

Artificial Insemination in the Human*

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The idea that impregnation might occur without coitus has aroused interest since ancient times. In the second century A.D., there was recorded a hypothetical discussion concerning a woman who had been inseminated by semen previously deposited in the bath water in which she bathed. In 1322, an Arab used artificial insemination with horses. A wad of wool was introduced into the vagina of a mare and left overnight. It was then held over the nostrils of a stallion, and with this stimulus the stallion ejaculated on a cloth held in readiness. The ejaculated material was then introduced into the vagina of a mare, which foaled after the appropriate length of time.

During the 1700's, isolated instances of human artificial insemination were reported, almost always using the husband's semen. In the United States, human artificial insemination using donor's semen was first practiced by Dr. Robert L Dickinson in 1890. His work was initially done in secrecy, although subsequently he did much to train others in the technique and to gain public acceptance of the procedure.

Artificial Insemination Using Donor's Sperm.

The most common indication for artificial insemination using donor's semen is sterility of the husband. This may be manifest as total azoospermia or severe oligospermia, demonstrated on repeated examinations. The husband should, of course, have a thorough medical and urologic examination to be certain that his sterility is not secondary to a treatable condition. Considerable judgment is required to decide when to treat cases of oligospermia this way. In most instances, this is done when appropriate medical therapy has failed to improve the quality of the ejaculate and artificial insemina-

tion using the husband's semen has not resulted in pregnancy.

When Rh incompatibility has already resulted in the birth of an erythroblastotic infant, and particularly when the husband is homozygous Rh positive, insemination of the wife with semen from an Rh negative donor will prevent the recurrence of erythroblastosis. Fortunately, the availability of Rh₀ (D) immune globulin (human) has dramatically reduced the number of Rh negative women who have been sensitized, and this is becoming a less common indication for artificial insemination.

When the husband has a family history of genetic disease which makes fatherhood inadvisable or when the couple has had affected offspring indicating abnormal recessive genes with the likelihood of producing serious congenital defects in subsequent pregnancies, artificial insemination with donor's semen may be used. Examples of this are Tay-Sachs disease, and cases where both husband and wife have AS hemoglobin.

The presence of agglutinating antibodies against the husband's sperm but not against donor sperm may occur in a woman with prolonged and otherwise unexplained infertility (Dukes and Franklin, 1968). The test for this is not an absolute one, as some women who agglutinate their husband's sperm have been of high fertility. In a situation where antibodies are present, in some cases the use of condom for a time to protect against repeated exposure to antigen will result in disappearance of agglutinating antibodies followed by pregnancy. Where condom therapy fails, artificial insemination using donor's sperm which are not agglutinated by the wife's antibodies may be used. The ABO relationship to infertility is even more unclear, although there seems to be a statistically significant effect when an ABO incompatibility exists and the husband is a "secretor," in which case donor semen from a male of appropriate blood type may result in pregnancy.

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Donor insemination has special emotional connotations, and both husband and wife should be aware of the possible emotional effect of the procedure on their future relationship. Both husband and wife should be reasonably well-adjusted individuals, and they should feel that they have a stable marriage. It is extremely important not to do this procedure to "save the marriage" as this is far too big a job to place upon the shoulders of an individual who weighs only seven pounds or so. Both husband and wife should have a desire for children, and the man should not feel that he is being coerced into this procedure in order to provide fulfillment of his wife's maternal instincts. In most cases, it is advisable to wait for a while after the husband has found out about his sterility problem before carrying out artificial insemination because he will need time to recover from the shock that such news is to every man. When it has been decided to use insemination with donor's semen, both husband and wife should sign a consent form.

In the past when there was no shortage of babies for adoption, we did not encourage artificial insemination, but did it only when the couple specifically requested it, feeling that adoption solved two social problems, that is, providing a family to the child and a child to the family. With the recent increase in therapeutic abortions, the supply of infants for possible adoption has so dwindled that artificial insemination is more often mentioned to the couple as a solution to their infertility or genetic problems. Aside from suggesting the availability of donor's semen to such couples, we do not try to encourage this where either partner expresses any hesitancy.

