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Deficiency of fibroblast growth factor 2 (FGF-2) leads to abnormal spermatogenesis and altered sperm physiology

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In previous studies, we described the presence of fibroblast growth factor 2 (FGF-2) and its receptors (FGFRs) in human testis and sperm, which are involved in spermatogenesis and in motility regulation. The aim of the present study was to analyze the role of FGF-2 in the maintenance of sperm physiology using FGF-2 knockout (KO) mice. Our results showed that in wild-type (WT) animals, FGF-2 is expressed in germ cells of the seminiferous epithelium, in epithelial cells of the epididymis, and in the flagellum and acrosomal region of epididymal sperm. In the FGF-2 KO mice, we found alterations in spermatogenesis kinetics, higher numbers of spermatids per testis, and enhanced daily sperm production compared with the WT males. No difference in the percentage of sperm motility was detected, but a significant increase in sperm concentration and in sperm head abnormalities was observed in FGF-2 KO animals. Sperm from KO mice depicted reduced phosphorylation on tyrosine residues (a phenomenon that was associated with sperm capacitation) and increased acrosomal loss after incubation under capacitating conditions. However, the FGF-2 KO males displayed no apparent fertility defects, since their mating with WT females showed no differences in the time to delivery, litter size, and pup weight in comparison with WT males. Overall, our findings suggest that FGF-2 exerts a role in mammalian spermatogenesis and that the lack of FGF-2 leads to dysregulated sperm production and altered sperm morphology and function. FGF-2-deficient mice constitute a model for the study of the complex mechanisms underlying mammalian spermatogenesis.

KEYWORDS
FGF-2 knockout (FGF-2 KO), fibroblast growth factor 2 (FGF-2), sperm, spermatogenesis

1 | INTRODUCTION

Fibroblast growth factor 2 (FGF-2) is the best-characterized member of the large family of fibroblast growth factors (FGFs; Itoh & Ornitz, 2011; Yu, Ferrari, Galloway, Mignatti, & Pintucci, 2007). This protein is translated by alternative initiation codons, giving rise to a low-molecular-weight (LMW) isoform of 18 kDa and 2–3 high-molecular-weight (HMW) isoforms (of 21 and 23 kDa in the mouse; Liao et al., 2009). FGFs bind to specific receptors (FGFRs) with tyrosine kinase activity, known as FGFR1, FGFR2, FGFR3, and FGFR4. The transcripts coding FGFR1, FGFR2, and FGFR3 are subjected to alternative splicing in the extracellular domain 3, generating different receptor isoforms (b or c) that confer FGF ligand specificity and are expressed in a tissue-specific fashion (Gong, 2014). Moreover, another FGFR (FGFR-like 1 or FGFR5), which lacks the intracellular kinase domain, has also been reported

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(Trueb, 2011). The interaction of FGF-2 with heparin or heparan sulfate proteoglycans allows their binding to FGFRs, triggering receptor dimerization and phosphorylation (Mohammadi, Olsen, & Ibrahimi, 2005). Activation of FGFRs leads to the stimulation of several intracellular transduction cascades, including the extracellular signal-regulated kinase, as well as the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB or Akt) signaling pathways. In somatic cells, components of these signal transduction cascades translocate to the nucleus, and phosphorylate specific transcription factors and induce the expression of FGF target genes (Eswarakumar, Lax, & Schlessinger, 2005).

The presence of FGFs and FGFRs has been reported in multiple tissues (Fon Tacer et al., 2010; Hughes, 1997), and they have been involved in several cellular processes, including proliferation, differentiation, adhesion, survival, apoptosis, motility, and chemotaxis. It has been described that this system has a role not only in the development and maintenance of normal tissues, but also in tumor progression (Belov & Mohammadi, 2013; Ornitz & Itoh, 2015; Turner & Grose, 2010). Components of the FGFs/FGFRs pathway have been found in both the female and male reproductive tracts from a variety of species, where they contribute to regulate the reproductive function (Chaves, de Matos, Buratini, & de Figueiredo, 2012; Jiang et al., 2013; Cotton, O'Bryan, & Hinton, 2008; Price, 2016). The ligand, FGF-2, has been reported in mammalian germ cells (Lahr et al., 1992; Mayerhofer, Russell, Grothe, Rudolf, & Gratzl, 1991; Steger, Tetens, Seitz, Grothe, & Bergmann, 1998); however, its role in the maintenance of sperm physiology remains unclear.

Sperm cells are produced in the seminiferous tubules of the testis by a complex process called spermatogenesis, with a last stage of differentiation or spermiogenesis. In mammals, testicular sperm are not competent to fertilize, and they need to undergo a series of changes, known as maturation and capacitation. Sperm maturation takes place in the epididymis and allows the development of sperm motility. Capacitation occurs after ejaculation, during sperm transit through the female reproductive tract. Capacitated sperm witness an increase in protein tyrosine phosphorylation, are able to respond to chemotactic stimuli, develop hyperactivated motility, and suffer the loss of the acrosomal content or acrosomal exocytosis. Acrosome-reacted sperm penetrate the oocyte vestments, reach the perivitelline space, and finally fuse with the oocyte plasma membrane (Vazquez-Levin & Marín-Briggiler, 2009; Wassarman, Jovine, & Litscher, 2001; Yanagimachi, 1994).

In previous studies, we have shown the presence of FGF-2 and FGFRs in human testis and sperm, and results suggest that this system is involved in the regulation of human spermatogenesis and sperm motility (Garbarino Azúa et al., 2017; Saucedo et al., 2015). In the present study, we aimed to further explore the relevance of FGF-2 in the maintenance of mammalian sperm physiology using FGF-2-deficient animals. Mice lacking FGF-2 have impaired cerebral cortex development and depict other alterations in the nervous system (Dono, Texido, Dussel, Ehmke, & Zeller, 1998; Korada, Zheng, Basilico, Schwartz, & Vaccarino, 2002; Ratzka, Baron, & Grothe, 2011; Rumpel et al., 2016; Timmer et al., 2007). These animals also show decreased blood pressure, delayed wound healing, and alterations in the heart development and infarct repair (Dono et al., 1998; Virag et al., 2007); however, there are no detailed studies on either their reproductive tissues or their sperm quality.

2 | MATERIALS AND METHODS

2.1 | Reagents and antibodies

All reagents were of tissue culture grade and molecular biology quality, purchased from Sigma Chemical Co. (St. Louis, MO), GE-Amersham Pharmacia (Piscataway, NJ), Thermo-Life Technologies (Carlsbad, CA), and Qiagen (Hilden, Germany), unless indicated.

Polyclonal anti-FGF-2 (sc-79; Santa Cruz Biotechnology Inc., Santa Cruz, CA) was used. Other antibodies used were rabbit immunoglobulin G (IgG; Sigma), anti-Ptyr (clone 4G10; Upstate Biotechnology, Lake Placid, NY), anti-tubulin (Sigma), anti-rabbit IgG conjugated with horseradish peroxidase (HRP; Sigma) and with Alexa Fluor® 488 (A-11008; Life Technologies), and anti-mouse IgG conjugated with HRP (Vector Laboratories, Inc., Burlingame, CA). Human recombinant FGF-2 (rFGF-2) was purchased from Peprotech (Hamburg, Germany).

2.2 | Animals

The FGF-2-deficient mouse strain (B6.Cg-FGF-$2^{tm1Zllr}$) was maintained on C57Bl6/J background (Dono et al., 1998). This mutation replaces the first exon of FGF-2 with a neomycin expression cassette, thereby CUG and AUG start-codons of HMW and LMW FGF-2 isoforms were removed. The homozygous FGF-$2^{-/-}$ (KO) mice and their wild-type (WT) FGF-$2^{+/+}$ littermates were obtained by cross-breeding of FGF-2 heterozygous FGF-$2^{+/-}$ animals. Genotypes were determined by polymerase chain reaction of the ear DNA, as described previously (Ratzka et al., 2011). Adult mice (2–6 months old) were used. The animals were housed in a room with controlled temperature (22 ± 2°C), ventilation, and lighting (14:10 hr light:dark cycle) and were provided ad libitum access to food and water. All experimental protocols followed the German animal protection act and were approved by the local authorities (Lower Saxony State Office for Consumer Protection and Food Safety LAVES Oldenburg, Germany, Approval number 33.12-42502-04-16/2296).

2.3 | Body and testis weight

Animals were killed by cervical dislocation, and the body weight was recorded immediately before the killing. Both left and right testes were excised and weighed after the removal of adipose tissues, and the mean testis weight was determined. The testis/body weight ratio was also calculated.

