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Research Article
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Research Article

L-amino acid oxidase 1 in sperm is associated with reproductive performance in male mice and bulls

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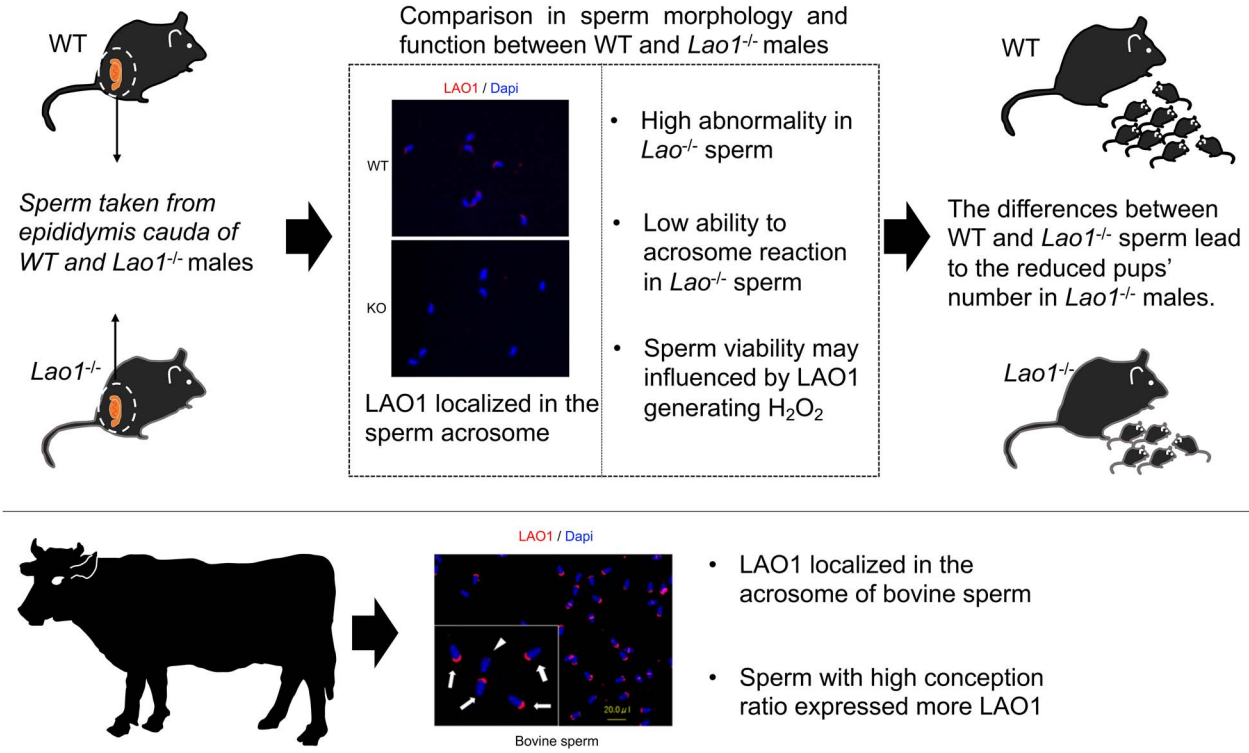
Abstract

Sperm quality is an important indicator of male fertility, and a suitable biomarker enables the selection of high-quality spermatozoa. We previously found that L-amino acid oxidase encoded by the L-amino acid oxidase 1 (*Lao1*) gene exerts biological roles in the mammary gland and brain by converting specific L-amino acids into keto acids, ammonia, and hydrogen peroxide (H_2O_2). Here, we describe the role of *Lao1* in male reproduction. *Lao1*-deficient (*Lao1*$^{-/-}$) male mice generated fewer pregnant embryos and pups as well as lower ratios of fertilized oocytes and even ovulated number was not different, suggesting that male subfertility caused the smaller litters. We found that LAO1 expressed in acrosomes is associated with high malformation ratios and low viability of *Lao1*$^{-/-}$ sperm. Wild-type (WT) sperm produced more H_2O_2 than *Lao1*$^{-/-}$ sperm, and 10 μM H_2O_2 restored knockout (KO) sperm viability in vitro. In addition, the sperm ratio of induced acrosome reaction was higher in WT than in *Lao1*$^{-/-}$ sperm incubated with the calcium ionophore A23187. Moreover, LAO1 expression was abundant in bovine sperm with high fertilization ratios. We concluded that LAO1 localized in the sperm acrosome influences sperm viability and morphology as well as the acrosome reaction, and that *LAO1*-deficient sperm might cause male subfertility. Thus, LAO1 might serve as a novel marker for selecting high-quality spermatozoa, especially for livestock reproduction.

