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·*Original Article*·

Immortalized Sertoli cell lines sk11 and sk9 and binding of spermatids *in vitro*

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Abstract

Aim: To determine the effectiveness of the sk11, sk9 and sk11 TNUA5 Sertoli cell lines in binding germ cells *in vitro*. **Methods:** The immortalized Sertoli cell lines sk9, sk11 and sk11 TNUA5 were used in co-culture experiments with germ cells in media with or without reproductive hormones and incubated for 44 h at 32ºC. The number of germ cells bound to Sertoli cells was then determined and statistically analyzed. Western blot analysis and reverse transcriptase-polymerase chain reaction (RT-PCR) studies were employed to investigate the presence of cell adhesion proteins and follicle stimulating hormone (FSH) receptor, respectively. **Results:** No statistical difference between the number of bound step-8 spermatids and bound pre-step 8 spermatids on Sertoli cells from any of the cell lines existed. After the addition of germ cells, Sertoli cells showed more lipid accumulation in their cytoplasm, indicating active phagocytosis. Western blot analysis in the sk11 TNUA5 line indicated the expression of N-cadherin. FSH-only and testosterone-only treatments increased N-cadherin expression, regardless of germ cell addition. The addition of germ cells to the sk11 TNUA5 Sertoli cells increased the expression of espin, as did the addition of FSH with germ cells. RT-PCR studies of the sk11 TNUA5 cells indicated that the mRNA for FSH receptor decreased with successive passages. **Conclusion:** *In vitro* binding between isolated germ cells and sk9, sk11 or sk11 TNUA5 Sertoli cells is not feasible, and therefore these cell lines are not useful for the *in vitro* investigation of Sertoli-germ cell interactions and primary Sertoli cell isolates must still be used. *(Asian J Androl 2007 May; 9: 312–320)*

Keywords: sk Sertoli cells; immortalized Sertoli cells; Sertoli-germ cell binding; Sertoli-germ cell co-culture; Sertoli-spermatid junctional complex; *in vitro* cell-cell binding

1 Introduction

Spermatogenesis is a complicated process occurring throughout the reproductive life of the male. It is a remarkable process in which germ cells undergo mitosis, meiosis and cellular differentiation to produce spermatozoa [1]. At any given point, several generations of germ cells develop at the same time in the seminiferous tubule of mammals [2]. The seminiferous epithelial cycle is made up of various stages, in which new generations of germ cells are connected to older generations, with their development coordinated via the presence of fixed cellular associations [2].

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Occluding junctions, adherens junctions and gap communicating junctions are thought to play crucial roles in spermatogenesis. Not only important in mechanical adhesion, the actin-based cell-cell adherens junctions between the Sertoli cell and the germ cell in the mammalian testis is also important in the morphogenesis and differentiation of the germ cells [3]. During the process of germ cell migration from the basal to the adluminal epithelial compartments, these junctions turn over [4]. However, their role in complete spermatogenesis is not yet fully understood. The Sertoli ectoplasmic specialization, a cytoskeletal structure of the Sertoli cell, is associated with Sertoli-germ cell binding [5]. The morphology of testicular junctions has been well described, but their molecular composition is still not well understood. Ectoplasmic specializations are found basally in the Sertoli cell near Sertoli-Sertoli tight junctions and between Sertoli cells and germ cells and consist of hexagonally packed bundles of actin filaments situated between the plasma membrane and a cistern of endoplasmic reticulum [3]. A reduction of mature sperm in semen has been associated with abnormal or absent Sertoli ectoplasmic specializations [6–8]. To ensure the retention of spermatids as they mature into spermatozoa, the ectoplasmic specialization is an important cell-cell adhesion mechanism in the seminiferous epithelium.

Espin is an actin binding protein found in the testis, specifically in the Sertoli cells and shows no resemblance to other actin-binding proteins [9]. In the seminiferous epithelium, espin appears to be concentrated around the heads of spermatids from mid to late spermiogenesis, as determined by immunoperoxidase immunocytochemistry [10]. It is also seen near the base of the seminiferous tubules [10]. Sertoli cells surrounding step-8 spermatids, where an organized ectoplasmic specialization is first seen, demonstrate espin immunostaining in a C-shaped cap near the area where the spermatid meets the Sertoli cell [9]. This is not seen around step-7 spermatids [9]. Nearing spermiation, espin immunostaining near the luminal edge of the seminiferous epithelium decreases and then disappears around the time of sperm release [9]. This change in localization appears to reflect the disassembly of the ectoplasmic specialization [9]. Through immunogold electron microscopy, espin has been localized to the parallel bundles of actin filaments present at the ectoplasmic specialization in Sertoli cells [10]. A smaller isoform of espin, termed "small espin", has been seen associated with parallel actin bundles found in brush border microvilli in the kidney and intestine [11]. This further supports the hypothesis that espin is involved in the bundling of actin at the ectoplasmic specialization.