2.4 | Tissue and sperm collection

Testicular and epididymal tissues were removed and were immediately used or stored at −80°C for RNA and protein extraction. To

recover sperm cells, both *cauda* epididymides from a mouse were collected, dissected, and placed in 400 µl of culture medium (Fraser & Drury, 1975) for 10 min to allow the release of motile cells (swim-out procedure). When indicated, sperm (at a concentration of 1–10 × 10^6/ml) were resuspended in a medium supplemented with 0.3% bovine serum albumin and incubated for 90 min at 37°C in an atmosphere of 5% CO_2 to promote sperm capacitation. For certain experiments, sperm were also recovered from the *caput* and *corpus* sections of the epididymis, using the swim-out procedure as described.

2.5 | RNA analysis

Total RNA from testis and epididymis was isolated using the Trizol™ reagent (Thermo-Life) following the protocol suggested by the manufacturer. Complementary DNA (cDNA) was synthesized and subjected to a standard end-point PCR followed by electrophoresis in 3% agarose gel. The following primers were used: for FGF-2, forward 5′-GAGAAGAGC GACCCACACG-3′ and reverse 5′-GGCACACACTCCCTTGATAGA-3′; and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward 5′-GAACATCATCCCTGCATCCA-3′ and reverse 5′-CCAGTGAGCTT CCCGTTCA-3′. The expected sizes of the amplified PCR fragments were 78 bp (base pairs) and 77 bp for FGF-2 and GAPDH, respectively. All samples were analyzed in triplicates; negative controls omitting the reverse transcriptase (RT control) were included.

To determine the expression levels of FGFs and FGFRs in the FGF-2 KO animals, quantitative PCR (qPCR) was done. Total RNA from testis of WT or FGF-2 KO animals was prepared using RNeasy Plus kit (Qiagen), according to manufacturer's protocol. Corresponding cDNA was prepared using the iScript™ cDNA Synthesis kit (Bio-Rad, Hercules, CA) and purified using a PCR-purification kit (Qiagen). The concentration of the obtained cDNA was measured on an Epoch plate reader (BioTek, Winooski, VT), and 10 ng cDNA per 15 µl reaction volume (in triplicates) was used for the subsequent qPCR analysis on a StepOnePlus qPCR system (Applied Biosystems, Foster City, CA). The primer sequences used are listed in Supporting Information Table S1. For PCR reactions, Power SYBR® Green PCR Master Mix (Applied Biosystems) was used. The relative expression of the selected FGF and FGFR family members in FGF-2 KO versus WT animals was calculated by the ΔΔCt-method using GAPDH as the reference gene.

2.6 | Protein extracts, sodium dodecyl sulfate polyacrylamide gel electrophoresis, and western immunoblotting

Protein extracts from testicular and epididymal tissues, as well as from sperm cells of WT animals, were obtained as described (Marín-Briggiler et al., 2008). Protein concentration was determined using the bicinchoninic acid assay. Extracted proteins were placed in the Laemmli sample buffer containing 5% 2-mercaptoethanol, boiled for 10 min, and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in 15% polyacrylamide gels and western immunoblotting. The anti-FGF-2 antibody or rabbit IgG was used at 2 µg/ml. To evaluate the phosphorylation on tyrosine residues, sperm from WT and KO animals were either used immediately after the swim-out or incubated under capacitating conditions for 90 min. Sperm proteins were extracted, subjected to SDS-PAGE in 10% polyacrylamide gels, and developed with anti-phosphotyrosine (pTyr) or anti-tubulin and anti-mouse HRP antibody (Marín-Briggiler et al., 1999). The reactive bands were detected by enhanced chemiluminescence (ECL kit; GE-Amersham Pharmacia) using standard procedures.

2.7 | Immunohistochemistry and immunocytochemistry

Testis and epididymis from adult WT and FGF-2 KO males (2 months old) were fixed and processed as previously described (Marín-Briggiler et al., 2008), using anti-FGF-2 antibody or rabbit IgG (1 µg/ml) and the LSAB + System HRP kit (K0690; Dako, Carpinteria, CA). Specimens were counterstained with hematoxylin, dehydrated, and mounted. Sections were evaluated at 600× magnification using an Eclipse E800 microscope (Nikon Instruments Inc., Tokyo, Japan).

After the swim-out procedure, *cauda* epididymal sperm from WT and KO animals were fixed with formaldehyde (2% in phosphate buffered saline) for 4 min and processed for staining with anti-FGF-2 antibody (20 µg/ml), or rabbit IgG as control, and Alexa Fluor® 488-conjugated secondary antibody. Nuclei were stained with propidium iodide, and slides were mounted with Vectashield (Vector Laboratories Inc., Burlingame, CA). Images were acquired with a Nikon laser confocal microscope C1, using an objective PlanApo 60×/1.40 oil, excitation/emission: 488 nm/515–530 nm and 544 nm/570 LP.

2.8 | Spermatogenesis analysis

Testis sections from WT and FGF-2 KO males were processed for immunohistochemistry and stained with hematoxylin and eosin following standard procedures. All seminiferous tubules present in each section were analyzed, and the stage of each tubule was assessed (Russell, Ettlin, Sinha, & Clegg, 1990). The percentage of tubules in each stage was determined for both the WT and KO animals.

Daily sperm production (DSP) assesses the number of spermatids produced in the testes per day and provides a measure of the male reproductive function (Johnson, Petty, & Neaves, 1980). DSP was determined as previously described (Kyjovska et al., 2013), with slight modifications. Testes were obtained during necropsy and frozen at −80°C until analysis. One testis was weighed, decapsulated, and placed in 1 ml of 0.05% Triton X-100 in 0.9% NaCl. The testis parenchyma was homogenized using an IKA ULTRA TURRAX® T25 at 13 000 rpm for 3 min. Samples were left on ice for 30 min, and a 200-µl aliquot was incubated with an equal volume of 0.08% Trypan blue (Sigma) in phosphate buffer for 5 min. Spermatids, which are

homogenization resistant, were counted using a Neubauer hemocytometer under light microscopy at 400× magnification. Samples were counted in two replicates and averaged. The number of spermatids per testis weight (SC/g) was calculated by multiplying the number of cells counted by the dilution factor. The DSP was calculated as the total number of elongated spermatids per testis divided by 4.84, which is the time in days for spermatids to develop through stages 14–16 (Kyjovska et al., 2013).

2.9 | Sperm analysis

Cauda epididymal sperm motility (progressive + nonprogressive sperm) was assessed by a microscopic observation of a 20-μm wet preparation, and sperm concentration was determined using a Neubauer hemocytometer. To assess sperm morphology, a 10-μl aliquot of the sperm suspension was smeared over a microscope slide. After drying in air, the smear was fixed with methanol for 5 min, washed with distilled water, stained with Harris hematoxylin for 15 min, and finally washed with tap water. Sperm morphology was evaluated using a Nikon microscope (Nikon Instruments Inc., Melville, NY) at 1000× magnification.

The sperm acrosomal status was determined in cells immediately after the swim-out (spontaneous, Spt T0) or after incubation under capacitating conditions (Spt T90). Capacitated sperm were also exposed to progesterone (30 μM), a physiological inducer of the acrosomal exocytosis, during the last 15 min of incubation (Prg T90). Cells were subjected to Coomassie brilliant blue staining as previously described (Busso, Goldweic, Hayashi, Kasahara, & Cuasnicú, 2007) and were analyzed under 400× magnification. Sperm were scored as acrosome intact when a bright blue staining was observed in the dorsal region of the head and as acrosome reacted when no labeling was observed in this region. To determine the effect of incubation with rFGF-2 on sperm acrosomal loss, cells were incubated under capacitating conditions in the presence of rFGF-2 (10 or 100 ng/ml) and processed as indicated above. In all cases, at least 200 sperm cells were evaluated.

2.10 | Sperm ultrastructural analysis

This analysis was performed not only on *cauda* sperm from WT and FGF-2 KO animals, recovered by the swim-out procedure, but also on sperm present in the *caput* and *cauda* epididymal lumen. Sperm or epididymal specimens were fixed with 2.5% glutaraldehyde (Polysciences, Warrington, PA) in 0.1 M sodium cacodylate at pH 7.3 (Th. Geyer, Hamburg, Germany) overnight at room temperature. Then, they were postfixed with 2% osmium tetroxide (Polysciences) in 0.1 M sodium cacodylate, dehydrated with a graded series of ethanol, and embedded in epoxy resin (Serva, Heidelberg, Germany). Ultrathin sections were stained with 2% uranyl acetate (Serva) and lead citrate (Serva). The micrographs were obtained using a transmission electron microscope (Tecnai G2 200 kV FEI, Eindhoven, The Netherlands) and were processed with Adobe Photoshop CS6.

2.11 | Fertility analysis

Female mice were individually housed and mated overnight with a WT or FGF-2 KO male. In one set of experiments, males were mated with females in their natural cycle. Females were checked for the presence of vaginal plug as evidence of successful mating and separated from the male. The time to delivery (from the beginning of mating), litter size, and pup weight at birth were determined. Each male was mated with two females, and the mean values of both matings were calculated. In another set of experiments, males were mated with hormone-stimulated females to challenge sperm against a higher number of oocytes. Adult WT females were treated with an intraperitoneal (i.p.) injection of equine chorionic gonadotropin (5 IU; Sigma), followed by an i.p. injection of human chorionic gonadotrophin (5 IU; Sigma) 48 hr later. Each female was caged with a WT or a FGF-2 KO male. The next morning, the presence of vaginal plug was checked, females were killed, and eggs were recovered from the oviducts. Eggs were cultured for 24 hr, and the fertilization rate was calculated as the percentage of two-cell stage embryos obtained.