Summary sentence

LAO1 localized in the sperm acrosome may influence sperm viability and acrosome reaction by metabolizing L-amino acid to produce H_2O_2.

Graphical Abstract



Key words: hydrogen peroxide, L-amino acid, acrosome reaction, sperm viability, male fertility.

Introduction

The testis produces male hormones (androgens) and germ cells (spermatozoa) that are released into seminiferous tubules from Sertoli cells. The spermatozoa then enter the epididymis, where they undergo further maturation to acquire motility [1]. As sperm enter the female reproductive tract, they pass through activation, capacitation, and acrosome reactions, which are necessary for engagement with oocytes [2]. Artificial insemination (AI) is now widely practiced, not only for commercial livestock, but also for humans. The complex physiological procedures influencing sperm maturation and function must be understood to select high-quality semen and/or spermatozoa.

Reactive oxygen species (ROS) signaling is one of the most important regulatory pathways associated with spermatozoon function [3]. At physiological concentrations, ROS modulate the morphological changes required for sperm maturation and the crucial processes involved in capacitation, hyperactivation, and acrosome reactions [4–6]. Conversely, an atypical increase in ROS concentrations causes oxidative stress that leads to the disruption of DNA integrity in spermatozoa via the induction of apoptosis [7, 8]. These findings suggest that a balance between the generation and elimination of ROS is critical for normal spermatozoon functions.

L-amino acid oxidase (LAAO) is a flavoenzyme that catalyzes the oxidation of L-amino acids to alpha-keto acid, ammonia, and hydrogen peroxide (H_2O_2) [9]. This enzyme is expressed and plays different roles in diverse organisms [10, 11]. The activity of LAAO in bovine and ovine spermatozoa is the suspected source of ROS-mediating sperm functions [12, 13]. Most mammals possess two related molecules encoded by L-amino acid oxidase 1 (*Lao1*) and *IL4-induced gene 1* (*Il4i1*), which are mainly expressed in lactating mammary glands and immune cells, respectively [14, 15]. In addition, spermatozoa of several species express *Il4i1*, and IL4I1 protein is more abundant in fertile sperm from boars and bulls [16–18]. Thus, *Il4i1* might be considered as a marker of functional spermatozoa. However, fertility issues have not been identified in *Il4i1*-deficient mice (Mouse Genome Informatics database; http://www.informatics.jax.org/marker/MGI:109552).

We previously generated *Lao1*-deficient mice ($Lao1^{-/-}$) and found that LAO1 is expressed in the mammary gland; it is involved in the anti-bacterial activity of milk and the colonization of gut microbiota during infancy [14, 19]. LAAO is also expressed in the brain and it is important for learning and memory [20]. As these studies were conducted, we found that fewer litters were generated when female mice were bred with $Lao1^{-/-}$ males. Therefore, the present study investigated the fertility and reproductive performance of $Lao1^{-/-}$ male mice. We show that LAO1 protein is localized in the acrosomes of spermatozoa and produces H_2O_2, which enhances sperm survival and acrosome reactions. We also compare LAO1 expression in bovine sperm with fertility rates. Our findings suggest that ROS generated by LAO1 play a positive role in increasing spermatozoon activity and that *LAO1* is associated with reproductive performance in male mammals.

