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REGULAR ARTICLE

# Regulation of expression of Sertoli cell glucose transporters 1 and 3 by FSH, IL1β, and bFGF at two different time-points in pubertal development

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**Abstract** Sertoli cells are necessary to provide adequate levels of lactate for germ cell development. Lactate production is hormonally regulated by follicle-stimulating hormone (FSH) and by a large set of intratesticular regulators such as interleukin-1β (IL1β) and basic fibroblast growth factor (bFGF). Little is known regarding the critical step in the production of this metabolite, viz., the entrance of glucose into the cell as mediated by GLUTs. The aim of the present study was to investigate the expression of the glucose transporters GLUT1 and GLUT3 and its possible regulation by FSH, IL1β, and bFGF in Sertoli cells at two different time-points in sexual development. Sertoli cells retaining the ability to undergo mitosis (obtained from 8-day-old rats) and in the process of terminal differentiation (obtained from 20-day-old rats) were examined. Testicular tissue sections and Sertoli cell monolayers obtained from 8- and 20-day-old rats showed positive immunostaining for GLUT1 and GLUT3 proteins. GLUT1 and GLUT3 mRNA levels were detected at the two ages analyzed. Treatment of Sertoli cells obtained from 8- and 20-day-old rats with FSH, IL1β, and bFGF for various periods of time (12, 24, and 48 h) increased GLUT1 without changing GLUT3 mRNA levels. Our results thus show that Sertoli cells express GLUT1 and GLUT3 throughout pubertal development, and that, in Sertoli cells, only GLUT1 is regulated by hormones during pubertal development. Hormonal regulation of GLUT1 expression and consequently glucose uptake and lactate production may be a key molecular event in the regulation of spermatogenesis by hormones.

**Keywords** GLUT1 · GLUT3 · Testis · Sertoli cell · Glucose transport · Rat (Sprague Dawley)

## Introduction

Spermatogenesis is an intricate process under endocrine, autocrine, and paracrine regulation, which results from multiple and complex interactions between different testicular cells (Parvinen 1982). In this context, Sertoli cells are absolutely necessary in order to provide an adequate and protected environment for germ cell development within the seminiferous tubules. This is best exemplified by lactate, which is produced by Sertoli cells, and may be delivered to and used by germ cells as an efficient energy substrate. The importance of lactate for normal spermatogenesis is highlighted in a report showing that spermatogenesis in adult cryptorchid testis is improved by intratesticular infusion of lactate (Courtens and Ploen 1999). In addition to follicle-stimulating hormone (FSH) and testosterone, interleukin-1β (IL1 β) and basic fibroblast growth factor (bFGF) are worth mentioning among

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the large number of hormones belonging to the set of intratesticular regulators that provide the fine-tuning of spermatogenesis (Gnessi et al. 1997). FSH, IL1β, and bFGF, among other hormones, increase lactate production in rat Sertoli cells (Riera et al. 2001, 2002).

Several biochemical mechanisms might contribute to an increase in lactate secretion in a cell; one of them is the transport of the main carbon source for lactate production, viz., glucose. The transport of this sugar through the plasma membrane (a rate-limiting step in glucose metabolism) is mediated by a set of homologous glycoprotein molecules (GLUTs) that are expressed in a tissue-specific manner (Gorovits and Charron 2003). Expression of GLUT1, GLUT2, GLUT3, and GLUT8 has been demonstrated in the testis (Ulisse et al. 1992; Burant and Davidson 1994; Carosa et al. 2005; Kokk et al. 2007). So far, only mRNAs for GLUT1, GLUT3, and GLUT8 have been observed in purified Sertoli cell preparations (Ulisse et al. 1992; Carosa et al. 2005; Galardo et al. 2007). Even though GLUT8 might be present in rat Sertoli cells, this glucose transporter has not been localized at the plasma membrane rendering unlikely a role for GLUT8 in glucose transport from the extracellular milieu (Reagan et al. 2000; Piroli et al. 2002).

With respect to the expression of GLUTs in other tissues, GLUT1 is highly expressed in the brain and also in the cells of the blood-tissue barriers such as the blood-brain/nerve barrier (Froehner et al. 1988), the placenta (Takata et al. 1992a), and the retina (Takata et al. 1992b). On the other hand, GLUT3 is highly expressed in the brain, with lower levels of expression in the kidney, liver, placenta, muscle, and adipose tissue (Yano et al. 1991). GLUT3 has been postulated as being specialized to act in tandem with GLUT1 to meet the high-energy demands of some tissues (Gould and Holman 1993).

In some cell types, such as trophoblasts, keratinocytes, and neurons, GLUT1 and GLUT3 are developmentally regulated. In rat choriocarcinoma cells, the expression of GLUT1 decreases when the cells begin differentiating to attain their mature functional phenotype (Okamoto et al. 2001). Moreover, GLUT1 expression decreases, whereas GLUT3 expression increases with differentiation in keratinocytes (Shen et al. 2000). Similarly, GLUT3 is predominantly expressed in neuronal processes of fully differentiated neurons (Burkhalter et al. 2003). With regard to the testis, Carosa et al. (2005) have shown that GLUT1 expression increases from 0- to 5-day-old rats, reaching adult levels at this time. No data are available on GLUT3 expression in the rat testis throughout development.

