

Regulation of lactate production and glucose transport as well as of glucose transporter 1 and lactate dehydrogenase A mRNA levels by basic fibroblast growth factor in rat Sertoli cells

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Abstract

By using cultured rat Sertoli cells as a model, both the action of basic fibroblast growth factor (bFGF) on lactate production and the site of this action were studied. bFGF stimulated Sertoli cell lactate production in a dose-dependent manner (basal: 7.3 ± 0.5 ; 0.1 ng/ml bFGF: 7.5 ± 0.5 ; 1 ng/ml bFGF: 7.5 ± 0.6 ; 10 ng/ml bFGF: 10.3 ± 1.0 ; 30 ng/ml bFGF: 15.2 ± 1.5 ; 50 ng/ml bFGF: 15.4 ± 1.6 $\mu\text{g}/\mu\text{g}$ DNA). Two major sites for the action of this growth factor were identified. First, bFGF was shown to exert short- and long-term stimulatory effects on glucose transport (basal: 1170 ± 102 ; 30 ng/ml bFGF for 120 min: 1718 ± 152 and basal: 718 ± 64 ; 30 ng/ml bFGF for 48 h: 1069 ± 69 d.p.m./ μg DNA respectively). Short-term bFGF stimulation of glucose transport was not inhibited by the protein synthesis inhibitor cycloheximide. These results indicate that short-term bFGF stimulation of glucose uptake does not involve an increase in the number of glucose transporters. On the other hand, stimulation with bFGF for periods of time

longer than 12 h increased glucose transporter 1 (GLUT1) mRNA levels. These increased mRNA levels were probably ultimately responsible for the increments in glucose uptake that are observed in long-term treated cultures. Secondly, bFGF increased lactate dehydrogenase (LDH) activity (basal: 31.0 ± 1.4 ; 30 ng/ml bFGF: 45.7 ± 2.4 mIU/ μg DNA). The principal subunit component of those LDH isozymes that favors the transformation of pyruvate to lactate is subunit A. bFGF increased LDH A mRNA levels in a dose- and time-dependent manner. In summary, the results presented herein show that glucose transport, LDH activity and GLUT1 and LDH A mRNA levels are regulated by bFGF to achieve an increase in lactate production. These observed regulatory actions provide unequivocal evidence of the participation of bFGF in Sertoli cell lactate production which may be related to normal germ cell development.

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Introduction

Spermatogenesis is an intricate process highly dependent on Sertoli cell function which is under endocrine (follicle-stimulating hormone (FSH) and testosterone) as well as under the autocrine and paracrine control which result from multiple and complex interactions between the different testicular cells (Parvinen 1982). A large number of growth factors belong to the set of intratesticular regulators which provide the fine tuning of cellular processes implicated in the maintenance of spermatogenesis (Gnessi *et al.* 1997). Among them are the fibroblast growth factors (FGFs), a family of structurally related peptides. First identified as mitogenic factors in brain extracts, they have been found to modulate various non-mitogenic biological processes in a wide range of tissues and organs, including the testis, where basic FGF

(bFGF) has been isolated (Ueno *et al.* 1987). Germ cells are a potential source of this peptide although other cells in the testis also express it (Mullaney & Skinner 1992, Han *et al.* 1993). While the functional role of bFGF in the testis is still under study, it is known to have several modulatory effects in Sertoli cell cultures, where bFGF receptors are constitutively expressed (Le Magueresse-Battistoni *et al.* 1994). Here, the growth factor modulates DNA synthesis (Jaillard *et al.* 1987), release of transferrin (Han *et al.* 1993), estradiol production, γ -glutamyl transpeptidase activity and lactate secretion (Schteingart *et al.* 1999). Additionally, bFGF modulates expression of FSH receptors (Smith *et al.* 1989), plasminogen activator inhibitor-1 (Le Magueresse-Battistoni *et al.* 1998), cfos, jun B (Smith *et al.* 1989) and Sertolin (Mruk & Cheng 1999).

Among Sertoli cell functions that might be of interest to germ cell development is the provision of adequate levels

of energy substrates. Studies on the metabolism of glucose have shown that Sertoli cells actively metabolize glucose but the majority of it is converted to lactate and is not oxidized via the citric acid cycle (Robinson & Fritz 1981, Grootegoed *et al.* 1986b). On the other hand, germ cells (particularly post-meiotic germ cells) are unable to use glucose for their energy metabolism and they do prefer lactate as an energy source (Jutte *et al.* 1981, Mita & Hall 1982, Grootegoed *et al.* 1986a). The importance of lactate for normal spermatogenesis was recently highlighted in a report showing that spermatogenesis in adult cryptorchid rat testis is improved by intratesticular infusion of lactate (Courtens & Plöen 1999).

Glucose transport into the cell and the lactate dehydrogenase (LDH) isoenzyme system (Plagemann *et al.* 1960, Pesce *et al.* 1961), which reversibly catalyzes the interconversion of pyruvate and lactate, are biochemical steps which participate in the regulation of lactate production. Facilitated Sertoli cell glucose transport across plasma membrane is mediated by the carrier protein termed glucose transporter 1 (GLUT1), the only glucose transporter so far demonstrated in this cell (Ulisse *et al.* 1992). As for the LDH isoenzyme system, increments in lactate production in Sertoli cells have been correlated with an increase in the LDH5 isoenzyme – containing four subunits A. Glucose transport through the plasma membrane and LDH A mRNA levels are regulated in a distinct manner by FSH (Riera *et al.* 2001), interleukin 1 (IL1) (Nehar *et al.* 1998), tumor necrosis factor- α (TNF α) (Nehar *et al.* 1997) and epidermal growth factor (EGF) (Boussouar & Benahmed 1999) – factors that modify Sertoli cell lactate production. No data are available on the mechanisms involved in the recently described effect of bFGF on lactate production (Schteingart *et al.* 1999).

In the present study, we have analyzed the biochemical steps that participate in the regulation exerted by bFGF on rat Sertoli cell lactate production. The results presented herein indicate that increments in glucose uptake and LDH activity, probably secondary to increased GLUT1 and LDH A mRNA levels, may be involved in the stimulatory effect of bFGF on lactate production.

