoints before routine quantitation outside the research laboratory becomes feasible

Progesterone stands beside LH and estradiol as the most important marker of ovarian function in the clinical laboratory Assays for Pd 3-G are well developed and require only a bit more field testing and final verification by a nonimmunological, direct measurement of the progesterone conjugate The levels of progesterone found in serum, saliva, and milk allow measurement with methods currently available Implementation of home test kits for assessment of progesterone in these fluids still requires identification of an optimal reagent system and assay protocol However, since the assay is important in monitoring both cyclic ovarian function and pregnancy, development of these assays should be encouraged Further improvements along these lines will probably require use of electronic meters to quantify colored or graded endpoints Alternative enhancements include development of biosensors that couple a redox endpoint directly to an alphanumeric readout of the assay result These might evaluate salivary progesterone as well as urinary steroids, LH or hCG

Enzymes, Ions, and Sugars

Changes in enzyme content and ionic composition of cervical mucus, saliva, and breast milk have been reported to coincide with the different phases of the ovarian cycle (1,33,49) The enzymes most often cited are common to many tissues They are susceptible to genetic variation and to alteration of activities by hormones or environmental influences The difficulty in fully controlling these influences during the evaluation of the methods may help explain the current lack of interest in pursuing them as reliable indices The ease with which they might be measured in home assay systems and their reported correlations with hormonal markers of ovarian function in some clinical tests suggest them as alternatives to CM or urinary steroids Appropriately controlled field studies using modern techniques need to be done

Hartmann and Prosser (33) report changes in the composition of breast milk (sodium, potassium and chloride ions, and lactose) that occur 5-6 days before the estimated time of ovulation and again 6-7 days after ovulation Two-fold increases in the sodium and chloride ion content of milk are mirrored by two-fold decreases in the content of potassium and lactose The changes occur over periods of 25-42 hours and are absent in anovulatory women and in those using progestational contraceptives These authors also reported 3-9 fold increases in salivary glucose that contrast with 1 3 fold decreases in milk glucose occurring at the same time as the ionic shifts during ovulatory cycles in lactating women (52) The salivary glucose increases took place only during the morning Increases in glucose also occurred in some nonlactating women just prior to ovulation Further work on the influence of dietary variation and the source(s) of the diurnal rhythm of the glucose phenomenon is justified since measurement of enzymes, ions, or sugars offers easily accessible alternative markers for ovarian function in lactating women

Pregnancy

Pregnancy is the final index of ovulation but the only definitive one For studies examining efficacy of contraceptive methods, pregnancy rates are the only endpoint capable of proving the validity of predictions based on hormonal or other indirect measurements Early detection of pregnancy is usually accomplished by immunoassay of hCG, which is within the range of assay detection by 1-3 weeks after conception Pregnancy can also be indicated by serum, salivary, or urinary measures of progesterone, which remains elevated and begins rising beyond the normal life-span of the corpus luteum Since these indicators normally fall about 10 days after ovulation, pregnancies of about 2 weeks' gestation can be detected Continued elevation of BBT may also indicate pregnancy

Two lactogenic hormones can also serve as indicators of pregnancy Prolactin (PRL) and human placental lactogen are formed by fetal or placental tissues Their titers rise smoothly throughout pregnancy in parallel with increases in placental weight Along with the major placental estrogen, estriol, they may be used to check the persistence and progress of a pregnancy past 2-4 weeks of gestation

Prolactin (PRL) in Lactating Women

After parturiton all of the indicators listed for pregnancy decline rapidly, except for PRL Prolactin declines slowly in parallel with frequency and intensity of breast feeding The decline is mirrored by resumption of ovarian cycles, but whether there is any direct causal relationship remains controversial (29,31,45,64,78) The inverse correlation between PRL and resumption of ovarian cycles suggests the importance of measuring PRL when monitoring lactating women for the return of fecundity Prolactin does not pass into saliva and cannot be assayed in urine, it must be measured in blood or serum

Gross (30) has attacked this problem by using a spring-loaded finger lance, the Autolet, and a home sampling scheme involving filter paper Drops of blood from the fingertip are applied to the filter paper and labeled After drying, they are stored in the refrigerator or mailed to the laboratory At the laboratory, uniform samples are punched out of the blood spots and assayed The stability of PRL, normally a labile protein, in the dried blood samples has proven encouraging Except for some samples mailed in hot, damp weather that stimulated mold growth on the paper, the results suggest little degradation of PRL during storage of up to 1 week at room temperature and >3 months under refrigeration The correlations of >0 95 with prolactin levels measured directly in serum suggest that further development of this methodology should be encouraged The use of paper spots may also allow more ready, inexpensive, minimally invasive access to other serum hormones such as LH, estradiol, and progesterone, especially if tests like those on prolactin prove favorable

RECOMMENDATIONS FOR FUTURE RESEARCH

Technological improvements of the endpoints used in immunoassays should be encouraged because they should lead to development of self-tests and will substantially decrease costs This will be realized both in field collections where sample storage and transportation costs can be minimized and in clinical studies where laboratory instrumentation, personnel, and materials costs can be reduced Production of direct assays, particularly those based on urine and saliva, which do not require prior treatment of the sample and do not necessitate complex separation of antibody-bound analyte from free analyte should probably be emphasized Similarly, the use of labels that can be disposed of readily will diminish costs, as will the use of simple dedicated optical reading systems such as microtiter plate readers and luminometers Development of biosensors with endpoints that can be coupled directly to electronic monitors should also be encouraged Improvements in techniques for obtaining serum samples will also improve the quality and quantity of the data that are accessible in any large field studies Further development and testing of the use of blood spot approaches therefore seems warranted Finally, teaching and support programs as well as method refinements must be worked out for each new technological development before its introduction into general use

Several areas of investigation of basic biological processes seem necessary to improve the effectiveness and utility of the physiologic and biochemical indicators discussed First, better estimates of the fertile life-spans of the gametes within the female tract are needed to delineate the range, variability, and timing of the limits of the fertile period Second, better estimates of spontaneous early embryonic loss are needed to improve the computation of method effectiveness Third, further study of lactational amenorrhea is needed to predict and or explain the disjunction of biochemical and physiologic indicators seen during this time and to improve the monitoring of the return to fecundity during prolonged lactation

This needed research could be facilitated by close cooperation between laboratory scientists trying to improve our knowledge of fertility and directors of family planning programs including those promoting NFP who are in a position to evaluate the acceptance of new technologies

REFERENCES

1. Anderson WA, Ahluwalia BS, Westney LS, Burnett CC and Rüchel R: Cervical mucus peroxidase is a reliable indicator for ovulation in humans. Fertil Steril 41:697-702, 1984.

2. Bäckström CT, McNeilly AS, Leask RM, Baird DT: Pulsatile secretion of LH, FSH, prolactin, oestradiol, and progesterone during the human menstrual cycle. Clin Endocrinol 17:29-42, 1982.

3. Baker TG: Oogenesis and ovulation. In Austin AV, Short RV (ed): Reproduction in Mammals. I. Germ Cells and Fertilization. London, Cambridge University Press, 1972, pp 14-45.

4. Baird DT: Prediction of ovulation: biophysical, physiological and biochemical coordinates. In Jeffcoate SL (ed): Ovulation: Methods for its Prediction and Detection. New York, John Wiley & Sons Ltd., 1983, pp 1-17.

5. Barrett JC, Marshall J: The risk of conception on different days of the menstrual cycle. Population Studies 23:455-461, 1969.

6. Billings EL, Billings JJ, Brown JB, Burger HG: Symptoms and hormonal changes accompanying ovulation. Lancet i:282-284, 1972.

7. Billings EL, Billings JJ, Catarinich M: Atlas of the Ovulation Method. The Mucus Pattern of Fertility and Infertility, 3rd ed. Melbourne, Advocate Press, 1977.

8. Bonnar J: Biological approaches to ovulation detection. In Jeffcoate SL (ed): Ovulation: Methods for its Prediction and Detection. New York, John Wiley & Sons Ltd., 1983, pp 33-47.

9. Brown JB, Blackwell L, Billings JJ, Billings EL, Burger HG, Cox RI, Bradlow E, Bascombe J, Bright MI, Harrison P, Martin M, Potter JB, Smith MA: Determination of ovarian hormone levels in urine for identifying the fertile and infertile phases of the menstrual cycle. In Proceedings of the 1984 Family Health International Conference on Methods of Monitoring Ovarian Function and Predicting Ovulation, in preparation.

10. Brown JB, Harrisson P, Smith MA: A study of returning fertility after childbirth and during lactation by measurement of urinary estrogens and pregnanediol excretion and cervical mucus production. J Biosoc Sci Suppl 9, 1985, in press.

11. Carson RS, Trounson A, Findlay JK: Successful fertilization of human oocytes *in vitro:* concentration of estradiol-17β, progesterone and androstenedione in the antral fluid of donor follicles. J Clin Endocrinol Metab 55:798-800, 1982.

12. Cedard L, Janssens Y, Tanquy G, Zorn JR: Radioimmunoassay of plasma and salivary progesterone during the menstrual cycle. J Steroid Biochem 20:487-490, 1984.

