Isolation of Sertoli Cells from Adult Rat Testes: An Approach to Ex Vivo Studies of Sertoli Cell Function

Matthew D. Anway, Janet Folmer, William W. Wright, and Barry R. Zirkin
Division of Reproductive Biology, Department of Biochemistry and Molecular Biology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland 21205

ABSTRACT

Much of what is known about the molecular regulation and function of adult Sertoli cells has been inferred from in vitro studies of immature Sertoli cells. However, adult and immature cells differ in significant ways and, moreover, many Sertoli cell functions are regulated by conditions that are difficult to replicate in vitro. Our objective was to develop a procedure to isolate Sertoli cells rapidly and in sufficient number and purity to make it possible to assess Sertoli cell function immediately after the isolation of the cells. The isolation procedure described here takes less than 4 h and does not require culturing the cells. From a single 4-mo-old adult rat, we routinely obtain \(7.0 \pm 0.4 \times 10^6\) Sertoli cells per testis, and from a 21-mo-old rat, \(7.2 \pm 0.4 \times 10^6\) Sertoli cells per testis. The purity, determined by morphologic analyses of plastic-embedded cells or after staining for tyrosine-tubulin or vimentin, averaged 80%. The contaminants typically included germ cells (10%) and myoid cells (10%). The germ cell-expressed genes protamine-2 and hemiferrin were not detected in the Sertoli cell preparations by Northern blot analyses, but the Sertoli cell-expressed genes clusterin, cathepsin L, and transferrin were highly expressed. Transferrin mRNA levels were greater in Sertoli cells isolated from aged than from young adult rats, consistent with previous analyses of whole testes; and cathepsin L mRNA levels were far more highly expressed in Sertoli cells isolated from stages VI–VII than from other stages of the cycle of the seminiferous epithelium, also consistent with previous analyses of whole testes and isolated tubules. These studies indicate that the freshly isolated cells retain differentiated function, and thus it should be possible to assess the in vivo function of adult Sertoli cells by isolating the Sertoli cells and immediately assessing their function.

aging, Sertoli cells, spermatogenesis

INTRODUCTION

Much of what is known about the molecular regulation and function of Sertoli cells has come from in vitro studies of Sertoli cells isolated from the testes of immature rats. Immature testes have been used as the source of Sertoli cells primarily because of the high purity that can be obtained [1]. However, the extent to which results obtained from studies of immature cells can be extrapolated to mature or aged cells is far from clear [2]. There are numerous examples in the literature that support this statement, including the following: 1) Structural changes have been shown to occur in Sertoli cells as they mature [2], 2) significant increases in cathepsin L [3, 4] and transferrin [5] expression occur during the maturation and aging of rat Sertoli cells, 3) immature Sertoli cells have been shown to be more responsive to follicle stimulating hormone (FSH) and less responsive to androgens than mature Sertoli cells [6, 7], and 4) Syed and Hecht [8] reported that Sertoli cells isolated from aged rat testes responded differently to germ cells in coculture studies than did Sertoli cells isolated from young adult rats.

There are some reports in the literature of the successful isolation of relatively pure Sertoli cells from mature rat testes [4, 8–11]. The protocols that are described in these reports involve culturing the isolated Sertoli cell preparations for several days, in part to reduce germ cell contamination and in part to allow the cells to recover from the isolation procedure for subsequent use in in vitro studies. A potential problem with culturing the cells is that isolated cells may lose differentiated function during the culture period, thus making it difficult to interpret results. In the case of Sertoli cells, differentiated function is not easy to define and thus loss of differentiated function is not easy to assess.

Herein we describe a method for the isolation of Sertoli cells from mature rat testes that is rapid and avoids cell culture. We show that the freshly isolated cells retain differentiated function. This method should make it possible to assess the in vivo function of Sertoli cells without subjecting the cells to culture conditions.

MATERIALS AND METHODS

Animals

Male brown Norway rats of ages 4–5 mo (young) and 18–21 mo (aged) were purchased from Harlan Sprague-Dawley (Indianapolis, IN) through the National Institute on Aging. Sprague-Dawley rats 60–70 days of age were purchased from Charles River (Indianapolis, IN). All rats were housed in a vivarium under a 14L:10D cycle and provided water and rat chow ad libitum. Animals were killed by decapitation under a protocol approved by the Johns Hopkins University Animal Care and Use Committee.