Materials and Methods

Materials

Ovine FSH (NIH-oFSH-S-16) was obtained from the National Hormone and Pituitary Program, NIDDK, Bethesda, MD, USA. Human recombinant bFGF and tissue culture media were purchased from GIBCO BRL (Life Technologies Ltd, Rockville, MD, USA). [2,6-³H]-2-deoxy-D-glucose ([2,6-³H]-2-DOG) was purchased from NEN (Boston, MA, USA). All other drugs and reagents were purchased from Sigma-Aldrich (St Louis, MO, USA).

Sertoli cell isolation and culture

Sertoli cells from 20-day-old Sprague–Dawley rats were isolated as previously described (Schteingart *et al.* 1989). Briefly, 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 consists of a 1:1 mixture of Ham's F12 and Dulbecco's modified Eagle's medium, supplemented with 20 mM HEPES, 100 IU/ml penicillin, 2.5 μ g/ml amphotericin B, 1.2 mg/ml sodium bicarbonate, 10 μ g/ml transferrin, 5 μ g/ml insulin, 5 μ g/ml vitamin E and 4 ng/ml hydrocortisone. Sertoli cells were cultured in 24-multiwell plates or 25 cm² tissue culture flasks (5 μ g DNA/cm²) at 34 °C in a mixture of 5% CO₂:95% air.

No myoid cell contamination was revealed in the cultures when an immunoperoxidase technique was applied to Sertoli cell cultures using a specific antiserum to α -smooth muscle 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; variable doses of bFGF were added on day 3 as indicated in the Figure legends. The 72-h conditioned media obtained on day 6 was used to evaluate lactate levels. In some experiments, 2-day-old Sertoli cell monolayers were treated with hypotonic solution (20 mM Tris/HCl, pH 7.4) for 2 min in order to eliminate residual contaminating germ cells (Wagle *et al.* 1986) and bFGF stimulation of lactate production was analyzed.

Cells harvested on day 6 were used to determine LDH activity. For glucose uptake studies, cells harvested on day 5 pretreated for variable periods of time with the different hormones were used. Cells pretreated for variable periods of time and harvested on day 6 were used for GLUT1 and LDH A mRNA studies.

Sertoli cell viability was higher than 98% in cells cultured for 6 days in all experimental conditions as evaluated by trypan blue exclusion test.

Lactate determination

Lactate was measured by a standard method involving conversion of NAD⁺ to NADH determined as the rate of

increase of absorbance at 340 nm. A commercial kit from Sigma-Aldrich with a 5% interassay coefficient of variation was used.

Measurement of 2-deoxy-D-glucose (2-DOG) uptake

Glucose transport was studied using the uptake of the labeled non-metabolizable glucose analogue 2-DOG. Cells that had received different treatments for variable periods of time (0.5–48 h), as indicated in the Figure legends, were used. After treatment, culture medium was discarded and cells were washed three times with glucose-free phosphate-buffered saline (PBS) at room temperature. Sertoli cells were then incubated at 34 °C in 0.5 ml glucose-free PBS containing [2,6-³H]-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, 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 µg DNA basis.

LDH activity measurement

After incubation of Sertoli cells in the absence or presence of the different stimuli, culture media were saved for lactate determinations and cells were disrupted by ultrasonic irradiation in NaCl (0.9%) and centrifuged (15 800 g, 10 min). The supernatant was used to measure total LDH activity. Total LDH activity was determined by a routinely used spectrophotometric method (Randox Laboratories, Crumlin, Antrim, UK). Interassay coefficient of variation for this method is 3%.

Analysis of GLUT1 and LDH A mRNA levels

Total RNA was isolated from Sertoli cells cultured in 25 cm² tissue culture flasks with TRI-Reagent (Sigma-Aldrich), a monophasic solution of phenol and guanidine isothiocyanate. This reagent is a modification of the single-step RNA isolation developed by Chomczynski & Sacchi (1987). The amount of RNA was estimated by spectrophotometry at 260 nm. About 20 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 × SSC (20 × stock solution: 3 M NaCl and 0.3 M sodium citrate, pH 7.4) and fixed with U.V. Stratalinker

(Stratagene Cloning Systems, La Jolla, CA, USA). cDNA probes (rat LDH A 3'UTR 0.4 kb insert, Pst I–Bgl II; rat GLUT1 2.6 kb insert, EcoRI; and a 28S oligonucleotide) were labeled with [α -³²P]deoxy-CTP (Amersham Pharmacia Biotech) using a random-primed labeling kit (Stratagene Cloning Systems). Blots were prehybridized for 5 h at 42 °C in 50% formamide, NaCl/Pi/EDTA (0.75 M NaCl, 20 mM sodium phosphate (pH 7.5) and 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 × 10⁶ c.p.m./ml ³²P-labeled probe. Membranes were washed twice in 2 × SSC–0.5% SDS (20 min, room temperature) followed by two washes in 1 × SSC–0.1% SDS (30 min, 65 °C). Membranes were exposed to Kodak X-Omat S films (Eastman Kodak, Rochester, NY, USA) for 1–2 days at –70 °C. The intensities of the autoradiographic bands were estimated by densitometric scanning using NIH Image (Scion Corporation, Frederick, MD, USA) software. The 28S signal was used to standardize LDH A and GLUT1 mRNA contents.

Other assays

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

Statistical analysis

To analyze data from glucose uptake studies, lactate production and LDH activity, one-way analysis of variance followed by the Tukey–Kramer test for multiple comparisons using the GB-STAT version 4.0 statistical program (Dynamic Microsystems Inc., Silver Spring, MD, USA) were performed. Densitometric data obtained in Northern blot analysis were normalized by log transformation and pooled data analyzed by Student's *t*-test. Probabilities <0.05 were considered statistically significant.

