CLINICAL ASSISTED REPRODUCTION

A Simplified Coculture System Using Homologous, Attached Cumulus Tissue Results in Improved Human Embryo Morphology and Pregnancy Rates During In Vitro Fertilization

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Purpose: *This study was undertaken to evaluate simplified methods of human embryo coculture using either attached or nonattached autologous cumulus tissue.*
Methods: *Eight hundred one zygotes were cultured for 48 hr in a prospective, randomized trial comparing culture of embryos either with intact cumulus tissue, with cumulus tissue added to the droplet of culture medium, or without any cumulus tissue. In a follow-up study, embryo quality, pregnancy rates, and implantation rates were compared in 120 consecutive patients undergoing in vitro fertilization with a coculture system using cumulus tissue compared to a cohort of 127 patients undergoing IVF immediately preceding the institution of the coculture protocol.*
Results: *Embryo morphology was significantly improved ($P < 0.05$) following culture with attached cumulus tissue (5.61 ± 0.29) and culture with added cumulus tissue (4.72 ± 0.31) compared to that of embryos grown in culture medium without cumulus tissue (3.95 ± 0.26). The clinical pregnancy rate improved from 39.4% (50/127) to 49.2% (59/120) following institution of a system of coculture with attached cumulus tissue.*
Conclusions: *These data indicate that a simple coculture system using autologous cumulus tissue can result in improved embryo morphology, implantation rates, and clinical pregnancy rates during in vitro fertilization. This coculture system is simple, is non-labor intensive, and eliminates many of the risks which may be present in other embryo coculture systems.*

KEY WORDS: coculture; cumulus tissues; embryo morphology; in vitro fertilization; pregnancy rates.

INTRODUCTION

Coculture of human embryos during in vitro fertilization (IVF) has been reported to improve embryo quality, blastocyst development, implantation rates, and clinical pregnancy rates (1–9). Various cell types, including commercially available cell lines, human epithelial and fibroblast cell lines, cancer cells, and autologous endometrial cell lines, have been used for IVF coculture (1,4,10,11). The mechanism by which coculture of embryos results in improved morphology, development, and implantation ability has not been clearly identified, however, two major hypotheses have been proposed. First, coculture of the embryo with another cell line may alter the chemical composition of the growth medium by altering the concentration of metabolites or detoxifying the culture medium. Second, the added cell line may contribute embryotrophic factors which directly affect the growth of the developing embryo.

Despite the abundance of data indicating the benefit of embryo coculture, coculture is not routinely used by most IVF laboratories. This is due primarily to the

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difficulty, cost, and time associated with maintaining a cell line and the potential risk of infection associated with the use of nonautologous cell lines. It has become increasingly evident that the use of autologous cell lines facilitate the routine use of coculture. Dirnfeld *et al.* recently demonstrated that a coculture system using autologous granulosa cells recovered during oocyte aspiration was beneficial in improving embryo quality and pregnancy rates in patients with a prior cycle of poor embryo development (12). Additionally, cumulus cells have been shown to improve blastocyst development in both the human and the mouse (13,14).

This study evaluated the effect of a simple, autologous coculture system on embryo morphology in a prospective randomized study using both attached and nonattached cumulus tissue. In a second study, embryo quality implantation rates, and pregnancy rates were evaluated before and after implementation of this coculture system for routine use during IVF.

MATERIALS AND METHODS

One hundred thirty-six patients underwent in vitro fertilization using standard techniques for ovarian stimulation and transvaginal, ultrasound-guided oocyte aspiration. Oocytes were identified and recovered from follicular aspirates in a regulated environment containing 5% CO_2 at 37°C, then placed in human tubal fluid culture medium (HTF) containing heparin and Hepes buffer. The oocytes were immediately rinsed in this culture medium, and bloody and/or highly condensed cumulus cells were removed using fine gauge needles. The cumulus oocyte complexes (COC) were then immediately transferred to 50 μl droplets of standard HTF culture medium supplemented with 10% maternal serum and covered with mineral oil. Following 4–6 hr of preincubation, each oocyte was inseminated with 100,000 to 250,000 progressively motile sperm prepared by percoll gradient centrifugation.