Sertoli Cell Isolation Procedure

Testes were removed and decapsulated. Two testes were placed in a 50-ml conical tube in 40 ml of 1× Hanks (20-031-CV; Medatech, Herndon, VA; calcium/magnesium-free, pH 7.4, adjusted with 7.5% sodium bicarbonate), washed twice, and allowed to settle. The seminiferous tubules were dispersed, but not fragmented, in a collagenase (C2674; Sigma, St. Louis, MO) solution (25 ml, 0.5 mg/ml in 1× Hanks, pH 7.4, 34°C, 10–15 min, shaking at 80 oscillations/min) and allowed to settle. It is important to not fragment the seminiferous tubules during the collagenase incubation because tubule fragmentation results in poor yield and purity. The supernatant, which contained interstitial cells, was decanted. The tubules were washed three times (40 ml of 1× Hanks) and then incubated in a trypsin (T5266; Sigma) solution (25 ml, 0.5 mg/ml trypsin in 1× Hanks, pH 7.4, 5–10 min, 37°C, without shaking) [1]. After two washes (40 ml of 1× Hanks), the tubules were washed a third time in a solution

Received: 4 June 2002.
First decision: 1 July 2002.
Accepted: 3 October 2002.
© 2003 by the Society for the Study of Reproduction, Inc.
ISSN: 0006-3363. http://www.bioreprod.org

996
containing trypsin inhibitor (T6522; Sigma; 20 ml, 0.3 mg/ml in 1× Hank's, pH 7.4). The tubes were then allowed to settle (2 min) [1].

To separate the Sertoli and germ cells from each other, the tubes were incubated in a solution (25 ml) containing a mixture of enzymes (0.1% collagenase [C2674; Sigma], 0.2% hyaluronidase [H6254; Sigma], 0.04% DNase I [D5024; Sigma], and 0.03% trypsin inhibitor [T6522; Sigma] in 1× Hank's, pH 7.4) at 34°C, shaking at 80 oscillations/min for 40 min [4]. The preparation then was centrifuged (500 rpm, 4 min in GPR Tabletop centrifuge; Beckman, Palo Alto, CA) to pellet Sertoli cells, and the pellet subsequently was washed three times (40 ml of 1× Hank's). At this step of the isolation procedure, the Sertoli cells were single cells, with very few multiclump cells and approximately 40% pure, with germ cells the major contaminating cell type. The Sertoli cells at this step of the procedure are referred to herein as before hypotonic shock. It is important at this point in the procedure for the majority of the Sertoli cells to be single cells because any clumps of cells will be lost during the following steps.

To increase the purity of the Sertoli cells, the Sertoli cell-containing pellet was subjected to hypotonic shock. To this end, the pellet was resuspended in 10 ml (total volume) of 1× Hank's, to which 25 ml of a 1:10 dilution of 1× Hank's in deionized water was added, bringing the total volume to 35 ml. Tubes were gently inverted three times to disperse the cells, after which the preparations were centrifuged at 500 rpm for 4 min and the supernatant decanted. The resulting Sertoli cell pellet was resuspended by gently pipetting up and down in a total volume of 25 ml of 1× Hank's. The Sertoli cell suspension was filtered through 53-μm pore-size nylon mesh (Small Parts Inc., Miami Lake, FL), and the cells were then washed three times with 40 ml of F12/DMEM (Invitrogen Corporation, Carlsbad, CA). After pelleting, the Sertoli cells were resuspended in a total volume of 10 ml of F12/DMEM. An aliquot (1 ml) of cells was used for cell number and purity analyses and the rest (9 ml) was immediately snap frozen in liquid nitrogen or homogenized for RNA isolations (see below).

For some Sertoli cell isolations, the starting point was tubules at stages VI-VII and XI-XII that were microdissected from the testes of 60- to 70-day-old Sprague-Dawley rats according to a previously described method [12]. Sertoli cells were isolated from 50 cm of microdissected tubules according to the isolation method described above but with one half of the volume of reagents.

Cell Number and Viability

Sertoli cell number was determined with a hemacytometer. Trypan blue exclusion assays were used to determine percent survival of the Sertoli cells following their isolation.