Reproductive hormones have been shown to play a role in the regulation of binding of spermatids at the Sertoli-spermatid junctional complex. The key regulators of spermatogenesis are follicle stimulating hormone (FSH) and testosterone [12]. FSH is thought to induce the binding competence of the Sertoli cell [13], whereas testosterone is believed to stimulate the actual binding between the two cell types [14, 15]. Cameron and Muffly [13] showed maximal binding of round spermatids to Sertoli cells *in vitro* in the presence of FSH and testosterone. Testosterone is also known to promote and maintain the maturation of round to elongated spermatids in the rat [16]. The withdrawal of testosterone has shown detachment of round spermatids between spermatogenic stages VII and VIII [17], the time when the ectoplasmic specialization forms.

Several Sertoli cell lines have been established from 10-day-old H-2Kb-tsA58 transgenic mice carrying a temperature inducible SV40 T-antigen, including the sk11 and sk9 cell lines [18, 19]. At a culture temperature of 33ºC these cells divide, and with switching the temperature to 39ºC, division stops. Little is known about the molecular phenotype of these cells, however, they have been reported to express mRNAs for α-inhibin, Steel factor, sulfated glycoprotein-2, transferrin, androgen receptor, steroidogenic factor-1 and FSH receptor [19]. Though the mRNA for the FSH receptor is found in these cells, it was down-regulated compared to *in vivo* levels, and the level of functional FSH receptor protein remains unknown. The sk11 cells were later transfected with human wild type FSH receptor, which allowed for continuously active FSH receptor expression [20]. These cells, sk11 TNUA5, showed a dose-dependent increase in cAMP production when stimulated with FSH [20].

This project was designed to determine the effectiveness of the sk11, sk9 and sk11 TNUA5 Sertoli cell lines in binding germ cells *in vitro*. To do this, an established Sertoli-germ cell co-culture system was used [13], and the number of spermatids bound to Sertoli cells was determined by morphometric analysis and correlated with the hormone treatments. The ectoplasmic specialization protein espin was also assayed in the co-cultures by immunocytochemistry and Western blot analysis, as was the cell adhesion protein N-cadherin. It was hypothesized that the sk11 TNUA5 Sertoli cell line would be

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suitable to study FSH effects on Sertoli-germ cell co-culture, as defined in the co-culture model using primary Sertoli cell isolates, and that the sk11 and sk9 cell lines would not.

2 Materials and methods

Germ cells were isolated from adult male mouse testes and co-cultured with the immortalized mouse Sertoli cells in the presence of FSH, testosterone (T), and a combination of these two reproductive hormones [13]. The number of spermatids bound to Sertoli cells was determined by morphometric analysis and correlated with the hormone treatments [13]. Espin, N-cadherin and FSH receptor were also assayed in these co-cultures.

2.1 Sertoli cell isolation, culture and pretreatment

Immortalized mouse sk9, sk11 and sk11 TNUA5 Sertoli cells were cultured on a Matrigel substrate (BD Europe, Heidelberg, Germany) in 24-well cell culture trays at either 32ºC or 39ºC, 5% CO_2–95% air and treated with FSH (NIDDK-oFSH-20, AFP7028D, 175xNIH-FSH-S1; Bethesda, MD, USA), T (Sigma, Taufkirchen, Germany) or FSH + T 24 h prior to the addition of mouse germ cells. The culture medium was DMEM (high glucose+L-glutamine) (CellGro, Fisher Scientific, Schwerte, Germany) supplemented with 10% fetal calf serum (PAA, Pasching, Austria), 0.01 µL/mL penicillin/streptomycin (Sigma, Taufkirchen, Germany), 0.01 µL/mL antibiotic/antimycotic (Sigma, Taufkirchen, Germany) and 5 µg/mL Plasmocin (Cayla–InvivoGen Europe, Toulouse, France). Cultures were not allowed to grow to confluence as there was lack of contact inhibition, and the cells did not maintain a monolayer configuration.

2.2 Mouse germ cell isolation and co-culture

Mouse germ cells were isolated from adult mice using a series of enzymatic treatments (0.5 mg/mL collagenase [Sigma, Taufkirchen, Germany] and 0.25 mg/mL trypsin [PAA, Pasching, Austria]) [21] and then filtered through a 74-µm nylon mesh. A total of 400 000 mouse germ cells were added to the Sertoli cell monocultures and incubated for 44 h at 32ºC, 5% CO_2–95% air. Most Sertoli cell cultures were near confluence at the time of plating. Controls included no hormone-treated co-cultures and germ cells preincubated for 30 min with the various hormone before being added to no hormone-treated Sertoli cells.