Eighteen to twenty hours following insemination the oocytes were gently denuded using a sterile micropipette with an inner diameter of 150 μm (Irvine Scientific, Santa Anna, CA). Oocytes were randomly allocated to one of three groups. For one-third of the oocytes a portion of cumulus was left attached to the oocyte when the oocyte was transferred into fresh HTF growth medium containing 15% heat-deactivated, maternal serum. The remaining oocytes were either transferred to growth medium without any cumulus tissue or transferred with a nonattached piece of cumulus tissue. Added cumulus was obtained from the denuding of the oocyte. Granulosa cells from the follicular fluid were not used. Care was taken to minimize the effect of reduced pipetting as a possible factor in the outcome of the study. Since some embryos denude more easily than others, the assignment to a treatment group was made prospectively, before initiation of the denuding process. The oocytes were evaluated for pronuclear development, and only embryos showing clear development of two pronuclei were evaluated in the study.

Embryos were evaluated after 48 hr of culture in the growth medium. The number of blastomeres and embryo quality were evaluated, and an embryo score based on the combination of cleavage rate and embryo quality was calculated. Briefly, 1 point was given for each blastomere present, and 1 to 2 points were subtracted for abnormal blastomere morphology. Three points were subtracted for blastomere fragmentation. Some patients received embryo transfers including embryos from different treatment groups, therefore, a comparison of implantation and pregnancy rates was not possible.

Based on the data from the above study, a second study was initiated in patients not undergoing intracytoplasmic sperm injection (ICSI), in which an attempt was made to culture all embryos with attached cumulus tissue. In rare instances in which cumulus did not remain attached to the oocyte during the denuding process, a portion of tissue was added to the growth medium microdroplet. Embryos were transferred into 120 recipients using this technique. Embryo scores, implantation rates, and pregnancy rates were compared for this group of patients with the data from 127 non-ICSI patients undergoing IVF immediately prior to initiation of this protocol.

Data were analyzed by analysis of variance (ANOVA) and chi-square analysis.

RESULTS

The mean embryo score was 5.61 ± 0.29 for embryos cultured with a portion of the cumulus tissue remaining attached to the embryo, significantly higher ($P < 0.05$) than for either of the other two study groups (Fig. 1). The mean embryo score for embryos cultured with cumulus tissue added to the microdrop was 4.72 ± 0.31, compared to 3.95 ± 0.26 for embryos cultured without any cumulus tissue in the microdroplet.

Based on the above data, embryo culture procedures were modified to include attached or added cumulus