Analyses of Purity by Morphology

Sertoli cells isolated from the testes of young or aged rats were fixed in 5% glutaraldehyde and postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer, dehydrated in a graded series of ethanol, and embedded in Epon 812 resin (Electron Microscopy Sciences, Fort Washington, PA). Sections of 1 μm were stained with 1% toluidine blue/1% sodium borate [13]. Sertoli cells were identified by nuclear morphology [14]. Estimates of purity were made by identifying and counting approximately 200 cells per testis. For these estimates, cells isolated from the testes of eight young (n = 8) and four aged (n = 4) rats were evaluated. Images were captured with a Nikon Microphot H-III automatic Camera System (Nikon Inc., Melville, NY) with a 63× Zeiss PlanApo lens (Zeiss, Inc., Thornwood, NY).

Analyses of Purity by Immunofluorescence Microscopy

Isolated Sertoli cells were dried to microscope slides and fixed with neutral buffered formalin for 10 min. The slides were washed three times in PBS and blocked in diluted (1:66) normal serum (20 min, room temperature). Slides were then incubated with mouse anti-tyrosine tubulin (1:500, T9028; Sigma), anti-mouse vimentin (1:200, V6630; Sigma), or mouse anti-alpha smooth muscle actin (1:100, A2547; Sigma) in blocking serum (1:66) for 1 h at room temperature. Bound primary antibodies were detected with a fluorescein isothiocyanate-conjugated anti-mouse IgM secondary antibody (1:100, FI-2000; Vector Laboratories, Burlingame, CA). Nuclei were stained with propidium iodide (H-1300; Vector Laboratories). Tyrosine tubulin and vimentin are known to be expressed in Sertoli cells and alpha-smooth muscle actin in peritubular myoid cells and lymphoepithelial cells [15]. Images were acquired and digitized using a Nikon Eclipse 800 Microscope System equipped with a Princeton Instruments Eclipse 800 Microscope System equipped with a Princeton Instruments

### TABLE 1. Isolated Sertoli cell number and purity.

<table>
<thead>
<tr>
<th></th>
<th>Testis weight (g)</th>
<th>Sertoli cell number (× 10⁶) per testis</th>
<th>Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>1.8 ± 0.04</td>
<td>7.0 ± 0.4</td>
<td>78 ± 1.2</td>
</tr>
<tr>
<td>Old</td>
<td>2.0 ± 0.05</td>
<td>7.2 ± 0.4</td>
<td>79 ± 1.2</td>
</tr>
</tbody>
</table>

* Each rat represents one Sertoli cell preparation with two testes per tube.
* All values are presented as mean ± SEM.

### RNA Isolation and Northern Blot Analyses

RNA was purified from isolated Sertoli cells or from tubule segments (30 cm) by the Trizol method (Invitrogen Corporation). Total RNA (10 μg) from each Sertoli cell preparation was fractionated in a 1% agarose/formaldehyde gel, transferred overnight to a nylon membrane (Hybond-N; Amersham Pharmacia, Piscataway, NJ), and UV cross-linked (UV Stratalinker; Stratagene Inc., La Jolla, CA). The cDNA fragments (see below) were radiolabeled with [α-32P]dATP using the Rapid Prud DNA Labeling Kit (Invitrogen Corporation). Northern blots were hybridized overnight at 65°C with labeled cDNA probes in ExpressHyb hybridization solution (Clontech, Palo Alto, CA) with 10 μg/ml of sheared salmon sperm DNA. Following hybridization, blots were washed in 2× saline sodium citrate (SSC)/1.0% SDS, 30 min at 65°C, 1× SSC/0.5% SDS for 30 min at 65°C, and 0.1% SSC/0.1% SDS for 30 min at 65°C. After blots were washed, they were placed in a phosphor screen cassette (Fuji Photo Film Co., Edison, NJ) for 8–12 h. The signals were detected and quantified using a Fuji Bio-Imaging Analyzer and MacBAS software version 2.2 (Fuji Photo Film Co.).