2.3 Germ cell viability and co-culture fixation

Following 44 h of incubation, the co-cultures were washed 5 times with warm medium, and the viability of the germ cells in the co-cultures was estimated using Trypan Blue assay. Co-cultures were fixed with 4% paraformaldehyde for 20 min at room temperature. Co-cultures for immunostaining were fixed with ice cold methanol : acetone (1:1) for 10 min at –20ºC and then allowed to air dry at room temperature.

2.4 Morphometry and statistics

Five digital images were taken in a systematic pattern from each well using 20 × and 40 × objectives. The number of germ cells was determined for each digital image using ImageJ (NIH, Bethesda, MD, USA). Germ cells were classified and counted based on size and appearance. Means of germ cell numbers counted for each treatment group for the three Sertoli cells lines used were statistically analyzed using one-way ANOVA followed by Scheffe's multiple-range analysis.

2.5 Immunocytochemistry

Sk11 TNUA5 Sertoli cell monocultures and Sertoli-germ cell co-cultures were fixed in methanol : acetone (1:1) for fluorescent immunostaining of the ectoplasmic specialization protein espin or fixed in 95% ethanol : 5% acetic acid for fluorescent immunostaining of the cell adhesion protein N-cadherin. The fixed co-cultures were incubated for 1 h at room temperature with espin (10 µg/mL; Transduction Laboratories, San Jose, CA, USA) or anti-N-cadherin (2 µg/mL; Zymed [InvitroGen, Toulouse, France]) followed by a 1-h incubation at room temperature with Cy3 (1:100; Jackson, Cambridgeshire, UK) as the secondary antibody. The antibody complex was visualized using a fluorescent microscope.

2.6 Gel electrophoresis and Western blot

Some Sertoli cell monocultures and Sertoli-germ cell co-cultures were collected for Western blot analysis. After 44 h of incubation, the cultures were washed 5 times with medium and the cells were lysed using a cell scraper and pooled in the various treatment groups. The protein was extracted using homogenization in Buffer A (10 mmol/L HEPES [KOH] pH 7.9, 10 mmol/L KCl, 1 mmol/L DTT, 0.2 mmol/L EDTA, 0.1% NP-40, protease inhibitors [Roche "Complete", Basel, Switzerland], 0.5 mmol/L PMSF) or Crash Buffer (1 mol/L Tris HCl [pH 6.8], 20% SDS, 1 mol/L DTT, protease inhibitors [Roche "Complete",

Basel, Switzerland], 0.5 mol/L EDTA , pH 8.0). The proteins were separated by SDS-PAGE gel electrophoresis and transferred onto 0.45 μm PVDF membrane. The blots were then stained for espin using the espin antibody mentioned above (1:1 000, 1 h room temperature), followed by a 1-h incubation with Cy5 (1:500; Jackson). Membrane bound antibodies were detected using a fluoroimager (Storm 860; Molecular Dynamics, Amersham, Germany) with a laser diode and emission filter for Cy5 (650 nm–670 nm). The image was viewed using Image Quant 5.0 (Molecular Dynamics, Amersham, Germany).

2.7 RNA Isolation

Monocultures of sk11 TNUA5 cells were washed once in serum-free medium and then lifted using a cell scraper and RNAPure (PeqLab, Erlangen, Germany). The cells were vortexed for 30 s and incubated at room temperature for 5 min, after which 600 μL chloroform was added and mixed well. The cell lysate was then centrifuged for 30 min at 3 360 × *g* at 4ºC. The supernatant was collected and added to 1.5 mL isopropanol. This was then placed on a shaker for 1 h at –20ºC, followed by centrifugation for 1 h at 3 360 × *g*. The supernatant was aspirated and discarded, and the total RNA was then further purified using the RNeasy Mini Kit (Qiagen, Düsseldorf, Germany), as per manufacturer's instructions. After isolation, RNA integrity was assessed using agarose/GITC gels. The purity was checked by UV-spectrometry in 10 mmol/L Na_2HPO_4/NaH_2PO_4-buffer (pH 7.0).