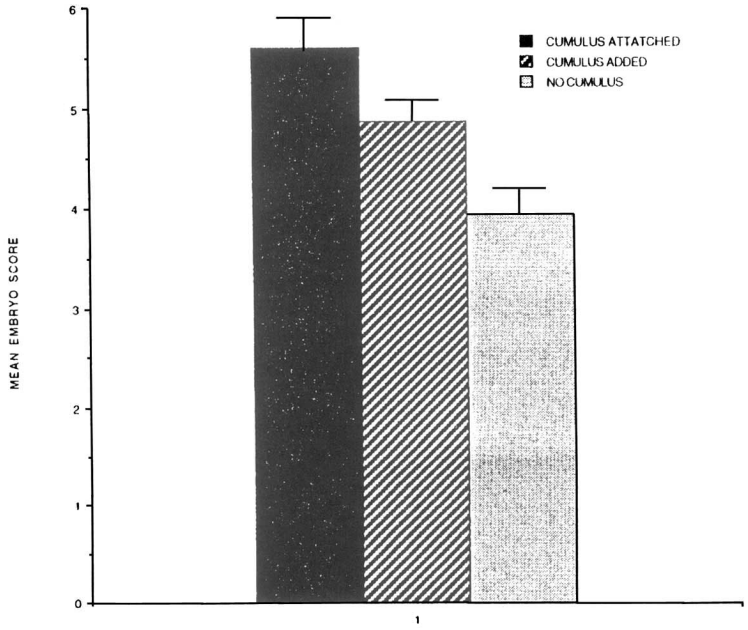

Fig. 1. Comparison of embryo quality in embryos cultured with attached cumulus, added cumulus, or no cumulus. $P < 0.05$ for both the attached and the nonattached groups compared to the no-cumulus treatment group.

tissue to all embryos when possible. A comparison of 127 consecutive IVF patients (excluding patients receiving ICSI) before the change of protocol with 120 patients using the cumulus coculture technique is shown in Table 1. Mean embryo scores, implantation rates, and clinical pregnancy rates were all significantly ($P < 0.05$) higher in the cumulus coculture technique and in the standard culture. The clinical pregnancy rate was 49.2% (59/120) with coculture, compared to 39.4% (50/127) with the standard culture technique.

DISCUSSION

This study demonstrated that a significant improvement in embryo quality, implantation ability, and clinical pregnancy rates was obtained using a simplified coculture technique with autologous cumulus tissue. Embryo quality was significantly improved when cumulus tissue was added to the microdrop culture medium, however, maximum improvement was observed when cumulus tissue remained attached to the zygote. This technique may be particularly advantageous compared to other coculture techniques because of its ease and safety. No cell lines need to be maintained in the laboratory, and the patients' embryos are not exposed to potentially harmful factors from other species or patients.

One explanation for these data may be a decrease in damage to the cocultured embryos due to less vigorous pipetting when leaving cumulus attached to the oocyte. While this cannot be disproved, two points are worth considering. First, care was taken to attempt to randomize the treatment, rather than assigning the treat-

Table I. Embryo Scores, Implantation Rates, and Pregnancy Rates in IVF Patients with Attached, Autologous Cumulus Coculture Versus Standard Culture Techniques

Treatment group	*n*	Mean embryo score (± SE)*	% implantation**	% clinical pregnancies*
Standard culture	127	4.7 ± 0.4	20.6 (76/368)	39.4 (50/127)
Cumulus coculture	120	5.8 ± 0.3	29.9 (98/327)	49.2 (59/120)

* Significantly different, $P < 0.05$.
** Significantly different, $P < 0.01$.

ment by the ease of cumulus removal. Second, it is interesting to note that embryo quality was improved when cumulus was simply readded to the microdrop of medium. These facts indicate a probable embryo trophic effect of the cumulus, rather than a reduction in damage to the oocyte.

Fukaya *et al.* have shown that direct cell-to-cell contact is not needed to obtain the benefits of coculture (15). Since gap junctions do not exist between the cumulus and the embryo at this stage of development, it is likely that the observed benefits are derived from a paracrine-like effect. Previous studies have shown paracrine actions in which cumulus-derived factors affect the embryo and, conversely, embryo-derived factors which affect the cumulus tissue (16–19). These paracrine interactions between the cumulus tissue and the embryo may be enhanced by the proximity of attached tissue to the embryo.

Differences were observed in the degree of attachment and spreading of the cumulus cells to the culture dish (Figs. 2 and 3). Generally, high embryo quality was associated with the highest degree of cumulus attachment to the culture dish, however, this study did not quantitate those differences. It is possible that interpatient differences in cumulus attachment and spreading may be reflective of the inherent developmental potential of the COC or may be reflective of differences in the maternal sera used to supplement the growth media. Interestingly, intrapatient differences in cumulus attachment and spreading were observed, indicating a likely variation in the cumulus tissues from the same patient.