Animal cells require both a basal and an up-regulable glucose transport. In insulin-dependent tissues, the rapid up-regulation of glucose transport is mediated mainly by GLUT4 translocation (Czech 1995), whereas the long-term action of this hormone may involve an increase in the expression of GLUT1 in addition to GLUT4 (Yu et al. 2001; Garcia de Herreros and Birnbaum 1989). In non-insulin-responsive tissues, the up-regulation of glucose transport has been subsumed by other GLUT isoforms. In rat Sertoli cells, no expression of GLUT4 has been demonstrated (Ulisse et al. 1992), and the hormone-responsive GLUT isoforms are barely known. In this context, only the regulation by thyroid hormone and by bFGF of GLUT1 expression has been shown (Ulisse et al. 1992; Riera et al. 2002). To our knowledge, no other reports about a possible hormonal regulation of GLUT1 and GLUT3 in rat Sertoli cells are available.

The aim of the present study has been to investigate the expression of GLUT1 and GLUT3 and its possible regulation by FSH, IL1β and bFGF in Sertoli cells. The study has been performed at two different time-points in Sertoli cell development: cells retaining the ability to undergo mitosis (obtained from 8-day-old rats) and cells in the process of terminal differentiation (obtained from 20-day-old rats).

## Materials and methods

### Materials

Tissue culture media were purchased from GIBCO BRL (Life Technologies, Rockville, Md., USA). Ovine FSH (NIH-oFSH-S-16) was obtained from the National Hormone and Pituitary Program (NIDDK, Bethesda, Md., USA). Human recombinant bFGF was purchased from Invitrogen (Argentina). Rat IL1β were purchased from Sigma-Aldrich (St. Louis, Mo., USA). The polyclonal antibody raised against a purified human erythrocyte glucose transporter (GLUT1) was the kind gift of Christin Carter-Su (University of Michigan, USA). Polyclonal antibody against mouse GLUT3 (AB1344) was purchased from Chemicon (Temecula, Calif., USA), and [2,6-$^3$H]-2-deoxy-D-glucose (2-DOG) was from NEN (Boston, Mass., USA).

### Sertoli cell isolation and culture

Rats of 8 and of 20 days of age were obtained from IBYME's animal care unit (Animal Care Laboratory, Instituto de Biología y Medicina Experimental, Buenos Aires). Animals were killed by decapitation according to protocols for animal laboratory use following the principles and procedures outlined in the National Institute of Health Guide for Care and Use of Laboratory Animals.

Sertoli cells from 8-day-old Sprague-Dawley rats were isolated as previously described (Schteingart et al. 1995). Briefly, 18 rats were decapitated, and their testes were removed, decapsulated, and incubated in culture medium containing 0.03% collagenase and 0.003% soybean trypsin

inhibitor, for 5 min at room temperature. Culture media consisted of a 1:1 mixture of Ham's F-12 and Dulbecco's modified Eagle's medium, supplemented with 0.1% bovine serum albumin, 100 IU/ml penicillin, 2.5 μg/ml amphotericin B, and 1.2 mg/ml sodium bicarbonate. After the initial dispersion, seminiferous tubules were sedimented, and supernatant was discarded to remove interstitial cells. Seminiferous tubules were submitted to 1 M glycine-2 mM EDTA (pH 7.4) treatment to remove peritubular cells. After several washes, a second collagenase treatment was performed. Tubules were treated for 10 min at room temperature with a solution of 0.03% collagenase and 0.003% soybean trypsin inhibitor and 0.03% DNase. The Sertoli cell suspension, collected by sedimentation, was resuspended in the culture medium described above with the following additions: 10 μg/ml transferrin, 5 μg/ml insulin, 5 μg/ml vitamin E, and 4 ng/ml hydrocortisone. Sertoli cells were cultured in 6- or 24-multiwell plates or in tissue culture chamber/slides (15 μg DNA/cm$^2$) at 34°C in a mixture of 5% $CO_2$:95% air.

Sertoli cells from 20-day-old Sprague-Dawley rats were isolated as previously described (Meroni et al. 2002). Briefly, six rats were decapitated, and their testes were removed. Decapsulated testes were digested with 0.1% collagenase and 0.006% soybean trypsin inhibitor in Hanks' balanced salt solution for 5 min at room temperature. Seminiferous tubules were saved, cut, and submitted to 1 M glycine-2 mM EDTA (pH 7.4) treatment to remove peritubular cells. The washed tubular pellet was then digested again with collagenase for 10 min at room temperature to remove germinal cells. The Sertoli cell suspension, collected by sedimentation, was resuspended in culture medium, which consisted of a 1:1 mixture of Ham's F-12 and Dulbecco's modified Eagle's medium, supplemented with 20 mM HEPES, 100 IU/ml penicillin, 2.5 μg/ml amphotericin B, 1.2 mg/ml sodium bicarbonate, 10 μg/ml transferrin, 5 μg/ml insulin, 5 μg/ml vitamin E, and 4 ng/ml hydrocortisone. Sertoli cells were cultured in 25-cm$^2$ flasks, 6- or 24-multiwell plates, or tissue culture chamber/slides (5 μg DNA/cm$^2$) at 34°C in a mixture of 5% $CO_2$:95% air.

No myoid cell contamination was revealed in the cultures when an immunoperoxidase technique was applied to Sertoli cell cultures by using a specific antiserum to smooth muscle α actin. Remaining cell contaminants were of germ cell origin, and this contamination was below 5% after 48 h in culture as examined by phase-contrast microscopy.