The cDNA clone for protamine-2 (Pro2) was generated for Northern blot analyses by reverse transcriptase-polymerase chain reaction (RT-PCR). First, total RNA was isolated from the testes of four 4-month-old rats. Testis total RNA (3 μg) was reverse transcribed in a 20-μl reaction at 46°C for 60 min using 0.2 units of Superscript II (Invitrogen Corporation) and 50 ng of oligo-dT primer in single-strength first-strand synthesis buffer according to the manufacturer’s specifications. PCR was done in a reaction volume of 50 μl containing 0.5 μl of the RT reaction, single-strength buffer, 20 μM dNTPs, 1.5 mM MgCl2, 400 μM forward primer, 400 μM reverse primer, and 0.5 units AmpliTaq DNA Polymerase (Perkin Elmer, Boston, MA). The PCR conditions were 35 cycles at 94°C/30 sec, 60°C/30 sec, and 72°C/1 min and a final extension of 72°C/3 min. PCR products were cloned into pGemT Easy Vector (Promega, Madison, WI) according to the manufacturer’s specifications and sequenced to verify the insert product. Germ cell-specific Pro2 cDNA was generated using forward primer 5′-GGTCTCGCTACCGAATGGAGGAG and reverse primer 5′-GCA-TTTCCTGCACCTGAGAGCT. Sertoli cells were probed with clusterin [16], cathespin L [17], or full-length transferrin, which detects both Sertoli cell transferrin and germ cell hemiferrin [18]. Ribosomal protein S2 (ChoB) cDNA was used as a control for RNA loading [19]. Each lane for the Northern blot analyses contained RNA from pooled Sertoli cells from two animals. Northern blots were repeated five times with five separate groups of rats. Northern blots also were prepared from Sertoli cells pooled from 50 cm of staged, microdissected tubules.

### Statistical Analyses

Data are expressed as the mean ± standard error of the mean (SEM). Statistical differences were determined by Student t-test.

### RESULTS

Methods were first established for the isolation of Sertoli cells from young adult rats and then applied to the isolation of Sertoli cells from aged rats. The testes of the aged rats that were used for Sertoli cell isolations were approximately the same weight as the testes from young adults (Table 1).

### Sertoli Cell Number and Viability

Individual Sertoli cells represented the dominant cell type in the final cell suspension. Small populations of germ...
Sertoli cells, myoid cells (usually in 2- to 3-cell clumps), and spermatozoon heads were also present. The number of Sertoli cells isolated from each testis was determined by hemacytometer counts. Sertoli cells were easily distinguished from peritubular myoid cells and from germ cells by their morphology under a phase-contrast microscope [14]. As seen in Table 1, of the approximately 25 million Sertoli cells per adult rat testis [20, 21], we obtained $7.0 \pm 0.4 \times 10^6$ Sertoli cells per testis from young mature rats (n = 30 rats, 2 testes per tube) and $7.2 \pm 0.4 \times 10^6$ Sertoli cells per testis from aged rats (n = 30 rats, 2 testes per tube).

Cell viability was determined by trypan blue exclusion following Sertoli cell isolation from the testes of young and aged rats. Approximately 2% of the isolated cells (which included Sertoli, germ, and myoid cells) failed to exclude trypan blue.

**Analyses of Purity by Morphology**

Sertoli cells were isolated from the testes of 30 young and aged rats. Preparations of isolated Sertoli cells were assessed for purity in whole-mount preparations under a light microscope. Sertoli cell purity was determined by dividing the number of Sertoli cells by the total number of cells present. The purity of Sertoli cells from young and aged testes was $78\% \pm 1.2\%$ and $79\% \pm 1.2\%$, respectively (Table 1).

Purity was also estimated by using embedded and sectioned preparations of isolated Sertoli cells. The use of these preparations had the added advantage of making it particularly easy to recognize Sertoli cells by their morphologic characteristics. For these analyses, cells were isolated from each of eight young and four aged rats, fixed and embedded in plastic (Fig. 1). Semithin (1-μm) sections were examined by light microscopy following toluidine blue staining. As illustrated in Figure 1, Sertoli cells were easily distinguished from germ cells (boxed) and other testicular cells by their extensive cytoplasm, large cytoplasmic vacuoles, and characteristic rabbit-ear nucleoli [14]. To estimate purity, Sertoli cell number was divided by the total number of cells counted per slide for each sample. For both young and aged testes, purity was estimated at about 80%, which was consistent with the results of estimating purity from whole-mount preparations.

**Analyses of Purity by Immunofluorescence Microscopy**

Sertoli cells but not germ or interstitial cells express tyrosine tubulin and vimentin [15, 22]. Antibodies to tyrosine tubulin (Fig. 2) and vimentin (Fig. 3) localized to Sertoli cell cytoplasm. We used these antibodies to further assess the purity of the isolated Sertoli cell preparations. Purity was determined by counting cells with tyrosine tubulin or vimentin-labeled (green) and dividing by the total number of cell propidium iodide-stained (red) nuclei in each of two distinct areas per slide, with approximately 800–1000 total cells per area. Each sample was from pooled cells from the two testes of a given rat. Six young and six aged rats were used for these analyses. With tyrosine tubulin antibody, the purity of the Sertoli cell samples was found to be $82\% \pm 1.1\%$ and $81\% \pm 1.6\%$ for young and aged rats, respectively. With the vimentin antibody, these figures were $82\% \pm 1.1\%$ and $82\% \pm 3.6\%$, respectively. These estimates of purity are close to those determined by morphologic criteria (whole mounts or semithin sections).

