Role of coculture in human in vitro fertilization: a meta-analysis

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Objective: To evaluate the role of coculture in human IVF.
Design: Meta-analysis.
Setting/Patient(s)/Intervention(s): A literature search was performed using the Cochrane Menstrual Disorders and Subfertility Group Trials register, the Cochrane Central register of Controlled Trials on the Cochrane Library (2006), and MEDLINE (January 1966 to March 2006).
Main Outcome Measure(s): Primary outcomes measured were implantation rates and pregnancy rates (clinical and ongoing). Secondary outcomes included evaluation of pre-embryo development based on average number of blastomeres per embryo.
Result(s): A total of 17 prospective, randomized trials were identified. There was an overall statistically significant effect of coculture on the implantation rate, clinical pregnancy rate, and ongoing pregnancy rate. The cocultured embryos had greater numbers of blastomeres, although the data were heterogeneous.
Conclusion(s): This is the first systematic, evidence-based review of randomized controlled trials to objectively determine the potential benefits of coculture in human IVF. The pooled data of human trials on coculture demonstrate a statistically significant improvement in blastomere number, implantation rates, and clinical and ongoing pregnancy rates. (Fertil Steril® 2008;90:1069–76. ©2008 by American Society for Reproductive Medicine.)

Key Words: Coculture, assisted reproduction, human IVF, embryo morphology

The landmark report in 1978 of the first human birth resulting from IVF–ET revolutionized reproductive medicine and the treatment of subfertile couples. Since its inception, numerous advances and modifications have occurred in IVF, leading to an increase in take-home baby rates (1, 2). These include optimizing controlled ovarian stimulation (3), the introduction of intracytoplasmic sperm injection (4), and advances in the in vitro culture environment and media (5). Despite these changes, <50% of all patients desiring conception achieve their goal of having a biological child (1).

One of the factors associated with poor success rates in human IVF is the suboptimal culture conditions in which fertilization and early embryonic growth occur. In their classic 1965 study (6), Cole and Paul, in an attempt to more closely mimic the in vivo environment, demonstrated improved blastulation rates for mouse embryos cultures cultured on an immortalized helper cell line. The use of feeder cell lines was then adapted to human IVF in 1989 (7–9). Wiemer et al., in a randomized trial, noted improved morphology, implantation rate, and clinical pregnancy rate for embryos cultured on bovine uterine epithelial cells compared with conventional media (7, 8). This led to a great deal of optimism that coculturing human embryos may optimize the in vitro environment and promote improved pregnancy rates. In the last 17 years a variety of human and nonhuman cell lines have been used, with conflicting clinical results.

A number of studies have evaluated the effect of coculture on human pre-embryo development. There seems to be an overall enhancement of both human and nonhuman pre-embryo development with the use of a variety of diverse cell types, suggesting that there is neither species nor tissue specificity (10). Improvements in pre-embryo grade, an increase in the average number of blastomeres, and a decrease in fragmentation rates have been demonstrated (7, 11, 12). In 1995 Tucker et al. (13) reported that cryopreserved cocultured embryos had improved postthaw blastomere survival, resulting in higher implantation rates. In addition, patients with multiple IVF failures who subsequently underwent coculture had a significant improvement in both the average number of cells and fragmentation rates on coculture compared with their previous non-coculture cycle (14–16). Conversely, other reports have demonstrated no statistically significant improvement in early embryogenesis (13, 17, 18) or clinical pregnancy rates (12, 18, 19). Unfortunately, most coculture studies have been poorly controlled and retrospective in nature and therefore have questionable clinical validity. Conflicting results have also been obtained even with the well-controlled studies. Wiemer et al. (20) and Morgan et al. (11) noted an almost twofold increase in clinical pregnancy rates with bovine oviductal epithelial cells, whereas Tucker et al. (13) found no differences in the pregnancy rates using the same cell line. Contrasting clinical

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outcomes have also been noted when different groups evaluated African Green Monkey kidney cells (Vero cells) (12, 17, 21). Major flaws of the randomized studies are that they typically fail to perform an a priori power analysis, routinely suffer from small sample sizes, and are at great risk for type 1 or type 2 statistical errors. Consequently, a lucid interpretation of the current body of literature on coculture is difficult, and the role of coculture in human IVF is controversial. Therefore, we decided to perform a systematic, evidence-based review of the randomized controlled trials to objectively determine the potential benefits of coculture in human IVF.