Materials and methods

Mice

We created *Lao1*$^{-/-}$ mice as described in [14]. Wild-type (WT) and *Lao1*$^{-/-}$ mice on a C57BL/6 genetic background were maintained at 23 ± 2°C under a 14-h light schedule (lights on 05:00–19:00 h) with free access to food and tap water. Fertility was evaluated in mice aged 10–16 weeks. Male and female mice of each genotype were mated and numbers of pups were recorded. Ovaries from post-mating WT and *Lao1*$^{-/-}$ female mice were used for histological analysis to confirm the number of ovulations. All experiments involving mice proceeded according to the guidelines of the University Animal Care and Use Committee of the Tokyo University of Agriculture and Technology (24-80).

Fertilization and pregnancy rates in vivo

The estrus cycle was confirmed by the observation of a vaginal smear, and WT or *Lao1*$^{-/-}$ male mice were introduced to WT female mice during proestrus. The presence of a vaginal plug in the morning (07:00–09:00) was taken as embryonic day 0.5 (E0.5). Oocytes were collected by uterine flushing at E3.5, fertilized (blastocysts) and nonfertilized oocytes were counted using an inverted microscope to determine fertilization ratios. Embryos in uteri of pregnant female WT and *Lao1*$^{-/-}$ mice were counted at E10.5.

Examination of in vitro fertilization

Female WT mice were injected with 5 IU of pregnant mare serum gonadotropin (ASKA Animal Health Co., Ltd, Tokyo, Japan), followed 48 h later by 5 IU of human chorionic gonadotrophin (ASKA Animal Health Co., Ltd) to induce superovulation. A total of 17 h later, cumulus–oocyte complexes from the ampulla oviducts were transferred to 200 µL of human tubal fluid (HTF) medium. Sperm were collected from the cauda epididymis of WT and *Lao1*$^{-/-}$ male mice and capacitated by incubation for 1 h in Krebs–Ringer bicarbonate (TYH) covered with liquid paraffin. Thereafter, the sperm were transferred to HTF medium containing oocytes and incubated in a humidified 5% CO_2 atmosphere at 37°C for 3 h. Thereafter, the oocytes were washed and cultured in K-modified simplex optimized medium (KSOM) containing 3 mg/mL of bovine serum albumin (BSA). The fertilization ratios (%) of two-cell embryos were calculated on the following day. Each experiment included 27–36 oocytes and was repeated four times.

Histological analysis

Ovaries were fixed in 4% paraformaldehyde, embedded in paraffin, and serially sectioned at a thickness of 6 µm. The sections were stained with hematoxylin and eosin, and then corpora lutea were counted in every 10th section.

Hormone analysis

Serum testosterone concentrations were measured using dissociation-enhanced lanthanide fluorescence immunoassay kits (Perkin-Elmer, Waltham, MA, USA) as described by the manufacturer. Hormones were analyzed in six serum samples each from WT or *Lao1*$^{-/-}$ mice. All samples were compared using the same assay, of which the intra-assay coefficient of variation was 5.2%.

Sperm morphology and viability

After dissecting the cauda epididymis, sperm counts in KSOM medium were determined by hemocytometry as described in [21]. Sperm morphology and viability were assessed by adjusting the sperm concentrations to 5 × 10^5/mL by dilution with KSOM medium. Sperm suspensions (one drop per glass slide) were spread on the slides, dried in air, and fixed with cold methanol–acetone (1:1, v/v). Morphology was assessed on some slides using a BZ-X700 microscope (Keyence, Osaka, Japan). Morphological abnormalities of sperm heads were classified as described in [22]. Ratios (%) of sperm with morphological abnormalities were determined.

Sperm were cultured in KSOM medium at 37°C for different durations. Thereafter, sperm suspensions were mixed with equal amounts of 1% aqueous eosin Y, followed by a 2-fold volume of 10% aqueous nigrosine. The viability of stained sperm suspensions on glass slides was identified by microscopy. Ratios of live sperm cells were determined by counting at least 200 sperm. In addition, the viability of sperm cultured with 10 µM H_2O_2 for 3 h were also analyzed.