A fertility investigation is done for each couple seeking artificial insemination, if this has not been previously accomplished. As a minimum, patency of the wife's fallopian tubes is established by a Rubin's test using carbon dioxide, and an endometrial biopsy is done to confirm ovulation. The patient is also instructed to begin keeping basal temperature charts, in order that the most optimal time for insemination may be selected. After reviewing temperature charts for several months, a day is selected. This day will likely be one or two days prior to the basal temperature rise. It has been our practice to perform insemination once a month for the first three months, and if pregnancy has not occurred in this time, then to perform several at two-day intervals until the temperature rise has definitely occurred. Some physicians use a rat

ovary hyperemia test to predict the time of ovulation. This test is said to be capable of determining the time of ovulation within six to twelve hours (Farris, 1948). More recently, the use of rapid radio immunoassay procedures for LH, in order to detect the pre-ovulatory LH peak, has been suggested as an aid in timing the deposition of sperm, whether it be by natural or artificial means.

Kleegman has found there is a sex differentiation in infants related to the time of artificial insemination and ovulation (Kleegman, 1967). She utilized basal temperatures somewhat to determine the time of ovulation, but put more emphasis on mittelschmerz (sometimes elicited with a "bounce test") and mucorrhea. Using this as evidence of ovulation, she has noted that exposure 2-24 hours before ovulation was more likely to result in a male infant and that exposure 36 or more hours before ovulation or 2-8 hours after ovulation was more likely to result in a female infant. She was able to predict sex in 77% of cases utilizing this method. She also noted that men who have severe oligospermia but whose wives have still become pregnant, have a preponderance of female children and that rhythm failures with conception occurring on cycle days 4 to 7 usually result in female infants. Certain other investigators have been unable to reduplicate this accuracy in prediction (Cohen, 1966), but in fairness to Kleegman, it should be noted that most have used only basal temperature as an index of ovulation and have not relied on mittelschmerz or cervical mucus changes.

Most of the donors which we use are either medical students or house officers, who are married and are already fathers of normal children. These men report no knowledge of hereditary disease and are of above average intelligence. If the wife has Rh negative blood, an Rh negative donor is also chosen. We do not make any effort to match blood types otherwise, because any good laboratory could readily establish the fact that the woman's husband is not the father of the child by utilizing other blood groups such as the MN, Kidd, Duffy, and Kell which establish a "fingerprint." We make an effort to match donor to husband by somatic type, skin, hair, and eye coloration, but this is not always possible. We would not, however, use a short, black-haired donor of Mediterranean origin for a couple where both husband and wife were tall, blonde, and fair-skinned.

Our experience has been entirely with fresh semen, and the donor is instructed to obtain a specimen by masturbation into a clean glass con-

tainer shortly before the couple's appointment. The donor then delivers the semen to a designated point, and it is then transferred to the patient area by other personnel. It is important that the donor not deliver the specimen himself to this office, in order to exclude the possibility that he and the recipients might meet. In order to preserve anonymity, the couple always pays the donor in cash. Our practice is to keep no record of which donor is used for insemination, and this precludes using the same donor for subsequent pregnancies. It is of note that some physicians do not even record in the patient's chart the fact that insemination was done.

On the selected day, the patient lies on an examining table, and an unlubricated vaginal speculum is inserted. The donor's semen is drawn into a syringe which has a small length of polyethylene tubing attached to it. The husband then comes to the examining room. The husband holds the syringe and deposits 0.1 to 0.5 ml. of semen about 1 cm. within the endocervical canal. The polyethylene tubing and speculum are then removed, and a plastic cap with an attached tubing is placed over the cervix. The remainder of the donor's semen is then inserted into the cervical cap by the husband. We feel that this active participation in the insemination of the wife by the husband is very important for him psychologically. The cervical cap is sometimes difficult for the patient to remove, so while the husband is in the examining room, we instruct both him and his wife on the removal of the cervical cap, and to date, between the two of them, this has always been accomplished at home.

We do not advocate the mixing of husband's ejaculate with donor's semen or that the couple have intercourse immediately prior to coming to the office for insemination. A cervical mucus-spermatozoa incompatibility is not always due to a cervical factor, and one case was reported where the donor's sperm but not the husband's sperm readily entered the wife's cervical mucus (Kunitake and Davajan, 1970). When the husband's sperm was separated from the seminal plasma and resuspended in seminal plasma from a donor who had had a vasectomy, good penetration of the wife's cervical mucus was obtained. Unless this seminal plasma factor is checked for, it would be inadvisable to mix husband's semen with donor's semen. After the cervical cap has been in place for six hours or so, the couple is advised to remove it, and it is suggested that they then have intercourse.

