Impaired Fertilizing Ability of Superoxide Dismutase 1-Deficient Mouse Sperm During In Vitro Fertilization

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ABSTRACT

The oxidative modification of gametes by a reactive oxygen species is a major deleterious factor that decreases the success rate of in vitro fertilization. Superoxide dismutase 1 (SOD1) plays a pivotal role in antioxidation by scavenging the superoxide anion, and its deficiency causes infertility in female mice, but the significance of the enzyme in male mice remains unclear. In the present study, we characterized Sod1−/− (Sod1-KO) male reproductive organs and compiled the first report of the impaired fertilizing ability of Sod1-KO sperm in in vitro fertilization. Insemination of wild-type oocytes with Sod1-KO sperm exhibited lower rates of fertilization compared with insemination by wild-type sperm. The low fertilizing ability found for Sod1-KO sperm was partially rescued by reductant 2-mercaptoethanol, which suggested the oxidative modification of sperm components. The numbers of motile and progressive sperm decreased during the in vitro fertilization process, and a decline in ATP content and elevation in lipid peroxidation occurred in the Sod1-KO sperm in an incubation time-dependent manner. Tyrosine phosphorylation, which is a hallmark for sperm capacitation, was also impaired in the Sod1-KO sperm. These results collectively suggest that machinery involved in sperm capacitation and motility are vulnerable to oxidative damage during the in vitro fertilization process, which could increase the rate of inefficient fertilization.

INTRODUCTION

Reactive oxygen species (ROS) are produced during vital activities, such as oxygen consumption, during mitochondrial respiration and under various stress conditions, such as inflammation [1]. Although locally produced ROS play a role in signal transduction in the cell [2], excessively produced ROS exert oxidative damage to proteins, lipids, and nucleic acids. Either augmented production of ROS or suppression of antioxidative systems causes oxidative stress, which results in harmful damage to many components of sperm [3–5]. The exposure of DNA to ROS causes oxidative modification of bases and DNA strand breaks [6], which transmits adverse genetic information to the offspring. Resonance double bonds of polyunsaturated fatty acids are the preferred target of ROS. In human sperm, ~40% (by weight) of the total fatty acid fraction is composed of polyunsaturated fatty acids, which are prone to peroxidation by ROS [7]. In the case of proteins, sulphydryl residues with low pKa values, such as catalytic cysteines of enzymes, are preferential targets of ROS [8]. Thus, ROS affects sperm in complex ways, depending on the physiologic conditions of the sperm and on environmental factors, notably oxygen and antioxidants.

Superoxide dismutase (SOD) converts superoxide radicals to hydrogen peroxide, which is a reactive oxygen but not a radical [9]. Superoxide radicals trigger radical chain reactions that exert a more serious impact compared with hydrogen peroxide. Hence, SOD functions as a primary antioxidative enzyme by terminating deleterious radical chain reactions. Among three isozymes, SOD1 is rich in the cytoplasm and partly in the intermembrane spaces of mitochondria [10]. Regarding the reproductive ability of Sod1−/− (Sod1-KO) mice, female infertility is prominent [11, 12] and is caused by embryonic death that occurs by the 10th day of pregnancy [11]. Recently, Noda et al. [13] demonstrated that decreased progesterone secretion from the ovary causes infertility in Sod1-KO female mice. We have reported that Sod1-KO oocytes showed normal fertilizing ability when fertilized with wild-type (WT) sperm in vitro. However, the resultant Sod1-heterozygote embryos were arrested at the two-cell stage [14], which suggests that SOD1 deficiency in oocytes exerts crucial effects on the development of the resultant Sod1-heterozygote embryos under in vitro fertilization (IVF) conditions. Because this developmental arrest occurs under normoxic culture at 20% oxygen but can be rescued by hypoxic culture at 1% oxygen, the metabolic use of molecular oxygen produces more ROS at 20% oxygen, which consequently causes developmental abnormalities. It is noteworthy, however, that the embryos arrested at the two-cell stage show normal mitochondrial membrane potential, oxygen consumption, and ATP production, suggesting that the target of ROS is the mitotic machinery of the embryonic cells rather than the mitochondria.

Spermatogenic cells are sensitive to various stress conditions, such as heat, and are prone to apoptosis-like cell death [15]. Although no abnormality has been found in the fertilizing ability of Sod1-KO male mice under conventional breeding conditions, the testes of Sod1-KO mice are more vulnerable to the oxidative insult caused by heat stress [16]. Because oxidative stress is considered to be a major cause for male infertility.
infertility [3–5], Sod1-KO sperm would be an ideal model for such studies to evaluate the causal mechanism in assisted reproduction technology. Here, we found a malfunction of Sod1-KO sperm regarding fertilizing ability during IVF and tried to elucidate its molecular basis.