Results

Effect of bFGF on lactate production

Figure 1 shows the dose–response curve to bFGF (0.1–50 ng/ml) for rat Sertoli cell lactate production. Significant increments in lactate production at doses equal to or higher than 10 ng/ml were observed as previously described (Schteingart *et al.* 1999). To ascertain the possible effect of residual contaminating germ cells on bFGF regulation of lactate production, Sertoli cell monolayers were hypotonically treated (HT) and compared with untreated controls in their ability to produce lactate. Identical results on lactate production were obtained under basal and bFGF (30 ng/ml)–stimulated conditions (basal:

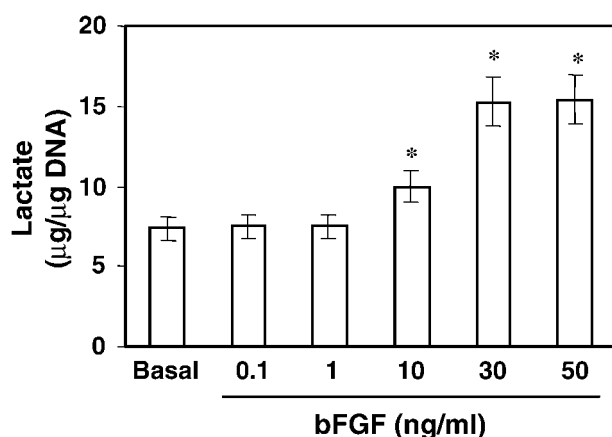


Figure 1 Dose–response curve to basic fibroblast growth factor (bFGF) for rat Sertoli cell lactate production. Sertoli cells were incubated for 72 h in the absence or presence of variable doses of bFGF (0.1, 1, 10, 30 and 50 ng/ml). Lactate was determined in the 72-h conditioned media recovered on day 6. Results are expressed as means \pm S.D. of triplicate incubations in one representative experiment out of three (* $P < 0.01$ vs basal).

5.5 ± 1.3^a ; bFGF: 8.5 ± 1.1^b ; basal (HT): 6.1 ± 1.0^a ; bFGF (HT): 8.9 ± 0.9^b $\mu\text{g}/\mu\text{g DNA}$; different superscripts indicate statistically significant differences, $P < 0.01$).

FSH (100 ng/ml) also stimulated lactate production and a simultaneous addition of bFGF (30 ng/ml) produced a synergistic effect (basal: 9.1 ± 1.0^a ; bFGF: 14.0 ± 1.4^b ; FSH: 16.7 ± 2.0^b ; bFGF+FSH: 40.1 ± 3.1^c $\mu\text{g}/\mu\text{g DNA}$; different superscripts indicate statistically significant differences, $P < 0.01$).

Short- and long-term effects of bFGF on glucose uptake

The possible short- and long-term effect of bFGF on Sertoli cell glucose uptake was investigated. Figure 2A shows 2-DOG uptake in rat Sertoli cells stimulated with 30 ng/ml bFGF for variable short periods of time (30, 60 and 120 min). bFGF rapidly (30 min) increased 2-DOG uptake and this transport remained elevated for the time-period analyzed. Figure 2B shows the dose–response curve to bFGF for glucose uptake in cells treated for 120 min. Significant increments in glucose uptake at doses equal to or higher than 10 ng/ml bFGF were observed. To determine whether *de novo* protein synthesis was required for this increase in glucose uptake, Sertoli cells were treated with bFGF (30 ng/ml) for 120 min in the absence or presence of the protein synthesis inhibitor cycloheximide (CHX; 5 μM). CHX did not modify basal or bFGF-stimulated glucose uptake (basal: 694 ± 70^a ; CHX: 810 ± 75^a ; bFGF: 1198 ± 73^b ; bFGF+CHX: 1266 ± 92^b d.p.m./ $\mu\text{g DNA}$; different superscripts indicate statistically significant differences, $P < 0.01$).

Sertoli cell cultures treated simultaneously with bFGF (30 ng/ml) and FSH (100 ng/ml) for 120 min showed an

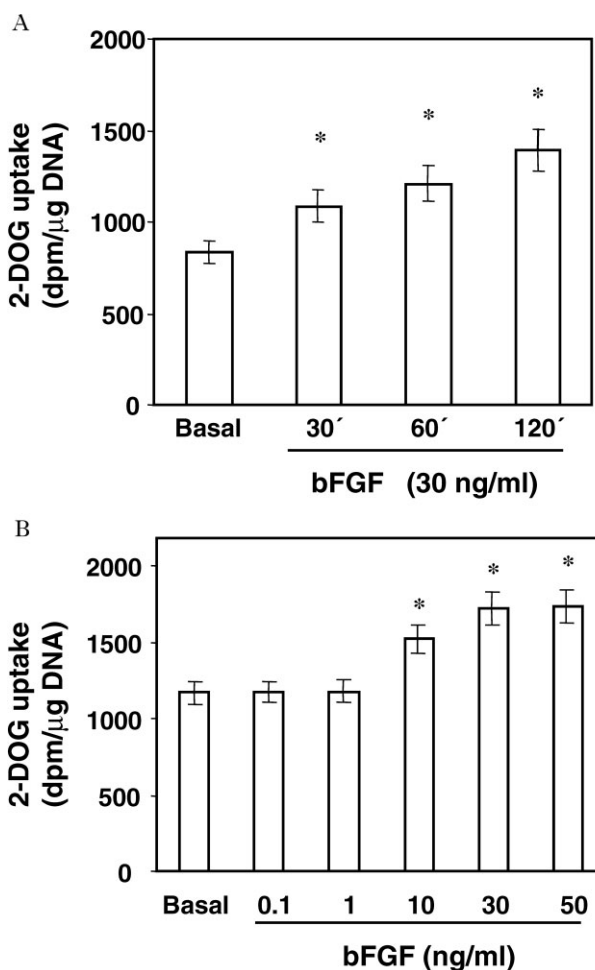


Figure 2 Effect of bFGF on 2-DOG uptake in rat Sertoli cells. (A) Cells were stimulated for variable short periods of time (30, 60 and 120 min) with 30 ng/ml bFGF. (B) Sertoli cells were incubated for 120 min in the absence or presence of variable doses of bFGF (0.1, 1, 10, 30 and 50 ng/ml). Glucose uptake assay was performed on day 5. Results represent the means \pm S.D. of triplicate incubations in one representative experiment out of three (* $P < 0.01$ vs basal).

additive effect of these hormones on glucose uptake (basal: 835 ± 59^a ; bFGF: 1475 ± 43^b ; FSH: 1573 ± 99^b ; bFGF+FSH: 2070 ± 207^c d.p.m./ $\mu\text{g DNA}$; different superscripts indicate statistically significant differences, $P < 0.01$).