2.12 | Statistical analysis

Data were expressed as mean ± standard error of the mean (*SEM*). To assume normal distribution, percentages were expressed as ratios and subjected to the arcsine square root transformation. Results were compared using the unpaired Student *t* test or one-way analysis of variance and the Dunnett's multiple comparison test. Statistical analyses were carried out using the GraphPad InStat program (GraphPad Software, San Diego, CA). Differences were considered significant at a level of $P < 0.05$.

3 | RESULTS

3.1 | FGF-2 expression and localization in the testis, epididymis, and sperm

The expression of FGF-2 messenger RNA in the murine testis and epididymis was evaluated by RT-PCR. The analysis revealed the presence of the FGF-2 transcript in the WT animals and its absence in tissues of the FGF-2 KO mice (Figure 1a). The expression of FGF-2 protein in WT reproductive tissues and sperm was assessed by western immunoblotting using a polyclonal anti-FGF-2 antibody. Bands of 18, 21, and 23 kDa were observed in testis, epididymis, and sperm protein extracts, (Figure 1b). Immunohistochemical studies showed FGF-2 localization in the interstitial cells of the testis, and in Sertoli cells and germ cells of the seminiferous epithelium (specifically in spermatogonia, preleptotene, leptotene, and zygotene spermatocytes, and elongating/elongated spermatids; Figure 1c). A strong immunoreactivity on the spermatid flagellum at stages I–VIII of the spermatogenic cycle was observed. FGF-2 was detected in the epithelium of all epididymal segments, with higher intensity in the epithelial cells of *corpus* and *cauda* (Figure 1c). This protein was also immunolocalized in the sperm acrosomal region and flagellum, mainly in the middle piece (Figure 1d).

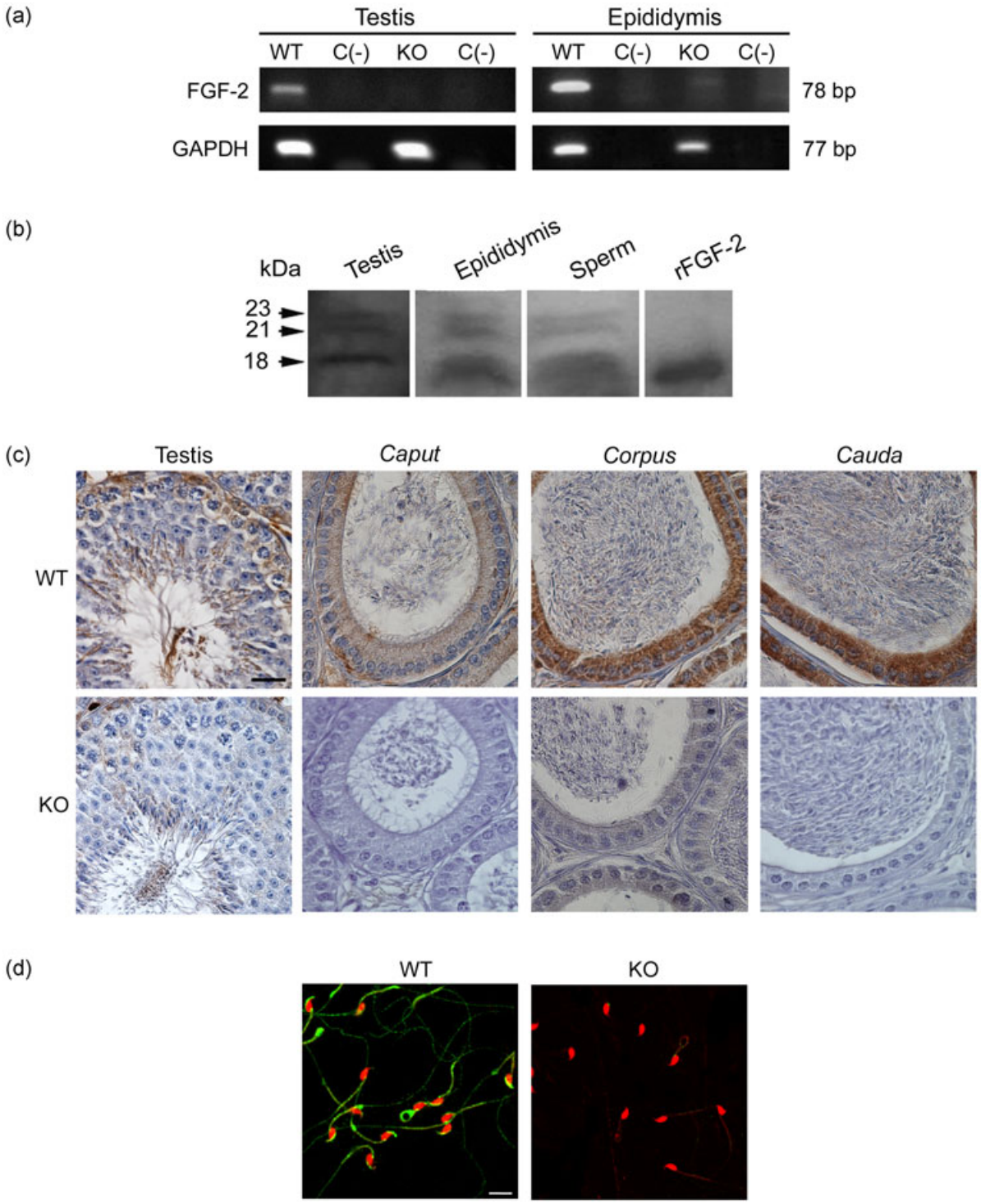

FIGURE 1 FGF-2 expression and localization in murine testis, epididymis, and sperm. (a) Messenger RNA expression of FGF-2 in the testis and epididymis in the wild-type (WT) and FGF-2 knockout (KO) animals assessed by RT-PCR. GAPDH was used as the housekeeper gene. The amplicon sizes are indicated on the right. The control omitting the reverse transcriptase was included (C (–)). (b) Detection of FGF-2 protein forms using western immunoblotting. Protein extracts from testis, epididymis (30 μg), or sperm (10×10^6) from WT animals were subjected to SDS-PAGE and western immunoblotting using a polyclonal anti-FGF-2 antibody. Recombinant FGF-2 protein (rFGF-2, 1 ng) was included as a control. The estimated molecular weights of the protein bands are indicated on the left. The experiment was performed at least three times, obtaining similar results. A typical result is shown. (c) Immunohistochemical analysis of FGF-2 in WT and KO testis and epididymis using a polyclonal anti-FGF-2 antibody. The specimens were counterstained with hematoxylin. Images are shown at 600× magnification. Scale bar: 20 μm. (d) Localization of FGF-2 in WT and KO sperm evaluated by indirect immunofluorescence. Sperm were stained with anti-FGF-2 and a secondary antibody labeled with Alexa Fluor® 488. Nuclei were stained with propidium iodide. Scale bar: 10 μm. FGF-2, fibroblast growth factor 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription-polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Testis and body weight in WT and KO mice

	Testis weight (g)	Body weight (g)	Testis/body weight ratio ($\times 10^{-3}$)
WT	0.10 ± 0.01	31.80 ± 1.10	3.30 ± 0.16
KO	0.11 ± 0.01	31.40 ± 1.50	3.54 ± 0.19

Note. FGF-2 KO and their WT littermates were weighed to determine the body weight. Animals were killed, both testes were weighed, and the values were averaged. Results are expressed as mean ± *SEM*, $n = 7$, no significant differences were observed between WT and KO mice. FGF-2 KO, fibroblast growth factor 2 knockout; *SEM*, standard error of the mean; WT, wild type

No FGF-2 reactivity was observed in testis, epididymis, and sperm from the KO animals (Figure 1c,d), indicating the specificity of the sc-79 antibody to FGF-2.

3.2 | Spermatogenesis in the FGF-2 KO mice

The FGF-2 KO mice showed no differences in the testis size, morphology, weight, or relative testicular weight compared with the WT (Table 1). Histological examination of testis sections was performed, and seminiferous tubules were classified according to their stages (Russell et al., 1990). The analysis revealed that KO animals depicted a significant increase ($P < 0.01$) in the percentage of seminiferous tubules in stages I–III of spermatogenesis. Conversely, a decrease ($P < 0.001$) in the percentage of tubules in stages VII and VIII (spermiogenesis stages) was found in the FGF-2 KO animals (Figure 2a). Moreover, the FGF-2 KO mice showed an increase ($P < 0.05$) in the number of spermatids per testis weight and in the DSP in comparison with the WT animals (Figure 2b).