13. Collins WP: An overview of hormone determinations used to validate or predict ovulation. In Proceedings of the 1984 Family Health International Conference on Methods of Monitoring Ovarian Function and Predicting Ovulation, in preparation.

14. Collins WP (ed): Alternative Immunoassays. Chichester, John Wiley & Sons, Ltd., 1984.

15. Collins WP: Biochemical approaches to ovulation prediction and detection and the location of the fertile period in women. In Jeffcoate SL (ed): Ovulation: Methods for its Prediction and Detection. New York, John Wiley & Sons Ltd., 1983, pp 49-66.

16. Collins WP, Branch CM, Collins PO, Sallam HN: Biochemical indices of the fertile period in women. Int J Fertil 26:196-202, 1981.

17. Collins WP, Branch CM, Collins PO: Ovulation prediction and detection by the measurement of steroid glucuronides. In Cortes-Prieto J, Campos da Paz A, Neves-e-Castro M (eds): Research on Fertility and Sterility. Lancaster, MTP Press Ltd., 1981, pp 19-33.

18. Collins WP, Collins PO, Kilpatrick MJ, Manning PA, Pike J, Tyler JPP: The concentrations of urinary oestrone-3-glucuronide, LH and pregnanediol-3α-glucuronide as indices of ovarian function. Acta Endocrinol (Copenhagen) 93:123-128, 1979.

19. Corrie JET: A direct radioimmunoassay for salivary progesterone using radiolabelled tracer. In Read GF, Riad-Fahmy D, Walker RF, Griffiths K (eds): Immunoassays of Steroids in Saliva. Cardiff, Alpha Omega Publishing Ltd., 1984, pp 127-133.

20. Donaldson A, Jeffcoate SL, Sufi SB: Rapid assays for oestradiol in saliva. In Read GF, Riad-Fahmy D, Walker RF, Griffiths K (eds): Immunoassays of Steroids in Saliva. Cardiff, Alpha Omega Publishing Ltd., 1984, pp 151-154.

21. Edwards RG: Test-tube babies. Nature 293: 253-256, 1981.

22. Edwards RG: Conception in the Human Female. New York, Academic Press, 1980.

23. Edwards RG, Anderson G, Pickering J, Purdy JM: Rapid assay of urinary LH in women using a simplified method of Hi-Gonavis. In Edwards RG, Purdy JM (eds): Human Conception *In Vitro*. London, Academic Press, 1982, pp 19-34.

24. Ekins RP: The role of binding proteins in hormone transport. In Read GF, Riad-Fahmy D, Walker RF, Griffiths K (eds): Immunoassays of Steroids in Saliva. Cardiff, Alpha Omega Publishing, Ltd., 1984, pp 64-86.

25. Eshhar Z, Kim JB, Barnard G, Collins WP, Gilad S, Lindner HR, Kohen F: Use of monoclonal antibodies of pregnanediol-3α-glucuronide to the development of a solid phase chemiluminescence immunoassay. Steroids 38:89-109, 1981.

26. Gougeon A: Rate of follicular growth in the human ovary. In Rolland R, vanHall EV, Hillier SG, McNatty KP, Schoemaker J (eds): Follicular Maturation and Ovulation. Princeton, Excerpta Medica, 1982, pp 155-163.

27. Gross BA: Natural family planning indicators of ovulation. In Proceedings of the 1984 Family Health International Conference on Methods of Monitoring Ovarian Function and Predicting Ovulation, in preparation.

28. Gross BA: Breastfeeding and the return to fertility. Int Rev Nat Fam Plann VIII:102-120, 1984.

29. Gross BA, Eastman CJ: Prolactin and the return of ovulation in breastfeeding women. J Biosoc Sci Suppl 9, 1985, in press.

30. Gross BA, Henry F, Eastman CJ: Filter paper blood spot technique for prolactin measurement. In Proceedings of the 1984 Family Health International Conference on Methods of Monitoring Ovarian Function and Predicting Ovulation, in preparation.

31. Habicht JP, Butts WP, Meyers L, DaVanzo J: The contraceptive role of breastfeeding. Pop Studies, 1985, in press.

32. Hartmann PE: Lactation and reproduction in Western Australian women. In Proceedings of the 1984 Family Health International Conference on Methods of Monitoring Ovarian Function and Predicting Ovulation, in preparation.

33. Hartmann PE, Prosser CG: Acute changes in the composition of milk during the ovulatory menstrual cycle in lactating women. J Physiol 324:21-30, 1982.

34. Hatherley L: Natural family planning after delivery. M.D. Thesis, Melbourne, Australia, University of Melbourne, 1983.

35. Hilgers TW, Abraham GE, Cavanagh D: Natural family planning. I. The peak symptom and estimated time of ovulation. Obstet Gynecol 52:575-582, 1978.

36. Jeffcoate SL (ed): Ovulation: Methods for its Prediction and Detection. New York, John Wiley & Sons Ltd., 1983.

37. Jeffcoate SL: Use of rapid hormone assays in the prediction of ovulation. In Jeffcoate SL (ed): Ovulation: Methods for its Prediction and Detection. New York, John Wiley & Sons Ltd., 1983, pp 67-82.

38. Jeffcoate SL: Efficiency and Effectiveness in the Endocrine Laboratory. New York, Academic Press, 1981.

39. Joshi UM, Sankolli GM: Oestrogens in saliva. Frontiers Oral Physiol 5:87-96, 1984.

40. Keefe EF: Cephalad shift of the cervix uteri: sign of the fertile time in women. Int Rev Nat Fam Plann 1:55-60, 1977.

41. Keefe EF: Self-observation of the cervix to distinguish days of possible fertility. Bull Sloane Hospital for Women 8:129-136, 1962.

42. Kerin JF, Edmonds DK, Warnes GM, Cox LW, Seamark RF, Matthews CD, Young GB, Baird DT: Morphological and functional relations of Graafian follicle growth to ovulation in women using ultrasonic, laparascopic and biochemical measurements. Br J Obstet Gynaecol 88:81-90, 1981.

43. Köhler G, Milstein C: Continuous cultures of fused cells secreting antibody of predetermined specificity. Nature 256:495-497, 1975

44. Kolata G: Fertility hormones cloned. Science 223:805, 1984.

45 Konner M, Worthman C Nursing frequency gonadal function and birth spacing among !Kung hunter gatherers Science 207 788 791 1980

46 Lardon J Mohmod S Distribution of drugs between blood and saliva In Read GF, Riad Fahmy D, Walker RF Griffiths K (eds) Immunoassays of Steroids in Saliva Cardiff, Alpha Omega Publishing Ltd 1984, pp 47 55

47 Liskin LS, Fox G Periodic abstinence how well do new approaches work? Population Reports IX 33 71 1981

48 McNeilly AS Howie PW Houston MJ, Cook A, Boyle H Fertility after childbirth adequacy of post partum luteal phases Clin Endocrinol 17 609 615, 1982

49 Moghissi KS Prediction and detection of ovulation Fertil Steril 34 89 98, 1980

50 O Herlihy C de Crespigny LJCh Robinson HP Monitoring ovarian follicular development with real time ultrasound Br J Obstet Gynaecol 87 613 618 1980

51 Parenteau Carreau S The return of fertility in breast feeding women Int Rev Nat Fam Plann VIII 34 43 1984

52 Prosser CG, Hartmann PE Saliva and milk composition during the menstrual cycle of women Aust J Exp Biol Med Sci 61 265 275, 1983

53 Queenan JT, O'Brien GD, Bains LM Simpson J Collins WP Campbell S Ultrasound scanning of ovaries to detect ovulation in women Fertil Steril 32 99 105, 1980

54 Read GF, Riad Fahmy D Walker RF Griffiths K (eds) Immunoassays of Steroids in Saliva Cardiff Alpha Omega Publishing Ltd , 1984

55 Riad Fahmy D Read GF, Walker RF, Truran PL Seaton B Determination of ovarian steroid hormone levels in saliva an overview In Proceedings of the 1984 Family Health International Conference on Methods of Monitoring Ovarian Function and Predicting Ovulation in preparation

56 Roger M, Grenier J, Houlbert C Castanier M Feinstein M C Scholler R Rapid radioimmunoassays of plasma LH and estradiol 17β for the prediction of ovulation J Steroid Biochem 12 403 410 1980

57 Royston JP Statistical approaches to the prediction and detection of ovulation detecting the signal among the noise In Jeffcoate SL (ed) Ovulation Methods for its Prediction and Detection New York, John Wiley & Sons Ltd , 1983, pp 19 32

58 Royston JP Basal body temperature, ovulation and the risk of conception with special reference to the lifetimes of sperm and egg Biometrics 38 397 406 1982

59 Royston JP Abrams RM An objective method for detecting the shift in basal body temperature in women Biometrics 36 217 224, 1980

60 Royston JP, Abrams RM, Higgins MP, Flynn AM The adjustment of basal body temperature measurements to allow for time of waking Br J Obstet Gynaecol 87 1123 1127, 1980

61 Sallam HN, Whitehead MI Collins WP Incidence of mature follicles in spontaneous and induced ovarian cycles Letter Lancet i 357, 1983