Immunofluorescence

Sperm slide smears were prepared as described above, fixed with cold acetone–methanol (volume ratio, 1:1), and then nonspecific protein binding was blocked with 5% BSA in phosphate-buffered saline (PBS) containing 1% Triton X-100 for 1 h. The slides were then incubated with rabbit antiserum against LAO1 [14] overnight at 4°C. Thereafter, the slides were incubated in Alexa Fluor 555 conjugated anti-rabbit immunoglobulin G for 1 h at room temperature, and then counterstained with 4',6-diamidino-2-phenylindole (DAPI). Negative controls were reacted against normal rabbit serum instead of primary antibodies. Images were captured using a BX-51 immunofluorescence microscope (Keyence).

Induced acrosome reaction

The acrosome reaction was induced as described in [23]. Briefly, sperm collected from the epididymal cauda of WT or *Lao1*$^{-/-}$ mice (n = 4) were cultured in HTF medium for 30 min, and then incubated with or without 10 µM (final concentration) calcium ionophore A23187 for 1 h. The acrosome status of sperm was determined by Coomassie blue staining, and the acrosomes were stained blue and it disappeared after acrosome reaction. For calculation, at least 250 sperm per sample were counted using a microscope.

Computer-assisted sperm analysis

The motility of sperm collected from the epididymal cauda of WT or *Lao1*$^{-/-}$ mice (n = 5) was determined using computer-assisted sperm analysis (CASA). The measured sperm motility parameters comprised average path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness of track (STR), and linearity of track (LIN). These parameters were determined in at least 250 sperm in at least five nonconsecutive fields within 30 s. The sperm in each field were selected by adjusting the grayscale threshold, and debris and round cells were manually deleted before analysis.

Measurement of H_2O_2

After dissecting the cauda epididymis, sperm were transferred into KSOM medium, the concentrations were adjusted, and then the sperm were incubated in KSOM medium at 37°C for 3 h. Thereafter, the amount of H_2O_2 in the culture medium was measured at 0 and 3 h using Hydrogen Peroxide Assay Kits (National Diagnostics, Atlanta, GA, USA).

Bovine semen samples

Cryopreserved semen from 26 Japanese Black bulls aged 12–134 months were obtained from the AI center of the Livestock Improvement Association of Japan, Inc. The sire conception rate (SCR) was determined by assessing the outcomes of the first AI of Japanese Black or Holstein cows and heifers maintained at private breeding farms in Japan. Over 32 females were inseminated with sperm from each bull. Conception was determined at 60 days by the lack of a return to estrus, rectal palpation, or ultrasonography. Individual SCR scores were calculated by dividing the number of pregnancies by the total number of first AI services. A total of 18 bulls were assigned to a normal fertile group (SCR, 40.4–71.0%). Eight bulls were assigned to a low fertile group (SCR, 2.3–20.0%).

Sample preparation

Cryopreserved semen straws were thawed at 38°C, and then the sperm were washed twice with PBS at room temperature by centrifugation for 5 min at 10 000 rpm. Sperm cells (1×10^7/mL) were fixed with 1% formaldehyde at room temperature for 15 min, and then the reaction was stopped by adding 10 volumes of 200 mM glycine. After centrifuging the sperm at room temperature for 10 min at 5000 rpm, the fixative and glycine were discarded. The sperm pellet was resuspended in PBS, and then sperm suspensions (10 µL) were smeared onto glass slides and dried before immunofluorescence analysis.

Statistical analyses

Data were statistically analyzed using Student *t*-test or one-way analysis of variance (ANOVA), followed by Dunnett multiple range comparisons or two-way ANOVA, with Bonferroni multiple range comparisons using Prism 5 (GraphPad Software, Inc., San Diego, CA, USA). Values with $P < 0.05$ were considered statistically significant.