3.3 | Expression levels of FGFs and FGFRs in the FGF-2 KO testis

The expression of several FGFs and FGFRs in the mammalian testis has been previously reported (Fon Tacer et al., 2010; Cotton et al., 2008). In the present study, to determine if FGF-2 deficiency affects the expression of other FGFs and/or FGFRs in the testis, qPCR analysis was performed. The results showed no significant changes in the expression levels of FGF-1, FGF-8, FGF-9, FGF-13, FGF-21, FGF-22, FGRF1c, FGRF2c, FGRF3c, FGRF4, and FGRF5 between the FGF-2 KO and WT testes. The expression of these FGFs and FGFRs in the KO, in relation to the WT testis, is depicted in Figure 3. No expression could be detected for FGF-4, as well as for FGFR1b, FGFR2b, or FGFR3b in either the WT or KO animals (data not shown).

3.4 | FGF-2 KO *cauda* epididymal sperm

The analysis of sperm recovered from the *cauda* of KO and WT mice showed no significant differences in the percentage of motile cells (Figure 4a). However, in the KO animals, a significantly ($P < 0.01$) higher sperm concentration was observed (Figure 4b). In addition, the assessment of sperm morphology revealed a significant ($P < 0.001$) increase in the percentage of abnormal sperm heads in the FGF-2 KO mice (WT: 6 ± 1% and KO: 28 ± 5%; $n = 9$; $P < 0.001$;

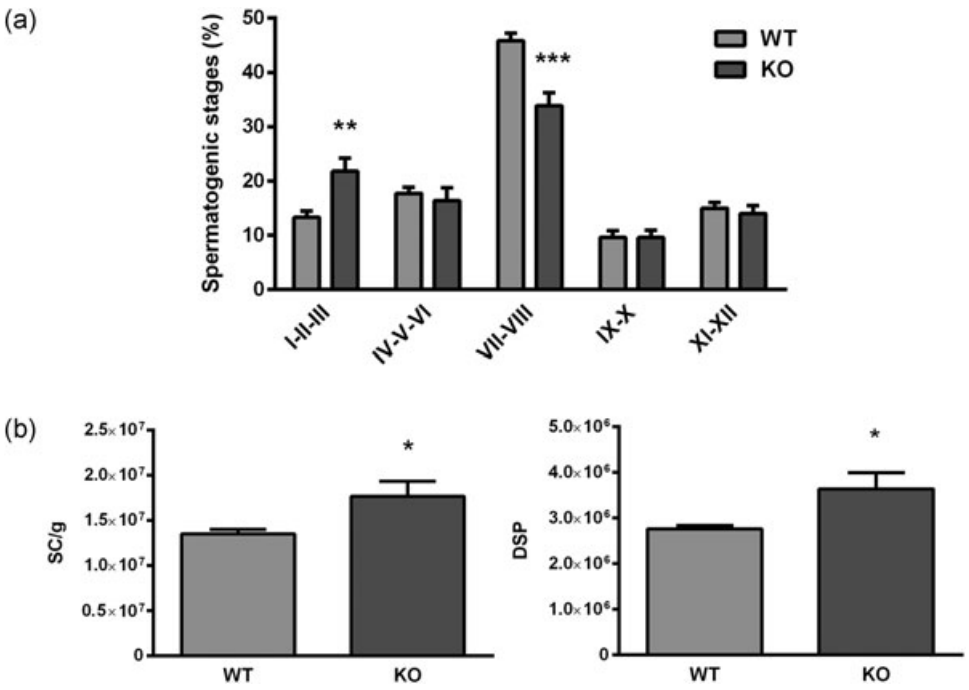

FIGURE 2 Analysis of the spermatogenic cycle in FGF-2 KO mice. (a) Percentage of seminiferous tubules in each stage of the spermatogenesis cycle in the WT and KO animals. Results are expressed as mean ± *SEM*, $n = 6$, $^{**}P < 0.01$; $^{***}P < 0.001$. (b) Number of spermatids per testis weight (SC/g) and daily sperm production per testis (DSP) in WT and KO animals, $n = 5$, $^{*}P < 0.05$. KO, fibroblast growth factor 2 knockout; *SEM*, standard error of the mean; WT, wild type

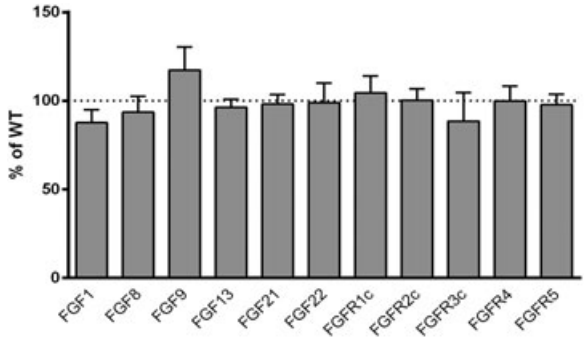

FIGURE 3 Expression analysis of selected FGFs and FGFRs in the testis of FGF-2 KO and WT animals. The expression of indicated FGF and FGFR family members in testis of FGF-2 KO ($n = 7$) and WT ($n = 4$) animals was determined by qPCR. The qPCR analysis for each sample was performed in triplicates. The relative expression level in the testis of KO animals was calculated by the ΔΔCt-method using GAPDH as the reference gene. Results are shown as the percentage (mean ± *SEM*) of the expression observed in WT animals (% of WT). FGF-2 KO, fibroblast growth factor 2 knockout; FGFRs, fibroblast growth factor receptors; qPCR, quantitative PCR; *SEM*, standard error of the mean; WT, wild type

Figure 4c). FGF-2 KO sperm depicted a wide variety of misshapen heads, lacking the typical hook-shaped appearance observed in the WT animals, but no defects in either their flagellum or neck were detected (Figure 4d,e). Individual analysis of sperm morphology in the FGF-2 KO mice revealed two subgroups: some animals ($n = 3$) exhibited a high percentage (48 ± 5%) of abnormal sperm morphology, whereas other animals showed a less severe phenotype (18 ± 2% abnormal sperm, which was still significantly higher than WT animals; Figure 4c). The increase in the percentage of abnormal heads in the KO mice was also detected in the sperm recovered from the *caput* and *corpus* sections (Figure 4f). It is worth mentioning that due to the increased sperm count of the FGF-2 KO mice, the total number of *cauda* sperm with a normal head morphology was similar between the WT and FGF-2 KO animals (WT: $8.6 \pm 2.3 \times 10^6$ and KO: $13.7 \pm 3.0 \times 10^6$ sperm; $n = 6$; $P = 0.21$).

The occurrence of capacitation-related events, as phosphorylation on tyrosine residues and acrosomal exocytosis, in sperm from FGF-2 KO mice was also evaluated. In WT sperm, an increase in tyrosine phosphorylation was observed after incubation under capacitating conditions, but reduced phosphorylation levels were detected in cells from the FGF-2 KO mice (Figure 5a). Regarding the acrosomal exocytosis, similar spontaneous loss of the acrosomal content (Spt T0) was observed in noncapacitated sperm from WT and KO mice (Figure 5b). However, after incubation under capacitating conditions, sperm from FGF-2 KO animals depicted a significant ($P < 0.01$) increase in the spontaneous acrosomal exocytosis compared with the WT (Spt T90; Figure 5b). Moreover, WT sperm showed an increase ($P < 0.05$) in the acrosomal exocytosis after exposure to progesterone (Prg 90), whereas FGF-2 KO sperm were unable to respond to this physiological stimulus (Figure 5b). To determine whether the elevated spontaneous acrosomal exocytosis observed in KO sperm could be prevented by the addition of exogenous FGF-2, mutant sperm were incubated for 90 min in the presence (10 and 100 ng/ml) or in the absence of the recombinant protein. Similar percentages of acrosome-reacted sperm were obtained in all the conditions (Figure 5c), suggesting that the lack of FGF-2 causes acrosomal instability that could not be reversed by the addition of the rFGF-2.

3.5 | Ultrastructural sperm analysis

To further analyze the morphology defects depicted by the FGF-2 KO sperm, a transmission electron microscopy study was carried out. The analysis showed that sperm recovered by swim-out from KO animals had comparable condensed chromatin and plasma membrane integrity with the WT sperm. However, membrane swelling at the acrosomal region was observed in some cells of the KO mice (Figure 6a). The evaluation was also done on sperm in the *caput* and *cauda* epididymal lumen. In the WT animals, the presence of cytoplasmic droplets was detected in *caput* sperm, but they were detached in the *cauda* sperm. On the contrary, several *cauda* KO sperm still presented cytoplasmic remnants with lysosome-like structures around the flagellum (Figure 6b), suggesting some defects in the maturation process in the FGF-2-deficient animals.