2.8 Real-time reverse transcriptase-polymerase chain reaction (RT-PCR)

Real-time RT-PCR was used to examine the mRNA expression of FSH receptor, N-cadherin and espin in sk11 TNUA5 Sertoli cells and was performed on a LightCycler instrument (Roche, Basel, Switzerland). cDNA was synthesized from 1 000 ng of total RNA using oligo $dT_{(12–18)}$ (Invitrogen, Carlsbad, CA, USA) with Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). PCR was performed using a PCR cocktail containing 10 pmol each gene specific primers (Table 1), 2 μL dNTP mix (25 mmol/L each; Takara Bio, Shiga, Japan), 0.5 μL SybrGreen I (1:1 000 in DMSO; Molecular Probes, Leiden, Netherlands), 0.25 μL BSA (20 mg/mL; Sigma), and 0.2 μL Ex-Taq HS (5 U/μL; Takara Bio, Shiga, Japan) in a total volume of 20 μL. Cycling conditions were as follows: denaturation (95ºC for 5 min), amplification and quantization (95ºC for 10 s, 60ºC for 10 s and 72ºC for 30 s, with a single fluorescence measurement at the end of the 72ºC segment) repeated 40 times, a melting curve program (60–95ºC with a heating rate of 0.2ºC/s and continuous fluorescence measurement) and a cooling step to 40ºC. The threshold cycle (crossing point [CP]) in which the fluorescence rises appreciably above the background level was determined by a second derivate maximum method with the use of the LightCycler Quantification Software (Roche, Basel, Switzerland). For exact comparison of mRNA transcription in the different samples the ribosomal gene RPS27a was used as reference gene. In addition to the verification of a single PCR product by the presence of only one melting peak, the PCR products were resolved by electrophoresis on a 1% agarose/TAE gel and checked for correct molecular size.

3 Results

Three cell lines reported to express mRNA for the FSH receptor–the sk9, sk11 and sk11 TNUA5 Sertoli cell lines – were used for Sertoli-germ cell co-culture.

Table 1. Sequences for real-time reverse transcriptase-polymerase chain reaction (RT-PCR) primers.

Gene	Sequence	Molecular weight (bp)	Temperature (ºC)
RPS27a-5′	CCA GGA TAA GGA AGG AAT TCC TCC TG	–	64.8
RPS27a-3′	CCA GCA CCA CAT TCA TCA GAA GG	–	62.4
FSHR-5′	GTG GTC ATC TGT GGT TGC TAC ACC	244	64.4
FSHR-3′	AAG GAT TGG CAC AAG AAT TGA TGG	244	59.3
N-cadherin-5′	CTG CCA ACT GGC TGA AAA TAG ACC	240	62.7
N-cadherin-3′	AGT TGG GTT CTG GAG TTT CAC AGG	240	62.7

After the addition of germ cells to subconfluent layers of sk9, sk11 and sk11 TNUA5, the lipid accumulation in these cells increased (figure not shown), indicating an increase in phagocytic activity. The sk11 cells appeared to contain the most lipids, although this was not quantified.

The number of bound spermatids per hormone treatment can be seen in Table 2. A one-way ANOVA ($P < 0.05$) determined no significant difference between the hormone treatments and number of pre-step-8 spermatids or step-8 spermatids bound to Sertoli cells from any of the cell lines. No difference was seen in the number of bound spermatids to Sertoli cells incubated prior to germ cell addition at 32ºC or 39ºC.

As the most promising cell line was thought to be the sk11 TNUA5 line, immunocytochemistry and Western blot were used to identify the presence of specific proteins involved in cell adhesion and the ectoplasmic specialization. The expression of espin in sk11 TNUA5 cells appeared to increase with the addition of FSH + T, as indicated by immunofluorescence staining intensity (Figure 1). Western blot analysis of this protein in these cells is inconclusive. Immunofluorescence of N-cadherin in the sk11 TNUA5 cells has thus far been unsuccessful. However, using Western blot analysis, N-cadherin was shown to be expressed in the sk11 TNUA5 cell line. While the FSH and T treatments alone appeared to increase N-cadherin expression in these cells, the combination of the two hormones, as well as the addition of germ cells, did not appear to affect the expression of this protein (Figure 2).

Real-time RT-PCR was used to examine the mRNA expression of FSH receptor and N-cadherin in sk11 TNUA5 Sertoli cells in comparison to a control sample (C14– mice testis, day 30). The CP for FSH receptor and N-cadherin in the cell line are shown in Table 3. The CP for the FSH receptor in sk11 TNUA5 passage 3 cells is 26.82, whereas in passage 17, it is 28.76, and for N-cadherin, sk11 TNUA5 passage 3 the CP is 17.79 and 16.22 in passage 17. The CP is defined as the point at which the fluorescence rises appreciably above the background fluorescence and is therefore a measure for the mRNA amounts.

