Success and failure of embryo transfer involves at least five major variables: (1) technique, (2) what is transferred, (3) when transfer occurs, (4) endometrial status at transfer, and (5) endometrial support subsequent to transfer. Because of these and other variables associated with programs of in vitro fertilization, it is quite impossible to develop impeccable data to support any of the various options available for each of the five major variables.

THE VARIABLES

Technique

The goal of transfer is to deposit the fertilized eggs near the top of the fundus equidistant from the cornua. Various types of catheters have been used, but in order to accomplish this goal, a rather stiff catheter is required. While this may have the disadvantage of gouging the endometrium, it has not been shown that damage to the endometrium is harmful. A bullet-tip to the catheter should facilitate transit through the cervical canal and a suitable carrier is useful to provide force along the axis of the canal (FIG. 1). The instrument and patient position for transfer used at Norfolk have been one of the few constant aspects of the program, having been used from the very first transfer. As of March 31, 1984, 501 transfers had been carried out and there has been no failed transfer at the initial attempt. No anesthesia or analgesia has been used.

Fortunately, there have been no ectopic pregnancies to date. It is, of course, unknown whether this is related to the transfer technique, which employs a rather stiff Teflon catheter which probably deposits the conceptuses equidistantly from the cornua.

The knee-chest position has been used routinely for patients whose uterus is in the normal anterior position. The rationale is simply to have the corpus of the uterus lower than the cervix. The transfer catheter is rotated as it is removed and the patient remains in the prone position for a minimum of 4 hours. A rather large amount of culture medium is used: 30 mcl, separated by an airlock from an additional 30 mcl in which the conceptuses are included, separated by a small airlock from yet another 30 mcl of culture medium. After injection of this bolus of material, a small amount of air is also injected to clear the catheter. At the end of the procedure, there may be a slight bubbling at the cervix, but observation of the cervix for a minute after removal of the catheter often fails to reveal any bubbling or regurgitation.

During 1983, a series of 33 patients underwent embryo transfer using 100% maternal inactivated serum as the medium in which the conceptuses were placed. The pregnancy rate for this series of patients was only 18% and the use of this high maternal serum medium has therefore been discontinued.
There has been a reluctance by some workers to transfer more than three conceptuses because of concerns about multiple pregnancies. This point of view has been strengthened by what seemed to be some diminution in the pregnancy rate with the high numbers of multiple transfers. However, this has not been the Norfolk experience. Except for a curious diminution with the transfer of five conceptuses, the pregnancy rate has constantly increased with the use of increasing numbers of conceptuses up to six (TABLE 1).

To be sure, multiple pregnancies have occurred. It is possible to calculate the anticipated number of multiple pregnancies by expansion of the simple binomial \((a + b)^n = 1\) where \(a\) is the expectancy of pregnancy with a single transfer and \(b\) is the expectancy of failure with a single transfer and where \(n\) represents the number of conceptuses transferred. By expanding the binomial, a table may be constructed that shows the expectancy of singleton pregnancies, twins, and so forth (TABLE 2). For such a calculation, a basic assumption is required and that is that the expectancy of pregnancy with the transfer of each conceptus is the same. In the case of the Norfolk experience, the expectancy of pregnancy for the transfer of a single conceptus is 20%. Therefore, in the expansion of the binomial, \(a = 20\%\) and \(b = 80\%\). If the actual experience with singletons, twins, triplets, and so forth is compared to this theoretical calculation, it can be seen that in the Norfolk experience there are fewer multiple pregnancies than expected by the theoretical calculation (TABLE 3). It is possible to use this evidence to support the notion that the conceptuses transferred are fundamentally

Table 1. Incidence of Pregnancy with Multiple Transfer: Norfolk, 1981–1983 (Series 1–12)

| No. of Conceptuses Transferred | No. of Transfers | % Pregnancy/Transfer |
|-------------------------------|------------------|----------------------|
| 1                             | 153              | 20%                  |
| 2                             | 147              | 23%                  |
| 3                             | 72               | 29%                  |
| 4                             | 33               | 39%                  |
| 5                             | 16               | 19%                  |
| 6                             | 8                | 50%                  |
TABLE 2. Probability of Multiple Pregnancy with Multiple Transfer