3.6 | Fertility of FGF-2 KO males

To study FGF-2 KO male fertility, either WT or KO males were mated overnight with WT females, and the time to delivery, litter size, and pup weight were analyzed. No differences in fertility parameters were observed between WT and KO males (Table 2). To dismiss a possible compensation from the female reproductive tract, FGF-2 KO males and females were mated, but similar results were obtained (Table 2). Next, to evaluate a possible subfertility of the FGF-2 KO males, WT, or FGF-2 KO males were mated with hormone-stimulated WT females. The eggs were recovered from the *ampulla*, and the development to two-cell embryo stage was determined. In this case, the percentage of fertilized eggs obtained for KO males (84 ± 6%) was comparable with that of WT animals (75 ± 7%; $n = 5$ females; $P = 0.33$). Altogether, these results suggest no apparent fertility defects in the FGF-2 KO mice and no in vivo fertilization impairment in the FGF-2 KO sperm.

4 | DISCUSSION

The present study aimed to analyze the role of FGF-2 in sperm physiology using FGF-2 KO mice as a model. Several alterations, mainly in the central nervous system, have been described in animals lacking FGF-2 (Dono et al., 1998; Korada et al., 2002; Ratzka et al., 2011; Rumpel et al., 2016; Timmer et al., 2007). However, to our knowledge, this is the first report describing the reproductive characteristics of FGF-2-deficient males.

Initially, we analyzed the presence and localization of FGF-2 in the murine male reproductive tissues and sperm cells. FGF-2

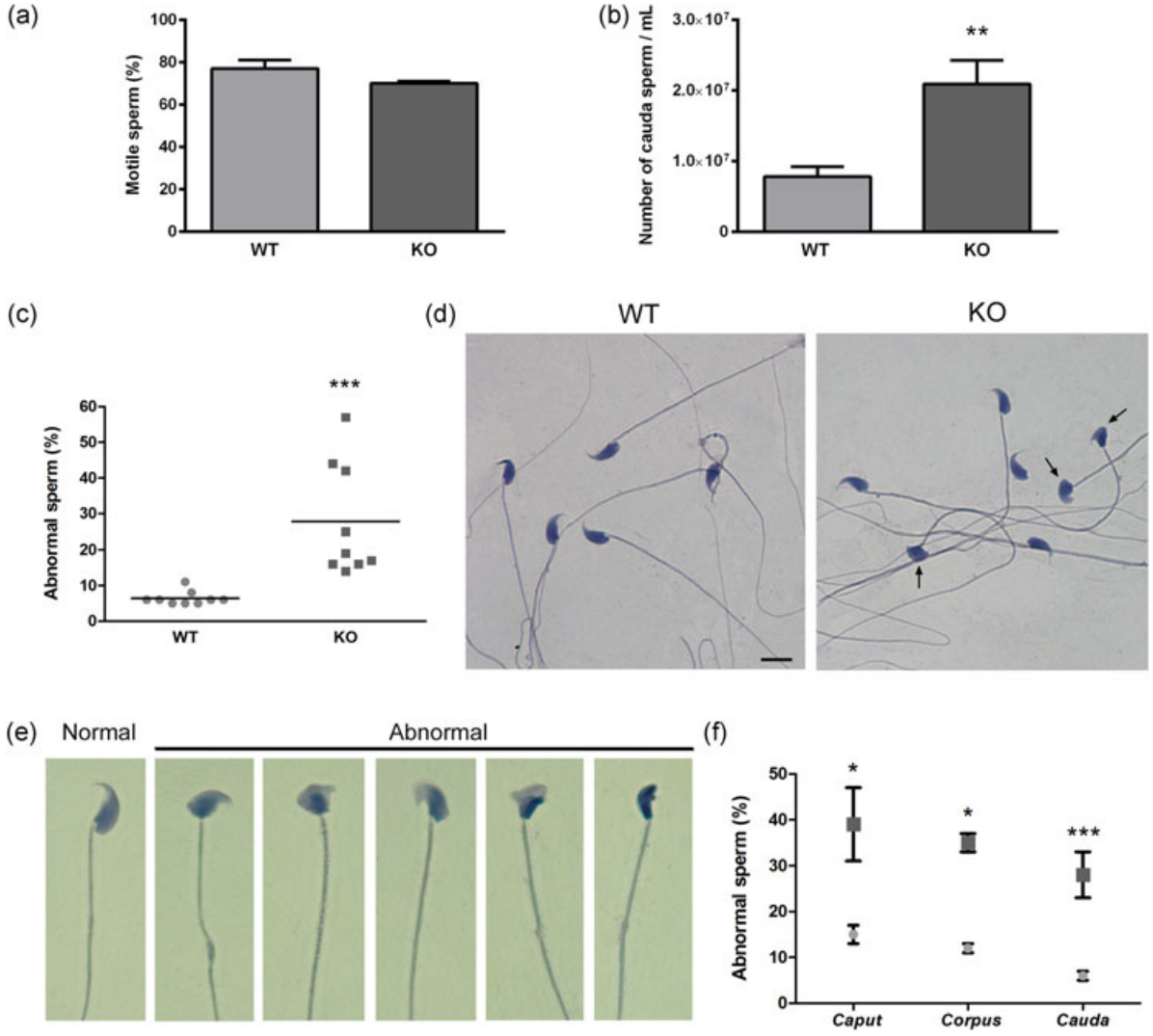

FIGURE 4 Sperm parameters in FGF-2-deficient mice. (a) Percentage of sperm motility in WT and KO animals. Results are expressed as mean ± *SEM*, $n = 3$, no significant differences. (b) Sperm concentration in WT and KO animals, $n = 6$, $^{**}P < 0.01$. (c) Percentage of abnormal sperm morphology in WT and KO animals (circles and squares, respectively), $n = 9$, $^{***}P < 0.001$. Lines indicate the mean values. (d) Images of WT and KO sperm stained with hematoxylin. The arrows indicate abnormal sperm forms. Scale bar: 10 μm. (e) Representative images of normal and abnormal sperm forms identified in FGF-2 KO animals. (f) Percentage of abnormal forms in sperm recovered from the *caput*, *corpus* ($n = 5$), and *cauda* epididymis ($n = 9$) of WT (circles) and KO animals (squares), $^{*}P < 0.05$, $^{**}P < 0.01$. KO, fibroblast growth factor 2 knockout; *SEM*, standard error of the mean; WT, wild type [Color figure can be viewed at wileyonlinelibrary.com]

transcripts were found in the WT testis and epididymis, and the expression of the 18-, 21-, and 23-kDa FGF-2 isoforms was detected in these tissues, as well as in sperm protein extracts. In the testis, FGF-2 was immunolocalized in the interstitial cells and in the seminiferous epithelium in a stage-dependent manner. The presence of FGF-2 in the mammalian testis has been previously reported (Cancilla, Davies, Ford-Perriss, & Risbridger, 2000; Fon Tacer et al., 2010; Garbarino Azúa et al., 2017; Lahr et al., 1992; Mayerhofer et al., 1991; Steger et al., 1998). Evidence in rodents describes the expression of FGF-2 by Sertoli and germ cell and suggests that the ligand secreted by the germ cells exerts a regulatory role on the functioning of Sertoli cells (Han et al., 1993; Regueira et al., 2015) and that Sertoli-cell-secreted FGF-2 regulates the metabolism of the germ cells (Gómez et al., 2012). Moreover, it has been reported that FGF-2 has a mitogenic and antiapoptotic effect on gonocytes recovered from perinatal testis (Van Dissel-Emiliani, De Boer-Brouwer, & De Rooij, 1996) and that the FGF-2-related signaling is involved in gonocytes-to-spermatogonia transition (Pui & Saga, 2017). Nevertheless, the relevance of FGF-2 in the maintenance and regulation of spermatogenesis in the adult testis has not been completely explored.