To investigate long-term effects of bFGF on glucose uptake, cells were incubated with the peptide for 48 h. Table 1 shows 2-DOG uptake in rat Sertoli cells stimulated with bFGF (30 ng/ml), FSH (100 ng/ml) and a combination of both. A significant increase in glucose uptake in all experimental conditions was observed. The effect of prolonged treatment with both hormones was synergistic.

Table 1 Long-term effect of basic fibroblast growth factor (bFGF) on glucose uptake. Sertoli cells were incubated for 48 h in the absence or presence of 30 ng/ml bFGF, 100 ng/ml FSH or the combination of both hormones. Glucose uptake assay was performed on day 5. Results represent the means \pm S.D. of triplicate incubations in one representative experiment out of three

	2-DOG uptake (d.p.m./ μ g DNA)
Basal	718 \pm 64 ^a
bFGF (30 ng/ml)	1069 \pm 69 ^b
FSH (100 ng/ml)	1010 \pm 91 ^b
bFGF+FSH	2172 \pm 221 ^c

Different superscripts indicate statistically significant differences ($P < 0.01$).

Effect of bFGF on GLUT1 mRNA levels

Additional experiments to analyze the possible role of bFGF on the expression of GLUT1 were performed. Figure 3 shows that bFGF increased GLUT1 mRNA levels in a time- and dose-dependent manner. Indeed, Fig. 3A shows that bFGF increased GLUT1 mRNA levels after 12 h of stimulation and that this stimulation was

maximal at 24 h. Results presented in Fig. 3B show that the stimulatory effect of bFGF on GLUT1 mRNA levels in 24 h-treated cultures was detectable at concentrations equal to or higher than 10 ng/ml. Densitometric data from six individual experiments (30 ng/ml bFGF for 24 h) were normalized by log transformation and analyzed by Student's *t*-test. Pooled data revealed a significant ($P < 0.01$) 2.4 \pm 0.7-fold stimulation of GLUT1 mRNA levels.

Effect of bFGF on LDH activity

To further investigate biochemical steps that may be involved in the regulation of lactate production, experiments to analyze the possible role of bFGF on the regulation of LDH activity were designed. Figure 4 shows the results obtained for LDH activity in rat Sertoli cells stimulated for 72 h with different doses of bFGF. LDH activity increased in cells treated with doses equal to or higher than 10 ng/ml bFGF. A 1.42 \pm 0.11-fold stimulation (means \pm S.D., $n = 5$) was observed in pooled data from five different experiments with 30 ng/ml bFGF

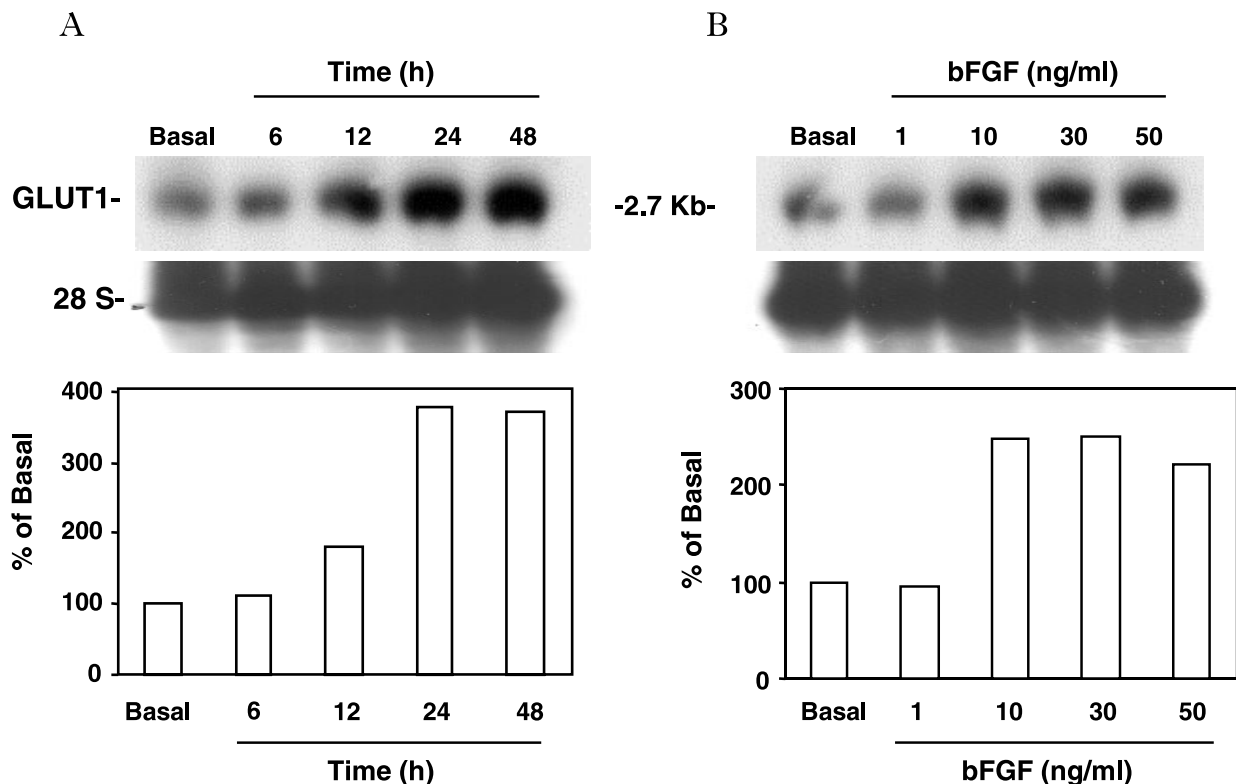


Figure 3 Effect of bFGF on glucose transporter 1 (GLUT1) mRNA levels in rat Sertoli cells. (A) Total RNA was extracted from Sertoli cells harvested on day 6 pre-incubated for different times (6, 12, 24 and 48 h) with bFGF (30 ng/ml). (B) Total cellular RNA was extracted from Sertoli cells harvested on day 6 pre-incubated for 24 h in the absence or presence of variable doses of bFGF (1, 10, 30 and 50 ng/ml). In the upper panels, representative Northern blots for GLUT1 are shown. In the lower panels, data yielded by scanning the autoradiographs and expressed as percentage of GLUT1 mRNA levels detected in basal cultures are shown. Blots correspond to a representative experiment out of two.