Results

Reduced pup numbers due to male infertility

Compared with WT mice, breeding *Lao1*$^{-/-}$ mice produced fewer pups (Figure 1A). The number of pups was also reduced when *Lao1*$^{-/-}$ male mice were bred with either WT or *Lao1*$^{-/-}$ female mice. The number of pups was not significantly reduced when heterozygous (*Lao1*$^{+/-}$) or WT male mice were bred with *Lao1*$^{-/-}$ females (Figure 1A). The number of embryos at E10.5 of *Lao1*$^{-/-}$ female mice mated with *Lao1*$^{-/-}$ males was decreased, whereas the number of ovulations indicated by the corpus luteum number remained unchanged (Figure 1B and C). The fertilized oocyte ratio was decreased in the oviduct at E3.5 in WT females mated with *Lao1*$^{-/-}$ males (Figure 1D). These results indicated that the reduction in pup numbers associated with the loss of *Lao1* is the result of a problem with male fertility.

LAO1 is expressed in sperm

We investigated serum testosterone levels and histological testicular changes to determine the mechanisms responsible for the decreased fertility of male *Lao1*$^{-/-}$ mice. However, testis weight, testosterone levels, and histological structures did not obviously differ (Figure 2A–C). Immunofluorescence analysis showed LAO1 protein expression in the regions of seminiferous tubules containing sperm (Figure 2C). We analyzed the concentration and morphology of sperm collected from the cauda epididymis of WT and *Lao1*$^{-/-}$ male mice to confirm LAO1 localization in sperm. Sperm counts did not significantly differ between WT and *Lao1*$^{-/-}$ male mice (Figure 3A). However, the ratio of abnormal to normal sperm in *Lao1*$^{-/-}$ male mice was increased, and misshapen heads were particularly obvious (Figure 3B). We investigated the ability of sperm acrosome reactions induced by A23187 in WT and *Lao1*$^{-/-}$ sperm. The ratios of sperm having acrosome were high level before the induced acrosome reactions and there is no difference between WT and *Lao1*$^{-/-}$ sperm. After the induced acrosome reactions, however, the ratio of sperm remaining acrosome in *Lao1*$^{-/-}$ sperm was significantly higher than in the WT sperm (Figure 3C). We also confirmed the expression of LAO1 protein in acrosomes of sperm in vitro, and that the expression of LAO1 protein was lost after the induced acrosome reaction by A23187 (Figure 3D). Moreover, assessment of in vitro fertilization (IVF) potential showed normal and comparable fertilization potential between WT and *Lao1*$^{-/-}$ sperm (Figure 3E). The results of CASA showed that none of the motility parameters VAP, VSL, VCL, ALH, BCF, STR, and LIN significantly differed between WT and *Lao1*$^{-/-}$ sperm (Table 1).

H_2O_2 produced by LAO1 supports sperm viability

The viability of *Lao1*$^{-/-}$ sperm cultured in KSOM medium for 3 and 4 h was decreased (Figure 4A). The H_2O_2 concentration was significantly increased in WT, but not in *Lao1*$^{-/-}$ sperm after 3 h of culture (Figure 4B). We incubated WT and *Lao1*$^{-/-}$ sperm with 10 µM H_2O_2 for 3 h, and then assessed their viability to determine the relationship between sperm viability and H_2O_2 production. We found that the viability ratio of *Lao1*$^{-/-}$ sperm approached that of WT sperm (Figure 4C).

Ratios of LAO1-positive spermatozoa are lower in bovine low-fertility sperm

Based on the SCR, 18 and 8 bulls were assigned to normal and low-fertility groups (Figure 5A). The immunofluorescence data showed that LAO1 was also localized in acrosomes of bovine sperm and that the ratio of LAO1-positive sperm was decreased in the low-fertility, compared with the normal group (Figure 5B). Multiple comparison tests showed that a cut-off of 31.9% LAO1-positive sperm yielded 94.4% sensitivity and a 100% specificity.