Our studies also showed the presence of FGF-2 in the epithelial cells of the murine epididymis, with a lower expression in the *caput* and a higher immunoreactivity in the *corpus* and *cauda* regions. These results are in agreement with a previous report describing increasing FGF-2 concentrations in homogenates of rat initial segment, *caput*, *corpus*, and *cauda* (Lan, Labus, & Hinton, 1998). Several growth factors are present in the *rete testis* fluid and can exert an effect on the

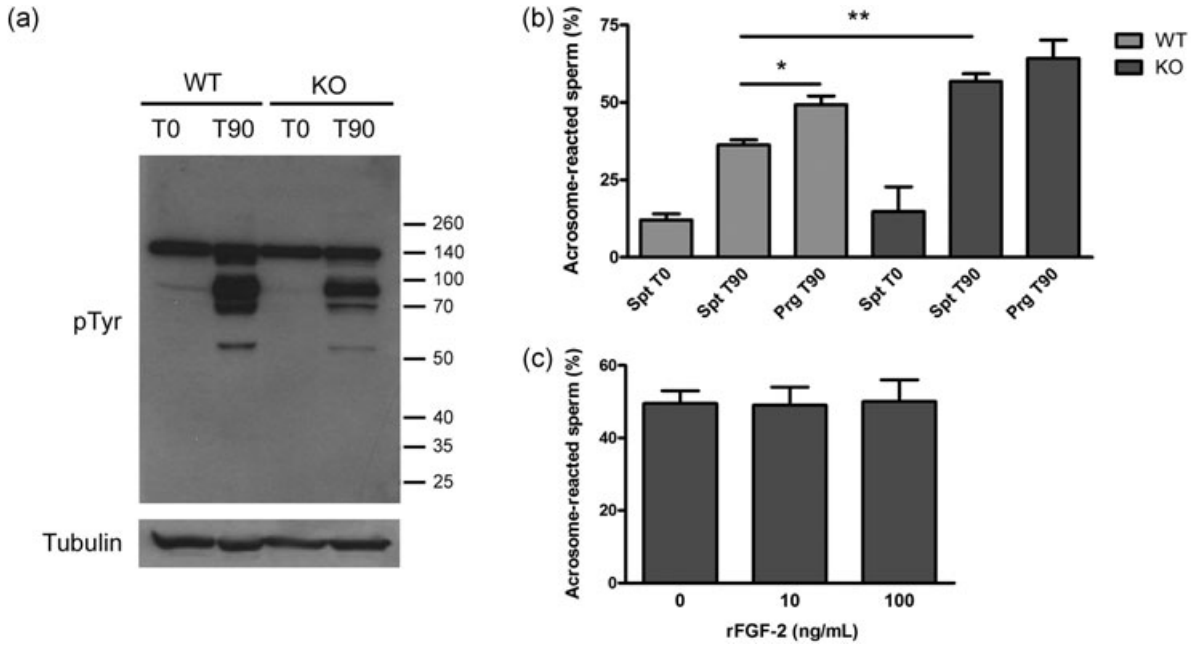

FIGURE 5 Capacitation-associated events in sperm from FGF-2 KO mice. (a) Protein tyrosine phosphorylation patterns in noncapacitated sperm (T0) from WT and KO animals or in sperm incubated under capacitating conditions for 90 min (T90). Membranes were developed using anti-phosphotyrosine antibody (pTyr) or anti-tubulin as a control. The molecular weight markers are shown on the right. The experiment was performed three times, obtaining similar results. A typical result is shown. (b) Spontaneous acrosomal exocytosis in noncapacitated sperm (Spt T0) from WT and KO animals or in sperm incubated under capacitating conditions for 90 min (Spt T90). Sperm were also incubated with progesterone (30 μM) for the last 15 min (Prg T90). Results are expressed as mean ± *SEM*, $n = 4$, $^{*}P < 0.05$; $^{**}P < 0.01$. (c) Spontaneous acrosomal exocytosis in sperm from KO mice incubated for 90 min in the absence or in the presence of rFGF-2, $n = 3$, no significant differences were observed. KO, fibroblast growth factor 2 knockout; rFGF-2, recombinant FGF-2 protein; *SEM*, standard error of the mean; WT, wild type

epithelial cells of the epididymal initial segment (Hinton, Lan, Rudolph, Labus, & Lye, 1998). Among them, it has been reported that FGF-2 stimulates the activation of Ras/mitogen-activated protein kinase in the first segments of the epididymis. This effect has also been described for other growth factors, such as epidermal growth factor and vascular epidermal growth factor (Tomsig, Usanovic, & Turner, 2006). A report suggests that FGF-2 stimulates the expression of γ-glutamyl transpeptidase, an enzyme that would protect sperm from oxidative stress damage by keeping low levels of glutathione and preventing protein oxidation (Lan et al., 1998). However, the secretion of FGF-2 by the epididymal epithelium, its differential expression, its role in each segment, and its function in sperm maturation requires further studies.

Immunocytochemical analysis revealed the presence of FGF-2 in the murine sperm flagellum and acrosomal region. A similar localization was found in human sperm cells (Garbarino Azúa et al., 2017), which is consistent with the detection of sperm FGFRs (Saucedo et al., 2015). Our results have shown that the activation of FGF-2/FGFRs-related signaling pathways is involved in the regulation of human sperm motility (Saucedo et al., 2015) and that human sperm FGF-2 levels correlate with sperm concentration and motility, suggesting the role of this system in the regulation of human sperm physiology, including spermatogenesis (Garbarino Azúa et al., 2017). The relevance of the activation of the FGF-2/FGFRs system in the maintenance of the WT sperm function is currently under evaluation in our laboratory.

Animals lacking FGF-2 constitute an invaluable model to analyze the involvement of this factor in the regulation of the male gamete physiology. In our study, the spermatogenesis of FGF-2 KO males was evaluated by histological analysis of the testis. The FGF-2 KO mice showed a significant increase in the percentage of seminiferous tubules in stages I–III, with a decrease in stage-VII and -VIII tubules compared with the WT. We also detected a higher number of spermatids per testis and an enhanced DSP in KO mice. The results suggest that FGF-2 KO mice show alterations in the spermatogenesis cycle kinetics in FGF-2 KO mice, with an accelerated release of the sperm from the seminiferous epithelium. The analysis of the expression levels of some FGFs and FGFRs in the testis indicated that, as in the nervous system (Ratzka et al., 2011), the loss of FGF-2 is not accompanied with changes in the expression of the FGFs or FGFRs tested. However, further studies are required to explore the possibility of compensation by other factors in the testis of the FGF-2 KO mice and to determine which mechanisms governing spermatogenesis are altered in these animals. It has been reported that mice devoid of FGF-2 show defects in the blood–brain barrier (BBB) permeability and alterations in the expression of proteins involved in cytoskeletal dynamics in the cerebral cortex (Baum, Vogt, Gass, Unsicker, & von Bohlen und Halbach, 2016; Reuss, Dono, & Unsicker, 2003). Considering the structural and functional similarities between the BBB and the blood–testis barrier (BTB; Wen, Tang, Gao, Jesus, & Chu, et al., 2018), it would be interesting to

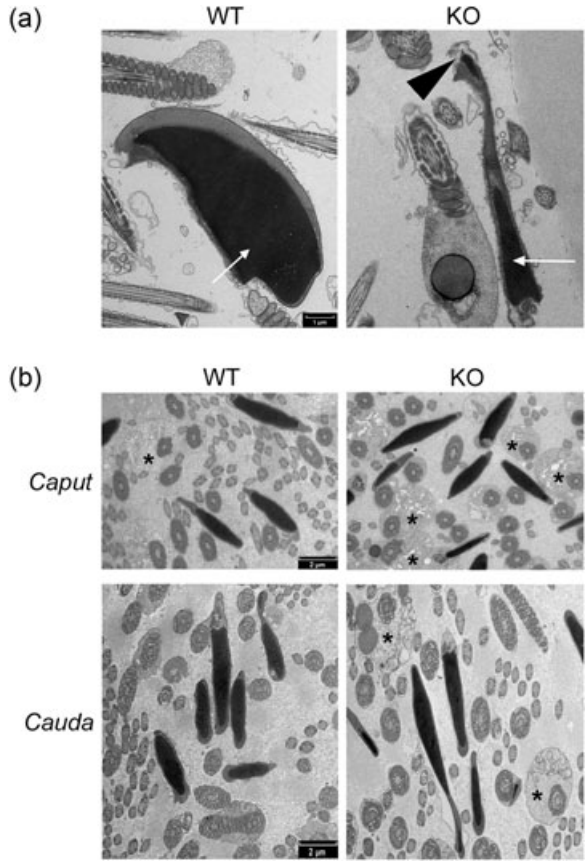

FIGURE 6 Ultrastructural analysis of sperm from FGF-2 KO mice. (a) Transmission electron microscopy study performed on *cauda* WT and KO sperm recovered by swim-out. The white arrows indicate the sperm chromatin and the arrowhead points out the swelling in the acrosomal region of the KO sperm. (b) Images of sperm inside the lumen of the *caput* and *cauda* epididymis. The asterisks indicate the cytoplasmic droplets around sperm flagella. KO, fibroblast growth factor 2 knockout; WT, wild type

analyze the integrity of the BTB in FGF-2 KO mice and determine whether an alteration at this level is involved in dysregulated spermatogenesis in these animals.

The analysis of *cauda* sperm recovered from FGF-2 KO mice compared with WT mice revealed no apparent differences in the motility, but an increase in sperm concentration and abnormal head morphology. In KO mice, two subgroups of animals were observed: one with higher percentages of abnormal sperm morphology, and another with a less severe phenotype. A similar variation has been described for other phenotypic characteristics of FGF-2 KO mice, such as regulation of blood pressure and neural progenitor migration (Dono et al., 1998). It has been suggested that this effect could be caused by an incomplete penetrance of the phenotype due to the compensation of other factors or by nonuniform segregation of unidentified genes that might regulate the FGF-2 expression (Dono et al., 1998).