## Culture conditions

Sertoli cells were allowed to attach for 48 h in the presence of insulin, and medium was replaced at this time with fresh medium without insulin. Stimulation with FSH (100 ng/ml), IL1β (50 ng/ml), or bFGF (30 ng/ml) was performed for various periods of time. Cells incubated for 12-, 24-, and 48-h with FSH, IL1β, or bFGF were used to evaluate GLUT1 and GLUT3 mRNA levels. For 2-DOG uptake studies, cells cultured for 48-h with FSH, IL1β, or bFGF were used. Lactate was measured in the 72-h conditioned medium collected on day 5. Cells maintained under basal conditions fixed on day 5 were used for immunocytochemistry.

## Immunohistochemistry and immunocytochemistry

Testicular tissue from 8- or 20-day-old rats was fixed in 4% paraformaldehyde in 0.1 mol/l phosphate buffer, dehydrated, and embedded in paraffin; 5-μm sections were incubated overnight at 4° C with specific rabbit polyclonal antibodies raised against human GLUT1 and mouse GLUT3 diluted 1:500 in TRIS-buffered saline (TBS). After incubation with the primary antibodies, sections were washed three times with TBS and the Dako Cytomation LSAB+System-HRP (Dako, Carpinteria, Calif., USA) was used with 3,3′-diaminobenzidine as the chromogen.

Immunocytochemistry was performed in 8- and 20-day-old rat Sertoli cell cultures. Cells were washed with TBS and fixed by immersion in methanol at −20°C. Fixed cells were incubated under identical experimental conditions with the specific antibodies described above. Thereafter, cells were washed three times with TBS, and the Super Sensitive Alkaline Phosphastase Ready-To-Use detection system (BioGenex, San Ramon, Calif., USA) was used. The reaction was revealed by incubation with Fast red. Negative controls were carried out by incubations with nonimmune rabbit serum replacing the primary antibody. Nuclear counterstaining was performed with hematoxylin.

## Northern blot analysis

Total RNA was extracted from Sertoli cells cultured in 6-multiwell plates (8-day-old cells) or 25-cm$^2$ tissue culture flasks (20-day-old cells) by the guanidinium isothiocyanate method (Chomczynski and Sacchi 1987). The amount of RNA was estimated by spectrophotometry at 260 nm. For Northern blot analysis, 10 μg total RNA was electrophoresed on a 1% agarose-10% formaldehyde gel. After migration, RNAs were transferred to Hybond-N nylon membrane (Amersham Pharmacia Biotech, Buenos Aires, Argentina) by capillary transfer with 20× standard sodium citrate (20× stock solution: 3 M NaCl, 0.3 M sodium citrate, pH 7.4) and fixed with U.V. Stratalinker (Stratagene Cloning Systems, La Jolla, Calif., USA). cDNA probes (rat GLUT1 2.6-kb insert, *Eco*RI; mouse GLUT3 0.6-kb insert, *Eco*RI-*Hin*dIII; 18S oligonucleotide) were labeled with [α-$^{32}$P]deoxy-CTP (Amersham Pharmacia Biotech) by

using a random-primed labeling kit (Prime-a-Gene Labeling System, Promega, Madison, Wis., USA). Blots were prehybridized for 5 h at 42°C in 50% formamide, 0.75 M NaCl, 20 mM sodium phosphate pH 7.5, 1 mM EDTA, 5× Denhardt's solution, 10% dextran sulfate, 0.5% SDS, and 100 μg/ml herring sperm DNA. Hybridization was then performed overnight at 42°C in the same hybridization buffer containing 1–4×$10^6$ cpm/ml $^{32}$P-labeled probe. Membranes were washed under different astringency conditions depending on the probe utilized. Membranes were exposed to Kodak X-Omat S films (Eastman Kodak, Rochester, N.Y., USA). The intensities of the autoradiographic bands were estimated by densitometric scanning and NIH Image (Scion Corporation, Frederick, Md., USA) software. The 18S signal was used to standardize mRNA contents.

## Measurement of 2-DOG

Glucose transport was studied by using the uptake of the labeled non-metabolizable glucose analog 2-DOG. Cells were washed three times with glucose-free phosphate-buffered saline (PBS) at room temperature and then incubated at 34°C in 0.5 ml glucose-free PBS containing [2,6-$^3$H]-2-DOG (0.5 μCi/ml) for 30 min. Unspecific uptake was determined in incubations performed in the presence of a 10,000-fold higher concentration of unlabeled 2-DOG. At the end of the incubation period, dishes were placed on ice and washed extensively with ice-cold PBS until no radioactivity was present in the washings. Cells were then dissolved with 0.5 M sodium hydroxide and 0.4% sodium deoxycholate and counted in a liquid scintillation spectrophotometer. Parallel cultures receiving identical treatments to those performed before the glucose uptake assay were destined for DNA determinations. Results are expressed on a per microgram DNA basis.

## Lactate determination

Lactate was measured by a standard method involving conversion of nicotinamide-adenine dinucleotide$^+$ ($NAD^+$) to NADH determined as the rate of increase of absorbance at 340 nm. A commercial kit from Sigma-Aldrich was used. Results were expressed on a per microgram DNA basis.

## Other assays

DNA was determined by the method of Labarca and Paigen (1980).