62 Samarajeewa P, Cooley G Kellie AE The radioimmunoassay of pregnanediol 3α glucuronide J Steroid Biochem 11 1165 1171, 1979

63 Shah HP, Joshi UM A simple rapid and reliable enzyme linked immunosorbent assay (ELISA) for measuring estrone-3 glucuronide in urine J Steroid Biochem 16 283 286, 1982

64 Short RV Breast feeding Scientific American 250 35 41 1984

65 Spieler JM Self detection of ovulation and the fertile period In Cortes Prieto J, Campos da Paz A, Neves e Castro M (eds) Research on Fertility and Sterility Lancaster, England, MTP Press Limited 1981 pp 35 51

66 Testart J Frydman R Feinstein MC, Thebault A, Roger M, Scholler R Interpretation of plasma luteinizing hormone assay for the collection of mature oocytes from women definition of a luteinizing hormone surge-initiating rise Fertil Steril 36 50 54 1981

67 Treloar AE Boynton RE, Behn BG Brown BW Variation of the human menstrual cycle through reproductive life Int J Fertil 12 77 126, 1967

68 Trounson AO Burger HG Kovacs GT Prediction of ovulation for *in vitro* fertilization In Jeffcoate SL (ed) Ovulation Methods for its Prediction and Detection New York, John Wiley & Sons Ltd 1983, pp 83 102

69 Usala SJ, Schumacher GFB Volumetric self sampling cervicovaginal fluid a new approach to ovulation timing Fertil Steril 39 304 309, 1983

70 Vining RF McGinley RA Transport of steroid from blood to saliva In Read GF, Riad Fahmy D Walker RF Griffiths K (eds) Immunoassays of Steroids in Saliva Cardiff, Alpha Omega Publishing Ltd 1984 pp 56 63

71 Vollman RF The Menstrual Cycle Philadelphia W B Saunders, 1977

72 Walker RF, Joyce BG, Dyas J, Riad Fahmy D Salivary cortisol I Monitoring changes in normal adrenal activity In Read GF Riad Fahmy D Walker RF, Griffiths K (eds) Immunoassays of Steroids in Saliva Cardiff Alpha Omega Publishing Ltd 1984 pp 308 316

73 Walker RF Read GF, Riad-Fahmy D Radioimmunoassay of progesterone in saliva application to the assessment of ovarian function Clin Chem 25 2030 2033, 1979

74 Walker RF, Read GF, Riad Fahmy D, Griffiths K The assessment of ovarian function by the radioimmunoassay of oestradiol 17β in saliva In Read GF, Riad Fahmy D Walker RF, Griffiths K (eds) Immunoassays of Steroids in Saliva Cardiff, Alpha Omega Publishing Ltd , 1984, pp 155 162

75 Weerasekera DA Kim JB Barnard GJ, Collins WP Multiple immunoassay the simultaneous measurement of two urinary steroid glucuronides as an index of ovarian function J Steroid Biochem 18 465 470 1983

76 Weerasekera DA, Kim JB, Barnard GJ Collins WP Kohen F and Lindner HR Monitoring ovarian function by a solid phase chemiluminescence immunoassay Acta Endocrinol (Copenhagen) 101 254 263 1982

77 Wetzels LCG Hoogland HJ Relation between ultrasonic evidence of ovulation and hormonal parameters luteinizing hormone surge and initial progesterone rise Fertil Steril 37 336 341, 1982

78 Wood JW Lai D, Johnson PL Campbell KL Maslar IA Lactation and birthspacing in Highland New Guinea J Biosoc Sci Suppl 1985, in press

79 World Health Organization Task Force on Methods for the Determination of the Fertile Period A prospective multicentre trial of the ovulation method of natural family planning IV The outcome of pregnancy Fertil Steril 41 593 598 1984

80 World Health Organization Task Force on Methods for the Determination of the Fertile Period A prospective multicentre trial of the ovulation method of natural family planning III Characteristics of the menstrual cycle and of the fertile phase Fertil Steril 40 773 778, 1983

81 World Health Organization Task Force on Methods for the Determination of the Fertile Period Temporal relationships between indices of the fertile period Fertil Steril 39 647 655 1983

82 World Health Organization Task Force The measurement of urinary steroid glucuronides as indices of the fertile period in women J Steroid Biochem 17 695 702, 1982

83 World Health Organization Task Force on Methods for the Determination of the Fertile Period A prospective multicentre trial of the ovulation method of natural family planning I The teaching phase Fertil Steril 36 152 158 1981

84 World Health Organization Task Force on Methods for the Determination of the Fertile Period A prospective multicentre trial of the ovulation method of natural family planning II The effectiveness phase Fertil Steril 36 591 598 1981

85 World Health Organization Task Force on Methods for the Determination of the Fertile Period Temporal relationships between ovulation and defined changes in the concentrations of plasma estradiol 17β luteinizing hormone follicle-stimulating hormone and progesterone II Histological dating Am J Obstet Gynecol 139 886 895, 1981

86 World Health Organization Task Force on Methods for the Determination of the Fertile Period Temporal relationships between ovulation and defined changes in the concentrations of plasma estradiol 17β, luteinizing hormone follicle stimulating hormone and progesterone I Probit analysis Am J Obstet Gynecol 138 383 390, 1980

87 Yen SCC Tsai CC Naftolin F Vandenber G Ajabor L Pulsatile patterns of gonadotrophin release in subjects with and without ovarian function J Clin Endocrinol Metab 34 671 675 1972

88 Zorn JR McDonough PG Nessman C Janssens Y Cedard L Salivary progesterone as an index of the luteal function Fertil Steril 41 248 253 1984

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RESEARCH FRONTIERS IN FERTILITY REGULATION

DO CONTRACEPTIVE METHODS POSE FETAL RISKS?

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That various contraceptive methods pose fetal risks is receiving increasing attention Initially, a relationship between congenital anomalies and oral contraceptives was claimed, but more recently relationships with other methods — spermicides, intrauterine devices — have been claimed

Both teratogenic as well as mutagenic effects have been alleged Here it may be useful to recall that a teratogen is an exogenous agent (drug, virus, irradiation) that deleteriously affects an embryo otherwise differentiating normal, its effect is exerted only during pregnancy By contrast, a mutagen deleteriously affects germ cells, producing mutant genes or chromosomal abnormalities that can result in anomalies in offspring conceived years later Principles of teratogenesis and mutagenesis have been reviewed elsewhere by the author, as have the types of anomalies that teratogens and mutagens can produce (75)

Fortunately, we shall be able to conclude that contraceptive methods do not pose substantive fetal risks In this review we shall consider for each method the initial claims of fetal risks, followed by evolving scientific consensus to the contrary (21,73,74,86,88)

TERATOGENICITY OF ORAL CONTRACEPTIVES
General Comments

Oral contraceptives have been implicated as both teratogens and mutagens Similar claims could logically extend to injectable or implantable progestins (medroxyprogesterone, norethisterone) In reality, there is no substantive evidence for contraceptive steroids being mutagenic or teratogenic It is true that certain progestins, namely the 19-nor-testosterone norethisterone and probably norgestrel, can virilize female fetuses when administered in high doses at susceptible periods of pregnancy (13,73) (Norethynodrel does not virilize humans) However, the doses required for virilization (e g , 20-40 mg/day of norethisterone) are considerably in excess of those present in oral contraceptives unwittingly ingested by women already pregnant Thus, virilization is not a practical consideration in the present context

It would clearly be desirable to analyze exposures separately on the basis of progestins only, estrogens alone, and combined oral contraceptives However, few if any studies permit such analysis The more typical experimental design has been to pool outcomes following exposure to a variety of sex hormones Fortunately, the necessity of pooling exposures is not likely to pose serious problems because the only estrogen implicated as a teratogen is diethylstilbestrol (DES) This compound is not a component in contraceptive agents Moreover, the specific progestin seems unimportant with respect to type of somatic (non-genital) defects

Samples that have been used to assess the teratogenicity of progestins include pregnancies characterized by 1) administration of progestins for pregnancy maintenance (20-40 mg/day), 2) administration of progestins for pregnancy diagnosis, based upon presence or absence of withdrawal uterine bleeding following 5 days of moderately high doses (10-20 mg/day), and 3) inadvertent progestin exposure (1 mg/day) during unrecognized gestation, as a result of continued oral contraceptive ingestion

Although the first two indications are no longer appro-

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priate, one can retrospectively identify populations of fetuses exposed when those indications were valid Table 1 summarizes some especially informative cohorts, with others cited elsewhere (73,74)