| No. of Conceptuses Transferred | Probability (%) of: | Total Rate |
|-------------------------------|---------------------|------------|
|                               | Singleton | Twins | Triplets | Quadruplets | Quintuplets | Sextuplets |          |
| 1                             | 20        |        |          |            |            |            | 20        |
| 2                             | 32        | 4      |          |            |            |            | 36        |
| 3                             | 38        | 10     | 0.8      |            |            |            | 49        |
| 4                             | 41        | 15     | 2.6      | 0.2        |            |            | 59        |
| 5                             | 41        | 20     | 5.1      | 0.6        | 0.03       |            | 67        |
| 6                             | 39        | 25     | 8.2      | 1.5        | 0.15       | 0.006      | 74        |

NOTE: It is assumed (1) that the probability of pregnancy with a single transfer = 20% and (2) that all conceptuses have an equal chance.

of different quality in spite of the fact that they may not exhibit such differences on morphologic examination.

When to Transfer

As judged by information from normal reproduction, the fertilized egg usually reaches the uterus approximately on the fifth day after ovulation, counting ovulation as day 1. There has been a reluctance to delay transfer to this normal physiological time because early workers in the field found that mitotic arrest was frequent after day 3. In addition to that, certain animal work has shown that the rate of mitosis in vivo is somewhat less than it is in vitro. For these reasons, there has been a tendency for transfer to occur earlier than would be indicated from available data. In the Norfolk program, and in many other programs, transfer usually occurs on day 3 (counting day 1 as the day of aspiration), but in a small group of cases, transfer in the Norfolk program has occurred on day 4. On day 3, the conceptus is usually 4 cells but may vary between 3 and 8 cells. On day 4, a preovulatory egg fertilized some 6 hours after aspiration will be at the 16-cell stage or thereabouts. In the Norfolk program, transfer frequently involves immature eggs that have been incubated up to 36 hours following aspiration and prior to insemination. If such eggs are transferred on day 3, that is, at about 48 hours, they are often transferred in the pronuclear stage. For this reason, in the last several months if there has been a preponderance of immature eggs, the transfer has been keyed to the immature eggs, that is, it has been carried out on day 4, when the immature eggs will be in about the 4-cell stage and any preovulatory eggs in

TABLE 3. Percentage of Observed Pregnancies versus Probability by Binomial Expansion*: Norfolk, 1981–1983 (Series 1–12)

| No. of Conceptuses Transferred | Singleton | Twins | Triplets | Quadruplets | Quintuplets | Sextuplets | Total Rate |
|-------------------------------|-----------|-------|----------|--------------|-------------|------------|------------|
| 1                             | 19(20)    | 1     |          |              |             |            | 20(20)     |
| 2                             | 20(32)    | 3(4)  |          |              |             |            | 23(36)     |
| 3                             | 26(38)    | 3(10) | 0(1)     |              |             |            | 29(49)     |
| 4                             | 27(41)    | 6(15) | 6(3)     | 0(0.2)       |             |            | 39(59)     |
| 5                             | 13(41)    | 6(20) | 0(5)     | 0(0.6)       | 0(0.03)     |            | 19(67)     |
| 6                             | 25(39)    | 25(25)| 0(8)     | 0(1.5)       | 0(0.15)     | 0(0.006)   | 50(74)     |

*Shown in parentheses.
that same transfer usually in the 16-cell or uncountable cell stage. When transfers were considered where there was at least one preovulatory egg, the pregnancy rate with transfer on day 3 was 26%, but the pregnancy rate with transfer on day 4 was 37%. This would seem to indicate that transfer on day 4 was better than day 3, but an examination of the details of what was transferred indicates that on day 4 there were a large number of patients with multiple egg transfers so that it is difficult to show statistically that one day is better than another (TABLE 4). Additional data on this point would be very welcome.