MATERIALS AND METHODS

Experimental Animals

Three pairs of C57BL/6 Sod1 heterozygous mice, originally established by Matzuk et al. [12], were purchased through Jackson Laboratories (Bar Harbor, ME) and bred at our institute, giving rise to Sod1-KO and WT littersmates. Mice backcrossed to C57BL/6 mice more than eight times [17] were used in the present study. For analysis, 10- to 16-wk-old male mice were used, and 5- to 10-wk-old WT female mice were used to collect oocytes. The animal room climate was kept under specific pathogen-free conditions at a constant temperature of 20–22°C with a 12L:12D cycle, and food and water were available ad libitum. Animal experiments were performed in accordance with the Declaration of Helsinki under the protocol approved by the Animal Research Committee of Yamagata University.

Measurement of Serum Testosterone by an Enzyme Immunoassay

Serum testosterone concentrations in mice were measured using a Testosterone EIA Kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s instructions. The blood collected from hearts was incubated at room temperature for 1 h to allow clotting and was centrifuged at 21,900 × g for 15 min. The serum samples were kept at −80°C until testosterone measurement.

Immunoblot Analysis of Antioxidative Enzymes

Testis, epididymis, seminal vesicle, and sperm collected from Sod1-KO and WT mice were lysed in RIPA buffer (25 mM Tris-HCl, pH 7.6; 150 mM NaCl; 1% NP-40; 1% sodium deoxycholate; and 0.1% SDS) containing 1 mM phenylmethylsulfonyl fluoride. Total proteins (30 µg) were separated on SDS-PAGE (15% gels) and elecroblotted onto polyvinylidene fluoride membranes. After blocking with 1% (w/v) skim milk in Tris-buffered saline (TBS) for 1 h, the membranes were incubated with the polyclonal anti-rat SOD1, anti-human SOD2 antisera [18], anti-human catalase (CarboBio-Merk Bioscience, Tokyo, Japan), anti-rat peroxiredoxin-1 (PRDX-1) [19], PRDX-2 (Ab Frontier, Seoul, Korea), PRDX-4 [20], or GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA) diluted in TBS overnight at 4°C. After three washes of 10 min each in 0.1% Tween-20 TBS, the membrane was incubated for 1 h at room temperature with a horseradish peroxidase (HRP)-conjugated secondary antibody (Dako, Glostrup, Denmark) diluted 1:20,000 in TBS. After washing, the presence of bound HRP activity was detected by chemiluminescence with a Western HRP Substrate (Millipore, Billerica, MA) and was exposed to x-ray film.

In Vitro Fertilization

Oocytes were collected from C57BL/6 female mice that had been superovulated with 7.5 IU of equine chorionic gonadotropin (ASKA Pharmaceutical, Tokyo, Japan), followed by 7.5 IU of human chorionic gonadotropin (ASKA Pharmaceutical) administered intraperitoneally 48 h later. Human chorionic gonadotropin (HTF) [21] was used for IVF, and potassium simplex optimization medium (KSON) [22] was used for zygote culture. Each medium was supplemented with 0.5% bovine serum albumin (BSA; fraction V; Sigma-Aldrich, St. Louis, MO). For IVF, sperm from Sod1-KO and WT male mice were collected by squeezing the cauda epididymis, and then the sperm were placed into 500 µl of HTF in 1.5-ml microtubes. These tubes were preincubated at 37°C in a humidified atmosphere of 5% CO₂ in air for 1 h, and then only swim-up sperm were collected and adjusted to a final concentration of 2 × 10⁵ sperm per milliliter for IVF. Ovulated cumulus-oocyte complexes (COCs) were collected by tearing the oviduct ampulla at 15 h after human chorionic gonadotropin injection and were pooled into 200-µl droplets with sperm. The COCs were inseminated with spermatozoa for 5 h. After IVF, zygotes were denuded of cumulus cells and sperm in KSON and were cultivated further. Cleavage rates were assessed at 24 h after the start of embryo culture. Normally cleaved embryos remained in culture for 4 days (up to the blastocyst stage). Each culture was kept in 50-µl droplets of medium overlaid with mineral oil in 35-mm diameter plastic culture dishes. Incubation was conducted at 37°C, 5% CO₂, and 95% air. The effects of antioxidant supplementation with 2-mercaptoethanol (2-ME) were also investigated. A total of 2 × 10⁵ sperm per milliliter was incubated with 100 µM 2-ME (Wako Pure Chemical Industries, Osaka, Japan) in HTF media for 1 h and then subjected to inactivation of COCs for 5 h without washing. The zygotes were incubated in KSON media without 2-ME as described above.