TABLE 2 Fertility of males mated with nonstimulated females

Female	Male	*N* (males)	Time to delivery (day)	Litter size (number of pups)	Pup weight (g)
WT	WT	5	20.8 ± 0.3	7.6 ± 1.0	1.38 ± 0.07
WT	KO	3	22.0 ± 0.9	8.3 ± 0.4	1.38 ± 0.03
KO	KO	4	21.0 ± 0.6	7.5 ± 0.9	1.39 ± 0.03

Note. Assessment of fertility in sexually mature animals. Each male was subjected to two rounds of mating with females in their natural cycle, and fertility was analyzed. Results are expressed as mean ± *SEM*, no significant differences were observed between WT and KO mice. KO, fibroblast growth factor 2 knockout; *SEM*, standard error of the mean; WT, wild type

Capacitated sperm from the FGF-2 KO animals depicted decreased tyrosine phosphorylation levels compared with the WT cells. Such observations would indicate that in addition to the morphological abnormalities, sperm from the FGF-2 KO animals show alterations in the biochemical events related to the capacitation process. Animals devoid of FGF-2 also had increased percentages of acrosome-reacted sperm, suggesting acrosomal instability that could not be prevented by sperm incubation with the recombinant protein in vitro. An unstable acrosome in the FGF-2 KO sperm was also evidenced by electron microscopy, suggesting defects in this organelle assembly, which occurs during spermiogenesis. Accordingly, immunohistochemistry results in the WT animals revealed a high expression of FGF-2 in the cytoplasm of elongating/elongated spermatids.

There are studies reporting that mice deficient of several proteins display abnormal sperm head morphology, which is associated with defects in the acrosome formation and nucleus condensation (Kimura et al., 2003; Zhou et al., 2009). Interestingly, sperm from animals deficient in protamine 1 had some characteristics similar to the FGF-2 KO sperm (Takeda et al., 2016). They showed an increased percentage of abnormal sperm forms, with enhanced acrosomal exocytosis after incubation under capacitating conditions (with no changes in the noncapacitated sperm), suggesting an involvement of this protein in the regulation of murine spermatogenesis (Takeda et al., 2016).

We also conducted experiments to evaluate FGF-2 KO male fertility using two approaches. First, males were mated with females in their natural cycle, and the in vivo fertility was assessed by analyzing the time to delivery, litter size, and pup weight. Second, WT or KO males were mated with WT females subjected to a superovulation protocol, and the development to two-cell embryo stage was analyzed. Results showed that FGF-2 KO males depict no apparent fertility deficiencies in comparison with WT animals, in agreement with previous reports indicating that FGF-2 KO animals are fertile (Dono et al., 1998; Ortega, Ittmann, Tsang, Ehrlich, & Basilico, 1998). Gene-manipulated animals, in particular gene-disrupted mice, have been widely used to evaluate the relevance of different sperm proteins in fertilization. The studies have shown that many sperm entities thought to be essential for fertilization (based of in vitro evidence) are dispensable for male fertility because the animals devoid of these proteins are fertile (Okabe, 2015). However, the deep analysis of the reproductive tissues and gametes of these KO animals may contribute to the understanding of the extremely

complex mechanisms underlying spermatogenesis, sperm maturation, capacitation, and interaction with the oocyte.

In summary, our study provides evidence that FGF-2 is required for normal spermatogenesis, since in FGF-2 KO animals there is a dysregulation of the spermatogenic cycle, leading to increased sperm production, aberrant sperm morphology, and altered sperm physiology. Such abnormalities seem not to be reflected in alterations in animal fertility because the total number of sperm with normal forms is comparable between FGF-2 KO and WT mice. Interestingly, transgenic mice expressing a dominant-negative variant of FGFR1 in the male haploid germ cells are subfertile and show impaired spermiogenesis and sperm capacitation (Cotton et al., 2006). Future studies of single or combined deletion of several members of this growth factor family and their receptors would shed light on the relevance of this system in mammalian spermatogenesis and in the maintenance of sperm function.

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REFERENCES

Baum, P., Vogt, M. A., Gass, P., Unsicker, K., & von Bohlen und Halbach, O. (2016). FGF-2 deficiency causes dysregulation of Arhgef6 and downstream targets in the cerebral cortex accompanied by altered neurite outgrowth and dendritic spine morphology. *International Journal of Developmental Neuroscience*, *50*, 55–64.

Belov, A. A., & Mohammadi, M. (2013). Molecular mechanisms of fibroblast growth factor signaling in physiology and pathology. *Cold Spring Harbor Perspectives in Biology*, *5*, a015958.

Busso, D., Goldweic, N. M., Hayashi, M., Kasahara, M., & Cuasnicú, P. S. (2007). Evidence for the involvement of testicular protein CRISP2 in mouse sperm-egg fusion. *Biology of Reproduction*, *76*, 701–708.

Cancilla, B., Davies, A., Ford-Perriss, M., & Risbridger, G. P. (2000). Discrete cell- and stage-specific localisation of fibroblast growth factors and receptor expression during testis development. *Journal of Endocrinology*, *164*, 149–159.

Chaves, R. N., de Matos, M. H., Buratini, J., Jr, & de Figueiredo, J. R., J. (2012). The fibroblast growth factor family: Involvement in the regulation of folliculogenesis. *Reproduction, Fertility, and Development*, *24*, 905–915.

Cotton, L., Gibbs, G. M., Sanchez-Partida, L. G., Morrison, J. R., de Kretser, D. M., & O'Bryan, M. K. (2006). FGFR-1[corrected] signaling is involved in spermiogenesis and sperm capacitation. *Journal of Cell Science*, *119*, 75–84.

Cotton, L. M., O'Bryan, M. K., & Hinton, B. T. (2008). Cellular signaling by fibroblast growth factors (FGFs) and their receptors (FGFRs) in male reproduction. *Endocrine Reviews*, *29*, 193–216.

Dono, R., Texido, G., Dussel, R., Ehmke, H., & Zeller, R. (1998). Impaired cerebral cortex development and blood pressure regulation in FGF-2-deficient mice. *EMBO Journal*, *17*, 4213–4225.

Eswarakumar, V. P., Lax, I., & Schlessinger, J. (2005). Cellular signaling by fibroblast growth factor receptors. *Cytokine and Growth Factor Reviews*, *16*, 139–149.

Fon Tacer, K., Bookout, A. L., Ding, X., Kurosu, H., John, G. B., Wang, L., … Kliewer, S. A. (2010). Research resource: Comprehensive expression atlas of the fibroblast growth factor system in adult mouse. *Molecular Endocrinology*, *24*, 2050–2064.

Fraser, L. R., & Drury, L. M. (1975). The relationship between sperm concentration on fertilization in vitro of mouse eggs. *Biology of Reproduction*, *13*, 513–518.

Garbarino Azúa, D. J., Saucedo, L., Giordana, S., Magri, M. L., Buffone, M. G., Neuspiller, F., … Marín-Briggiler, C. I. (2017). Fibroblast growth factor 2 (FGF2) is present in human spermatozoa and is related with sperm motility. The use of recombinant FGF2 to improve motile sperm recovery. *Andrology*, *5*, 990–998.

Gómez, M., Manzano, A., Figueras, A., Viñals, F., Ventura, F., Rosa, J. L., … Navarro-Sabaté, À. (2012). Sertoli-secreted FGF-2 induces PFKFB4 isozyme expression in mouse spermatogenic cells by activation of the MEK/ERK/CREB pathway. *American Journal of Physiology, Endocrinology and Metabolism*, *303*, E695–E707.

Gong, S. G. (2014). Isoforms of receptors of fibroblast growth factors. *Journal of Cellular Physiology*, *229*, 1887–1895.

Han, I. S., Sylvester, S. R., Kim, K. H., Schelling, M. E., Venkateswaran, S., Blanckaert, V. D., … Griswold, M. D. (1993). Basic fibroblast growth factor is a testicular germ cell product which may regulate Sertoli cell function. *Molecular Endocrinology*, *7*, 889–897.

Hinton, B. T., Lan, Z. J., Rudolph, D. B., Labus, J. C., & Lye, R. J. (1998). Testicular regulation of epididymal gene expression. *Journal of Reproduction and Fertility*, *3*, 47–57.

Hughes, S. E. (1997). Differential expression of the fibroblast growth factor receptor (FGFR) multigene family in normal human adult tissues. *Journal of Histochemistry and Cytochemistry*, *45*, 1005–1019.

Itoh, N., & Ornitz, D. M. (2011). Fibroblast growth factors: From molecular evolution to roles in development, metabolism and disease. *Journal of Biochemistry*, *149*, 121–130.

Jiang, X., Skibba, M., Zhang, C., Tan, Y., Xin, Y., & Qu, Y. (2013). The roles of fibroblast growth factors in the testicular development and tumor. *Journal of Diabetes Research*, *2013*, 489095.

Johnson, L., Petty, C. S., & Neaves, W. B. (1980). A comparative study of daily sperm production and testicular composition in humans and rats. *Biology of Reproduction*, *22*, 1233–1243.

