Human Embryo Culture: Back to Nature

INTRODUCTION

Research to improve mammalian embryo culture media has taken off in recent years following the development of medium CZB by Chatot *et al.* in 1989 (1). Three research strategies have tended to be adopted: (i) empirical trial and error, (ii) simplex optimization, and (iii) embryo-somatic cell coculture. There have been successes, notably in culturing mouse embryos (2); while for others, for example, the bovine, there has been no obvious improvement (3).

One strategy, which has tended to be ignored, is (iv) to base embryo culture media on the environment in the female reproductive tract.

There is a strong argument for adopting strategy iv, particularly in improving *human* embryo culture media, because the numbers of embryos available to conduct meaningful experiments using strategies i and ii are likely to be limited, and strategy iii raises a fundamental problem: embryos and somatic cells have different nutrient requirements. As I and others have discussed (4), embryos initially develop in a relatively dilute medium, oviduct fluid, while somatic cells are normally bathed in extracellular fluid, which, by comparison, is rich in nutrients and serum constituents. It is arguable whether human embryos should be exposed to such an environment in view of the potential consequences of such exposure in domestic animals (5).

So what information about the composition of human oviduct (tubal) fluid is available?

IONS

The best data are those of Borland *et al.* (6), who, using electron probe microanalysis, measured the concentration of seven elements in samples of human tubal fluid obtained by micropuncture from women attending for surgical operations.

The results are shown in Table I, with the composition of Earle's salts included for comparison. The data indicate that while the concentrations of Na, Cl, and Ca are fairly similar in human tubal fluid

Table I. Electron Probe Microanalysis to Measure Human Tubal Fluid Concentrations

	Human tubal fluid (mM)	Earle's salts (mM)
Na	130	143
Cl	132	125
K	21.2	5.4
Ca	1.13	1.8
Mg	1.42	0.81
S	12.3	0.81
P	8.69	1.01

and Earle's medium, K concentrations in tubal fluid are much higher, as is the case for oviduct fluid in other species (7). Less well known, perhaps, is that the same is true for S and P.

NUTRIENTS

Gardner *et al.* (8) were able to sample oviduct and uterine fluid from women in different stages of the cycle. During the follicular phase, pyruvate, lactate, and glucose concentrations were 0.25, 4.87, and 3.11 mM, respectively, but at midcycle, they were 0.32, 10.5, and 0.5 mM, respectively. Mean pyruvate and lactate values were similar to those reported by Lopata *et al.* (9). The fall in glucose concentration is particularly interesting, since Nichol *et al.* (10) also reported a precipitous drop in glucose concentration in pig oviduct fluid following ovulation.

Dickens *et al.* (11) applied the technique of vascular perfusion to human fallopian tubes removed at hysterectomy. An artery serving the tube was cannulated and vascularly perfused with medium 199 for up to 2 hr. At the same time, a cannula was tied into the lumen, enabling tubal fluid to be collected. Mean values for pyruvate, lactate, and glucose in such tubal fluid were 0.17, 8.58, and 0.53 mM, respectively.

Taken together, these data suggest that the pyruvate concentration in most human embryo culture media, 0.3–0.5 mM, is fairly close to physiological, while that of glucose should be reduced from 5.55

mM in most media to 0.5–1.0 mM or, for day 2 or 3 transfers, possibly to 0. A large-scale trial to assess this is in progress (12).

Glucose should be present at fertilization, because Mahadevan *et al.* (13) showed that, in its absence, human fertilization and sperm movement characteristics were decreased. The group of Sakas (14) has also shown that glucose is required for successful fertilization in the mouse.

Human embryo culture media should contain pyruvate (Conaghan *et al.*) (15), but should they contain lactate? The answer is probably "yes" since the extracellular ratio of lactate/pyruvate is likely to determine the intracellular ratio, which is in equilibrium with the cytoplasmic NAD⁺/NADH ratio. A concentration of 5 mM L-lactate is a suitable compromise.

AMINO ACIDS

My view is that it is prudent to add a full mixture of 20 amino acids. My colleague Jackie Tay, using the vascular perfusion technique applied to human tubes removed at hysterectomy, has obtained data on the amino acid composition of tubal fluid generated in this way (16).

MACROMOLECULE

One consequence of the work of Kaye's group (17) is that serum albumin should be considered a physiological constituent of embryo culture media. It is also the major protein in oviduct fluid (7). In the case of the human, I would add human serum albumin.

GAS TENSION

The oxygen concentration in the oviduct lumen in a variety of species is approximately 5% (18). Although it has not, to my knowledge, been measured in the human, it is likely to be similar.

GROWTH FACTORS

Any requirement for growth factors, for day-2 or -3 transfers, is beyond the scope of this short review, but I would agree with Kane [cited by Kane *et al.*

(19)] that "*the beneficial effects of growth factors are most likely to emerge at blastocyst formation*" and that they are not required for culture prior to day 2 transfers.

THE CUMULUS MASS

In the spirit of the title of this article, there is a further factor to mention: the cumulus mass. Ideally, the cumulus cells should be retained up to the point of transfer and not removed. Cumulus cells provide a locally high concentration of pyruvate (20–22) together with estrogen and progesterone (23) and may protect embryos from complement in the female tract (24). Of course, retention of cumulus cells, or at least those of the corona, would render the detection of fertilization difficult, and intracytoplasmic sperm injection impossible, but that is another story!

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