Cardiac Anomalies

Studies Claiming Teratogenic Effects. The somatic anomaly most often alleged to be teratogenic as a result of progestins involves the heart That progestins could be cardiac teratogens was initially claimed by Levy et al (43) in 1973 In a case-control study, 7 of 76 mothers delivered of infants with transposition of the great vessels received "hormones" in the first trimester Significantly fewer (0 76) controls were exposed ($p<0$ 007) Nora and Nora later claimed similar findings in a series of overlapping studies that were initially retrospective but later more prospective in design In one case-control study (54), 20 of 224 mothers giving birth to infants with cardiac defects recollected receiving an estrogen progestin compound, compared to only 4 of 262 controls ($p<0$ 001) Nora then began a prospective study (56) After no significant differences were observed between the first 60 mothers and their controls, a second study was conducted with 2 controls per subject In the second study, 31 of 176 mothers with affected offspring received hormones, compared to only 21 of 352 control mothers ($p<0$ 001) Although labelled as prospective, exposure interval was not well defined, and controls were not selected in prospective fashion At time of delivery of exposed women, a "control infant without exposure [was] selected " Recall and memory biases invalidate this approach

Similar criticisms can be levelled at the Israeli study of Harlap et al (25) This study was potentially marked by recall and memory biases because interviews were conducted months after exposure A slight increase in major anomalies was observed among women exposed to "hormones," however, no increase was observed when analysis was restricted to cardiac anomalies alone

Case-control studies by several other groups have also revealed significantly positive correlations, but again recall bias is likely Janerich et al (30) identified infants with cardiac defects through birth certificates Of 104 mothers of affected infants, 18 women received hormones, in 16 women, the hormones were prescribed for pregnancy diagnosis, and 2 women inadvertently used contraceptives Significantly fewer controls reported exposure Cardiac anomalies were also among the anomalies said to be responsible for the findings by Greenberg et al (22) of a relationship between progestins and generalized anomaly rates

The major impetus for claims of teratogenicity was the prospective U S Collaborative Perinatal Project (26,27), a study whose original conclusions with respect to progestins have recently been invalidated Of 1,042 offspring said to have been exposed to "sex hormones" during their gestation, 19 had cardiac defects (1 82%), 385 of 49,240 (0 78%) unexposed offspring were affected (relative risk 2 3, $p<0$ 05) There were too few cases to permit analysis by specific hormones, however, exposure to progestins only had a lower but still significantly increased relative risk (1 8, $p<0$ 05) Interestingly, continued contraceptive use during the second and third lunar months was associated with a relative risk of 2 4, yet exposure to progestogens only in the same interval was associated with a risk of 1 5 and exposure to estrogens only with a risk of 1 4

In 1984 Wiseman and Dodds-Smith (87) reevaluated the U S Collaborative Perinatal Project, presenting new data that invalidated the previous conclusions Of the 19 progestin-associated "cardiac defects," reanalysis revealed that 2 represented coding errors, no progestogen exposure ever occurred Two (2) other infants with cardiac defects had trisomy 21, the aneuploidy and not the progestins surely causing the cardiac defect Two more were exposed during the first lunar month However, the first lunar month includes the two weeks before conception and the two weeks after During this interval (all or-none period), anomalies cannot ordinarily be produced Five (5) other infants were not exposed until after cardiac embryogenesis was complete (42 days embryogenesis) Thus, only 8 of the 19 cardiac defects could not plausibly have been caused by progestogen teratogenicity The conclusions of Heinonen et al (26,27) should now be considered disproved

Other Studies Not Confirming Teratogenicity. In contrast to most of the studies cited above, other case-control reports (10,52,63,67 90) have reached contrary conclusions Of greatest importance are the many prospective studies that found no evidence for progestins being cardiac teratogens Table 1 summarizes representative findings in larger, more recent studies Other smaller cohorts can also be cited, again none showing deleterious effects (58,60,65,77,85)

In France, Spira et al (79) followed 20,000 French women throughout pregnancy Almost half (9,566) received hormones, usually for pregnancy diagnosis or pregnancy maintenance The anomaly rate among exposed subjects did not differ from that in the unexposed group In a later tabulation of the same population (12,764 women) by Goujard and Rumeau-Rouquette (19), cardiac anomalies proved not significantly more frequent in exposed (43%) than unexposed (41%) mothers

In Sweden, Kullander and Kallen (37) followed 6,379 pregnancies, from which 194 mothers had abnormal

Investigator	*Sample*	*Control*	*Anomalies*	*Comment*
Spira et al (79)	9 566 women interviewed in the 3rd month who most often received hormones for pregnancy support or diagnosis (France)		171 9 566 (1 8)	Anomalies equally frequent in exposed and unexposed pregnancies
		6 387 not receiving hormones	168 6 387 (2 0)	
Harlap et al (25)	11 468 women 432 receiving hormones (Israel)		47 432 (10 9) all anomalies 21 432 (4 9) major anomalies only	Small increase (25) (p< 0 02) observed recall or memory bias possible because interviews were months after exposure
		11 036 unexposed	925 11 036 (8 4) 426 11 036 (3 9%) major only	
Kullander and Kallen (37)	6 379 pregnancies from which 194 mothers had abnormal infants (Sweden)		5 194 exposed to progestogen (2 6)	Exposure rates similar in both groups
		5,002 women delivered of normal infants	98 5,002 not exposed to progestogen (2 0%)	
Royal College of General Practitioners (69)	136 pregnancies conceived during oral contraceptive therapy (Great Britain)		2 136 (1 5)	No differences among groups
		11,009 pregnancies in nonusers, 5 530 pregnancies in previous contraceptive users	177 11 009 (1 6%) 865 5 530 (1 6%)	
Goujard and Rumeau Rouquette (19)	12 895 mothers interviewed in the first trimester of whom 1,165 were exposed (France) (same population as Spira et al)		5 335 (1 5) testosterone derivatives 15 830 (1 8%) 'progesterone derivatives'	Chromosomal anomalies excluded from analysis no differences observed either overall or after separate analysis
		9 822 nonexposed	160 9 822 (1 6) nonexposed	
Heinonen et al (26 27), Wiseman and Dodds Smith (87)	Collaborative Perinatal Project (50,282 women) in 1958-66, of whom 1,042 were exposed to sex hormones' and 866 to progestogens only (United States)		19 1 042 (1 8) cardiac defects associated with sex hormone exposure 75 866 (8 7%) exposures to progestins only associated with anomalies	In original analysis no significant differences for total anomalies but relative risk significantly increased for cardiac anomalies alone (relative risk 2 3 p< 0 05) Relative risk of cardiac defects with progestins alone 1 8 (p< 0 05)
		49,240 women not exposed to sex hormones, 49,416 not exposed to progestogens	385 49 240 (0 8%) cardiac 3 172 49 416 (6 5%) of women not exposed to progestogens had anomalous infants	Wiseman and Dodds Smith (87) later showed that 11 of the 19 cardiac cases could not plausibly have been caused by hormonal exposure (2 cases never exposed 2 cases trisomy 21 2 exposed only during first lunar month 5 exposed only during fourth lunar month)
Torfs et al (83)	Over 18 000 women, of whom 203 had 'hormonal pregnancy tests' (United States)		9 203 (4 4)	No significant differences among groups
		689 with serum pregnancy tests 332 with urine pregnancy tests 17 057 with no pregnancy tests	30 617 (4 4%) 9 332 (2 7) 650 17 047 (3 8)	
Goujard et al (20)	3 451 women, of whom 133 used progestins (France)		5 133 (3 8)	Four of the 5 anomalies occurred in subset of 35 women exposed to testosterone derivatives
		3 318 nonexposed	76 3 318 (2 3)	
Savolainen et al (70)	3 002 mothers of malformed infants of whom 38 conceived while receiving 'pills (Finland)			Anomaly rates similar in sample and control both for previous and for concurrent contraceptive usage
		3,002 matched controls		
Michaelis et al (47)	In 13,643 pregnancies about 10% of women received hormones for pregnancy diagnosis or support (West Germany)		4 320 (1 3) in progesterone exposures 11 610 (1 8) in cases exposed to progesterone and estradiol	No significant difference between exposed cases and their unexposed matched controls
		Matched controls within same population who were not exposed		

Table 1 Prospective studies evaluating effects of progestin exposure during pregnancy The clear consensus is that progestins in the doses received were not teratogenic

infants Of the 194, only 5 (2 5%) were exposed to progestogens, 2 0% of controls (normal infants) were exposed In Great Britain (69), anomaly rates were 1 5% among 136 pregnancies in women exposed to oral contraceptives, 1 6% among 11,009 women never using oral contraceptives, and 1 6% among 5,530 women discontinuing oral contraceptives prior to pregnancy

Other cohort studies failing to show an association were those of Torfs et al (83), Vessey et al (85), Savolainen et al (70), Michaelis et al (47), and Ressegue et al (62) *(Table 1)*

Several other studies deserve special comment One is that of Nishimura et al (53), who detected no cardiac anomalies in 108 microdissected embryos exposed to hormones Several controls had cardiac defects A second is a case-control study of Katz et al (35) Both subjects and controls were women presenting with vaginal bleeding Anomalies were not increased in those women exposed to progestogens This study takes into account the confounding variable of bleeding, believed by some but not most investigators to bear an association with anomalies

Conclusion. In view of the consensus that progestins are not cardiac teratogens, it is worth reconsidering why a minority of studies arrived at ostensibly contradictory conclusions Unavoidable statistical vicissitudes and differing genetic susceptibilities could explain some discrepancies However, methodological shortcomings furnish the predominant explanations for those studies purporting to show positive associations between progestins and cardiac defects