Benefits of coculture may result from a modification or conditioning of the culture medium, expression of growth factors, or expression of other factors which may guard against oxidative stress (16,20–24). Autologous cumulus cell coculture appears to provide such benefits. Studies are under way to determine the mechanisms which result in the improved embryo development seen in this study. Additionally, the effect of this

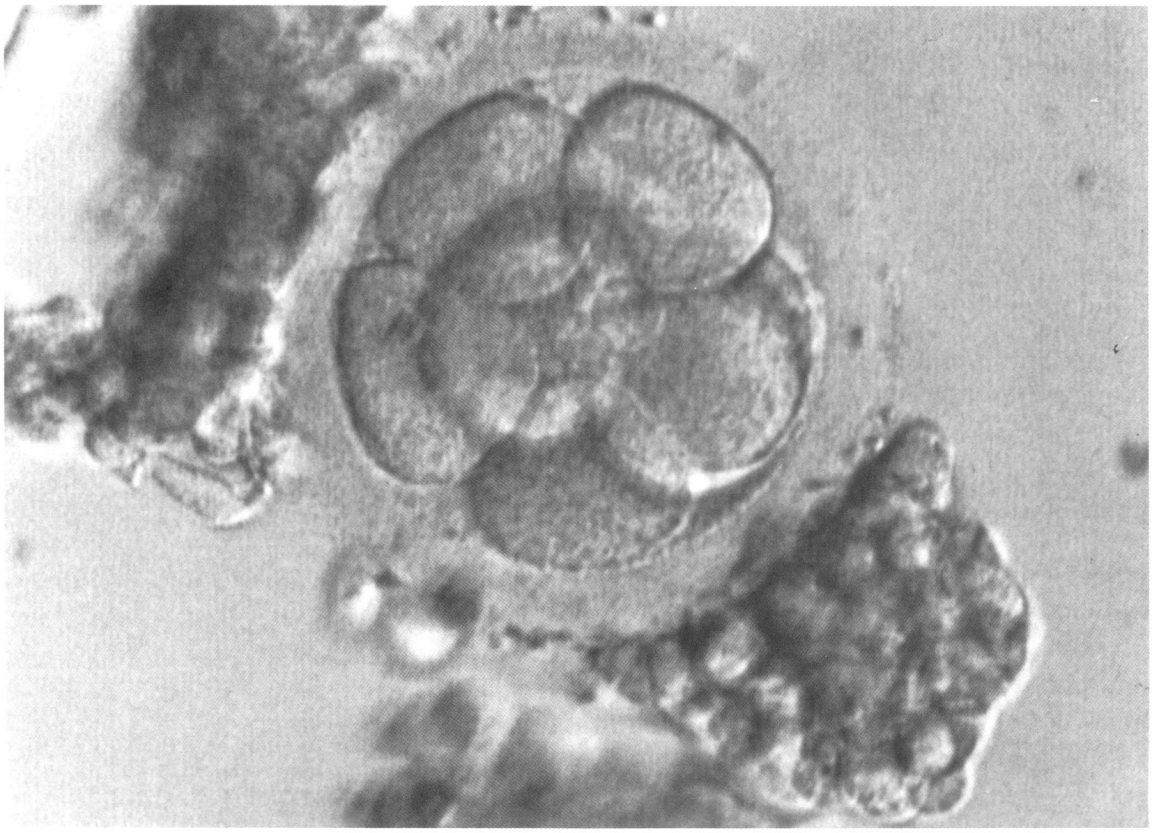

Fig. 2. Embryo with the minimal quantity of attached cumulus acceptable for the initial study comparing embryo quality following coculture with attached, added, or no cumulus. In the subsequent study evaluating implantation and pregnancy rates, as much cumulus tissue was left attached as possible while not impeding embryo morphology evaluation.