## Statistical analysis

All experiments were run in triplicates and repeated three to four times. Results are expressed as means±SD. One-way analysis of variance with the Tukey-Kramer post test was

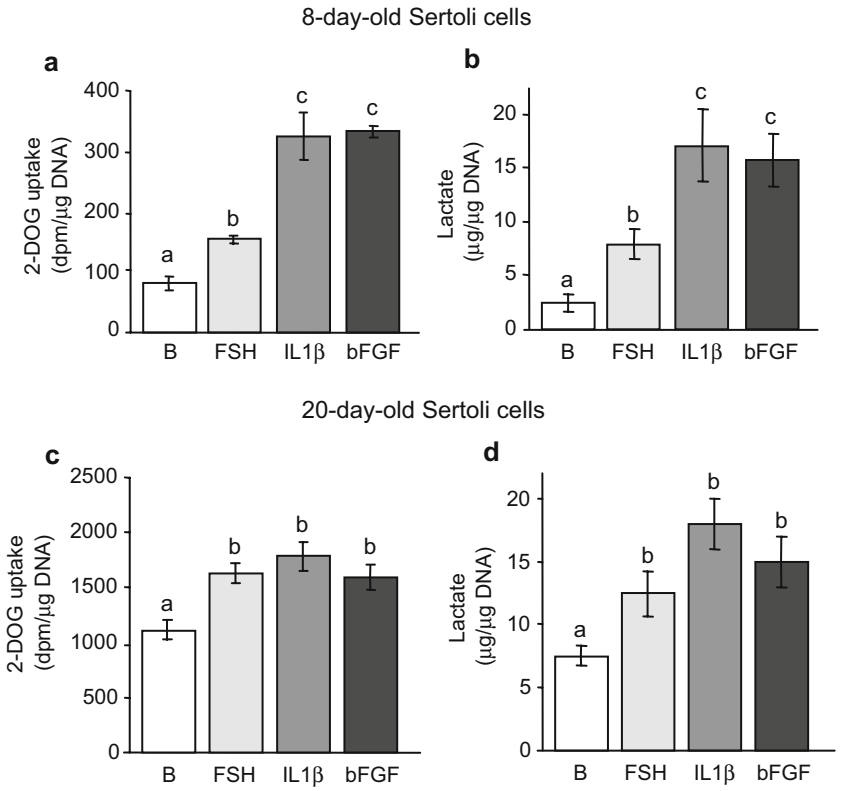
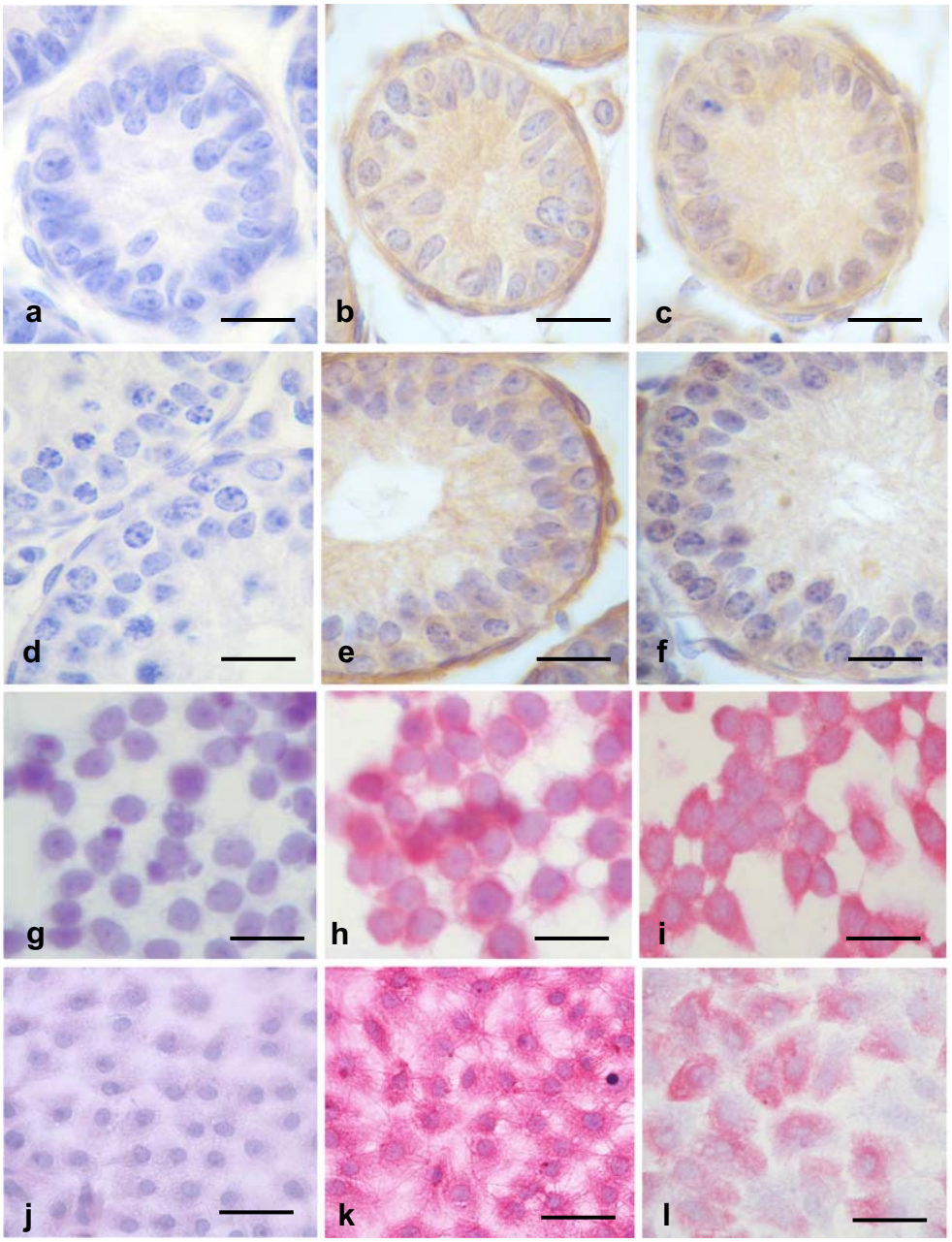