First, it is axiomatic that recall biases are inherent in all retrospective (case-control) studies Investigations of Janerich et al (30), Levy et al (43), and the Noras (54,56) all potentially suffer from this bias

Second, prior pregnancy outcome was rarely, if ever, taken into account In fact, the birth of one child with a cardiac defect confers an increased risk (1-4%) in subsequent pregnancies (75) The increased risk might even pass unrecognized if a cardiac defect had caused an infant to be stillborn Even more relevant is that occurrence of a stillborn infant in a previous pregnancy could tempt some obstetricians to administer hormones empirically

Third, reasons for attempting to maintain pregnancies, often the reason for administering progestins, were not sought

Fourth, hormones could have been administered in pregnancies already manifesting problems (e g , bleeding) indicative of underlying defects Indeed, Matsunaga and Shioto (45) believe that not progestins, but rather the bleeding for which progestins were administered was responsible for cardiac defects

Fifth, few studies restrict analysis to the inverval in embryogenesis during which exposure could produce cardiac defects A good example of this fallacy is the U S Collaborative Perinatal Project (26,27), already cited In that study the entire first 4 lunar months were considered to be that interval during which exposure could have produced anomalies

We can thus conclude that not only do most retrospective and almost all prospective studies fail to support the hypothesis that progestins are cardiac teratogens, but serious methodological flaws also exist in the few reports claiming positive associations The magnitude of the observed differences in the few positive case-control studies seems consistent with recall biases, and the few prospective studies claiming effects are marred by methodological shortcomings The logical conclusion is that progestins are not cardiac teratogens

Hypospadias

In 1971, Aarskog (1) claimed that progestins and medroxyprogesterone in particular cause penile or perineoscrotal hypospadias Aarskog's data (1) were uncontrolled, but two other studies later supported his hypothesis A Latin American case-control study (51) showed a relative risk of 2 4 for hypospadias being associated with progestin exposure Of 314 cases, 24 (7 6%) were exposed to one of the various progestins, 12 of 319 controls (3 8%) ($p<0$ 05) were exposed However, details concerning the time of exposures were not available A second study, from Hungary, reported that 28 of 294 mothers delivered of males with hypospadias received sex hormones, compared to 12 of an unspecified number of controls (15) This difference was said to be significant, however, controls may not have been well matched, and a high prevalence of hypospadias in the proband's male relatives suggests selection bias

In contrast to these two case-control studies, better-designed studies failed to show an association between hypospadias and medroxyprogesterone or progestins (5,10,81) Moreover, not a single prospective study (*Table 1*) has shown a relationship between progestins and hypospadias

In conclusion, progestins seem unlikely to adversely affect male genital development

Limb Reduction Deformities

Shortening or absence of a limb, finger, or a toe (limb reduction defects) was alleged to be associated with

progestin exposure Janerich et al (32) reported that 15 of 108 women with an affected infant received hormones (inadvertent oral contraceptive exposure, hormone pregnancy test, or hormones for pregnancy maintenance) Only 4 of 108 controls were exposed ($p<0.05$) Greenberg et al (22) claimed an overall increase in anomalies following progestin exposure, apparently contributed in part by limb reduction defects However, these studies invite invalidation because of recall bias Interviews were conducted long after birth In the study of Janerich et al (32) some exposures may also have occurred during the all-or-none period

By contrast, other case-control investigations failed to show an association between limb reductions and maternal hormonal exposure Bracken et al (10) showed no statistically significant association, and Oakley et al (57) failed to observe a relationship in an especially well-constructed case-control study Controls in the latter study included women delivered of offspring with chromosomal abnormalities, a design presumably obviating recall biases

No prospective studies have found an association (*Table 1*) Strengthening the value of prospective studies is that missing digits or severe limb shortening should be obvious to even the casual observer Finally, Nishimura et al (53) failed to observe limb reduction deformities in their microdissection of 108 embryos recovered from progestin-exposed mothers

In conclusion, the well-publicized report of Janerich et al (31) has been followed by a variety of studies failing to confirm a relationship between progestin exposure and limb reduction defects This reassurance is enhanced by its being derived in part from studies in which exposure levels were much higher than those expected with oral contraceptives More recently (31), Janerich himself has modified his earlier opinion

Neural Tube Defects (NTD)

Neural tube defects (anencephaly, spina bifida, encephalocele and other rarer defects) were once claimed to be associated with hormone exposure However, the claim is now almost completely discounted

Using a case-control design, Gal et al (18) reported that 19 of 100 women delivered of infants with myelomeningocele or hydrocephalus received hormones (estradiol plus ethisterone or norethisterone) for pregnancy diagnosis, only 4 of 100 controls recalled hormone exposure ($p<0.01$) Unfortunately ignored in this study was prior pregnancy history This is especially relevant in the United Kingdom, where NTD recurrence risk is 5% for first-degree relatives Likewise not considered was the stage of embryogenesis at which exposure occurred The neural tube closes at 28 embryogenic days, earlier than attempts to diagnose pregnancy are usually made and, hence, earlier than hormones would ordinarily have been administered for pregnancy diagnosis A second study showing a possible relationship to NTD was that of Greenberg et al (22) The anomaly rate in the hormone-exposed group was increased, with 25 of 93 malformed infants showing NTD However, this study is of uncertain validity because only a small and hence possibly unrepresentative proportion of eligible subjects participated

Other case-control studies showed no association between NTD and progestins Illustrative is the United Kingdom study of Laurence et al (39), who showed no significant increase in a sample much larger than that of Gal et al (18) Another large negative case-control study is that of Bracken et al (10) Furthermore, not a single prospective study showed an association between progestins and NTD There were no NTDs in 108 progestin-exposed embryos microdissected by Nishimura et al (53)

The hypothesis that progestins cause NTDs thus currently receives no support

Other Anomalies

A final consideration is whether a generalized (nonspecific) increase in anomalies occurs after exposure to oral contraceptives Evaluating an overall increase in anomaly rates is hazardous because of the etiologic heterogeneity inherent in pooling different anomalies Moreover, it is unlikely that a generalized increase would exist without at least one organ system subsequently being identified as primarily responsible

Nonetheless, an excess in anomaly rates of nonspecific type has been claimed following oral contraceptive exposures One case-control study claiming an association is that of Greenberg et al (22), whose validity we have already questioned because so few eligible subjects participated By contrast, no significant associations were found in large case-control studies of Bracken et al (10) and Oakley et al (57) The frequency of anomalies was also not increased in 541 "pill-failure" pregnancies pooled by Harlap and Eldor (24)

Only 2 of 17 prospective studies showed any possible association One is the previously cited U S Collaborative Perinatal Project (26,27) There existed a significant association with "hormones, hormone antagonists, and contraceptives," but not with "progestational agents" alone A second study is that of Harlap et al (25), who showed a small increased relative risk All other prospective studies failed to show any generalized increase (see *Table 1*)

We conclude that progestins do not cause a generalized increase in anomalies

VACTERL Complex

VACTERL is an acronym referring to the complex of vertebral, anal, cardiac, tracheoesophageal, renal and limb anomalies Defects involving any 3 of the 7 organ systems are said to justify the diagnosis

Nora et al (55) reported significantly increased frequency of progestin exposure in 30 VACTERL probands (11 exposures), compared to 60 controls (5 exposures) However, the sample size is small, and recall bias would surely be amplified in mothers whose infants had multiple malformations Moreover, knowledge of the purported relationship to progestin exposure may have tempted referring physicians to diagnose the VACTERL association Even more importantly, we have already refuted individually the claims of cardiac and limb reduction components of VACTERL Other case-control studies failed to reveal a significant relationship between progestin exposure and other VACTERL components, such as esophageal atresia (16) In prospective studies, investigators have specifically sought and failed to observe the VACTERL complex A further variant, likewise not substantiated, is the claim by Lorber et al (44) for an "EFESSES syndrome" (embryo-fetal exogenous sex steroid exposure syndrome), characterized by various dysmorphic features

MUTAGENICITY OF ORAL CONTRACEPTIVES

In addition to direct effects on the developing embryo (teratogenesis), oral contraceptives have been said to induce mutations in individual germ cells Induction of both gene mutations and chromosomal abnormalities have been claimed

Gene Mutations

Whether progestins or estrogens result in mutations responsible for mendelian or polygenic disorders should ideally be assessed locus by locus, for mutability almost certainly varies However, this proves mathematically impossible, given baseline mutation rates of 10^{-5} to 10^{-6}/locus, gamete generation (75) One must be content to compare overall anomaly rates in women exposed and women not exposed to hormones In fact, studies usually pool anomalies of mendelian, polygenic, chromosomal, and environmental etiology This approach is obviously less than ideal

Offspring of over 20,000 women who used oral contraceptives prior to conception have been studied (3,64,68,69, 84,85,88) There is no evidence for any increased anomaly rates Other data in support of safety of oral contraceptives can be cited National surveillance reports record no increase in any mendelian disorder after the introduction of oral contraceptives, and there is also no alteration in sex ratio The latter is significant because induction of lethal X-linked recessive mutations would decrease the proportion of liveborn males Mutations at any X-linked loci would contribute to an altered sex ratio Moreover, such an observation would have general applicability because agents inducing X-linked mutations would surely also induce autosomal mutations In aggregate, the large cohort studies cited above provide no evidence of an altered sex ratio, contradicting a limited number of studies of much smaller sample size