The ribosomal gene *RPS27a* was used as reference gene for exact comparison of mRNA levels in the different samples. The CP values for the *RPS27a* show that this gene is expressed at a constant level in the sk11 TNUA5 Sertoli cells and in the control sample C143, which indicates that there are nearly equal amounts of mRNA starting material (Table 3). Figure 3 demonstrates the

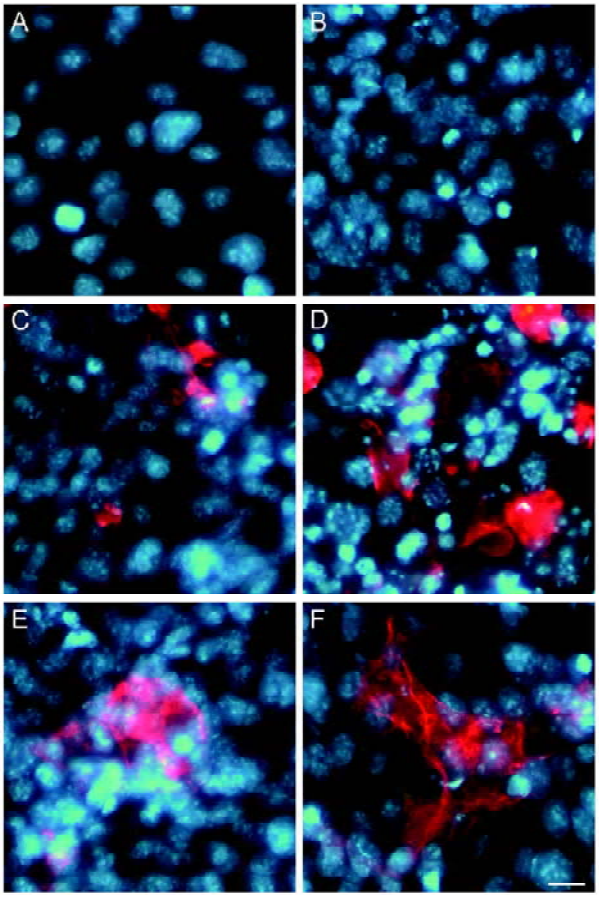

Figure 1. Fluorescent immunostaining of espin in immortalized mouse Sertoli cell-spermatid co-cultures plated on Matrigel. (A): Sk11 TNUA5 monoculture immunostained for espin. (B): Sertoli-germ cell co-culture using sk11 TNUA5 cells. Negative control for espin immunostaining. (C): Sertoli-germ cell co-culture using sk11 TNUA5 cells immunostained for espin in the absence of hormones. (D): Sertoli-germ cell co-culture using sk11 TNUA5 cells immunostained for espin in the presence of follicle stimulating hormone (FSH). (E): Sertoli-germ cell co-culture using sk11 TNUA5 cells immunostained for espin in the presence of testosterone. (F): Sertoli-germ cell co-culture using sk11 TNUA5 cells immunostained for espin in the presence of FSH and testosterone (T). Blue, nucleus; Red, espin. Bar = 10 μm.

LightCycler PCR results of the sk11 TNUA5 Sertoli cells for the FSH receptor and the N-Cadherin gene. While N-cadherin could be detected, it was not possible to quantify mRNA levels for the FSH receptor gene in these cells.

4 Discussion

In vitro studies of the interactions between Sertoli

Table 2. Total number of spermatids bound to immortalized mouse Sertoli cells. Total number of spermatids bound to sk9 cells from 7 co-cultures (24-well plates), bound to sk11 cells from 5 co-cultures (24-well plates), and bound to sk11 TNUA5 cells from 9 co-cultures (24-well plates). FSH, follicle stimulating hormone; T, testosterone.

Cell lines	No hormone	FSH	T	FSH + T
sk9				
Pre-step-8 spermatid	51	38	35	43
Step-8 spermatid	19	19	16	18
sk11				
Pre-step-8 spermatid	1	0	3	1
Step-8 spermatid	1	0	0	2
sk11 TNUA5				
Pre-step-8 spermatid	1	2	6	3
Step-8 spermatid	3	2	5	5

Table 3. Crossing points (CP) for the FSH receptor and N-cadherin transcripts in the sk11 TNUA5 cell line. The CP for the investigated genes in the sk11 TNUA5 cells, using real time reverse transcriptase-polymerase chain reaction (RT-PCR) and performed on a LightCycler instrument. The CP is defined as the point at which the fluorescence rises appreciably above the background fluorescence and is therefore a measure for the mRNA amounts. FSH-R, follicle stimulating hormone receptor; p, passage.