**Fig. 1** Effect of follicle-stimulating hormone (*FSH*), interleukin-1β (*IL1β*), and basic fibroblast growth factor (*bFGF*) on 2-DOG uptake and lactate production in 8- and 20-day-old rat Sertoli cells. Results are expressed as means±SD, $n$=3. Different *letters* indicate statistically significant differences ($P$<0.01)



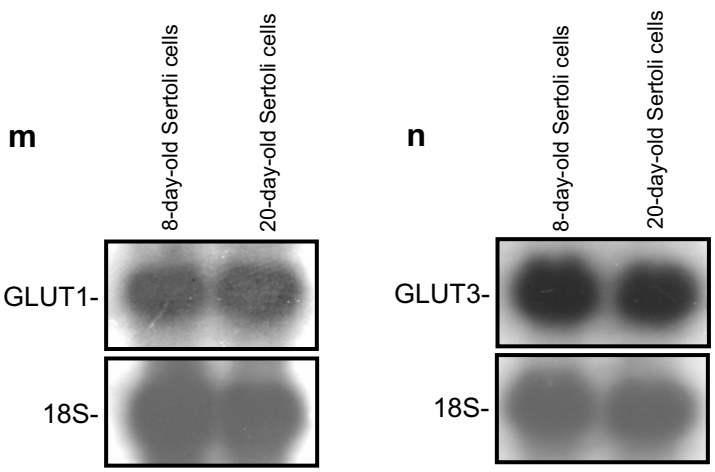




**Fig. 2** Expression of GLUT1 and GLUT3: immunohistochemical, immunocytochemical, and Northern blot analysis. Testicular sections from 8-day-old (**a–c**) or from 20-day-old rats (**d–f**) showing positive GLUT1 (**b**, **e**) and GLUT3 (**c**, **f**) staining and negative controls (**a**, **d**). Sertoli cell monolayers from 8-day-old (**g–i**) or from 20-day-old rats (**j–l**) showing positive staining for GLUT1 (**h**, **k**) and GLUT3 (**i**, **l**) and negative controls (**g**, **j**). *Bars* 1.9 μm (**a–i**), 6.5 μm (**j–l**). mRNA GLUT1 (**m**) and GLUT3 (**n**) Sertoli cell in 8- and 20-day-old rats

performed by using GraphPad InStat version 3.00 for Windows 95 (GraphPad Software, San Diego, Calif., USA). Probabilities of <0.05 were considered statistically significant.

## Results

### Effect of FSH, IL1β, and bFGF on glucose uptake and lactate production

The effect of long-term incubations with FSH (100 ng/ml), IL1β (50 ng/ml), bFGF (30 ng/ml), and testosterone (1 μM) on glucose uptake and lactate production in Sertoli cells obtained from 8- and 20-day-old rats was investigated. Figure 1 shows that basal glucose uptake and lactate production were higher in Sertoli cells obtained from 20-day-old animals than in those obtained from 8-day-old ones and that, at both maturational stages of the Sertoli cells, FSH, IL1β, and bFGF produced a significant increase in glucose uptake and lactate production. On the other hand, treatment of the cultures with testosterone did not modify glucose uptake or lactate production (data not shown).

### Expression of GLUT1 and GLUT3 in 8- and 20-day-old rat Sertoli cells

Testicular tissue sections and Sertoli cells monolayers obtained from 8- and 20-day-old rats were processed for immunostaining to detect GLUT1 and GLUT3 proteins by using specific antibodies. Positive staining for GLUT1 and GLUT3 was seen at both time points in tissue sections and in Sertoli cell monolayers. No staining was seen in the controls (Fig. 2a–l).

Furthermore, we analyzed GLUT1 and GLUT3 mRNA levels in 8- and 20-day-old rat Sertoli cells. Both GLUT1