Finally, neither progestins nor estrogens are representative of those classes of compounds known to be mutagens For example, progestins do not yield mutations in the Ames test (38) That progestins do not induce mutations in germ cells causing anomalies offers reassurance, incidentally, against mutagenic effects in somatic cells, causing cancer later in life

Numerical Chromosomal Abnormalities

That prior hormonal exposure could induce chromosomal abnormalities is not an unreasonable hypothesis Oocytes remaining in dictyotene of meiosis I until ovulation might complete meiosis sluggishly as a result of hormone exposure, resulting in abnormalities that would be chromosomal

Initial concerns were generated by Carr (12), who reported an increase in chromosomally abnormal abortuses among women previously using oral contraceptives In such women, 48% of abortuses were chromosomally abnormal, only 22% of controls were abnormal Most of the excess was due to polyploidy However, these observations were not confirmed in several later studies Boue and Boue (9) reported 66% abnormal complements (16% polyploidy) among 243 previous contraceptive users, compared to 63% (18% polyploidy) among 604 controls Lauritsen (40) found a slight excess in those women previously using oral contraceptives (61% v 49%) However, even this insignificant increase was due to monosomy X and structural abnormalities, and not polyploidy as Carr (12) had reported Alberman et al (2) observed 32% abnormalities in 524 prior users, compared to 26% in 428 controls Dhardial et al (17) found differences of a similar, but again insignificant, magnitude In induced abortuses, Klinger et al (36) noted an insignificantly higher frequency in prior contraceptive users (1%), compared to controls (0 5%)

Failure to confirm Carr's initial findings (12) is especially noteworthy because either of two biases might spuriously yield increased abnormality rates in exposed groups First, unrecognized induced abortuses are more likely to be included inadvertently among controls than among women stopping contraception in order to achieve

pregnancy Indeed, surreptitious illicit abortions furnish a likely explanation for the unusually low (22%) frequency of chromosomally abnormal abortuses in Carr's controls (12) Second, ovulation may be delayed immediately after contraception is discontinued This delay will produce pregnancies of less advanced gestation than those of controls, who are more likely to have normal cycles Because the frequency of chromosomal abnormalities is inversely related to gestational age, unrecognized earlier gestation would lead to ostensibly higher abnormality rates among prior contraceptive users

We can cite yet other data suggesting that oral contraceptives do *not* cause either numerical or structural chromosomal abnormalities If prior contraceptive use leads to chromosomal abnormalities, we would expect the following conditions

1) The frequency of spontaneous abortions should increase after hormonal discontinuation because 50-60% of first-trimester abortuses show cytogenetic abnormalities To the contrary, pooled data from several large prospective studies show no increase in abortion rates (88)

2) An increased frequency of liveborns with Down syndrome should be evident because trisomy in both abortuses and liveborns is presumably caused by the same cytologic mechanism (nondisjuction) Neither case-control nor prospective studies have shown any increase

3) An increase in liveborns with cytogenetic abnormalities would be reflected by a generalized increase in anomalies following cessation or oral contraceptives, a possibility not confirmed (3,64,69,85,88)

Worth noting as well is that several *in vitro* studies have claimed increased chromosomal breakage in lymphocytes of contraceptive users (8,29,46) This raised the possibility that progestins or estrogens could be clastogenic and lead to liveborns with structural chromosomal abnormalities However, breakage studies are notoriously hazardous without rigorous "blind" analysis Confounding experimental variables are also legend

In conclusion, concern generated by observations of an apparent excess of polyploid abortuses following cessation of oral contraceptives has abated The consensus is that progestins do not predispose to chromosomally abnormal abortuses

INJECTABLE AND IMPLANTABLE PROGESTINS

Few data specifically applicable to injectable/implantable progestins exist The injectable and implantable progestins (medroxyprogesterone, norethisterone) are the same compounds already considered in our discussion of oral contraceptives (73) Moreover, maternal serum hormone levels are usually lower with injectables and implantables (73) Thus, conclusions drawn earlier in this report are appropriate here

One study providing data specific for injectable agents was conducted in Thailand (89) Between 1975 and 1978, 190 of 8,816 infants born in Chiang Mai were anomalous The mothers of the 190 infants used medroxyprogesterone in the same proportion as mothers of the 8,626 normal infants, but whether any fetuses were exposed during gestation is not known Even fewer direct data exist concerning injectables or implantable norethisterone or norgestrel, but similarly no direct claims of teratogenicity or mutagenicity exist

TERATOGENICITY OF INTRAUTERINE DEVICES (IUD)

That intrauterine devices could cause congenital malformations was proposed in 1976 by Barrie (7), who reported two infants with limb reduction defects whose mothers were wearing IUDs at conception Both devices (one Grafenberg ring, one probable Dalkon Shield) had remained *in situ* throughout pregnancy, and both infants showed the same type of limb reduction defects (upper and lower extremities) Leighton et al (42) added a further case associated with a copper-containing IUD

Hypothesizing that limb reduction defects result from a coexisting IUD is not unreasonable Presumably the mechanism would be related to direct mechanical action Moreover, at least one animal study (6) is consistent with a teratogenic relationship Fetuses born of rats wearing a Silastic device showed increased anomaly rates compared to animals without devices However, further reflection recalls that an IUD would need to penetrate both chorion and amnion in order to exert a direct mechanical action on the early embryo Infections could arise, but the likely outcome of infection in early pregnancy is abortion Even if the developing pregnancy envelops the IUD later in gestation, limb reduction defects would not seem particularly likely to arise Indeed, amniotic bands and their sequelae would be a more plausible outcome

Longitudinal studies of pregnancies conceived despite a coexisting IUD have later confirmed that IUDs are not teratogenic Tatum et al (82) evaluated the outcome of 918 women conceiving while wearing a Copper T device After excluding the 465 who chose elective abortion, abortion rates and anomaly rates were assessed The spontaneous abortion rate was 20 3% (24/118) with the device removed or expelled and 54 1% (85/157) with the device *in situ* The stillbirth rate was 0 9% with removal and 1 9% without removal However, only 1 anomaly (vocal cord fibroma) was observed among 166 embryos that developed to the stage at which anomalies could be

detected Albert (4) also failed to observe increased anomalies in women conceiving with copper devices Snowden (78) reported no anomalies in 317 pregnancies conceived in the United Kingdom with IUDs (20 centers, 25,000 insertions) Guillebaud (23) surveyed 20,684 insertions and 714 pregnancies There were 5 anomalies among 167 full-term infants whose mothers had an IUD *in situ* throughout pregnancy All 5 anomalies were dissimilar (bald spot, eyelid ptosis, lipoid tumor, congenital hip dislocation, spina bifida) In at least four other studies, investigators have also failed to detect an association between IUDs and anomalies (50,59,80,85)

Because numbers of exposed subjects are inevitably small in longitudinal studies, case-control designs have also been utilized Layde et al (41) conducted a case-control study of 96 mothers delivered of infants with limb reduction defects There existed no significant increase in IUD usage at time of conception The exposure frequency was 2 1% (2 96) in the limb reduction group, 1 6% (15 915) in mothers of infants with all other major anomalies, and 1 2% (1 169) in mothers of infants with chromosomal abnormalities (The latter group served as an "anomaly group" to obviate recall bias)

A second case-control study is that of Bracken and Vita (11), who studied malformed and control infants in Connecticut Of 2,191 anomalous infants, 1,417 mothers were interviewed, 3,001 controls were identified Contraceptive usage of all types was assessed The only significant association among 154 categories was that between multiple malformations and IUD usage, however, only 2 mothers wearing IUDs (i e , numerator of 2) at conception were in this category

In conclusion, pregnancies in women wearing an IUD are known to be associated with increased frequencies of ectopic pregnancy and spontaneous abortions However, there is no increased frequency of anomalies

MUTAGENICITY AND TERATOGENICITY OF VAGINAL SPERMICIDES

A relationship between vaginal spermicides and congenital anomalies has been claimed Indeed, both mutagenic and teratogenic effects are possible

A teratogenic effect is actually not particularly plausible because women conceiving while using spermicides will probably appreciate their pregnancy and stop using contraception Early use, such as until the "missed" menses, would generally result only in exposures during the "all-or-none" period, an event not likely to result in anomalies Of course, a few women might fail to recognize pregnancies until several months later Maternal storage of alcohol, mercury, nonoxynol and other spermicidal agents is also a formal consideration

In 1981 Jick et al (34) were the first to claim an association between spermicides and anomalies This well-publicized report involved a study of prescriptions filled by participants in a health maintenance organization in Seattle Of 4,772 pregnant women, 790 (17%) filled prescriptions for spermicidal contraceptives Among 4,665 liveborns were 84 anomalous infants Eighteen (18) of the 84 infants were excluded because their defects were "familial, minor, or positional " Of the remaining 66, the frequency of spermicide prescription was 2 2%, compared to only 1 0% in the remaining women An excess of "exposed" cases existed in four groups limb reduction defects, neoplasms, chromosomal abnormalities, and hypospadias