Cell	RPS27a	FSH-R	N-cadherin
sk11 TNUA5 p3	10.44	26.82	17.79
sk11 TNUA5 p17	10.37	28.76	16.22
sk11 TNUA5 p17 +FSH	10.66	28.93	16.20
C143 (control)	9.69	22.65	20.71

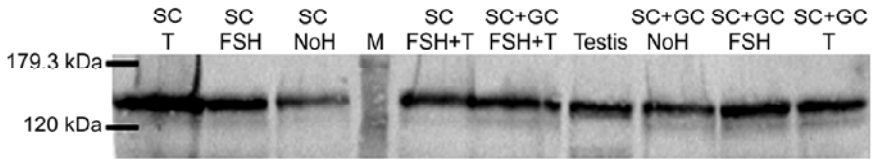

Figure 2. Immunodetection of N-cadherin in Sertoli cell, germ cell and Sertoli-germ cell co-culture lysates. Western blot for N-cadherin in sk11 TNUA5 cell cultures. SC, sk11 TNUA5 Sertoli cells; GC, germ cells; M, marker; NoH, no hormone; FSH, follicle stimulating hormone; T, testosterone.

cells and germ cells are time-consuming and expensive, in that the current established method consists of using primary Sertoli cell isolates [22]. Very few Sertoli cell lines exist and most do not possess the receptor for FSH and are therefore insufficient for studying how FSH is involved in the binding dynamics between germ cells and Sertoli cells. The development of a Sertoli cell line that expresses functional FSH receptor protein and supports germ cell binding is of great interest. Three cell lines have been reported to express the mRNA for the FSH receptor – the sk9, sk11 and sk11 TNUA5 Sertoli cell lines [18–20], all established from H-2Kb-tsA58 transgenic mice. Sneddon *et al*. [23] have demonstrated that the sk11 Sertoli cell line maintains the Sertoli cell phenotype in relation to androgen and estrogen receptors, in that the expressed androgen receptor and estrogen receptor-β induces expression of reporter gene constructs in the presence of a range of steroid ligands [23]. As a result of these studies, these cells would appear to be good candidates for use in Sertoli-spermatid binding