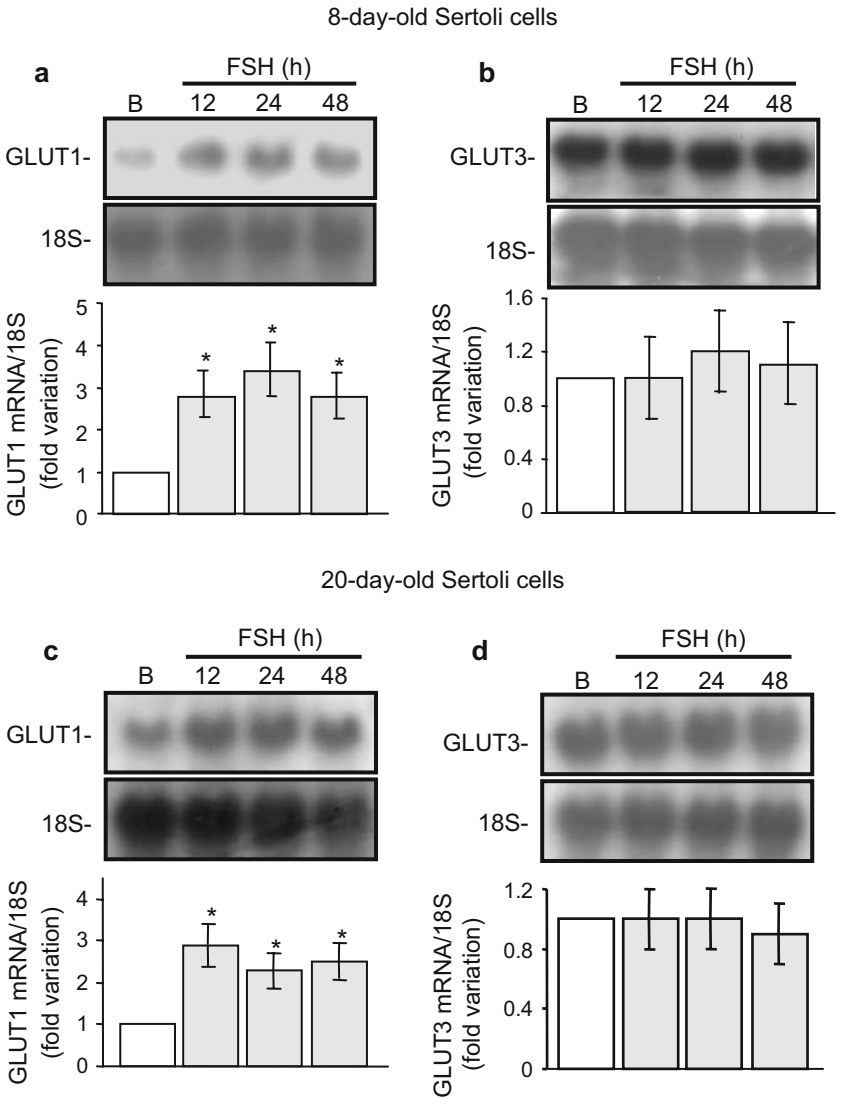



**Fig. 3** Effect of FSH on GLUT1 (**a**, **c**) and GLUT3 (**b**, **d**) mRNA levels in Sertoli cells from 8- and 20-day-old rats. Bar graphs show the fold variation in mRNA levels (ratio of GLUT1 mRNA to 18S and of GLUT3 mRNA to 18S in each sample) relative to the basal level (*B*). Results are expressed as means±SD, $n=3$, $^{*}P<0.05$ versus basal

and GLUT3 mRNA were detected at the two ages analyzed (Fig. 2m,n).

Effect of FSH on GLUT1 and GLUT3 mRNA levels

Treatment of Sertoli cells obtained from 8-day-old rats with FSH (100 ng/ml) for various periods of time (12, 24, and 48 h) increased GLUT1 (Fig. 3a) without changing GLUT3 (Fig. 3b) mRNA levels. Similar results were obtained in Sertoli cells isolated from 20-day-old rats (Fig. 3c,d).

Effect of IL1β and bFGF on GLUT1 and GLUT3 mRNA levels

Treatment of Sertoli cells obtained from 8-day-old rats with IL1β (50 ng/ml) for various periods of time (12, 24, and 48 h) increased GLUT1 (Fig. 4a) without changing GLUT3 (Fig. 4b) mRNA levels. Once again, similar results were obtained in Sertoli cells isolated from 20-day-old rats (Fig. 4c,d).

Finally, experiments performed to analyze a possible role of bFGF on the expression of GLUT1 and GLUT3 showed identical results to those observed with FSH and IL1β (Fig. 5).

## Discussion

The aim of the present study was to analyze the existence of possible changes in the expression of GLUT1 and GLUT3 and its regulation by FSH, IL1β, and bFGF at two different time-points in the sexual maturation of Sertoli cells. For this purpose, we utilized cultures of Sertoli cells obtained from 8- and 20-day-old rats that offered a unique experimental model to analyze cell physiology at two different developmental stages of these cells.

Our results show a three-fold increase in basal Sertoli cell lactate production when comparing Sertoli cells from 8- and 20-day-old rats. The changes in lactate production observed with age and in response to hormonal stimulation

