Effect of an Extracellular Matrix on the Hormonal Regulation of Sertoli Cell Function

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An extracellular matrix has been used in an effort to enhance the quality of the *in vitro* environment for a number of systems including primary cultures of Sertoli cells. Dramatic changes in Sertoli cell morphology have been demonstrated with the use of the extracellular matrix.[1] We were interested in investigating the effects of the matrix on selected parameters of Sertoli cell function. Of primary concern was whether or not a matrix substratum would enhance the secretion of transferrin and androgen-binding protein (ABP) by primary cultures of Sertoli cells.

Sertoli cells were obtained from 20-day-old rat testes by a series of enzymatic digestions.[2] The rinsed cells were plated either on a plastic substratum alone or over an extracellular matrix and cultured for five days. The culture media contained either no hormones as a control (C), follicle-stimulating hormone (FSH) (F), or a combination of agents (FSH, insulin, retinol, and testosterone) designated FIRT. Secreted transferrin and ABP were assayed by RIA, and the data were normalized for DNA content.[3,4] The matrix employed was a commercially available product (Matrigel) obtained from Collaborative Research and used as previously described.[1]

For cells plated over plastic, maximal transferrin secretion was obtained using the combined treatment FIRT, whereas FSH resulted in 50% of the maximal transferrin secretion. The presence of an extracellular matrix resulted in a slight increase in the levels of secreted transferrin for control, FSH, and FIRT-treated cells, but no dramatic increase was noted in any treatment group. TABLE 1. Similar results were obtained when ABP was assayed from the same culture media.

The effect of the matrix on the *in vitro* synthesis and secretion of proteins was also examined. Sertoli cells were plated either on plastic or matrix and then radiolabeled. The media were applied to a polyacrylamide SDS gel for electrophoresis and processed for fluorography. The Sertoli cells were maximally stimulated (FIRT) throughout the experiment. Peritubular cells, plated in separate wells, were treated similarly except that 10% fetal calf serum was used instead of hormonal stimulation. The presence of matrix did not appear to influence the levels of secreted proteins by cultured Sertoli cells, but increased levels of secreted proteins were noted when cultured peritubular cells were plated over the extracellular matrix (data not shown). Cell density can dramatically affect the transferrin signal, especially in maximally stimulated Sertoli cells. We observed that there appears to be an optimal plating density at which hormonal stimulation showed its maximal effect. Plating densities above and below the optimal levels resulted in

TABLE 1. Effect of the Extracellular Matrix on Transferrin Secretion by Sertoli Cells[a]

Treatment	Control	FSH	FIRT
(Substratum)			
Plastic	23 ± 4.6	55 ± 4.6	99 ± 7.2
Matrix (Matrigel)	29 ± 1.6	57 ± 3.2	107 ± 7.4

[a] Effect of substratum and hormone treatment on transferrin production by Sertoli cells was determined from a 72 hr medium collection on day 5 of culture and represented as ng transferrin/μg Sertoli cell DNA. Values are the mean ± SEM from three different experiments done in triplicate.

hormones (either FSH or FIRT) being less effective in stimulating transferrin secretion.

In summary, although previous work demonstrated that dramatic morphologic effects could be achieved in Sertoli cells with the use of an extracellular matrix, the data presented here indicate that the extracellular matrix does not have dramatic effects on the hormonal regulation of Sertoli cell function. This was determined by a direct measurement of transferrin and ABP production by Sertoli cells and by fluorography of radiolabeled secreted proteins. On the other hand, we have found that cell density may alter the responsiveness of cells to hormonal stimulation *in vitro*. This often overlooked environmental factor may be responsible for the lack of hormonal responsiveness and/or the apparent differential hormonal stimulation of transferrin and ABP secretion by Sertoli cells previously reported.[5]

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