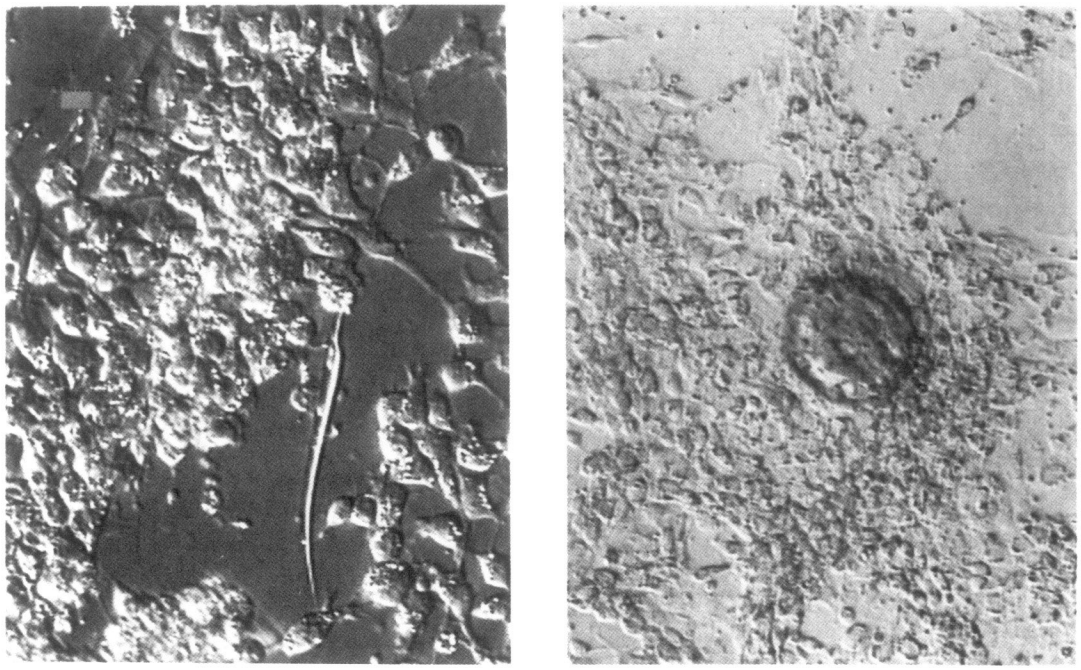

Fig. 3. Abundant cumulus attached to the culture dish and spreading. Generally, this type of cumulus attachment and spreading was associated with a higher level of embryo quality for the patient, though not necessarily specific for that embryo.

technique on embryo development following ICSI is being studied.