Staining of Oocyte Cortical Granule

After 5 h of inactivation, oocytes were denuded of cumulus cells and sperm and on the surface of zona pellucida by pipetting several times. Denuded zygotes were washed in PBS containing 1 mg/ml polyvinyl alcohol (PVA; Sigma-Aldrich) and were fixed with 2.5% glutaraldehyde in PBS-PVA at room temperature for 30 min. After fully rinsing with PBS-PVA, oocytes were permeabilized with 0.1% Triton X-100 in PBS-PVA for 5 min. After washing three times for 5 min each, oocytes were incubated in PBS-PVA containing 50 µg/ml fluorescein isothiocyanate (FITC)-conjugated Pisum sativum agglutinin (FITC-PSA; Sigma-Aldrich) at room temperature for 30 min. After washing, oocyte nuclei were stained with 20 µg/ml propidium iodide (Sigma-Aldrich) for 10 min. After washing, samples were observed by laser scanning confocal microscopy (LSM510 META; Carl Zeiss, Oberkochen, Germany).

Preparation of Zona Pellucida-Free Oocytes and IVF

The COCs derived from WT female mice were denuded by hyaluronidase treatment (300 µg/ml in HTF; type IVs; Sigma-Aldrich) and then incubated in acidic Tyrode solution, pH 2.5, for several seconds to remove the zona pellucida. Zona-free oocytes were sufficiently rinsed with HTF and incubated with Sod1-KO or WT sperm (1 × 10⁵ sperm per milliliter). After 5 h of inactivation, the fertilization rate was determined by pronuclei staining.

Analyses of Sperm Motility

Parameters of sperm motility were quantified by computer-assisted semen analysis (CASA) using integrated visual optical system software (Hamilton-Thorne Biosciences, Beverly, MA). Briefly, cauda epididymal sperm of Sod1-KO male mice, with WT mice as the control, were incubated for 1, 3, and 7 h in a 50-µl HTF medium drop at 37°C under 5% CO₂ in air. An aliquot of the incubated sperm suspension was transferred into a prewarmed counting chamber (20-µm depth), and more than 200 cells per sample were analyzed using standard settings, as described previously [23, 24].

Assay for Lipid Peroxidation Products in Sperm

Lipid peroxidation products in sperm were assessed as thiobarbituric acid-reactive substances (TBARS). A total of 1 × 10⁵ sperm were collected and washed twice with PBS. Then the cell pellet was suspended in 0.3 ml of PBS and combined with 0.6 ml of a reagent containing 15% trichloroacetic acid, 0.375% (w/v) thiobarbituric acid, 0.25 M HCl, and 1.8 mM butylhydroxytoluene, and mixed thoroughly. The solution was heated for 15 min in boiling water, cooled in ice-cold water, and centrifuged at 10,000 × g for 10 min. After extraction with 250 µl of n-butanol, the fluorescence was measured at 515/553 nm for excitation/emission wavelengths.

Measurement of the ATP Content of Sperm

The ATP content of sperm was measured using a BacTiter-Glo Microbial Cell Viability Assay Kit (Promega, Madison, WI), which is based on the luciferin-luciferase reaction. Briefly, 1 × 10⁵ sperm were collected and washed twice with PBS, suspended in 250 µl of PBS, and stored at −80°C until use. After the addition of 50 µl of the reaction mixture followed by incubation at 25°C for 5 min, the chemiluminescence of the sample was measured using a luminometer (Berthold Lumat LB9507, Bad Wildbad, Germany). A five-point standard curve (0–100 pmol per tube) was routinely included in each assay. The ATP content was determined from the formula for a standard curve (linear regression). A linear relationship was observed between luciferin luminescence and ATP content based on 0.01–10 pmol per assay, which fully covered the ATP contents of the sperm.