MATERIALS AND METHODS

We searched the Cochrane Menstrual Disorders and Subfertility Group Trials register (searched March 22, 2006), the Cochrane Central Register of Controlled Trials on the Cochrane Library (2006), and MEDLINE (January 1966 to March 2006) using the key words "coculture," "assisted reproduction," "human IVF," and "embryo morphology." Two reviewers independently assessed eligibility and quality of the studies. Prospective randomized trials were included if they were relevant to the clinical question posed and reported data in treated (coculture) and untreated (conventional media) groups. Quantitative assessments and data extraction were also performed independently by two of the authors (N.K., L.I.B.). The primary outcomes measured were implantation rates and pregnancy rates (clinical and ongoing). Secondary outcomes included evaluation of pre-embryo development based on the average number of blastomeres per embryo. No institutional review board approval was obtained for our study because research involving the collection or study of publicly available existing data is exempted from institutional review board review, as per 45 CFR 46.101(b). Statistical analysis and calculations were performed with commercial software (Comprehensive Meta-Analysis; Biostat, Englewood, NJ).

The meta-analysis was performed to [1] take a closer look at whether the studies compared were homogenous, [2] illustrate the magnitude of effect of our comparison, and [3] possibly characterize any individual factors contributing to variations of results if heterogeneity came into play. We used the statistical software to display results with forest plots demonstrating implantation rate, clinical pregnancy rate, and ongoing pregnancy rate.

Within each forest plot, parameters were listed by "Citation" (principal investigator, year published), "Effect" (standardized mean of the coculture minus standardized mean for the control), "Lower" and "Upper" limits of a 95% confidence interval (CI) for effect, "Ntotal" (sample size for each study), and "Pvalue" (significance of coculture effect on outcome, with an indicating scale to illustrate how the effect was driven [either toward being influenced by coculture or control]).

Potential bias of studies was checked by the use of a funnel plot technique looking at effect estimates vs. sample sizes. Plot symmetry and concordance appeared among the included previously published research and was supplemented by a further bias check using logistic regression analysis, which yielded no significant detectable publication bias, to the best of our knowledge.

Heterogeneity was checked using the Q statistic, part of the software algorithm set. The test represents a way of assessing consistency or inconsistency of study findings, looking at the pattern of effect from study to study that may be due to random variation. Analysis of variance was also used to assess the impact of the group of studies on effect (as well as an assumption of homogeneity).

RESULTS

In total, 50 studies were identified in the English literature, of which 17 matched the inclusion criteria. Wiemer et al. reported two studies in two different journals in 1989 using the same data (7, 8). Only the study published in *Fertility and Sterility* was included for meta-analysis (7). Table 1 describes the distribution of the studies according to the feeder cell layer used for the coculture.

There were six prospective randomized trials evaluating implantation rates that had appropriate data for analysis. Two of these suggested a statistically significant improvement in implantation rate, three showed a trend of improved implantation rate for cocultured embryos, and one favored an improved implantation rate for the non-cocultured embryos. When the data were pooled, there was an overall statistically significant effect of coculture on the implantation rate (P=.027) (Fig. 1). Specifically, we can expect an average increase of 3.0 (95% CI 0.3–5.7) implantations per 100 women by using coculture. There was no statistically significant evidence of a violation in the assumption that the effect is homogeneous across all the available citations (Q [implantation rate] = 9.72; P=.084).

For clinical pregnancy rates, nine prospective randomized trials had the required data. A statistically significant improvement in clinical pregnancy rate for the cocultured embryos was reported by three studies, whereas the remaining six reported trends toward an increased clinical pregnancy rate. Overall, there was a statistically significant effect of coculture on the clinical pregnancy rate (P=.003) (Fig. 1). Specifically, we can expect an average increase of 8.1 (95% CI 2.7–13.4) clinical pregnancies per 100 women by using coculture. There was no statistically significant evidence of a violation in the assumption that the effect is homogeneous across all the available citations (Q [clinical pregnancy rate] = 8.21; P=.41).

Also notable was an overall statistically significant effect of coculture on the ongoing pregnancy rate (P=.004) (Fig. 1). Specifically, we can expect an average increase of 8.7 (95% CI 2.8–14.6) ongoing pregnancies per 100 women by using coculture. There was no statistically significant evidence of a violation in the assumption that the effect is