History of KSOM, A Single Medium for Embryo Culture

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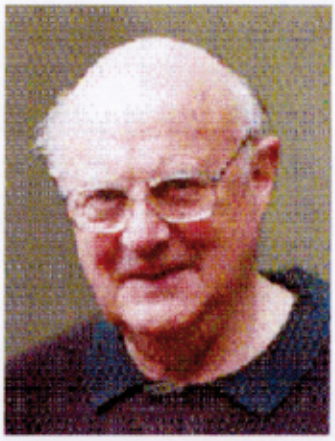
Since 1993 a family of media for the culture of preimplantation embryos has been designed that has acquired the generic name KSOM (Biggers, 2002). The initial work on the design of these media was done as part of the National Cooperative Program on Non-Human In Vitro Fertilization and Preimplantation Development, sponsored by the NICHD between 1986 and 1996, with the objective of improving embryo culture. Since the original formulation of a medium called SOM (Lawitts and Biggers, 1991), four modifications have been published:

KSOM (Lawitts and Biggers, 1993)
mKSOM (now called $KSOM_g$) (Summers et al., 1995)
$KSOM^{AA}$ (Ho et al., 1995, Biggers et al., 2000)
$KSOM_g^{AA}$ (Biggers and McGinnis, 2000).

In the early days of preimplantation mouse embryo culture, heralded by the pioneer paper of Whitten (1956), a major problem had been an arrest of development at the two-cell stage, called the two-cell block. Over the years several seemingly unrelated ways of overcoming this block were described (review: Biggers, 1993), which led to the notion that the media being used were unbalanced and hence not optimal. Optimizing culture media in general is a complex matter because of the need to take account of the possible interaction of effects between its components (Biggers et al., 1957). This is a general problem in many areas and was first studied in the optimization of industrial processes such as the manufacture of chemicals. One solution that was introduced was called sequential simplex optimization (see sidebar). Our research involved determining the proportion of embryos that developed beyond the two-cell stage as the concentrations of constituents in a start medium were simultaneously varied, according to the rules determined by the optimization protocol. After twenty iterations, taking about two years, a medium called SOM was developed which overcame the two-cell block (Lawitts and Biggers, 1991). Soon after, the composition of the medium was slightly modified to formulate KSOM (Lawitts and Biggers, 1993), based on measurements of the intracellular ionic composition of the blastomeres determined by electron probe microanalysis. An unexpected bonus of this research was the fact that KSOM supported a high yield of mouse blastocysts from zygotes that were capable of developing into fetuses after transfer into the uterus of surrogate mothers (Erbach et al., 1995). The medium was greatly improved when Ho et al. (1995) supplemented KSOM with 19 natural amino acids (glutamine was already in the medium). In another study very high yields of expanded blastocysts were obtained containing nearly double the numbers of inner cell mass and trophectoderm cells compared to KSOM alone (Biggers et al. (2000a). This medium is called $KSOM^{AA}$.



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A medium developed by Sequential Simplex Optimization

A variant of KSOM (mKSOM) was introduced to support IVF in the mouse. The original version of KSOM contains a low concentration of glucose (0.2mmol/l), which does not support the viability of sperm and therefore cannot be used for IVF. When the concentration of glucose in KSOM was raised to that found in blood (5.56mmol/l), IVF in the mouse was successful (Summers et al., 1995). This result was particularly surprising since this concentration of glucose did not prevent the subsequent cleavage divisions, contrary to the widespread dogma asserting that glucose inhibits the development of the preimplantation embryo. Subsequent studies have confirmed that glucose in a concentration as high as 5.56mmol/l in $KSOM^{AA}$ does not inhibit the early cleavage divisions of the mouse embryo (Biggers et al., 2000b). This medium is now called $KSOM_g$.

The inclusion of glutamine in media used for the culture of cell lines has always been of concern because its instability leads to the

accumulation of ammonium in the media. Gardner and Lane (1993) alerted the IVF community to the putative dangers of including glutamine in embryo culture media by reporting a disturbing incidence of exencephaly in mice whose preimplantation embryos had been cultured in a medium containing the compound. More recent studies, by two independent laboratories, have failed to detect any incidence of gross abnormal development in fetuses and newborn mice whose preimplantation embryos have been cultured in KSOM-type media. Possible reasons for the discrepancies have been discussed by Biggers et al. (2004a). Nevertheless, concern about the potential adverse effects of glutamine in media can be alleviated by replacing glutamine with a dipeptide containing glutamine. Alanylglutamine is commonly used for this purpose. However, glycylglutamine may be preferable since there is evidence that it favors the development of the ICM (Biggers et al., 2004b). Glycylglutamine is therefore included in all currently used variants of KSOM in our laboratory.

It has become almost universal to use a two-step protocol when it is desired to culture human zygotes to the blastocyst stage following the early recommendations of Gardner (1998). The protocol involves the sequential culture of the embryos in media of different chemical composition. There are several pairs of such media available commercially. The justification put forward for changing the medium in the middle of the culture period is either to remove putative toxic substances that have accumulated, or to imitate the natural environment which changes as the embryos pass from the oviduct into the uterus. Unfortunately there have been few experimental investigations to verify the need for a two-step protocol. A paper that has been submitted for publication describes results which show that there is no gain in renewing $KSOM_g^{AA}$ during the culture of the mouse preimplantation embryo. The studies of Biggers and Racowsky (2002) using $KSOM^{AA}$, and Macklon et al. (2002) using the so-called "Rotterdam" medium, failed to show an advantage of the two-step procedure, for the culture of human preimplantation embryos, suggesting that a more thorough examination of the practical advantage of two-step procedures should be undertaken.

The ultimate medical objective of treatment for infertility is the production of normal healthy babies. The techniques used to evaluate treatment protocols has involved such parameters as the rates of embryo development before transfer, and the rates of biochemical pregnancies and delivery rates. There has always been a lingering concern that some constituents of media may have deleterious effects (review: Summers and Biggers, 2003), particularly epigenetic effects that may be passed on to later generations (review: Johnson, 2005). Advances in molecular genetics are beginning to open up studies on these questions. For example, Rinaudo and Schultz (2004) have clearly shown that media can effect the expression of genes in mouse blastocysts cultured from the zygote to the blastocyst stage; 114 genes were mis-expressed in Whitten's medium and only 29 genes in $KSOM^{AA}$. Whether this difference can account for the better development in $KSOM^{AA}$ needs detailed analysis.

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EDITOR'S NOTE: global® medium is a derivative of KSOM-AA.