Studies like that of Jick et al (34) are never intended to do other than generate hypotheses, which in this case was certainly accomplished Most glaring among the deficiencies of experimental design is lack of verification that ostensibly exposed women even used the spermicides, especially during the cycle of conception or early pregnancy Nonetheless, two other reports also claimed a relationship between spermicidal usage and anomalous liveborns Smith et al (76) observed an association between limb reduction defects and spermicides Rothman (66) found increased spermicidal exposure in Down syndrome infants having cardiac defects, compared to normal controls However, spermicide use was not increased in mothers of infants who had cardiac defects for reasons other than Down syndrome Thus, Rothman's conclusions (66) are probably invalidated by recall bias

In contrast to the above reports are at least seven studies failing to show such an effect (11,14,28,48,49,61,72) Several are well designed We have already described the study of Bracken and Vita (11), which showed no association between anomalies and spermicide use A second noteworthy study is the review by Shapiro et al of the U S Collaborative Perinatal Project data (72), a sample also described earlier In that sample, 462 women used spermicides other than phenylmercuric acetate Usually the active compounds were nonoxynol-9 (74%) or octoxynol (18%) These 462 women used spermicides during the first 4 lunar months, with 438 also reporting use during the month preceding the last menstrual period Thus, both mutagenic and teratogenic effects were possible The 462 exposed women were delivered of 23 anomalous offspring (5%), compared to 2,254 anomalies among 49,825 nonexposed controls (4 5%) The difference is not significant, nor was it when anomalies were grouped according to those claimed significant by Jick et al (34) Down syndrome, hypospadias, and limb reduction defects Negative findings were also observed when analysis was restricted to women exposed only to phenylmercuric acetate

Polednak et al (61) used the case-control approach to

assess those anomalies implicated by Jick et al (34) The relative risks were 0 43 for limb reduction defects (n=108), 1 10 for hypospadias (n=99), and 1 17 for Down syndrome (n=103) No significant differences were observed as well for neural tube defects (n=201), cardiovascular defects, or "multiple defects"

An important search for deleterious effects of spermicides has been made by Mills and colleagues (48,49) In the most extensive study (49), a case-control design was used to evaluate 34,660 women Of these, 3,146 had used spermicides before but not after their last menstrual period. 2,282 used spermicides only after their last menstrual period Confounding variables and other contraceptive usages were documented, and analysis by time of exposure (before or after last menstrual period) was conducted No significant differences between controls and users of spermicides were noted for 157 types of malformations, for infants with 3 or more anomalies or for specific patterns of anomalies Mills et al also noted no change in the sex ratio following spermicidal exposure, offering reassurance concerning lethal X-linked recessive mutations and thus lethal mutations in general (49)

A few other fetal effects of spermicides have been claimed, but again none has been confirmed Two studies (33,71) found an increase in fetal losses, but this was likewise not confirmed by Mills et al (49) A significant reduction in female (but not male) birth weight was found by Polednak et al (61) but again not by others (49)

In conclusion, a clear scientific consensus exists that vaginal spermicides are not associated with increased malformations This applies both to offspring of women who become pregnant while using these agents as well as to those who discontinue usage prior to pregnancy

BARRIER AND OTHER METHODS

For barrier methods, there have been few if any serious claims of a relationship to anomalies Indeed, postulating a relationship between anomalies and diaphragm or condom usage seems almost frivolous Nonetheless, it is worth referencing one study that assessed and failed to observe an association The case-control study of Bracken and Vita (11) found no associations between anomalies and any of these contraceptive methods

Rhythm (natural family planning) poses no maternal risks and few *direct* fetal risks However, the method could indirectly lead to fetal abnormalities as a result of delayed fertilization — fertilization of a moribund yet still viable ovum This complicated and poorly studied topic is reviewed elsewhere by the author (74)

CONCLUSION

Pregnancies resulting from contraceptive failures — oral contraceptives, intrauterine devices, spermicides, or barrier methods — do not appear to be at increased risk for congenital anomalies A similar conclusion applies to pregnancies occurring after discontinuation of the methods Thus, neither mutagenic nor teratogenic risks exist Earlier studies claiming deleterious effects can now be refuted by more numerous and better designed studies showing no such effects

REFERENCES

1 Aarskog D Maternal progestins as a possible cause of hypospadias N Engl J Med 300 75, 1971

2 Alberman E Creasey M Elliott M, Spicer C Maternal factors associated with fetal chromosomal anomalies in spontaneous abortions Br J Obstet Gynaecol 83 261, 1976

3 Alberman E Pharoah P, Chamberlain J Roman E, Evans S Outcome of pregnancies following the use of oral contraceptives Int J Epidemiol 9 207 1980

4 Albert A Study of pregnancies in women with intrauterine devices of copper Reproduction 7 25, 1983

5 Avellan L On aetiological factors in hypospadias Scand J Plast Reconstr Surg 11 115, 1977

6 Barlow SM, Knight AF Teratogenic effects of Silastic intrauterine devices in the rat with or without added medroxyprogesterone acetate Fertil Steril 39 224, 1983

7 Barrie H Congenital malformation associated with intrauterine contraceptive device Br Med J 1 488, 1976

8 Bishun NP Chromosomes and oral contraceptives Proc Roy Soc Med 69 353, 1976

9 Boue A, Boue I Actions of steroid contraceptives on genetic material Geburtsh Frauenheilk 33 77, 1973

10 Bracken MB, Holford TR, White C, Kelsey JL Role of oral contraception in congenital malformations of offspring Int J Epidemiol 7 309, 1978

11 Bracken MB Vita K Frequency of non hormonal contraception around conception and association with congenital malformations in offspring Am J Epidemiol 117 281, 1983

12 Carr DH Chromosome studies in selected spontaneous abortions 1 Conception after oral contraceptives J Can Med Assoc 103 343, 1970

13 Carson SA, Simpson JL Virilization of female fetuses following maternal ingestion of progestational and androgenic steroids In Mahesh VB, Greenblatt RB (eds) Hirsutism and Virilization Boston, John Wright PSG, 1983, p 177

14 Cordero JF, Layde PM Vaginal spermicides, chromosomal abnormalities and limb reduction defects Fam Plann Perspect 15 16, 1983

15 Czeizel A, Toth J, Erodi E Aetiological studies of hypospadias in Hungary Hum Hered 29 166, 1979

16 David TJ, O'Callaghan SE Birth defects and oral hormonal preparations Lancet 1 1238, 1974

17 Dhardial RK Machin A, Tait SM Chromosome anomalies in spontaneously aborted human fetuses Lancet 2 20 1971

18 Gal I, Kirman B, Stern J Hormonal pregnancy tests and neural tube defects Nature 216 83, 1967

19 Goujard J Rumeau Rouquette C First trimester exposure to progestogen estrogen and congenital malformations Lancet 1 482 1977

20 Goujard J Rumeau Rouquette C Saure Cubizalles MJ Tests hormonaux de grossesse et malformations congenitales J Gynecol Obstet Repr 8 489 1979

21 Gray RH Progestins in therapy Teratogenesis In Benagiano G (ed) Progestogens in Therapy New York Raven Press 1983 p 109

22 Greenberg G Inman WHW Weatherall JAC Adelstein AM Haskey JC Maternal drug histories and congenital abnormalities Br Med J 2 853 1977

23 Guillebaud J IUD and congenital malformations Br Med J 1 1016, 1976

24 Harlap S Eldor J Births following oral contraceptive failure Obstet Gynecol 55 447, 1980

25 Harlap S Prywes R Davies AM Birth defects and oestrogens and progesterones in pregnancy Lancet 1 682 1975

26 Heinonen OP Slone D Monson RR Hook EB Shapiro S Cardiovascular birth defects in antenatal exposure to female sex hormones N Engl J Med 296 67, 1976

27 Heinonen OP Slone D, Shapiro S Birth Defects and Drugs in Pregnancy Littleton Mass, Pub Sciences Group 1977

28 Huggins G Vessey M, Flavil R Vaginal spermicides and outcome of pregnancy Findings of a large cohort study Contraception 25 219, 1982

29 Jagiello G, Lin JS Oral contraceptive compounds and mammalian oocyte meiosis Am J Obstet Gynecol 120 390 1974

30 Janerich DT Dugan JM Standfast SJ Strite L Congenital heart disease and prenatal exposure to exogenous sex hormones Br Med J 1 1058, 1977

31 Janerich DT, Polednak AP Epidemiology of birth defects Epidemiol Rev 5 16, 1983

32 Janerich DT Piper JM Glebatis DM Oral contraceptives and congenital limb reduction defects N Engl J Med 291 697 1974

33 Jick H Shicta K Shepard T Hunter J Stergachis A Madsen S, Porter JB Vaginal spermicides and miscarriage seen primarily in the emergency room Teratogen Carcinogen Mutagen 2 205 1979