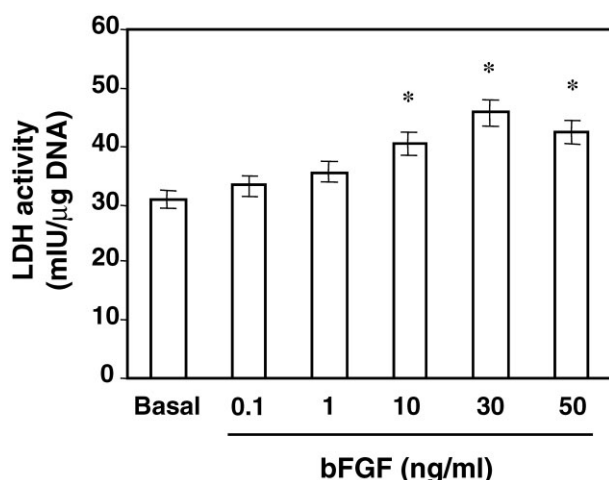


Figure 4 Effect of bFGF on lactate dehydrogenase (LDH) activity in cultured rat Sertoli cells. Sertoli cells were incubated for 72 h in the absence or presence of variable doses of bFGF (0.1, 1, 10, 30 and 50 ng/ml). LDH activity was determined on the cells harvested on day 6. Results represent the means \pm s.d. of triplicate incubations in a representative experiment out of three (* $P < 0.01$ vs basal).

performed in triplicate incubations. Cultures stimulated simultaneously with FSH (100 ng/ml) and bFGF (30 ng/ml) showed that the effect of both hormones on LDH activity was additive (basal: 30.9 ± 1.4^a ; bFGF: 47.7 ± 1.5^b ; FSH: 53.0 ± 5.2^b ; bFGF+FSH: 67.6 ± 5.8^c mIU/ μ g DNA; different superscripts indicate statistically significant differences, $P < 0.05$).

Effect of bFGF on LDH A mRNA levels

Finally, to characterize the action of bFGF on Sertoli cell LDH activity, LDH A mRNA levels were analyzed. Figure 5 shows that bFGF increased LDH A mRNA levels in a time- and dose-dependent manner. Figure 5A shows that bFGF (30 ng/ml) increased LDH A mRNA levels after 6 h of exposure and that maximal increments were obtained at 24 h. Figure 5B shows that doses equal to or higher than 10 ng/ml bFGF in 24 h-treated cultures increased LDH A mRNA levels. Densitometric data from six individual experiments (30 ng/ml bFGF for 24 h) were normalized by log transformation and analyzed by Student's *t*-test. Pooled data revealed a significant ($P < 0.01$) 2.1 \pm 0.6-fold stimulation of LDH A mRNA levels.

Discussion

Lactate, a glucose metabolite considered to be the preferred energy substrate for germ cells, is mainly produced in Sertoli cells to be consumed by germ cells. This

constitutes an interesting example of metabolic co-operation among the different cells present in an organ (Jutte *et al.* 1981). Interestingly, a similar metabolic co-operation between astrocytes and neurons has been demonstrated (Pellerin & Magistretti 1994).

As noted in the Introduction, FSH and a plethora of locally produced factors regulate Sertoli cell function. Several observations have been made on the regulation of lactate production by different locally produced factors. In this regard, EGF (Nehar *et al.* 1993), IL1 (Nehar *et al.* 1998) and TNF α in porcine Sertoli cells (Nehar *et al.* 1997) and insulin, insulin-like growth factor-I (Oonk *et al.* 1989), EGF (Mallea *et al.* 1986), PModS (Mullaney *et al.* 1994), FSH (Mita *et al.* 1982) and IL1 β (Riera *et al.* 2001) in rat Sertoli cells, act as regulators of lactate secretion. A distinct participation of these hormones on the stimulation of the different biochemical steps that will ultimately lead to increased lactate production has been observed. We have recently observed that not only the above-mentioned hormones but bFGF (Schteingart *et al.* 1999) regulate lactate production. However, the biochemical steps involved in this stimulation have not been previously analyzed.

As a consequence, the aim of the present study was to analyze the possible regulation by bFGF of those biochemical steps that may be involved in the stimulation of lactate secretion in rat Sertoli cells. The present investigation focused on two major steps: glucose transport and LDH activity.

Glucose transport through the plasma membrane is produced by a set of homologous glycoprotein molecules (GLUTs) that are expressed in a tissue-specific manner. GLUT1 is widely expressed in normal tissues and highly represented in erythrocytes and brain (Gould & Holman 1993). This glucose transporter is the only member of this family that has been unequivocally identified in rat Sertoli cells (Ulisse *et al.* 1992).

Several hormones – EGF, TNF α , IL1 and FSH – are involved in the regulation of glucose transport in Sertoli cells. The results presented herein demonstrate that bFGF also belongs to the set of factors with the ability to increase glucose transport in rat Sertoli cells. This peptide exerts short- and long-term effects on glucose transport. A rapid increase (30 min) in glucose transport with bFGF similar to that previously described for FSH and IL1 β was observed (Riera *et al.* 2001) and suggests that these hormones regulate glucose transporter activation or translocation to the membrane. The lack of effect of the protein synthesis inhibitor CHX on short-term bFGF stimulation of glucose uptake supports the latter hypothesis.