Discussion

The production of viable offspring requires high-quality oocytes and sperm. The smaller litters of crossbred WT and *Lao1*$^{-/-}$ mice suggested that LAO1 functions in reproduction. The numbers of corpora lutea obtained from the WT and *Lao1*$^{-/-}$ mouse ovaries confirmed that the decreased numbers of pups were not associated with ovulation. Moreover, embryo numbers and fertilized oocyte ratios were significantly decreased in female mice mated with *Lao1*$^{-/-}$ males compared with WT males, indicating that low fecundity of *Lao1*$^{-/-}$ mice caused the decreased numbers of offspring. Our investigations of the differences in the reproductive organs between WT and *Lao1*$^{-/-}$ male mice included assessment of testicular weight and histology, as well as the levels of circulating testosterone. However, reproductive parameters including testicular weight, circulating testosterone levels, and histological findings did not significantly differ between WT and *Lao1*$^{-/-}$ male mice. We localized LAO1 at the inner face of seminiferous tubules, specifically in the acrosomes of mouse spermatozoa. Our findings were consistent with those of three studies of LAO1 or its homolog IL4I1 in human, horse, and boar sperm [16, 17, 24]. We also found morphological abnormalities

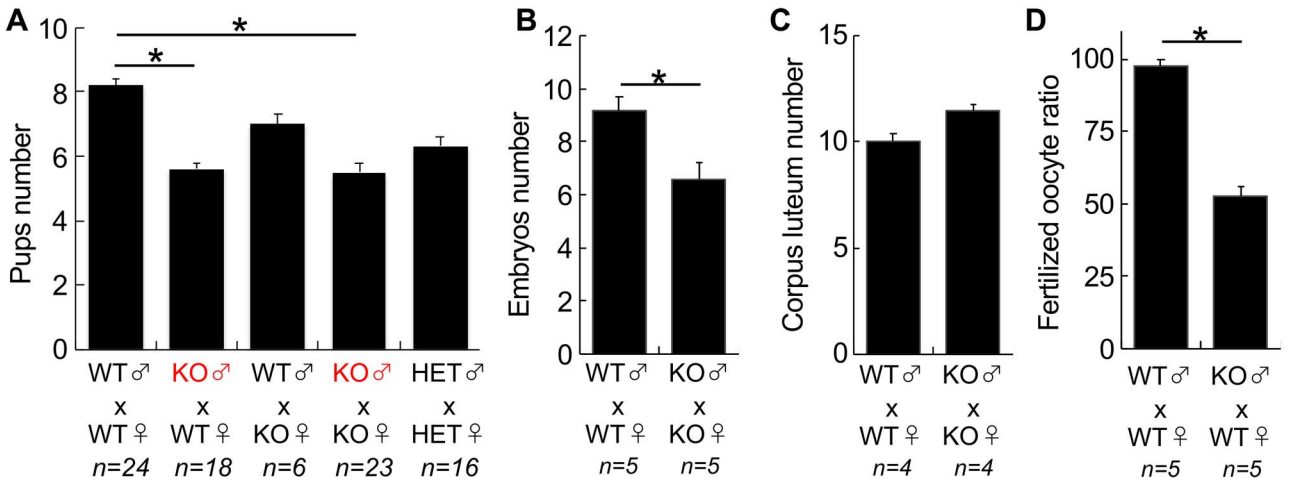


Figure 1. (A) Pup numbers from cross-bred WT, *Lao1*$^{-/-}$, and *Lao*$^{+/-}$ mice. (B) Numbers of embryos in WT and *Lao1*$^{-/-}$ female mice at E10.5 after mating with WT or *Lao1*$^{-/-}$ male mice. (C) Numbers of corpora lutea in WT and *Lao1*$^{-/-}$ female mice. (D) In vivo fertilization ratio of WT or *Lao1*$^{-/-}$ males mated with WT female mice at E3.5; $*P < 0.05$.