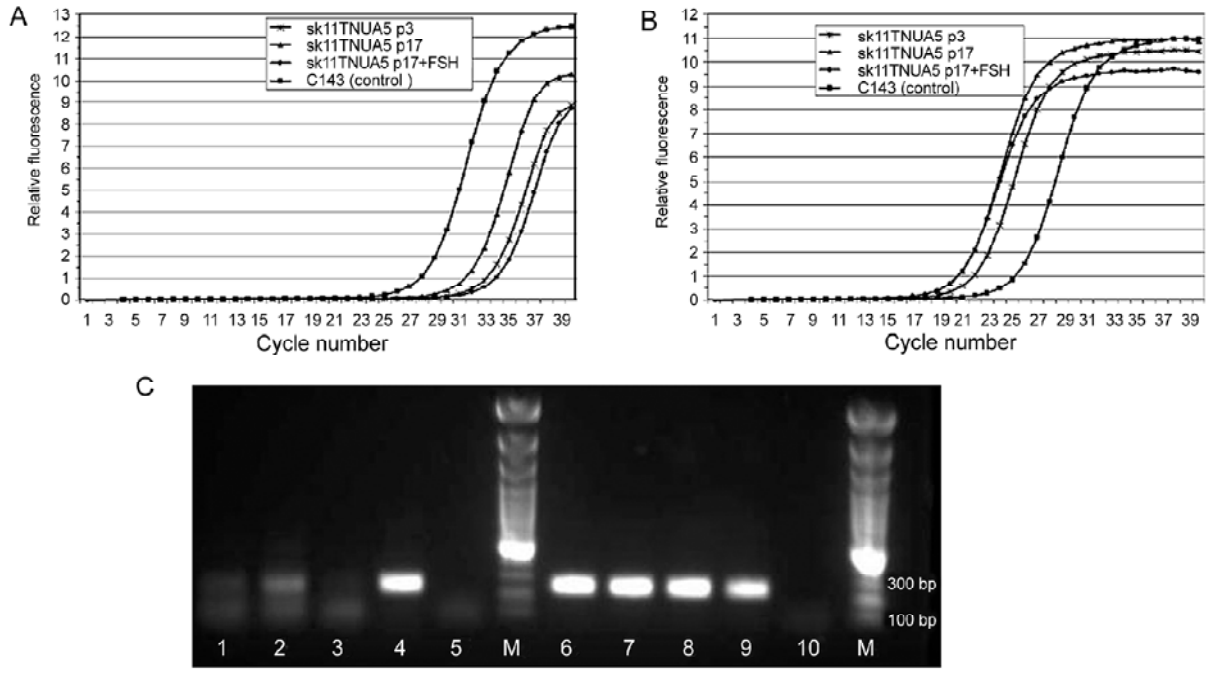

Figure 3. Reverse transcriptase-polymerase chain reaction (RT-PCR) results for the follicle stimulating hormone (FSH) receptor and N-cadherin genes. RT-PCR results for sk11 TNUA5 Sertoli cells and a control (C143) for the FSH receptor (A) and the N-cadherin gene (B). (C): 1% agarose/TAE gel of the RT-PCR products. lanes 5 and 10: no cDNA, water control; p, passage. 1: Nr 1-FSH-R; 2: Nr 2-FSH-R; 3: Nr 3-FSH-R; 4: Nr 4-FSH-R; 5: H_2O-FSH-R; M, 100 bp molecular weight marker; 6; Nr 1-N-cadherin; 7: Nr 2-N-cadherin; 8: Nr 3-N-cadherin; 9: Nr 4-N-cadherin; 10: H_2O-N-cadherin.

studies.

The accumulation of lipids in the sk9, sk11 and sk11 TNUA5 cells was evident following the addition of germ cells, indicating, therefore, the retention of the well-defined phagocytic activity of Sertoli cells. Results from the current study, however, indicate that these cell lines have limited value for the investigation of Sertoli-germ cell binding dynamics *in vitro*.

There was no apparent binding *in vitro* between the Sertoli cells and the added germ cells. It is possible that the presence of serum in the culture medium, necessary to maintain the viability of the immortalized Sertoli cells, inhibited FSH binding and/or receptor activation [24]. This being the case, there would not likely be FSH-induction of Sertoli cell binding competency, and therefore an inability to bind germ cells [13]. When cultured in medium without serum, but supplemented with retinol, insulin, transferrin and selenium, the cells appeared fusiform and not suitable for binding studies. The RT-PCR results indicate that the sk11 TNUA5 Sertoli cells have almost non-existent levels of FSH receptor mRNA with each passage, thereby providing an explanation for the lack of specific spermatid binding. This is in contrast to Strothmann *et al*. [20], who claim continuous active FSH receptor expression in these cells, showing a dose-dependent increase in cAMP production when stimulated with FSH.

With the addition of germ cells, the expression of espin in the sk11 TNUA5 cells, as analyzed using immunofluorescence, appeared to increase in the presence of FSH, but the organization of this protein appeared to be random. Still, in the presence of both FSH and T, espin appeared to be at the periphery of the Sertoli cells, suggesting that this actin binding protein was not involved in cell-cell binding activity in our co-culture model.

Using western blot analysis, N-cadherin was also detected in the sk11 TNUA5 cell line. Whereas FSH or T treatment alone appeared to increase N-cadherin expression in these cells, the combination of the two hormones, as well as the addition of germ cells, did not appear to affect the expression of this binding protein.

Although the sk11 TNUA5 cells were stably trans-

fected with a human FSH receptor construct [20], these cells lines have limited value for the investigation *in vitro* of Sertoli-germ cell binding interactions. First, the mRNA for the FSH receptor decreases in amount with successive passages, so that by passage 17 the message is almost non-existent. Second, the actin binding protein espin was expressed with the addition of germ cells, appeared to increase in the presence of FSH and became peripheralized in the presence of both FSH and T together. Still, this response was not associated with germ cell binding. Finally, the sk11 TNUA5 Sertoli cell line also expressed the binding protein N-cadherin, which appeared enhanced by the presence of either FSH or T alone. The combination of these hormones, as well as the addition of germ cells, did not appear to affect the expression of this binding protein, and, like espin, was not associated with the binding of germ cells.

Although the sk9 and sk11cells originally expressed mRNA for the FSH receptor, and the sk11 TNUA5 cells are thought to have a functional FSH receptor, these cells are not useful for *in vitro* investigation of Sertoli-germ cell interactions. However, they should not be ruled out for *in vitro* work, such as Sertoli cell biology and/or Sertoli cell interactions with non-germ cell types not requiring FSH.