Detection of Tyrosine Phosphorylation in Sperm

Cauda epididymal sperm were dispersed in HTF medium containing 5 mg/ml BSA. After 30, 60, and 90 min of incubation at 37°C under 5% CO₂ in air, 1 × 10⁵ sperm were collected, washed twice with PBS, and resuspended in a Laemmli sample loading buffer. The sample was boiled for 5 min and centrifuged at 10,000 × g for 5 min. The resultant supernatant was reboled in 2× sample buffer.
The presence of 5% (v/v) 2-ME for 5 min. Protein samples were subjected to immunoblot analysis using an anti-phosphotyrosine monoclonal antibody (PY20, Santa Cruz Biotechnology). The membrane was blocked with 5% BSA instead of skim milk, followed by immunoblot analysis as described above.

Statistical Analysis

Data are presented as mean values ± SD. Statistical analyses of the data were carried out mainly using a Student t-test. A Fisher exact test was performed to evaluate the developmental ability among individual groups. *P < 0.05, **P < 0.01.

RESULTS

Properties of the Male Reproductive System in Sod1-KO Mice

We first examined the fecundity of Sod1-KO male mice but found no significant difference from WT mice (data not shown), which was consistent with previous reports. However, in addition to body weight, male genital and accessory organs were significantly smaller in Sod1-KO male mice than those of the WT mice (Supplemental Fig. S1, all Supplemental Data are available online at www.biolreprod.org). Sperm content in the seminal vesicle (data not shown) was significantly smaller in Sod1-KO mice than those of WT mice (data not shown), which was consistent with previous reports. However, we found no significant difference from WT mice (data not shown).

Expression of Antioxidative Enzymes in Male Reproductive Organs

We then examined the protein levels of major antioxidative enzymes in male reproductive organs and sperm by immuno-

FIG. 1. Immunoblot analysis of major antioxidative enzymes in male reproductive tracts. SOD2, mitochondrial superoxide dismutase; CAT, catalase; PRDX, peroxiredoxin. GAPDH was used as a loading control. Representative images of two experiments are shown.

TABLE 1. Success rates of fertilization and development of blastocysts in IVF.

<table>
<thead>
<tr>
<th>Genotype + treatment</th>
<th>No. of oocytes inseminated</th>
<th>No. (%) of embryos developed to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Two-cell*</td>
<td>Four-cell*</td>
</tr>
<tr>
<td>WT</td>
<td>98</td>
<td>79 (80.6)*</td>
</tr>
<tr>
<td>KO</td>
<td>97</td>
<td>32 (33.0)*</td>
</tr>
<tr>
<td>WT + 2-ME†</td>
<td>89</td>
<td>76 (85.4)*</td>
</tr>
<tr>
<td>KO + 2-ME†</td>
<td>84</td>
<td>44 (52.4)*</td>
</tr>
</tbody>
</table>

Values with different superscript letters within each developmental stage are significantly different. Fisher exact test with Bonferroni correction, P < 0.05 (n = 5).

* Percentage of the number of inseminated oocytes.
† Percentage of the number of two-cell embryos.
2-ME: 100 μM 2-mercaptoethanol.

Low Fertilizing Ability of Sod1-KO Sperm in IVF

In order to examine fertilization ability, we inseminated the oocytes collected from WT female mice with the sperm from Sod1-KO as well as WT male mice. We regarded fertilization to be successful only when the zygotes developed to the two-cell stage in this experiment. Based on this criterion, the success rates using the Sod1-KO sperm were remarkably lower compared with those using the WT sperm in IVF (Table 1). However, once fertilized, the embryos developed normally to the blastocyst stage. The low fertilization rate of Sod1-KO sperm was improved by adding reducing agent 2-ME to 100 μM medium during insemination, but hypoxic culture (1% O₂) did not improve the efficiency (Supplemental Fig. S2).

Inability of Sod1-KO Sperm to Penetrate Zona Pellucida

Next, we investigated which stages of the fertilization process were affected in Sod1-KO sperm. The sperm appeared to pass through clumps of the cumulus cells and reach zona pellucida (Fig. 2). After 1 h of insemination, more Sod1-KO sperm than WT sperm were bound to the zona pellucida, and the number of bound Sod1-KO sperm was consistently larger than that of WT sperm thereafter. To examine whether fertilization was successful or not, the cortical granules of the oocytes were visualized by reacting with FITC-PSA. A meiotic second metaphase plate was absent in the oocytes inseminated with WT sperm but still present in those with Sod1-KO sperm, suggesting that the fertilization was unsuccessful with Sod1-KO sperm.