Tyrosine tubulin localized to the apical region of the Sertoli cells isolated from young (Fig. 2A) and old (Fig. 2B) rats. This is the region in which tyrosine tubulin localizes in sections as well [15]. Vimentin has been shown to label the perinuclear region of Sertoli cells in sections [15, 22] and also did so in the isolated cells from young (Fig. 3A) and old (Fig. 3B) rats. Sertoli cells labeled before (Fig. 2, C and D) and after (Fig. 2, E and F) hypotonic shock showed no differences in the apical labeling of the tyrosine tubulin, suggesting that the microtubule environment is not disrupted with the swelling of the Sertoli cells that takes place during the hypotonic shock. The pattern of staining for vimentin also showed no differences before (Fig. 3, C and D) or after (Fig. 3, E and F) hypotonic shock.

We identified the contaminating cells in the Sertoli cell preparations in part from their morphology and in part from their staining characteristics. Peritubular myoid cells, identified by labeling with an antibody to alpha-smooth muscle actin, constituted $9.2\% \pm 0.5\%$ of cells isolated from the testes of young rats and $9.3\% \pm 0.2\%$ of the cells isolated from testes of aged rats. Germ cells, the cells that did not label with tyrosine tubulin, vimentin, or smooth muscle actin constituted approximately 10% of the cells in typical preparations from young and aged testes. Among these cells was a small number of spermatozoon heads (approximately 1% of the total cell number).

**Northern Blot Analyses**

We analyzed the isolated Sertoli cells for expression of specific genes by Northern blot analyses. These analyses were done for two reasons: first, to determine the normalcy of Sertoli cell gene expression following the isolation of...
the cells, and second, to further assess Sertoli cell purity by examining the possible expression of genes known to not be expressed by Sertoli cells. As seen in Figure 4, cells isolated from the testes of young (4-mo-old) and aged (21-mo-old) rats expressed the Sertoli cell-specific transcript clusterin but did not express detectable levels of the germ cell-specific transcripts hemiferrin or Pro2. This is in contrast with gene expression by the far less pure before-hypotonic-shock young cells, which expressed Sertoli cell clusterin mRNA but also the germ cell-specific transcripts hemiferrin and Pro2.

**Maintenance of Differentiated Function**

As one means by which to assess the differentiated function of the freshly isolated Sertoli cells, clusterin and transferrin gene expression were assessed in Sertoli cells isolated from young and aged rat testes. The rationale for this approach was a previous study showing that the steady-state clusterin mRNA levels do not change with aging but that transferrin mRNA levels increase [3]. As seen in Figure 5, the steady-state levels of clusterin mRNA in freshly isolated Sertoli cells from young and aged rats did not differ, but transferrin levels increased significantly in the old cells.

Differentiated function was further assessed by analyzing Sertoli cell-specific genes relative to the stages of the cycle at which they are known to be expressed. Cathepsin L, e.g., has been shown to be highly expressed during stages VI–VII and expressed at far lower levels at all other stages, whereas clusterin expression has been shown to not vary with the stage of the cycle [17, 23]. With the isolation methods described above, we found it possible to isolate 600,000–800,000 Sertoli cells from 50 cm of tubule fragments and thus to isolate Sertoli cells from specific stages of the cycle of the seminiferous epithelium. Figure 6 shows clusterin and cathepsin L mRNA expression in Stage VI–VII and XII-I tubule fragments and in Sertoli cells isolated from these tubules; clusterin mRNA levels did not change with the stage of the cycle, but cathepsin L mRNA was
expressed far more highly at stages VI–VII than at XII-I. Note that Pro2 was expressed when the RNA was derived from whole tubules, reflecting the presence of spermatids in the tubules, but not when the RNA was from isolated Sertoli cell preparations.