REFERENCES

1. Bongso A, Fong CY: The effect of co-culture on human zygote development. Curr Opin Obstet Gynecol 1993;5(5):585–593
2. Guerin J-F, Nicollet B: Interest of co-cultures for embryos obtained by in-vitro fertilization: A French collaborative study. Hum Reprod 1997;12(5):1043–1046
3. Lai YM, Chang MY, Chang FH, Lee CL, Lee JD, Chang SY, Huang HY, Wang ML, Chan PJ, Soong YK: The effects of Vero cell co-culture on human zygotes resulting from in vitro fertilization and oocytes following subzonal insemination. Chang Keng I Heush 1996;19(3):203–210
4. Menezo YJ, Sakkas D, Janny L: Co-culture of the early human embryo: Factors affecting human blastocyst formation in vitro. Microsc Res Tech 1995;32(1):50–56
5. Nieto FS, Watkins WB, Lopata A, Baker HWG, Edgar DH: The effects of co-culture with autologous cryopreserved endometrial cells on human in vitro fertilization and early embryo morphology: A randomized study. J Assist Reprod Genet 1996;13:386–389
6. Vlad M, Walker D, Kennedy RC: Nuclei number in human embryos co-cultured with human ampullary cells. Hum Reprod 1996;11(8):1678–1686
7. Wiemer KE, Cohen J, Wiker SR, Malter HE, Wright G, Godke RA: Co-culture of human zygotes on fetal bovine uterine fibroblasts: embryonic morphology and implantation. Fertil Steril 1989;52(3):503–508
8. Wiemer KE, Garrisi J, Steuerwald N, Alikani M, Reing AM, Ferrara TA, Noyes N, Cohen J. Beneficial aspects of co-culture with assisted hatching when applied to multiple-failure in-vitro fertilization patients. Hum Reprod 1996;11(11):2429–2433
9. Yeung WSB, Lau EYL, Chan AYF, Ho PC: The production of interleukin-1 alpha immunoreactivity by human oviductal cells in a co-culture system. J Assist Reprod Genet 1996;13:762–767.
10. Thibodeaux JK, Godke RA: Potential use of embryo co-culture with human in vitro fertilization procedures. J Assist Reprod Genet 1995;12:665–677
11. Ben-Chetrit A, Jurisicova A, Casper RF: Co-culture with ovarian cancer cell enhances human blastocyst formation in vitro. Fertil Steril 1996;65(3):664–666
12. Dirnfeld M, Goldman S, Gonen Y, Koifman M, Calderon I, Abramovici H: A simplified co-culture system with luteinized granulosa cells improves embryo quality and implantation rates: A controlled study. Fertil Steril 1997;67(1):120–122
13. Quinn P, Margalit R: Beneficial effects of co-culture with cumulus cells on blastocyst formation in a prospective trial with supernumerary human embryos. J Assist Reprod Genet 1995;13:9–14
14. Roudebush WE, Levine AS, Lodge JS, Tsai CC, Butler WJ: Human follicular fluid and mouse cumulus cells act synergistically to enhance preimplantation mouse Balb/cJ embryo development. J Assist Reprod Genet 1995;12:733–737
15. Fukaya T, Chida S, Murakami T, Yajima A: Is direct cell-to-cell contact needed to improve embryonic development in co-culture? Tohoku J Exp Med 1996;180(3):225–232

16. Abeydeera LR, Wang WH, Cantley TC, Rieke A, Day BN: Co-culture with follicular shell pieces can enhance the developmental competence of pig oocytes after in vitro fertilization: relevance to intracellular glutathione. Biol Reprod 1998; 58:213–218
17. Desai NN, Goldfarb JM: Growth factor/cytokine secretion by a permanent human endometrial cell line with embryotrophic properties. J Assist Reprod Genet 1996;13(7):546–550
18. Lim JM, Hansel W: Improved development of in vitro-derived bovine embryos by use of a nitric oxide scavenger in a cumulus-granulosa cell co-culture system. Mol Reprod Dev 1998; 50(1):45–53
19. Seifer DB, Freeman MR, Gardiner AC, Hill GA, Schneyer AL, Vanderhyden BC: Autologous granulosa cell co-culture demonstrates zygote suppression of granulosa cell steroidogenesis. Fertil Steril 1996;66(3):425–429
20. de Matos DH, Furnus CC, Moses DF: Glutathione synthesis during in vitro maturation of bovine oocytes: Role of cumulus cells. Biol Reprod 1997;57:1420–1425
21. Bongso A, Ng SC, Fong CY, Ratnam S: Co-cultures: A new lead in embryo quality improvement for assisted reproduction. Fertil Steril 1991;56(2):179–191
22. Leppens G, Sakkas D: Differential effect of epithelial cell-conditioned medium fractions on preimplantation mouse embryo development. Hum Reprod 1995;10(5):1178–1183
23. Lim JM, Hansel W: Roles of growth factors in the development of bovine embryos fertilized in vitro and cultures singly in a defined medium. Reprod Fertil Dev 1996;8(8):1199–1205
24. Liu LPS, Chan STH, Ho PC, Yeung WSB: Human oviductal cells produce high molecular weight factor(s) that improves the development of mouse embryo. Hum Reprod 1995; 10(10):2781–2786