By this time, active sperm should be well on their way up the female reproductive tract and any lethal activity in the seminal plasma would be of no consequence.

When a woman has somewhat irregular ovulation, she is given an injection of 5000 international units of human chorionic gonadotrophin to act as an LH surge, hopefully to cause an impending ovulation to occur while viable sperm are still present (Fuchs, *et al*, 1966).

The effectiveness of insemination using donor semen has been shown in many studies. In an analysis of seven reported series which included 630 couples who had used artificial insemination with donor semen, the proportion becoming pregnant varied from 55-78%. Of the women who eventually became pregnant, between 31 and 46% became pregnant during the first month of insemination. The number of inseminations per menstrual cycle appeared to be more significant in obtaining an early pregnancy than the method of semen deposit (Potter, 1958). About 90% of the women who became pregnant did so in 6 months, and if a pregnancy has not resulted within 12 months, the chances of success are very remote.

Artificial Insemination Using Husband's Semen.

Indications for insemination using husband's semen include failure to deposit the semen in the posterior vaginal fornix, inadequate cervical invasion, and a moderate but irreversible degree of male infertility.

The failure to deposit the semen may be due to penile or vaginal malformations, impotence, or retrograde ejaculation. Penile hypospadias was one of the first-recorded indications for artificial inseminations, and this is still valid. The cases of impotence are best individualized, and decisions should be made only after consultation with the husband's psychiatrist. The therapy for male impotence is psychiatric, and in some instances a pregnancy in the wife and impending fatherhood will exert a beneficial psychiatric effect, while in others this is best postponed until psychotherapy has progressed further. Retrograde ejaculation may follow injury to the internal bladder sphincter due to surgery such as transurethral resection. However, retrograde ejaculation may also be due to congenital anatomical abnormalities or neurologic problems, including diabetes and chemical sympathectomy achieved with guanethidine. Typically, there is not only azoospermia but also markedly reduced semen volume, although an ejaculatory sensation is experienced with orgasm. To corroborate the diagnosis, the patient empties his bladder and

then ejaculates in one glass, after which he collects in a separate glass whatever urine is in the bladder.

Artificial insemination using husband's semen is also sometimes used in an attempt to bypass the cervical mucus when it is found that normal sperm cannot migrate through this or when postcoital tests reveal no living sperm in the presence of apparently normal mucus and a good sperm count. The achievement of pregnancy in these last two instances is quite rare in our hands, although Kleegman reported a success rate of around 85% in the small group where the only abnormal factor was a cervical secretion impenetrable to sperm (Kleegman and Kaufman, 1966). She stated that most cases of cervical impenetrability are due to endocrine dysfunction rather than cervicitis, and the results of husband insemination in this group are poor. Others have found consistently poor results in all attempts to bypass the cervix (Balin, 1967).

When intrauterine insemination is done, the cannula is placed in the endometrial cavity and only 0.5 ml. or less of semen is instilled. This is done very slowly with practically no pressure. The physician must be careful not to inject the semen through the fallopian tubes into the peritoneal cavity where it will cause a peritonitis, and he must be ready to stop the installation if the patient complains of any cramping.

Insemination with husband's semen may be tried when the wife is of apparently good fertility and the husband's sperm consistently has a low count with the postcoital test showing only a few sperm in the cervical canal. This may also be utilized when the husband's sperm count is within normal limits, but the motility of the sperm is less than normal. The split ejaculate offers a concentrated number of better sperm, and is often employed when husband's semen is to be inseminated.

Split ejaculates are obtained simply by using one clean container for the first part of the ejaculate and another for the remainder. It is most useful when the volume of the ejaculate is over 2 ml. In one study of 86 husband donors, the count was significantly higher in the first portion than in the second portion of the total specimen in 88%, equally distributed in both in about 8%, and significantly higher in the second portion than in the first in 6%. Sperm motility was greater in the first portion in 77%. In 25 cases of increased viscosity of the total ejaculate, the second portion was consistently the most viscous (Amelar and Hotchkiss, 1965).

Biochemical studies of the split ejaculate have

indicated that the first half contains the main bulk of acid phosphatase, which comes from the prostate, along with the products of the testes, epididymis, and vas deferens. Since lactic acid accumulates as an end product of the metabolism of spermatozoa, it is also found in greater concentration here. In the second half of the split ejaculate, the higher concentration of fructose is found, a substance specific to the seminal vesicles (Amelar and Hotchkiss, 1965; MacLeod and Hotchkiss, 1942).