In rat Sertoli cells, an increase of glucose transport with L-tri-iodothyronine was observed and this increase was correlated with an increase in GLUT1 mRNA levels (Ulisse *et al.* 1992). The question arose as to whether the observed long-term effects of bFGF could be accounted for by the ability of this hormone to increase the synthesis of

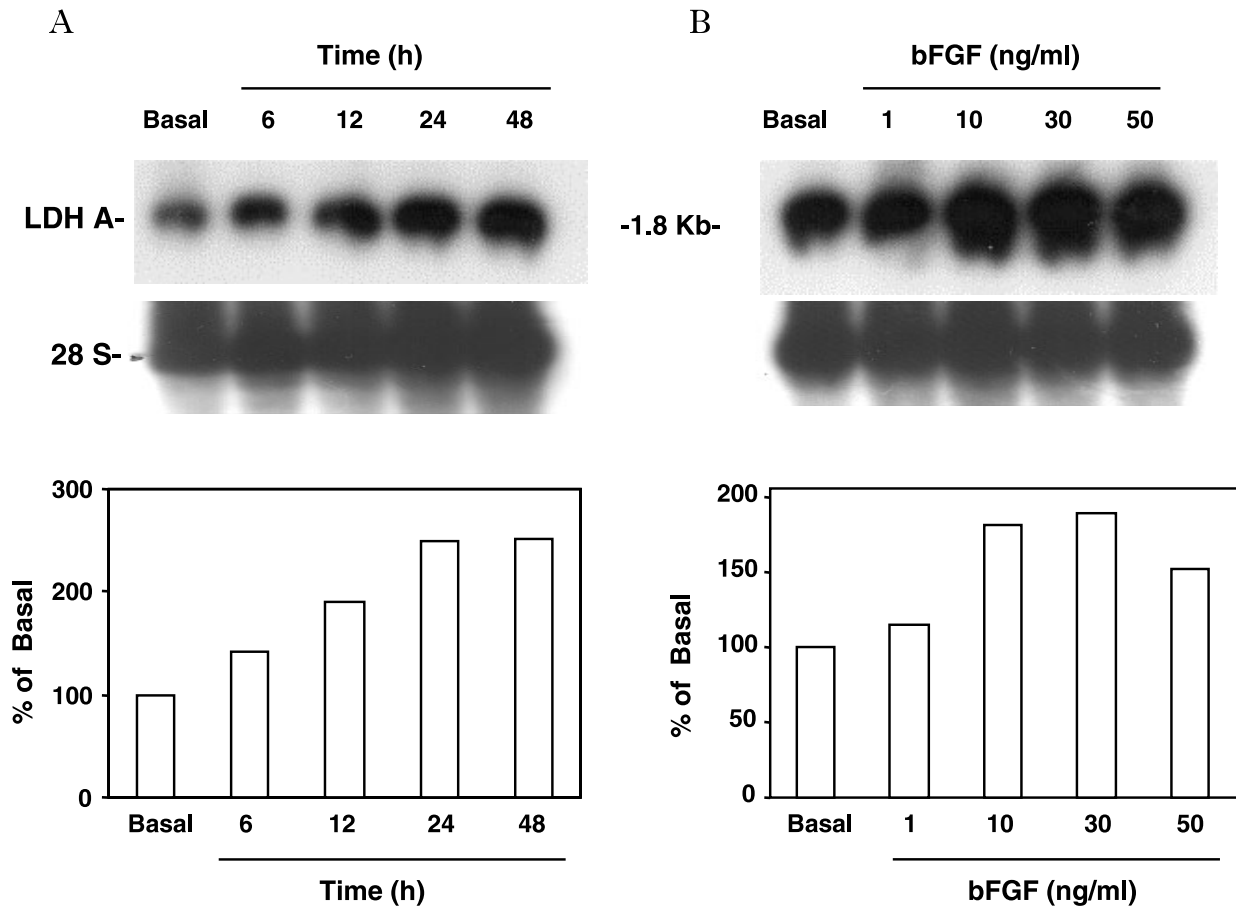


Figure 5 Effect of bFGF on LDH A mRNA levels in rat Sertoli cells. (A) Total RNA was extracted from Sertoli cells harvested on day 6 pre-incubated for different times (6, 12, 24 and 48 h) with bFGF (30 ng/ml). (B) Total cellular RNA was extracted from Sertoli cells harvested on day 6 pre-incubated for 24 h in the absence or presence of variable doses of bFGF (1, 10, 30 and 50 ng/ml). In the upper panels, representative Northern blots for LDH A are shown. In the lower panels, data yielded by scanning the autoradiographs and expressed as percentage of LDH A mRNA levels detected in basal cultures are shown. Blots correspond to a representative experiment out of two.

new glucose transporter units. To investigate this hypothesis, the mRNA levels of the glucose transporter present in rat Sertoli cells – GLUT1 – was investigated. It was demonstrated that after a 12-h treatment with bFGF, GLUT1 mRNA levels increase. This result suggested that increased synthesis *de novo* of glucose transporter units may be responsible for the increment in glucose uptake observed in Sertoli cells treated for 48 h with bFGF.

The LDH isozymes are encoded by three different genes, *ldh a* (muscle type), *ldh b* (heart type) and *ldh c* (testis type) (Li *et al.* 1989). The latter gives rise to LDH C4 isozyme present only in the mature testis, specifically in spermatozoa (Markert *et al.* 1975). The combinations of the other two gene products result in five tetrameric LDH isozymes, LDH-1 (B4), LDH-2 (A1B3), LDH-3 (A2B2), LDH-4 (A3B1) and LDH-5 (A4) that are present in variable proportions in different somatic tissues including rat Sertoli cells (Santemma *et al.* 1987). LDH-1 (heart) has

a lower K_m for pyruvate but it is inhibited by high concentrations – like those existing in Sertoli cells – of this substrate. On the other hand, LDH-5 (muscle) has a K_m one order of magnitude higher than LDH-1 and can convert high amounts of pyruvate to lactate (Pesce *et al.* 1961). Thus, an increment of the latter isozyme may be responsible for the increased lactate production observed. In rat Sertoli cells, variations in LDH activity and in the distribution profile of isozymes are accompanied by an increment in *ldh a* gene expression after FSH and IL1 β treatments (Riera *et al.* 2001). On the other hand, similar results were obtained in porcine Sertoli cells after EGF (Boussouar & Benahmed 1999), IL1 α (Nehar *et al.* 1998) and TNF α treatments (Nehar *et al.* 1997). The present study shows that bFGF increases LDH activity and the levels of mRNA for the LDH A subunit, suggesting that the LDH isoenzyme system is another potential site of action for bFGF. Increments in LDH A mRNA levels

may be related to increased transcription of *ldh a* gene. However, considering that Huang *et al.* (1995) have shown that pKc – a signal transduction pathway that may be utilized by bFGF – plays an active functional role in regulating LDH A mRNA stability in rat C6 glioma cells, an mRNA stabilizing mechanism cannot be ruled out. Thus, the possibility exists that, in rat Sertoli cells, bFGF controls the expression of the LDH subunit A at a transcriptional and/or post-transcriptional level.