**DISCUSSION**

Although studies of Sertoli cells isolated from the testes of immature rats have provided a great deal of information about Sertoli cell function and regulation, there are changes in Sertoli cell structure, function, and regulation that occur through adulthood and beyond [2, 3, 5, 8], making extrapolation of results obtained with immature cells to the adult potentially misleading. There are some reports of the successful isolation of Sertoli cells from mature testes [4, 9–11]. The protocols that have been described to date involve culturing the isolated cell preparations for several days. Doing so reduces germ cell contamination, and thus previous studies have reported purities of greater than 90% [4, 9–11]. An underlying problem with this approach is that the isolated Sertoli cells may lose differentiated function during lengthy (days-long) culture periods, making it difficult to interpret results obtained. This potential problem is exacerbated in the case of Sertoli cells because we do not have good criteria by which to assess Sertoli cell differentiated function, and thus loss of differentiated function in vitro, if it occurred, would be difficult, if not impossible, to assess.

The rationale for the work presented herein, therefore, is the need for a method by which to isolate Sertoli cells rapidly with a high degree of purity and without the need for cell culture. The method that we describe, which can be used equally well for the isolation of Sertoli cells from adult or aged rat testes, is rapid (hours) and produces large numbers of cells per testis (about 7 million) with reasonable purity (about 80%) even without cell culture. The procedure that we describe involves the use of enzymatic digestions (i.e., collagenase and hyaluronidase), which also have been used extensively by others to isolate Sertoli cells from rats and mice [1, 4, 9–11]. What sets our procedure apart is the application of hypotonic shock to Sertoli cells in suspen-
FIG. 4. Northern blot analyses of Sertoli cells isolated from mature testes. The lanes were loaded with total RNA (10 μg per lane) from Sertoli cells isolated from the testes of 4-mo-old rats before hypotonic shock (Bef) and from the testes of 4- and 21-mo-old rats at the completion of the isolation procedure. Membranes were probed for Sertoli cell-expressed gene clusterin and germ cell-expressed genes hemiferrin and protamine 2 (Pro2). ChoB is a loading control.

FIG. 5. Northern blot analyses of clusterin and transferrin gene expression in Sertoli cells isolated from 4- and 21-mo-old rats. A) Lanes were loaded with total RNA (10 μg per lane) from Sertoli cells isolated from the testes of 4-mo-old and 21-mo-old rats. Membranes were probed for clusterin and transferrin. B) Graphic representation of transferrin gene expression levels normalized to ChoB expression. B) Graphic representation of clusterin (C) and transferrin (T) gene expression levels normalized to ChoB expression from Sertoli cells isolated from 4- and 21-mo-old rats. *Significant increase from the 4-mo-old level (P ≤ 0.05).

FIG. 6. Northern blot analyses of Sertoli cells isolated from staged tubules. The lanes were loaded with total RNA from Sertoli cells isolated from tubule segments from stages VI-VII and XII-I (3 μg per lane) and from whole tubule segments from the same stages (6 μg per lane). Membranes were probed for Sertoli cell-expressed genes clusterin and cathepsin L and for germ cell-expressed gene protamine 2 (Pro2). ChoB is a loading control.
hemiferrin were not detectable (10 μg total RNA) despite the fact that germ cells constituted about 10% of the total cells in the isolated preparations. Pro2 and hemiferrin were detectable in the isolated Sertoli cell preparations with 15 μg total RNA and cell preparations that were <70% pure (before hypotonic shock).

As indicated above, a major drawback of in vitro analyses of Sertoli cells is that changes in gene expression may occur during the culturing of these cells. Indeed, the expression of a number of Sertoli cell genes has been found to change over time in culture. For example, plasminogen activator inhibitor-1 and TGFβ have been shown to be up-regulated [25], while α2-microglobulin and cathepsin L have been shown to decrease in expression [26]. Such culture-related changes in Sertoli cell gene expression might occur because of changes in the Sertoli cells themselves or because of the absence of germ cells. Thus, although in vitro analyses may provide valuable clues about function and/or regulation, such analyses may or may not serve to provide understanding of the in vivo condition. We show herein that, immediately after their isolation, Sertoli cells from aged rats expressed higher levels of transferrin than those from young rats and that cathepsin L expression was higher at stages VI–VII of the cycle than at other stages. In both cases, the results obtained with the isolated cells mirrored the results of previous studies in which testes or tubule fragments were studied [17, 23], indicating that the isolated cells retain differentiated function. This should make it possible to treat animals in vivo and then determine treatment effects on Sertoli cells without subjecting the cells to culture conditions.

ACKNOWLEDGMENT

We thank Alice Karl at Washington State University for her suggestions and critical comments.

REFERENCES