Culturing human embryos with and without glucose

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Objective: To review published data and to compare pregnancy rates (PRs) after culturing human embryos with and without glucose and phosphate.

Design: Comparison of results from various programs.

Setting: Assisted Reproductive Technology Program.

Patient(s): Patients were enrolled in various studies.

Intervention(s): Human embryos were cultured with and without glucose and phosphate.

Main Outcome Measure(s): Pregnancy rates after different techniques of embryo culture.

Result(s): Some studies reported higher PRs in patients undergoing IVF after embryos were cultured in media without glucose and phosphate versus media with glucose and phosphate. One study showed that PRs were lower when embryos were cultured in media lacking glucose and phosphate compared with media containing glucose and phosphate. Some studies have also shown similar PRs with the two types of culture media.

Conclusion(s): The PRs in IVF patients will not necessarily be enhanced if the embryos are cultured in media without glucose and phosphate. (Fertil Steril 1998;69:970–1. ©1998 by American Society for Reproductive Medicine.)

Key Words: Human embryos, glucose, phosphate, pregnancy rates

IVF programs, particularly those in the United States, have recently become interested in using culture media lacking glucose and phosphate for human embryo cultures. One reason for this may be the consensus that mammalian embryos develop much better in a culture medium lacking glucose and phosphate than in a culture medium containing them. The most convincing evidence supporting this concept comes from the studies of Schini and Bavister (1), who showed that the two-cell block normally occurring during in vitro development of the hamster embryo could be prevented if glucose and phosphate were removed from the culture medium. The objective of this article is to summarize current information pertaining to the use of culture media with and without glucose in human IVF programs.

MATERIALS AND METHODS

A culture medium without glucose and phosphate has been developed in the United States for human embryos. This culture medium, referred to as preimplantation stage one (P-1), has been formulated by Dr. Thomas B. Pool of the Fertility Center in San Antonio, Texas. The P-1 medium can be purchased from Irvine Scientific (Santa Ana, CA). A similar culture medium has been developed by Dr. Patrick Quinn, who also formulated human tubal fluid (HTF). This medium, referred to as Quinn’s Basal XI HTF medium, which lacks glucose and phosphate, can be purchased from Conception Technologies (San Diego, CA).

The P-1 and Quinn’s Basal XI HTF media are modifications of the original HTF medium, which contains glucose and phosphate. The essential difference is that P-1 and Quinn’s Basal XI HTF lack glucose and phosphate. The P-1 medium is supplemented with taurine (0.05 mm), and Quinn’s Basal XI HTF medium is supplemented with ethylenediaminetetraacetic acid (EDTA, 0.1 mm) and glutamine (1.0 mm).

Rawlins et al. (2) compared pregnancy rates (PRs) after culturing human embryos in P-1 and HTF. In this comparison, the authors used two different types of proteins. With HTF, they used 10% synthetic serum and with P-1 they used 7% human serum albumin. Quinn et al. (3) compared HTF with Quinn’s Basal X1 HTF.
medium in 10 patients. Lee et al. (4) compared P-1 medium with Menezo B2 medium (which contains glucose and phosphate). Barak et al. (5) compared PRs after culturing embryos in HTF, P-1, or M-3 (Menezo, Inc., Copenhagen, Denmark). During a 5-year period (Michigan Reproductive & IVF Center, Grand Rapids, MI), I used HTF supplemented with 0.5% bovine serum albumin (BSA; Irvine Scientific, Santa Ana, CA) in GIFT, zygote intrafallopian transfer (ZIFT), IVF-ET, and donor IVF-ET cycles.

RESULTS

Rawlins et al. (2) showed that when embryos were cultured in P-1 medium, PRs were significantly higher (33%) than PRs (19%) when embryos were cultured in HTF. Quinn et al. (3) observed a significant increase in PRs with Quinn’s Basal XI HTF medium (4 of 5 women pregnant) compared with HTF (2 of 5 pregnant). Lee et al. (4) obtained similar PRs after culturing embryos in P-1 medium or Menezo B2 medium. Using HTF supplemented with 0.5% BSA, I obtained clinical PRs of 52%, 52%, 33%, and 50% for GIFT, ZIFT, IVF-ET, and donor IVF-ET, respectively (unpublished data). Barak et al. (5) observed that PRs with the P-1 medium were significantly lower (22%) than PRs with HTF and M3 (33% and 32%, respectively).

DISCUSSION

In comparing P-1 with HTF, Rawlins et al. (2) used two different types of proteins. Therefore, it is not possible strictly to compare the media because the proteins themselves may have caused the difference in PRs. It is difficult to assess these differences because of the nature of the experimental design. An additional concern is that the taurine present in the P-1 medium could have a beneficial effect on embryo development. Bavister and McKiernan (6) have shown that taurine improved the development of one-cell hamster embryos in culture. The HTF medium, on the other hand, did not contain taurine. Therefore, the beneficial effects of the P-1 medium could result from the absence of taurine and not from the lack of glucose or phosphate.

In the experiment done by Quinn et al. (3), comparing HTF with Quinn’s Basal XI HTF medium, the sample size was extremely small. In addition, the beneficial effects of Quinn’s Basal XI HTF medium could result from the presence of EDTA and glutamine and not from a lack of glucose and phosphate. Both EDTA and glutamine have shown a positive influence on embryo development (7). As I have shown in data from our program, high PRs can be achieved with HTF and 0.5% BSA. In other words, the presence of glucose and phosphate in the culture medium did not appear to have a negative effect on PRs.

Perhaps an important question to address in developing an optimal culture medium is whether the energy requirements of the embryo change dramatically after embryonic gene activation. If this is the case, then a culture medium providing specific sources of energy before embryonic gene activation and other sources of energy after embryonic gene activation may be optimal for development of the pre-embryo. In fact, Medi-Cult Inc. in Denmark has marketed two separate culture media, one to be used from the time of insemination up to day 2 (IVF medium) and another if cultures are performed beyond day 2 (M3 medium).

It must be remembered that a culture medium suitable for one species may not be entirely appropriate for another species. Until these issues are resolved, one must be careful not to make abrupt changes in culture media. Researchers must be encouraged to present new ideas and to improve existing media for human embryo cultures. However, such efforts must be pursued with good scientific hypotheses, proper design of experiments, and valid interpretations. In comparing media with or without glucose, one should eliminate other confounding variables (e.g., amino acids, different protein sources, EDTA) and conduct prospective randomized trials, with adequate numbers of patients. Investigators should note the different outcomes (e.g., biochemical, ectopic, clinical, or multiple pregnancies, miscarriages) when different culture media are used with human embryos.

On the basis of the information presented here, it is not possible to conclude that elimination of glucose and phosphate from the culture medium will lead to higher PRs in women undergoing IVF. Each program should take a careful look not only at the culture medium, but also at all other factors influencing the outcome of IVF, before they decide to change their culture medium. For those programs attempting to change from a glucose-based medium to a glucose-free medium, a reasonable approach would be to compare PRs using the two types of media in prospective, randomized trials. A change in media is prudent only if investigators are convinced that one medium has performed better than the other.

References