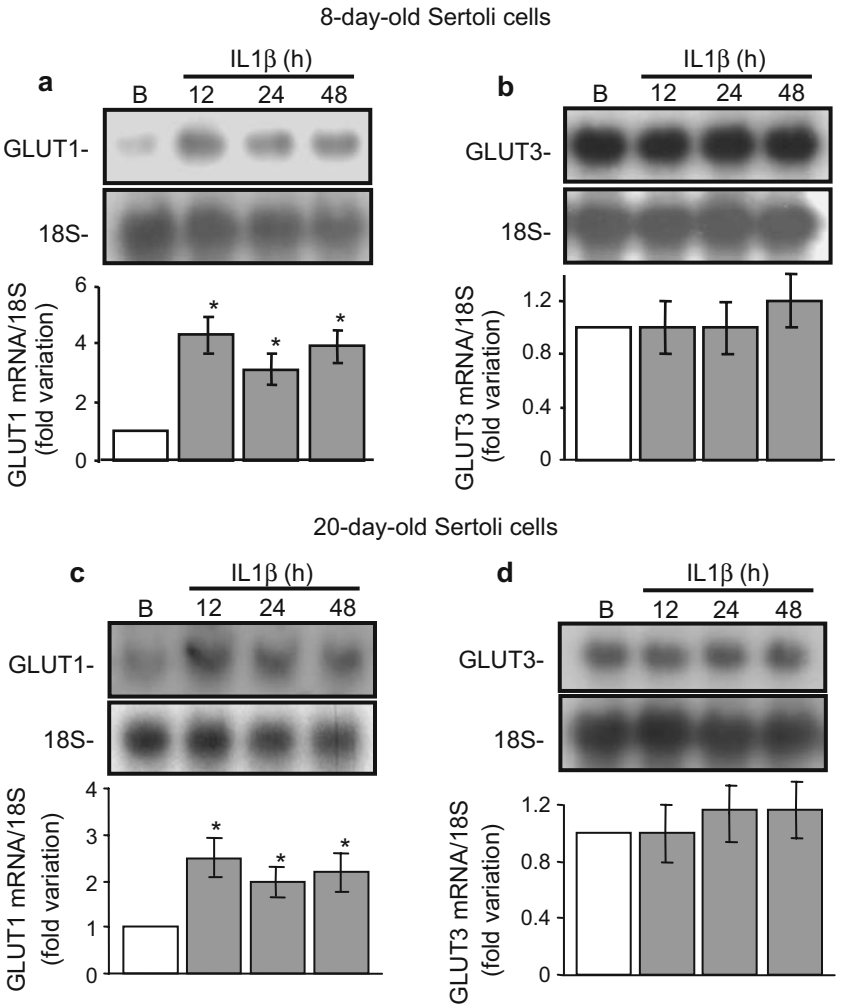



**Fig. 4** Effect of IL1β on GLUT1 (**a**, **c**) and GLUT3 (**b**, **d**) mRNA levels in Sertoli cells from 8- and 20-day-old rats. Bar graphs show the fold variation in mRNA levels (ratio of GLUT1 mRNA to 18S and of GLUT3 mRNA to 18S in each sample) relative to the basal level (*B*). Results are expressed as means±SD, $n=3$, $^{*}P<0.05$ versus basal

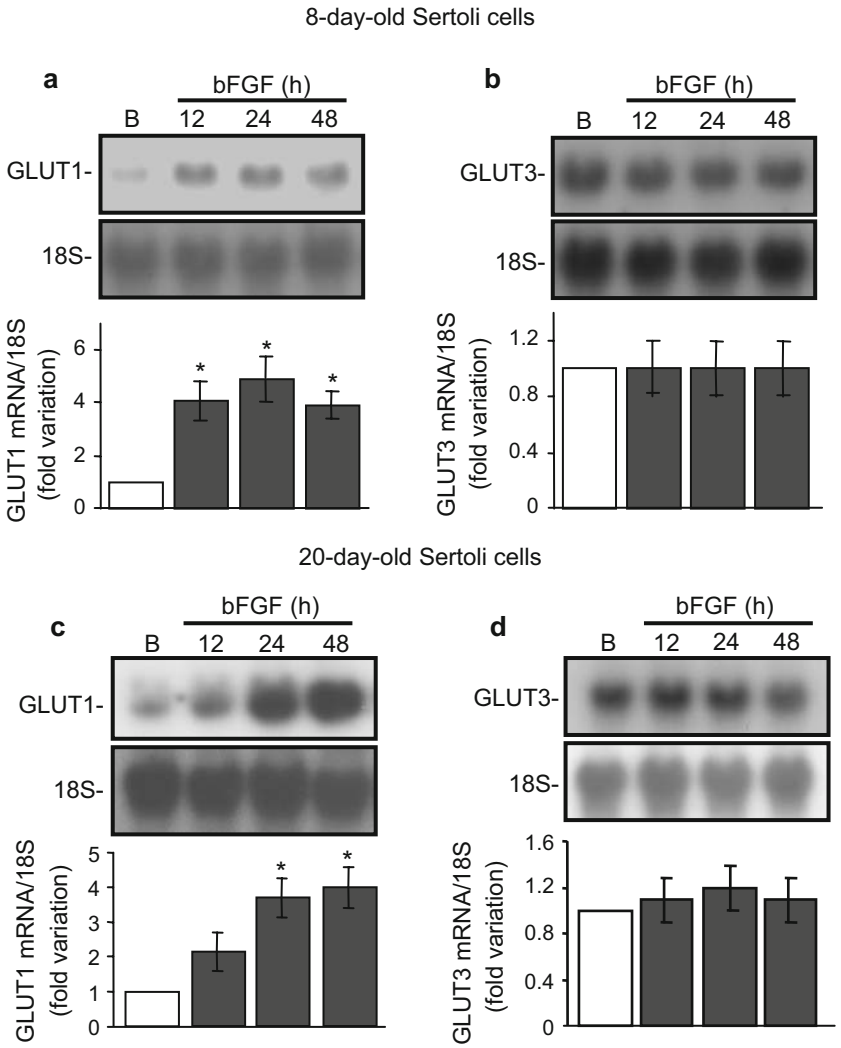



**Fig. 5** Effect of bFGF on GLUT1 (**a**, **c**) and GLUT3 (**b**, **d**) mRNA levels in Sertoli cells from 8- and 20-day-old rats. Bar graphs show the fold variation in mRNA levels (ratio of GLUT1 mRNA to 18S and of GLUT3 mRNA to 18S in each sample) relative to the basal level (*B*). Results are expressed as means±SD, $n=3$, $^*P<0.05$ versus basal