We then compared fertilization rates of the sperm using WT oocytes with and without zona pellucida (Fig. 3). Here we regarded fertilization successful when pronuclei were detected in the zygotes after IVF. Although the fertilizing rates of the oocytes with zona pellucida were lower with Sod1-KO sperm than with WT sperm, no difference was observed between WT and Sod1-KO sperm when zona pellucida-free oocytes were used.
Incubation Time-Dependent Decline in the Sod1-KO Sperm Motility

The sperm were incubated in a drop medium at indicated times, and their motility and progressing abilities were evaluated by CASA (Fig. 4, A and B). Although the contents of the motile and progressive sperm were similar between Sod1-KO and WT sperm following a 3-h incubation, these parameters had markedly declined in Sod1-KO sperm thereafter. When ATP contents were measured, they were markedly decreased in Sod1-KO sperm compared with WT sperm during incubation (Fig. 4C). Lipid peroxidation products, as judged by TBARS, adversely increased in Sod1-KO sperm in an incubation time-dependent manner (Fig. 4D).

Impaired Tyrosine Phosphorylation in the Sod1-KO Sperm

Finally, we examined levels of phosphotyrosine in sperm, which is a hallmark of sperm capacitation. Proteins were extracted from the collected sperm and subjected to immunoblot analysis using anti-phosphotyrosine antibody (Fig. 5). Tyrosine phosphorylation of the protein at 110 kDa in size showed only slight changes in both WT and KO sperm throughout the incubation time. One protein band was observed around 65 kDa at time 0 in both WT and KO sperm to a similar extent, but it disappeared at the beginning of the incubation. In the case of the protein at 70 kDa, the tyrosine phosphorylation increased during the course of incubation as reported previously [24]. It is noteworthy that the levels of phosphorylation were lower in Sod1-KO sperm than those in WT sperm. Thus, the process of phosphotyrosine formation was impaired in the Sod1-KO sperm.

DISCUSSION

In this communication, we showed how the oxidative insult that is caused by a SOD1 deficiency results in decreased sperm fertilizing ability during the IVF process. Because the abnormality accompanied declining ATP levels, elevated levels of lipid peroxidation products, and malfunction of tyrosine phosphorylation in sperm, these oxidative damages together...
would impart mobility and result in a decreased fertilization rate of Sod1-KO sperm.

Sod1-KO male mice typically do not show an abnormality in fertility under conventional breeding conditions [11, 12], which was also confirmed in the present study. Although Sod1-KO mice were small (Iuchi et al. [17] and the present study) and possessed small male reproductive organs compared with WT mice (Supplemental Fig. S1), there were no significant differences in the organ size per body weight, with the exception of the seminal vesicle. In addition, serum levels of testosterone, a male hormone essential for sexual maturation, were similar between Sod1-KO and WT male mice, which was consistent with normal male reproductive ability.

We found, however, that Sod1-KO sperm showed significantly low fertilizing ability in IVF (Table 1). Lower oxygen concentrations (1%) in culture did not improve the fertilizing ability of the Sod1-KO sperm (Supplemental Fig. S2), although the same manipulation rescued the impaired development of Sod1-heterozygote embryos [14]. Heterozygous embryos produced with Sod1-KO oocytes were totally arrested in two cells, as reported previously [14], but those produced with Sod1-KO sperm, once fertilized, developed normally, as observed in this study (Table 1). The difference would be attributable to amounts of SOD1 in the two kinds of embryos. The embryos from Sod1-KO oocytes have no SOD1 before expression of the sperm gene and start producing SOD1 only after fertilization. The amounts of such a low level of SOD1 would not be sufficient for supporting further development. On the other hand, WT oocytes originally have a certain level of the SOD1, which is carried over from primary oocytes, and hence would be able to go through the two-cell stage and advance further development. In a similar way, because Sod1-KO sperm do not possess SOD1, they would be highly sensitive to ROS produced during the fertilizing process. The oxidative damage impaired Sod1-KO sperm and caused the decreased fertilizing ability, but the resultant heterozygous embryos developed normally. However, although administration of 2-ME significantly increased the fertilizing ability of Sod1-KO sperm, the reductant had no effect on the development of the Sod1-heterozygote embryos derived from Sod1-KO oocytes [14]. Thus, molecules that were potential targets of the elevated ROS due to SOD1 deficiency appeared to differ between sperm and oocytes/embryos during IVF.