Kimura, T., Ito, C., Watanabe, S., Takahashi, T., Ikawa, M., Yomogida, K., … Nakano, T. (2003). Mouse germ cell-less as an essential component for nuclear integrity. *Molecular and Cellular Biology*, *23*, 1304–1315.

Korada, S., Zheng, W., Basilico, C., Schwartz, M. L., & Vaccarino, F. M. (2002). Fibroblast growth factor 2 is necessary for the growth of glutamate projection neurons in the anterior neocortex. *Journal of Neuroscience*, *22*, 863–875.

Kyjovska, Z. O., Boisen, A. M. Z., Jackson, P., Wallin, H., Vogel, U., & Hougaard, K. S. (2013). Daily sperm production: Application in studies of prenatal exposure to nanoparticles in mice. *Reproductive Toxicology*, *36*, 88–97.

Lahr, G., Mayerhofer, A., Seidl, K., Bucher, S., Grothe, C., Knöchel, W., & Gratzl, M. (1992). Basic fibroblast growth factor (bFGF) in rodent testis. Presence of bFGF mRNA and of a 30 kDa bFGF protein in pachytene spermatocytes. *FEBS Letters*, *302*, 43–46.

Lan, Z. J., Labus, J. C., & Hinton, B. T. (1998). Regulation of gamma-glutamyl tranpeptidase catalytic activity and protein level in the initial

segment of the rat epididymis by testicular factors: Role of basic fibroblast growth factor. *Biology of Reproduction*, *58*, 197–206.

Liao, S., Bodmer, J., Pietras, D., Azhar, M., Doetschman, T., & Schultz, J. E. J. (2009). Biological functions of the low and high molecular weight protein isoforms of fibroblast growth factor-2 in cardiovascular development and disease. *Developmental Dynamics*, *238*, 249–264.

Marín-Briggiler, C. I., Vazquez-Levin, M. H., Gonzalez-Echeverría, F., Blaquier, J. A., Tezón, J. G., & Miranda, P. V. (1999). Strontium supports human sperm capacitation but not follicular fluid-induced acrosome reaction. *Biology of Reproduction*, *61*, 673–680.

Marin-Briggiler, C. I., Veiga, M. F., Matos, M. L., Gonzalez-Echeverría, M. F., Furlong, L. I., & Vazquez-Levin, M. H. (2008). Expression of epithelial cadherin in the human male reproductive tract and gametes and evidence of its participation in fertilization. *Molecular Human Reproduction*, *14*, 561–571.

Mayerhofer, A., Russell, L. D., 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.

Mohammadi, M., Olsen, S. K., & Ibrahimi, O. A. (2005). Structural basis for fibroblast growth factor receptor activation. *Cytokine and Growth Factor Reviews*, *16*, 107–137.

Okabe, M. (2015). Mechanisms of fertilization elucidated by gene-manipulated animals. *Asian Journal of Andrology*, *17*, 646–652.

Ornitz, D. M., & Itoh, N. (2015). The fibroblast growth factors signaling pathway. *Wiley Interdisciplinary Reviews. Developmental Biology*, *4*, 215–266.

Ortega, S., Ittmann, M., Tsang, S. H., Ehrlich, M., & Basilico, C. (1998). Neuronal defects and delayed wound healing in mice lacking fibroblast growth factor 2. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 5672–5677.

Price, C. A. (2016). Mechanisms of fibroblast growth factor signaling in the ovarian follicle. *Journal of Endocrinology*, *228*, R31–R43.

Pui, H. P., & Saga, Y. (2017). Gonocytes-to-spermatogonia transition initiates prior to birth in murine testes and it requires FGF signaling. *Mechanisms of Development*, *144*(Pt B), 125–139.

Ratzka, A., Baron, O., & Grothe, C. (2011). FGF-2 deficiency does not influence FGF ligand and receptor expression during development of the nigrostriatal system. *PLoS One*, *6*(8), e23564.

Regueira, M., Riera, M. F., Galardo, M. N., Camberos, M. C., Pellizzari, E. H., Cigorraga, S. B., & Meroni, S. B. (2015). FSH and bFGF regulate the expression of genes involved in Sertoli cell energetic metabolism. *General and Comparative Endocrinology*, *222*, 124–133.

Reuss, B., Dono, R., & Unsicker, K. (2003). Functions of fibroblast growth factor (FGF)-2 and FGF-5 in astroglial differentiation and blood-brain barrier permeability: Evidence from mouse mutants. *Journal of Neuroscience*, *23*, 6404–6412.

Rumpel, R., Baron, O., Ratzka, A., Schröder, M. L., Hohmann, M., Effenberg, A., … Grothe, C. (2016). Increased innervation of forebrain targets by midbrain dopaminergic neurons in the absence of FGF-2. *Neuroscience*, *314*, 134–144.

Russell, L., Ettlin, R., Sinha, A., & Clegg, E. (1990). *Histological and histopathological evaluation of the testis*. Clearwater, FL: Cache River Press.

Saucedo, L., Buffa, G. N., Rosso, M., Guillardoy, T., Góngora, A., Munuce, M. J., … Marín-Briggiler, C. (2015). Fibroblast growth factor receptors (FGFRs) in human sperm: Expression, functionality and involvement in motility regulation. *PLoS One*, *10*(5), e0127297.

Steger, K., Tetens, F., Seitz, J., Grothe, C., & Bergmann, M. (1998). Localization of fibroblast growth factor 2 (FGF-2) protein and the receptors FGFR 1–4 in normal human seminiferous epithelium. *Histochemistry and Cell Biology*, *110*, 57–62.

Takeda, N., Yoshinaga, K., Furushima, K., Takamune, K., Li, Z., Abe, S., … Yamamura, K. (2016). Viable offspring obtained from Prm1-deficient sperm in mice. *Scientific Reports*, *6*, 27409.

Timmer, M., Cesnulevicius, K., Winkler, C., Kolb, J., Lipokatic-Takacs, E., Jungnickel, J., & Grothe, C. (2007). Fibroblast growth factor (FGF)-2 and FGF receptor 3 are required for the development of the substantia nigra, and FGF-2 plays a crucial role for the rescue of dopaminergic neurons after 6-hydroxydopamine lesion. *Journal of Neuroscience*, *27*, 459–471.

Tomsig, J. L., Usanovic, S., & Turner, T. T. (2006). Growth factor-stimulated mitogen-activated kinaase (MAPK) phosphorylation in the rat epididymis is limited by segmental boundaries. *Biology of Reproduction*, *75*, 598–604.

Trueb, B. (2011). Biology of FGFRL1, the fifth fibroblast growth factor receptor. *Cellular and Molecular Life Science*, *68*, 951–964.

Turner, N., & Grose, R. (2010). Fibroblast growth factor signalling: From development to cancer. *Nature Reviews Cancer*, *10*, 116–129.

Van Dissel-Emiliani, F. M., De Boer-Brouwer, M., & De Rooij, D. G. (1996). Effect of fibroblast growth factor-2 on Sertoli cells and gonocytes in coculture during the perinatal period. *Endocrinology*, *137*, 647–654.

Vazquez-Levin, M. H., & Marín-Briggiler, C. I. (2009). An overview of the molecular mechanisms involved in human fertilization. In L. T. Lipshultz, S. S. Howards, & C. S. Niederberger (Eds.), *Infertility in the male*. (pp. 104–121). Cambridge, U.K.: Cambridge University Press.

Virag, J. A. I., Rolle, M. L., Reece, J., Hardouin, S., Feigl, E. O., & Murry, C. E. (2007). Fibroblast growth factor-2 regulates myocardial infarct repair: Effects on cell proliferation, scar contraction, and ventricular function. *American Journal of Pathology*, *171*, 1431–1440.

Wassarman, P. M., Jovine, L., & Litscher, E. S. (2001). A profile of fertilization in mammals. *Nature Cell Biology*, *3*, E59–E64.

Wen, Q, Tang, E. I., Gao, Y, Jesus, T. T., Chu, D. S., Lee, W. M., … Cheng, C. Y. (2018). Signaling pathw ays regulating blood-tissue barriers—Lesson from the testis. *Biochimica et Biophysica Acta*, *1860*, 141–153.

Yanagimachi, R. (1994). Mammalian fertilization. In E. Knobil, & J. D. Neill (Eds.), *The physiology of reproduction* (pp. 189–317). New York, NY: Raven Press.

Yu, P. J., Ferrari, G., Galloway, A. C., Mignatti, P., & Pintucci, G. (2007). Basic fibroblast growth factor (FGF-2): The high molecular weight forms come of age. *Journal of Cellular Biochemistry*, *100*, 1100–1108.

Zhou, J., Du, Y. R., Qin, W. H., Hu, Y. G., Huang, Y. N., Bao, L., … Xu, G. L. (2009). RIM-BP3 is a manchette-associated protein essential for spermiogenesis. *Development*, *136*, 373–382.

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