34 Jick H, Walker AM Rothman KJ, Hunter JR, Holmes LB, Watkins RN, D'Ewart DC Danford A, Madsen S Vaginal spermicides and congenital disorders JAMA 245 1329 1981

35 Katz Z, Lancet M Skornik J, Chemke J, Mogilner BM Klinberg M Teratogenicity of progestogens given during the first trimester of pregnancy Obstet Gynecol 65 775 1985

36 Klinger HP Glaser M Kava HW Contraceptives and conceptus 1 Chromosome abnormalities of the fetus and neonate related to maternal contraceptive history Obstet Gynecol 48 40, 1976

37 Kullander S Kallen B A prospective study of drugs and pregnancy Acta Obstet Gynecol Scand 55 221 1976

38 Lang R Redman U Non mutagenicity of some sex hormones in the Ames salmonella microsome mutagenicity test Mutation Res (Amsterdam) 67 361, 1979

39 Laurence M, Miller M, Vowles M, Evans K, Carter C Hormonal pregnancy tests and neural tube defects Nature 233 495, 1971

40 Lauritsen JG The significance of oral contraceptives in causing chromosome anomalies in spontaneous abortions Acta Obstet Gynecol Scand 64 261 1975

41 Layde PM Goldberg MF Safra MJ, Oakley GP Jr Failed intrauterine device contraception and limb reduction deformities A case control study Fertil Steril 31 18, 1979

42 Leighton PC Evans DG, Wallis SM IUD and congenital malformations Br Med J 1 959, 1976

43 Levy EP Cohen A, Fraser FC Hormone treatment during pregnancy and congenital heart disease Lancet 1 611, 1973

44 Lorber CA Cassidy SB Engel E Is there an embryo fetal exogenous sex steroid exposure syndrome (EFESSES)? Fertil Steril 31 21 1979

45 Matsunaga E, Shioto K Threatened abortion hormone therapy, and malformed embryos Teratology 20 469, 1977

46 McQuarrie HG Cytogenic studies on women using oral contraceptives and their progeny Am J Obstet Gynecol 107 659 1970

47 Michaelis J Michaelis H, Gluck E, Koller S Prospective study of suspected associations between certain drugs administered during early pregnancy and congenital malformations Teratology 27 57 1983

48 Mills JL Harley EE Reed GF Berendes HW Are spermicides teratogenic? JAMA 248 2148, 1982

49 Mills JL, Reed GF, Nugent RP, Harley EE, Berendes HW Are there adverse effects of periconceptional spermicide use? Fertil Steril 43 422, 1985

50 Mishell D Pregnancy related complications and bleeding problems with IUDs In Sciarra JJ Zatuchni GI, Speidel JJ (eds) Risks Benefits, and Controversies in Fertility Control Hagerstown, Harper & Row, 1978, p 428

51 Monteleone RN, Castilla EE, Paz JE Hypospadias An epidemiologic study in Latin America Am J Med Genet 10 5, 1981

52 Mulvihill JJ, Mulvihill CG Neill CA Prenatal sex hormone exposure and cardiac defects in man Teratology 9 A30, 1976

53 Nishimura H Uwabe C Semba R Examination of teratogenicity of progestogens and or estrogens by observation of the induced abortuses Teratology 10 93 1974

54 Nora JJ Nora AH Preliminary evidence for a possible association between oral contraceptives and birth defects Teratology 7 A24, 1973

55 Nora AH, Nora JJ A syndrome of multiple congenital anomalies associated with teratogenic exposure Arch Environ Health 30 17, 1975

56 Nora JJ Nora AH Blum J, Ingram J, Fountain A, Peterson M, Lortscher RH, Kimberling WJ Exogenous progestogen and estrogen implicated in birth defects JAMA 240 837, 1978

57 Oakley GP, Flynt JW Falek A Hormonal pregnancy tests and congenital malformations Lancet 2 256, 1973

58 Ortiz Peres HE Fuertes-De la Habra A, Bangdiwala IS Rowie CA Abnormalities among offspring of oral and non-oral contraceptive users Am J Obstet Gynecol 134 512, 1979

59 Perlmutter JF Pregnancy and the intrauterine device J Reprod Med 20 137, 1978

60 Peterson WF Pregnancy following oral contraceptive therapy Obstet Gynecol 34 363, 1969

61 Polednak AP, Janerich DT, Glebatis DM Birth weight and birth defects in relation to maternal spermicide use Teratology 26 27, 1982

62 Resseguie LJ, Hick JF, Bruen JA, Noller KL O'Fallon WM, Kurland LT Congenital malformations among offspring exposed in utero and progestins Olmstead County, Minnesota, 1936 1974 Fertil Steril 43 514, 1985

63 Rock JA, Wentz AC, Cole KA Kimball AW Jr Lazar HA, Early SA, Jones GS Fetal malformations following progesterone therapy during pregnancy A preliminary report Fertil Steril 44 17, 1985

64 Rice Wray E, Cervantes A Gutierrez J, Marquez Monter H Pregnancy and progeny after hormonal contraceptives — genetic studies J R Soc Med 6 101, 1971

65 Rice Wray E Corren S, Gastelum H Erfahrungen mit dem oralen Kontrazeptivum Anovlar Med Klin 61 24, 1966

66 Rothman KJ Spermicide use and Down's syndrome Am J Publ Health 72 399, 1982

67 Rothman KJ, Fyler DDC, Goldblatt A, Kreidberg MB Exogenous hormones and other drug exposures of children with congenital heart disease, Am J Epidemiol 109 433, 1979

68 Rothman KJ Louik C Oral contraceptives and birth defects N Engl J Med 299 522, 1978

69 Royal College of General Practitioners The outcome of pregnancy in former oral contraceptive users Br J Obstet Gynaecol 83 608, 1976

70 Savolainen E, Saksela E Saxen L Teratogenic hazards of oral contraceptives analyzed in a national malformation register Am J Obstet Gynecol 140 521, 1981

71 Scholl TO, Sobel E, Tanfer K, Soefer EF, Saidman B Effects of vaginal spermicides on pregnancy outcome Fam Plann Perspect 15 244 1983

72 Shapiro S, Slone DH, Heinonen OP Kaufman DW, Rosenberg L, Mitchell AA, Helmrich SP Birth defects and vaginal spermicides JAMA 247 2381, 1982

73 Simpson JL Mutagenicity and teratogenicity of injectable and implantable progestins Probable lack of effect In Zatuchni GI, Goldsmith A, Shelton JD, Sciarra JJ (eds) Long Acting Contraceptive Delivery Systems Philadelphia, Harper & Row, 1984, p 334

74 Simpson JL Relationship between congenital anomalies and contraception Adv Contracep 1 3, 1985

75 Simpson JL Golbus MS, Martin AO, Sarto GE Genetics in Obstetrics and Gynecology New York, Grune & Stratton, 1982

76 Smith ESO, Dafoe CS, Miller JR An epidemiological study of congenital reduction deformities of the limbs Br J Prev Soc Med 31 39, 1977

77 Smithells RW, Chen ER The problem of teratogenicity Practitioner 198 104, 1965

78 Snowden R IUD and congenital malformation Br Med J 1 770, 1976

79 Spira N Goujard J, Huel G, Rumeau Rouquette C Etude teratogene des hormones sexuelles Premiers resultats d une enquete epidemiologique portant sur 20 000 femmes Rev Red Fr 41 2683, 1972

80 Stevens JD, Fraser IS The outcome of pregnancy after failure of an intrauterine contraceptive device J Obstet Gynaecol Br Commonw 81 282, 1974

81 Sweet RA, Schroot HG Kurland R, Cupl OS Study of the incidence of hypospadias in Rochester, Minnesota, 1940 .970, and a case control comparison of possible etiologic factors Mayo Clin Proc 49 52, 1974

82 Tatum HJ Schmidt FH, Jain AK Management and outcome of pregnancies associated with the Copper T intrauterine contraceptive device Am J Obstet Gynecol 126 869 1976

83 Torts CP, Milkovich L, Van Den Berg BJ The relationship between hormonal pregnancy tests and congenital anomalies A prospective study Am J Epidemiol 113 563, 1981

84 Tuchman Dupleisses H, Mercier Parot L Action d'un steroide anticonceptionnel sur la descendance J Gynecol Obstet Biol Reprod 1 141 1972

85 Vessey M, Meisler L, Flavil R Outcome of pregnancies in women using different methods of contraception Br J Obstet Gynecol 86 548 1979

86 Wilson JG, Brent RL Are female sex hormones teratogenic? Am J Obstet Gynecol 141 567, 1981

87 Wiseman RA, Dodds Smith IC Cardiovascular birth defects and antenatal exposure to female sex hormones A reevaluation of some base data Teratology 30 359, 1984

88 World Health Organization The effect of female sex hormones on fetal development and infant health World Health Organization (WHO) Technical Report Series 657 Geneva, WHO 1981

89 World Health Organization Facts about injectable contraceptives Memorandum from a WHO meeting Bull WHO 60 199, 1982

90 Yasuda M, Miller JR Prenatal exposure to oral contraceptives and transposition of the great vessels in man Teratology 12 239, 1975