*Endometrial Status at Transfer*

In normal reproduction, the conceptus reaches the uterus when the endometrium exhibits the first morphologic evidence of secretion. This is on day 18 or 19 of an idealized 28-day cycle, with ovulation presumed to have occurred on day 14. Therefore, transfer on day 3 of the luteal phase, with day 1 counted as the day of aspiration, is early with respect to the norm and deposits the conceptus in an endometrium that is 48–72 hours “earlier” than the endometrium that receives the conceptus under normal circumstances. A biopsy study of the endometrium on day 3 in patients stimulated with human chorionic gonadotropin who had not undergone embryo transfer showed a considerable variation in secretory activity. The secretory activity had a very good correlation with the peripheral serum progesterone values. When the serum progesterone value exceeded 12 ng/ml on luteal day 3, the endometrium was advanced and showed evidences of early secretion, that is, it had changes consistent with those of day 18. A study of the serum progesterone values in patients who became pregnant as compared with values in patients who did not suggests that pregnancy occurs in patients with serum progesterone values of approximately 12 ng/ml on the day of transfer. The presumption is that in those patients who become pregnant, the endometrium on the day of transfer was advanced to about day 18 of the idealized cycle. In patients stimulated with hMG, the luteal-phase progesterone values and indeed the estradiol values are considerably higher than those in the normal cycle (FIG. 2). It is an open question as to whether these elevated values are beneficial, but the evidence just cited suggests that they are.

Not only has the correlation between the pregnancy rate and the serum progesterone values been impressive, but also the ratio between serum progesterone and estradiol seems to be important.

### TABLE 4. Pregnancies by Day of Transfer and Number of Conceptuses Transferred: Norfolk, 1981–1983 (Series 1–12)

| No. of Conceptuses Transferred | Transfer on Day 3 | Transfer on Day 4 |
|-------------------------------|------------------|------------------|
|                               | No. | Pregnancies | Pregnancies/ Transfer | No. | Pregnancies | Pregnancies/ Transfer |
| 1                             | 119 | 26          | 22                  | 2   | 1           | 50                  |
| 2                             | 119 | 30          | 25                  | 7   | 1           | 14                  |
| 3                             | 44  | 14          | 32                  | 19  | 6           | 32                  |
| 4                             | 23  | 8           | 35                  | 7   | 5           | 71                  |
| 5                             | 8   | 2           | 25                  | 7   | 1           | 14                  |
| 6                             | 3   | 1           | 33                  | 4   | 3           | 75                  |
| Total                         | 316 | 81          | 26                  | 46  | 17          | 37                  |
FIGURE 2. Serum progesterone values by day of luteal phase; normalized day of aspiration is regarded as day 1.

**Endometrial Support**

It has been shown by numerous studies that aspiration of follicles does not seem to cause luteal-phase deficiencies. However, it is to be noted that in these studies the luteal phase has been judged adequate in terms of the luteal phase of the normal cycle. It has previously been indicated that in in vitro fertilization the luteal phase characterized by higher than normal progesterone values may be advantageous. Therefore, the evaluation of normalcy in the luteal phase in an in vitro program does not have validity if the comparison is with the luteal phase of the normal cycle. For this reason, even though in vitro luteal phases are normal as judged by the normal cycle, it has been the policy of the program at Norfolk to support the luteal phase by the use of intramuscular progesterone beginning at or before the time of transfer. With the use of 12.5 mg of progesterone intramuscularly beginning on day 3, luteal-phase lengths are not increased over the norm, but if greater amounts of progesterone are used, such as 25 or 50 mg per day, the luteal-phase length may be artificially prolonged in the absence of pregnancy.

**DISCUSSION**

It has long been noted that the transfer step seems to be the most inefficient of the various steps in programs of in vitro fertilization. Pregnancy rates in 1984 of about 25% after transfer must be considered the minimal expectancy. However, this rate is greatly influenced by the incidence of multiple transfers in any particular program. While it is natural to examine the technique of transfer and all of the other circumstances surrounding the transfer to attempt to account for the relatively great inefficiency at this step, it might well be that failures are an expression of the inefficiency of human reproduction. Numerous studies have shown that the expectancy of pregnancy after normal exposure in a normal menstrual cycle is probably no greater
than about 25%. The reason for the inefficiency has been difficult to document, but where studies have been done, it has been found that when fertilization occurs there has been a large number of chromosomal abnormalities that have resulted either in failure of implantation or in early miscarriage. There have been a limited number of studies of the chromosomal constitution of eggs fertilized in vitro, and the numbers to date are inadequate to arrive at a binding conclusion as to the abnormalities that are occurring in vitro. At the present time the chief avenue of hope in increasing the pregnancy rate after transfer is the use of multiple conceptuses at transfer. The obvious concern is that the incidence of multiple pregnancies will be troublesome and self-defeating.

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