The Sod1-KO sperm produced less ATP than the WT sperm after 5 h of incubation (Fig. 4C), which influenced the motility of sperm flagella. Although the reduction in ATP in the Sod1-KO sperm was not great and was evident only after long periods of incubation, quick movements of the sperm during the fertilizing process consumed ATP more rapidly. This energy consumption resulted in a decrease in the success rate and increased the number of sperm that adhered to the oocyte. Because polyunsaturated fatty acids support the rapid movement of sperm flagella [7], the elevation in the lipid peroxidation of Sod1-KO sperm (Fig. 4D) also was involved in the impaired sperm motility.

Capacitation is required for freshly ejaculated sperm to acquire fertilizing capability, such as penetration of the zona pellucida of oocytes. Among several factors, tyrosine phosphorylation is a hallmark of capacitation [25, 26]. We found impaired tyrosine phosphorylation of the 70-kDa proteins (Fig. 5), which were likely to be protein kinase A-anchoring proteins, such as AKAP-3 and AKAP-4 [27], in the Sod1-KO sperm. SOD1 deficiency caused an elevation of ROS to excess levels and resulted in the oxidative modification of sperm molecules, such as lipids (Fig. 4D) and essential thiols for sperm function.

The elevated ROS would also affect kinases and phosphatases involved in capacitation, lead to the malfunction of tyrosine phosphorylation, and cause a decline in fertilization ability [28]. Although cAMP-dependent protein kinase, which phosphorylates serine/threonine residues, plays an essential role in capacitation, protein kinases and phosphatases responsible for tyrosine phosphorylation during capacitation are not well understood [29]. In general, levels of phosphotyrosine in proteins depend on balances between tyrosine kinases and phosphotyrosine phosphatases in response to stimuli [30]. Because phosphotyrosine phosphatases have a highly reactive cysteine residue at the catalytic site and are prone to oxidative inactivation, the increased ROS would cause inactivation of them, and consequently this would elevate tyrosine phosphorylation in sperm [31]. The data regarding the 70-kDa protein, however, disagree with this conclusion (Fig. 5), which suggests that levels of the phosphorylated tyrosines were determined in a more complicated manner during sperm capacitation. Thus, it is hard to explain logically the decreased levels of tyrosine phosphorylation in the SOD1-deficient sperm in the context of sperm capacitation under situations without sufficient information on the tyrosine kinases and the phosphotyrosine phosphatases responsible for sperm capacitation.

Moreover, although the addition of antioxidants to a culture medium raises the success rates of IVF, excessive antioxidants tend to reduce the rate by supererogatory scavenging of ROS [32, 33]. These conflicting results may be explained by disturbing the signaling function of ROS that plays a role in both tyrosine phosphorylation and cholesterol release during the capacitation process of the sperm [32, 34, 35]. Thus, further studies are required to clarify the causal association between impaired tyrosine phosphorylation and elevated oxidative stress.

When we examined the fertilizing ability of Sod1-KO sperm in IVF, large numbers of sperm were found to bind the zona pellucida after insemination compared with WT sperm (Fig. 2). The presence of a cortical granule on the inseminated oocytes, as judged by FITC-conjugated PSA staining, and the oocytes remaining at the meiotic second metaphase clearly indicated that the oocytes were not fertilized. Because zona pellucida is

![Image](https://www.biolreprod.org)
involved in avoiding polyspermy in conjunction with cortical granules released from the cell surface of the oocyte [36, 37], it is essential for sperm to pass through zona pellucida and reach the oocyte surface for fertilization. Among several possible explanations, we hypothesized that the Sod1-KO sperm were exhausted and could not accomplish the journey to the oocyte because they consumed ATP quickly, and consequently lost motility (Fig. 4). This notion was also supported by the experiment using the oocytes without zona pellucida, which showed the same success rate for Sod1-KO sperm as WT sperm in IVF (Fig. 3). Because unsaturated lipids in the plasma membrane support quick movement of the sperm, elevated lipid peroxidation in Sod1-KO sperm would affect their motility [38]. Collectively, the data show that the fertilization disorder of Sod1-KO sperm in IVF was at least partially due to the inability to penetrate the zona pellucida. Although we did not examine the acrosomal reaction in this study, this could also exhibit a malfunction.

This is the first observation that an intrinsically produced ROS due to a SOD1 deficiency caused a defect in the fertilizing ability of sperm. Because tyrosine phosphorylation, which is required for capacitation, was impaired in Sod1-KO sperm, this process appears to be highly vulnerable to oxidation by ROS. Because SOD1 can rescue sperm with appropriate reductants, we can rescue the impaired sperm that show a low fertilizing ability in IVF, the Sod1-KO sperm may be a useful model to screen such reductants for the purpose of increasing the success rate.

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REFERENCES