Additive or synergistic effects of bFGF and FSH treatments on lactate production, glucose transport and LDH activity were observed. A similar synergistic effect of both hormones has already been observed for transferrin secretion in rat Sertoli cells (Schteingart *et al.* 1999) and for plasminogen activator secretion in porcine Sertoli cell cultures (Jaillard *et al.* 1987). bFGF actions are mediated through specific receptors belonging to the transmembrane tyrosine kinase family. In rat Sertoli cells, gene expression of FGF-R-1 is upregulated by FSH as well as by dbcAMP (Le Magueresse-Battistoni *et al.* 1994). On the other hand, Jaillard *et al.* (1987) have shown that bFGF treatment increases the number of FSH receptors. The observed synergistic effects of bFGF and FSH on lactate production and glucose uptake after long-term treatments and the additive effects on LDH activity might reflect the combined effects of these peptides on their receptors. Alternatively, the additive effect observed for glucose uptake in short-term treatment might be the reflection of different signal transduction pathways that interact positively.

As for lactate production at different moments of sexual maturation, the work done by Mullaney *et al.* (1994) shows that while a 4-fold increase in lactate secretion occurs between 10- and 20-day-old Sertoli cell cultures, only a small increase is observed between 20- and 35-day-old Sertoli cell cultures, the age at which spermatogenesis has advanced to the mid-stage of spermatid differentiation. These results suggest that lactate production increases as the Sertoli cell differentiates during pubertal development and that, by 20 days of age, Sertoli cells are able to produce enough lactate to sustain germ cell development.

Several Sertoli cell functions and their regulation by FSH vary with the stage of the spermatogenic cycle. Minimal binding of FSH and FSH-stimulated cAMP production occurs at stages VI to VIII (Kangasniemi *et al.* 1990). In addition, expression of bFGF – one germ cell product that may exert regulatory functions on Sertoli cells – varies with the different stages of the cycle. Mayerhofer *et al.* (1991) analyzed the presence of bFGF in adult rodent testis and found that the peptide is localized predominantly in the cytoplasm of pachytene spermatocytes and that the staining becomes more prominent in stage VII through IX of the cycle. Stage-specific lactate secretion may not be expected as this metabolic product has been shown to be important for the survival of both spermatocytes and spermatids that are present along all stages of the cycle.

Based on the above-mentioned observations on FSH responsiveness and on bFGF distribution in adult testis, which suggest a differential action of these hormones at stages VII and VIII of spermatogenesis, it is tempting to speculate that the action of bFGF on lactate production may be particularly important at stages VII and VIII, which show decreased responsiveness to FSH, in order to maintain the energy requirements of germ cells.

In summary, in rat Sertoli cell cultures, bFGF increases lactate production utilizing at least the following regulatory steps: (a) an increase in glucose uptake probably through the activation or translocation of pre-existing GLUT1 units at the plasma membrane, (b) an increase in glucose uptake mediated by increased number of GLUT1 units secondary to increased levels of GLUT1 mRNA and (c) an increase in LDH activity which is probably associated with increased levels of LDH 5 secondary to increased LDH A mRNA levels.

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References

- Boussouar F & Benahmed M 1999 Epidermal growth factor regulates glucose metabolism through lactate dehydrogenase A messenger ribonucleic acid expression in cultured porcine Sertoli cells. *Biology of Reproduction* **61** 1139–1145.
- Courtens JL & Plöen L 1999 Improvement of spermatogenesis in adult cryptorchid rat testis by intratesticular infusion of lactate. *Biology of Reproduction* **61** 154–161.
- Chomczynsky P & Sacchi N 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Analytical Biochemistry* **162** 156–159.
- Gnessi L, Fabbri A & Spera G 1997 Gonadal peptides as mediators of development and functional control of the testis: an integrated system with hormones and environment. *Endocrine Reviews* **18** 541–609.
- Gould GW & Holman GD 1993 The glucose transporter family: structure, function and tissue-specific expression. *Biochemical Journal* **295** 329–341.
- Grootegoed JA, Jansen R & van der Molen HJ 1986a Effects of glucose on ATP dephosphorylation in rat spermatids. *Journal of Reproduction and Fertility* **77** 99–107.