Centrifugation of the full specimen concentrates the sperm, but frequently depresses the motility and also concentrates the debris and mucus. Concentration of the sperm can be obtained using the split ejaculate without injuring the cells and without concentrating debris also.

Kleegman reports that with 100 consecutive women who had intrauterine insemination of the split ejaculate in cases where there was subnormal husband's sperm quality, 17 women became pregnant (Kleegman and Kaufman, 1966). Other reports include 15% pregnancies in 86 women with insemination of husband's semen (Kaskarelis and Comminos, 1959). A number of women who failed with insemination conceived subsequently with normal intercourse.

Insemination with husband's sperm suspended in donor's seminal plasma may be considered in the rare cases where the husband's own seminal plasma contributes to cervical mucus-spermatozoa incompatibility or where there is excessively low fructose in the husband's seminal plasma (Moon and Bunge, 1968).

Frozen Semen. Much has been written about the practical applications of frozen human semen, with the establishment of human semen banks. For example, by utilizing such a bank, couples could be suitably matched according to many physical characteristics and by blood type, regardless of the time and place. Another possible use of frozen semen entails its use in insemination with husband's semen where the husband is oligospermatic. Several split ejaculates could be pooled, and then delivered to the wife at the calculated fertile time. It has been proposed that men might deposit semen in a bank for possible future use prior to having a vas ligation. Other possible uses of semen banks sound like science fiction stories. They include (1) having a supply safely stored behind thick walls in case of nuclear attack which might sterilize the entire male population of the country, (2) making it possible for a man to sire offspring

many years after he had died; and (3) complete population control where all pregnancies are from bank sperm and only those sperm known to transmit characteristics thought to be useful to the state are dispensed, thus enabling the development of either a "super race" or a "leader-slave culture."

In any event, various techniques have been used to freeze sperm. Most employ glycerol as a protecting medium. One study from the University of Michigan used a protective medium of egg yolk, glycerol, glucose plus sodium citrate solution, and glycine, which was buffered at pH 7.3. The semen sample was mixed with the protective medium, cooled slowly to -80°C and then stored at -196.5°C . Thawing was performed in a 37°C . water bath, and inseminations were then done within 30 minutes of thawing the samples (Behrman and Sawada, 1966).

A conception rate of 40–50 % may be expected when frozen semen is used, and this compares unfavorably with the 75% which may be achieved by using fresh specimens for donor insemination. This would be an indication that the fertilizing capacity of the frozen-preserved sperm is relatively low. In the experience which was reported from the University of Michigan in 1968, conception had never been achieved when a frozen-preserved specimen had been administered more than 24 hours prior to ovulation as determined by basal temperature records. This was thought to indicate a relatively short term of viability. Human sperm have an apparently good motility after thawing, but despite this, fertility potential is reduced. Ackerman was able to demonstrate a pronounced effect upon sperm metabolism by cold-shock and believes that the metabolic changes occurring in frozen-preserved human sperm may account for its lowered fertility capacity (Ackerman, 1968).

Pedersen and Lebech found that most cells showed varying and often pronounced ultrastructure changes after a routine method of freezing for semen storage (Pedersen and Lebech, 1971). The most conspicuous changes were found in the acrosome region, the anterior segment of the acrosome becoming increasingly swollen, the content thinned out, and the outer acrosome membrane becoming tortuous and discontinuous. As a result, the anterior end of the spermatozoa loses the cell membrane and the anterior segment of the acrosome. Changes in the mitochondrial matrix and in the endpiece were also noted. As the acrosome has previously been shown to contain enzymes which probably play a role in lysing the zona pellucida during pene-

tration, it seems probable that morphologic changes in this region result in enzyme changes. It is possible that the changes reported here were influenced by the protective substance in which the spermatozoa were mixed rather than by the freezing itself, nevertheless, the structural changes did follow an accepted method of sperm preservation.

Because of the lowered conception rate and the ultrastructure changes associated with frozen semen, we do not think that this technique is ready for routine clinical use, but believe that its present usefulness is in an investigational setting.

Artificial insemination in appropriate cases, using fresh human semen from either husband or donor, will result in a high pregnancy rate and will successfully treat infertility.

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