correlate roughly with the changes in glucose uptake, reinforcing the idea that the entrance of the substrate constitutes a major limiting step for the production of the energetic metabolite, viz., lactate. Our data are different from those of Ulisse et al. (1992) showing no changes in basal glucose uptake throughout sexual maturation in rat Sertoli cells. The discrepancy may be attributable to slightly different methodological approaches. First, whereas Ulisse et al. (1992) carried out the glucose uptake assay in subconfluent cultures, we have performed the assay in confluent cultures. Under the latter conditions, autocrine effects of certain growth factors may become more relevant and contribute to the increment in glucose uptake observed with age. Second, whereas Ulisse et al. (1992) express their results on a per microgram protein basis, we have expressed our results on a per microgram DNA basis. As the protein to DNA relationship may increase with the differentiation process of these cells, this increase may be responsible for the underestimation of glucose uptake when expressed on a microgram protein basis.

With regard to the expression of glucose transporter isoforms, we have not observed differences in GLUT1 and GLUT3 mRNA levels when comparing 8- and 20-day-old Sertoli cells. So far, we have not been able to detect the corresponding proteins by Western blot analysis, and immunocytochemisty only allows us to conclude that GLUT1 and GLUT3 proteins are present at both ages analyzed. Even in the case that similar levels of both glucose transporters are present at both ages, other phenomena may explain the increment in glucose transport observed in older animals. In this respect, Kumar et al. (2004) have shown that the composition of the plasma membrane, particularly the lipid rafts, plays an important role in glucose transporter activities, and Sweeney et al. (1999) have demonstrated that specific signal transduction pathways may also regulate the activity of the glucose transporters present at the plasma membrane. Alternatively, other mechanisms, such as those regulating the translation of GLUT mRNAs or others, involved in the translocation of glucose transporters to the membrane may also participate

in the increase of glucose uptake with age. Further studies will be necessary to clarify this complex regulation in Sertoli cells throughout sexual maturation.

Another controversial point concerning the expression of GLUTs is related to the existence of a specific isoform distribution in the testis. The results presented herein show that GLUT3 is expressed in 8- and 20-day-old Sertoli cells. In addition, other authors utilizing immunohistochemical techniques have shown the presence of this glucose transporter in Sertoli cells of adult rats (Burant and Davidson 1994; Kokk et al. 2007). Together, the results indicate that GLUT3 is present in Sertoli cells throughout their life span. Regarding GLUT1 expression, the results obtained so far by different authors do not allow a clear conclusion to be reached. Burant and Davidson (1994) have claimed that GLUT1 expression is low in rat testes, probably representing expression in vascular and stromal elements, and they have ruled out Sertoli cell expression of the protein. The latter observations are in agreement with those of Kokk et al. (2007), who have only found positive immunoreaction for GLUT1 in peritubular myoid cells, macrophage-like interstitial cells, testicular endothelial cells, and early spermatocytes. We have observed the immunoreaction of GLUT1 in testicular sections obtained from 8- and 20-day-old rats with a staining consistent with Sertoli cell localization. Moreover, the protein has been revealed in purified 8-day-old and 20-day-old Sertoli cell cultures and, as previously mentioned, mRNA levels for GLUT1 are easily detectable in cultures obtained at both ages. These results demonstrate that GLUT1 is expressed in Sertoli cells at two different time-points in sexual maturation.

Finally, the results presented herein deal with the identification of glucose transporter isoforms that may be regulated by hormones in Sertoli cells. We have hypothesized that the hormonal up-regulation of glucose uptake is mediated either by GLUT1 or GLUT3. In this context, we have observed that FSH, IL1β, and bFGF, which increase glucose uptake and lactate production, increase GLUT1 and not GLUT3 mRNA levels. The differential regulation of GLUT1 and GLUT3 by hormones has been observed in various cell types. GLUT1 is up-regulated by estrogen and progesterone, but the same hormonal treatments do not affect GLUT3 protein levels in Ishikawa endometrial cancer cells (Medina et al. 2004). Similar results have been observed in human articular chondrocytes stimulated by IL1β and TNFα (Shikhman et al. 2001). However, in other cell types, GLUT3 is the glucose transporter isoform apparently responsible for the increment in glucose transport in response to hormones. This is the case in placental cells obtained from 13-day-pregnant mice that have undergone sialoadenectomy and that have been treated with epidermal growth factor (Kamei et al. 1999). In other cell types, such us ovarian cells stimulated by IL1, both GLUT1 and GLUT3 protein levels increase in response to the hormone (Kol et al. 1997). Our results suggest that, in Sertoli cells, although both transporters may be responsible for basal glucose transport, GLUT1 constitutes the glucose transporter molecule responsible for the hormonal up-regulation of glucose entrance into the cell.

In summary, the results obtained herein show that Sertoli cells express GLUT1 and GLUT3 throughout pubertal development. They also show that, in Sertoli cells, only GLUT1 is regulated by hormones. This hormonal regulation of GLUT1 expression and consequently of glucose uptake and lactate production may be a key molecular event in the regulation of spermatogenesis by hormones.

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