- Grootegoed JA, Oonk RB, Jansen R & van der Molen HJ 1986b Metabolism of radiolabelled energy-yielding substrates by rat Sertoli cells. *Journal of Reproduction and Fertility* **77** 109–118.
- Han IS, Sylvester SR, Kim KH, Schelling ME, Venkateswaran S, Blanckaert VD, McGuinness MP & Griswold MD 1993 Basic fibroblast growth factor is a testicular germ cell product which may regulate Sertoli cell function. *Molecular Endocrinology* **7** 889–897.
- Huang D, Hubbard CJ & Jungmann RA 1995 Lactate dehydrogenase A subunit messenger RNA stability is synergistically regulated via the protein kinase A and C signal transduction pathways. *Molecular Endocrinology* **9** 994–1004.
- Jaillard C, Chatelain PG & Sáez JM 1987 *In vitro* regulation of pig Sertoli cell growth and function: effects of fibroblast growth factor and somatomedin-C. *Biology of Reproduction* **37** 665–674.
- Jutte NH, Grootegoed JA, Rommerts FFG & van der Molen HJ 1981 Exogenous lactate is essential for metabolic activities in isolated rat spermatocytes and spermatids. *Journal of Reproduction and Fertility* **62** 399–405.
- Kangasniemi M, Kaipia A, Mali P, Toppari J, Huhtaniemi I & Parvinen M 1990 Modulation of basal and FSH-stimulated cyclic AMP production in rat seminiferous tubules staged by an improved transillumination technique. *Anatomical Record* **227** 62–76.
- Labarca C & Paigen K 1980 A simple, rapid and sensitive DNA assay procedure. *Analytical Biochemistry* **102** 344–352.
- Le Magueresse-Battistoni B, Wolff J, Morera AM & Benahmed M 1994 Fibroblast growth factor receptor type 1 expression during rat testicular development and its regulation in cultured Sertoli cells. *Endocrinology* **135** 2404–2411.
- Le Magueresse-Battistoni B, Pernod G, Sigillo F, Kolodíe L & Benahmed M 1998 Plasminogen activator inhibitor-1 is expressed in cultured rat Sertoli cells. *Biology of Reproduction* **59** 591–598.
- Li SSL, O'Brien D, Hou EW, Versola J, Rockett DL & Eddy EM 1989 Differential activity and synthesis of lactate dehydrogenase isozymes A (muscle), B (heart), and C (testis) in mouse spermatogenic cells. *Biology of Reproduction* **40** 173–180.
- Mallea LE, Machado AJ, Navaroli F & Rommerts FFG 1986 Epidermal growth factor stimulates lactate production and inhibits aromatization in cultured Sertoli cells from immature rats. *International Journal of Andrology* **9** 201–208.
- Markert CL, Shakelee JB & Whitt GS 1975 Evolution of a gene. *Science* **189** 102–114.
- Mayerhoffer A, Russell L, Grothe C, Rudolf M & Gratzl M 1991 Presence and localization of a 30-kDa basic fibroblast growth factor-like protein in rodent testes. *Endocrinology* **129** 921–924.
- Mita M & Hall PF 1982 Metabolism of round spermatids from rats: lactate as the preferred substrate. *Biology of Reproduction* **26** 445–455.
- Mita M, Price JM & Hall PF 1982 Stimulation by follicle-stimulating hormone of synthesis of lactate by Sertoli cells from rat testis. *Endocrinology* **110** 1535–1541.
- Mruk DD & Cheng CY 1999 Sertolin is a novel gene marker of cell–cell interactions in the rat testis. *Journal of Biological Chemistry* **274** 27056–27058.
- Mullaney BP & Skinner MK 1992 Basic fibroblast growth factor (bFGF) gene expression and protein production during pubertal development of seminiferous tubule: follicle-stimulating hormone-induced Sertoli cell bFGF expression. *Endocrinology* **131** 2928–2934.
- Mullaney BP, Rosselli M & Skinner MK 1994 Developmental regulation of Sertoli cell lactate production by hormones and the testicular paracrine factor, PModS. *Molecular and Cellular Endocrinology* **104** 67–73.
- Nehar D, Mauduit C, Revol A, Morera AM & Benahmed M 1993 Effect of epidermal growth factor/transforming growth factor α on lactate production in porcine Sertoli cells: glucose transport and lactate dehydrogenase isozymes as potential sites of action. *Molecular and Cellular Endocrinology* **92** 45–53.
- Nehar D, Mauduit C, Boussouar F & Benahmed M 1997 Tumor necrosis factor- α -stimulated lactate production is linked to lactate dehydrogenase A expression and activity increase in porcine cultured Sertoli cells. *Endocrinology* **138** 1964–1971.
- Nehar D, Mauduit C, Boussouar F & Benahmed M 1998 Interleukin 1- α stimulates lactate dehydrogenase A expression and lactate production in cultured porcine Sertoli cells. *Biology of Reproduction* **59** 1425–1432.
- Oonk RB, Jansen R & Grootegoed JA 1989 Differential effects of follicle-stimulating hormone, insulin and insulin-like growth factor I on hexose uptake and lactate production by rat Sertoli cells. *Journal of Cellular Physiology* **139** 210–218.
- Parvinen M 1982 Regulation of the seminiferous epithelium. *Endocrine Reviews* **3** 404–417.
- Pellerin L & Magistretti PJ 1994 Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *PNAS* **91** 10625–10629.
- Pesce A, McKay RH, Stolzenbach F, Cahn RD & Kaplan NO 1961 The comparative enzymology of lactic dehydrogenases. *Journal of Biological Chemistry* **239** 1753–1761.
- Plagemann PGW, Gregory KF & Wróblewski F 1960 The electrophoretically distinct forms of mammalian lactic dehydrogenase. *Journal of Biological Chemistry* **235** 2288–2293.
- Riera MF, Meroni SB, Gomez GE, Scheingart HF, Pellizzari EH & Cigorraga SB 2001 Regulation of lactate production by FSH, IL1 β and TNF α in rat Sertoli cells. *General and Comparative Endocrinology* **122** 88–97.
- Robinson R & Fritz I 1981 Metabolism of glucose by Sertoli cells in culture. *Biology of Reproduction* **24** 1032–1041.
- Santemma V, Salfi V, Casasanta N & Fabbrini A 1987 Lactate dehydrogenase and malate dehydrogenase of Sertoli cells in rats. *Archives of Andrology* **19** 49–64.
- Scheingart HF, Rivarola MA & Cigorraga SB 1989 Hormonal and paracrine regulation of γ -glutamyl transpeptidase in rat Sertoli cell. *Molecular and Cellular Endocrinology* **67** 73–80.
- Scheingart HF, Meroni SB, Canepa DF, Pellizzari EH & Cigorraga SB 1999 Effects of basic fibroblast growth factor and nerve growth factor on lactate production, γ -glutamyl transpeptidase and aromatase activities in cultured Sertoli cells. *European Journal of Endocrinology* **141** 539–545.
- Smith EP, Hall SH, Monaco L, French FS, Wilson EM & Conti M 1989 A rat Sertoli cell factor similar to basic fibroblast growth factor increased c-fos messenger ribonucleic acid in cultured Sertoli cells. *Molecular Endocrinology* **3** 954–961.
- Ueno N, Baird A, Esch F, Ling N & Guillemin R 1987 Isolation and partial characterization of bFGF from bovine testis. *Molecular and Cellular Endocrinology* **49** 189–194.
- Ulisse S, Jannini EA, Pepe M, De Matteis S & D'Armiento M 1992 Thyroid hormone stimulates glucose transport and GLUT1 mRNA in rat Sertoli cells. *Molecular and Cellular Endocrinology* **87** 131–137.
- Wagle JR, Heindel JJ, Steinberger A & Sanborn BM 1986 Effect of hypotonic treatment on Sertoli cell purity and function in culture. *In Vitro Cellular and Developmental Biology* **22** 325–331.

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