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References

1 Leblond CP, Steinberger E, Roosen-Runge EC. Spermatogenesis. In: Hartman C, editor. Mechanisms concerned with conception. New York: MacMillan Co; 1963. p1–72.
2 Courot M, Hochereau-de-Reviers M, Ortavant R. Spermatogenesis. In: Johnson AD, Gomes WR, Vandemark NL, editors. The Testis. vol. 1. New York: Academic Press; 1970. p339–432.
3 Russell LD. Morphological and functional evidence for Sertoli-germ cell relationships. In: Russell LD, Griswold MD, editors. The Sertoli cell. Clearwater: Cache River Press. 1993.
4 Lui WY, Mruk D, Lee WM, Cheng CY. Sertoli cell tight junction dynamics: their regulation during spermatogenesis. Biol Reprod 2003; 68: 1087–97.
5 Russell L. Sertoli-germ cell interrelations: a review. Gamete Res 1980; 3: 179–202.
6 Russell LD, Goh JC, Rashed RM, Vogl AW. The consequences of actin disruption at Sertoli ectoplasmic specialization sites facing spermatids after *in vivo* exposure of rat testis to cytochalasin D. Biol Reprod 1988; 39: 105–18.
7 Boekelheide K, Neely MD, Sioussat TM. The Sertoli cell cytoskeleton: a target for toxicant-induced germ cell loss. Toxicol Appl Pharmacol 1989; 101: 373–89.
8 O'Donnell L, Stanton PG, Bartles JR, Robertson DM. Sertoli cell ectoplasmic specializations in the seminiferous epithelium of the testosterone-suppressed adult rat. Biol Reprod 2000; 63: 99–108.
9 Chen B, Li A, Wang D, Wang M, Zheng L, Bartles JR. Espin contains an additional actin-binding site in its N terminus and is a major actin-bundling protein of the Sertoli cell-spermatid ectoplasmic specialization junctional plaque. Mol Biol Cell 1999; 10: 4327–39.
10 Bartles JR, Wierda A, Zheng L. Identification and characterization of espin, an actin-binding protein localized to the F-actin-rich junctional plaques of Sertoli cell ectoplasmic specializations. J Cell Sci 1996; 109: 1229–39.
11 Bartles JR, Zheng L, Li A, Wierda A, Chen B. Small espin: a third actin-bundling protein and potential forked protein ortholog in brush border microvilli. J Cell Biol 1998; 143: 107–19.
12 McLachlan RI, Wreford NG, O'Donnell L, de Kretser DM, Robertson DM. The endocrine regulation of spermatogenesis: independent roles for testosterone and FSH. J Endocrinol 1996; 148: 1–9.
13 Cameron DF, Muffly KE. Hormonal regulation of spermatid binding. J Cell Sci 1991; 100: 623–33.
14 Perryman KJ, Stanton PG, Loveland KL, McLachlan RI, Robertson DM. Hormonal dependency of neural cadherin in the binding of round spermatids to Sertoli cells *in vitro*. Endocrinology 1996; 137: 3877–83.
15 Lampa J, Hoogerbrugge JW, Baarends WM, Stanton PG, Perryman KJ, Grootegoed JA, *et al*. Follicle-stimulating hormone and testosterone stimulation of immature and mature Sertoli cells *in vitro*: inhibin and N-cadherin levels and round spermatid binding. J Androl 1999; 20: 399–406.
16 O'Donnell L, McLachlan RI, Wreford NG, Robertson DM. Testosterone promotes the conversion of round spermatids between stages VII and VIII of the rat spermatogenic cycle. Endocrinology 1994; 135: 2608–14.
17 O'Donnell L, McLachlan RI, Wreford NG, de Kretser DM, Robertson DM. Testosterone withdrawal promotes stage-specific detachment of round spermatids from the rat seminiferous epithelium. Biol Reprod 1996; 55: 895–901.
18 Walther N, Jansen M, Ergun S, Kascheike B, Ivell R. Sertoli cell lines established from H-2Kb-tsA58 transgenic mice differentially regulate the expression of cell-specific genes. Exp Cell Res 1996; 225: 411–21.
19 Walther N, Jansen M, Ergun S, Kascheike B, Tillmann G, Ivell R. Sertoli cell-specific gene expression in conditionally immortalized cell lines. Adv Exp Med Biol 1997; 424: 139–42.
20 Strothmann K, Simoni M, Mathur P, Siakhamary S, Nieschlag E, Gromoll J. Gene expression profiling of mouse Sertoli cell lines. Cell Tissue Res 2004; 315: 249–57.
21 Romrell LJ, Bellve AR, Fawcett DW. Separation of mouse spermatogenic cells by sedimentation velocity. A morpho-

logical characterization. Dev Biol 1976; 49: 119–31.
22 Enders GC, Millette CF. Pachytene spermatocyte and round spermatid binding to Sertoli cells *in vitro*. J Cell Sci 1988; 90: 105–14.
23 Sneddon SF, Walther N, Saunders PT. Expression of androgen and estrogen receptors in sertoli cells: studies using the mouse SK11 cell line. Endocrinology 2005; 146: 5304–12.
24 Schipper I, Fauser BC, ten Hacken PM, Rommerts FF. Application of a CHO cell line transfected with the human FSH receptor for the measurement of specific FSH receptor activation inhibitors in human serum. J Endocrinol 1996; 150: 505–14.

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