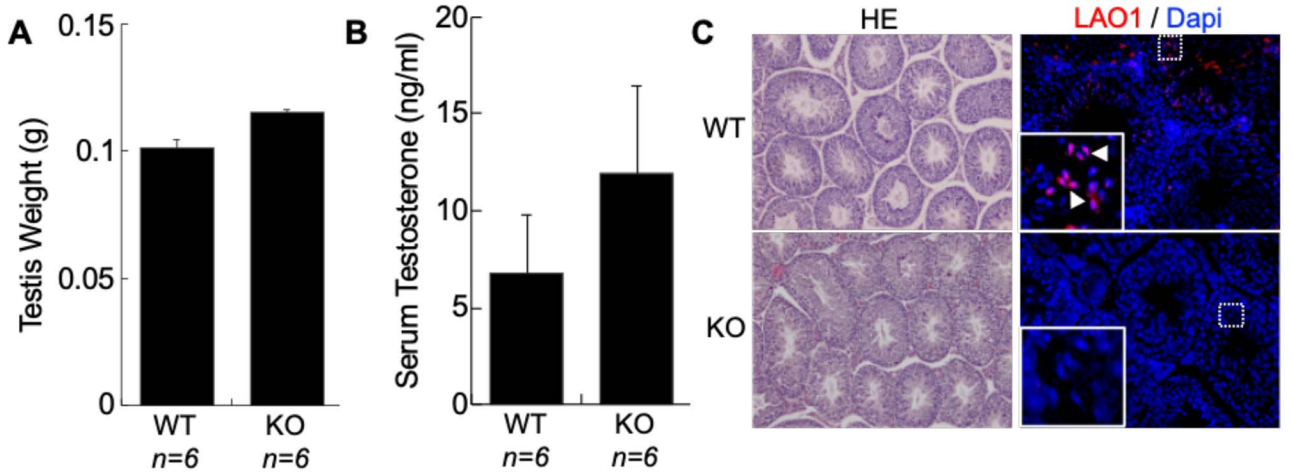


Figure 2. Comparison of (A) testicular weight, (B) circulating testosterone concentration, and (C) histological morphology and LAO1 localization in testes between WT and *Lao1*$^{-/-}$ male mice. Insertions are enlarged views of square area with white dash line. White arrows indicate positive LAO1 staining in sperm.

Table 1. The comparison in sperm motility parameters between WT and *Lao*$^{-/-}$ sperm

Sperm motility parameters	WT sperm	*Lao* $^{-/-}$ sperm	Significance
Average path velocity (VAP)	80.41 ± 1.05 μm/s	77.58 ± 1.02 μm/s	No
Straight line velocity (VSL)	56.95 ± 0.99 μm/s	55.40 ± 0.93 μm/s	No
Curvilinear velocity (VCL)	144.1 ± 1.6 μm/s	142.9 ± 1.8 μm/s	No
Amplitude of lateral head displacement (ALH)	7.960 ± 0.122 μm	7.947 ± 0.123 μm	No
Beat cross frequency (BCF)	24.19 ± 0.41 Hz	25.02 ± 0.42 Hz	No
Straightness of track (STR)	69.48 ± 0.59 (%)	69.95 ± 0.58 (%)	No
Linearity of track (LIN)	41.43 ± 0.57 (%)	40.98 ± 0.55 (%)	No

such as misshapen heads in sperm from *Lao1*-deficient mice. Overall, these data indicated a connection between the biological roles of LAO1 and sperm function.

Early studies of the biological function of LAO1 can be traced back to the isolation and biochemical characterization of LAAO in snake venom [9]. LAAO is involved in H_2O_2 production and it catalyzes the oxidation of L-amino acids. In line with this function, the present findings showed that less H_2O_2 was produced in culture medium of *Lao1*$^{-/-}$ than WT sperm. Generally, H_2O_2 is considered to induce oxidative stress that negatively impacts cell function [25]. However, H_2O_2 also serves as a messenger in some signal transduction pathways. H_2O_2 is involved in the promotion of cell survival

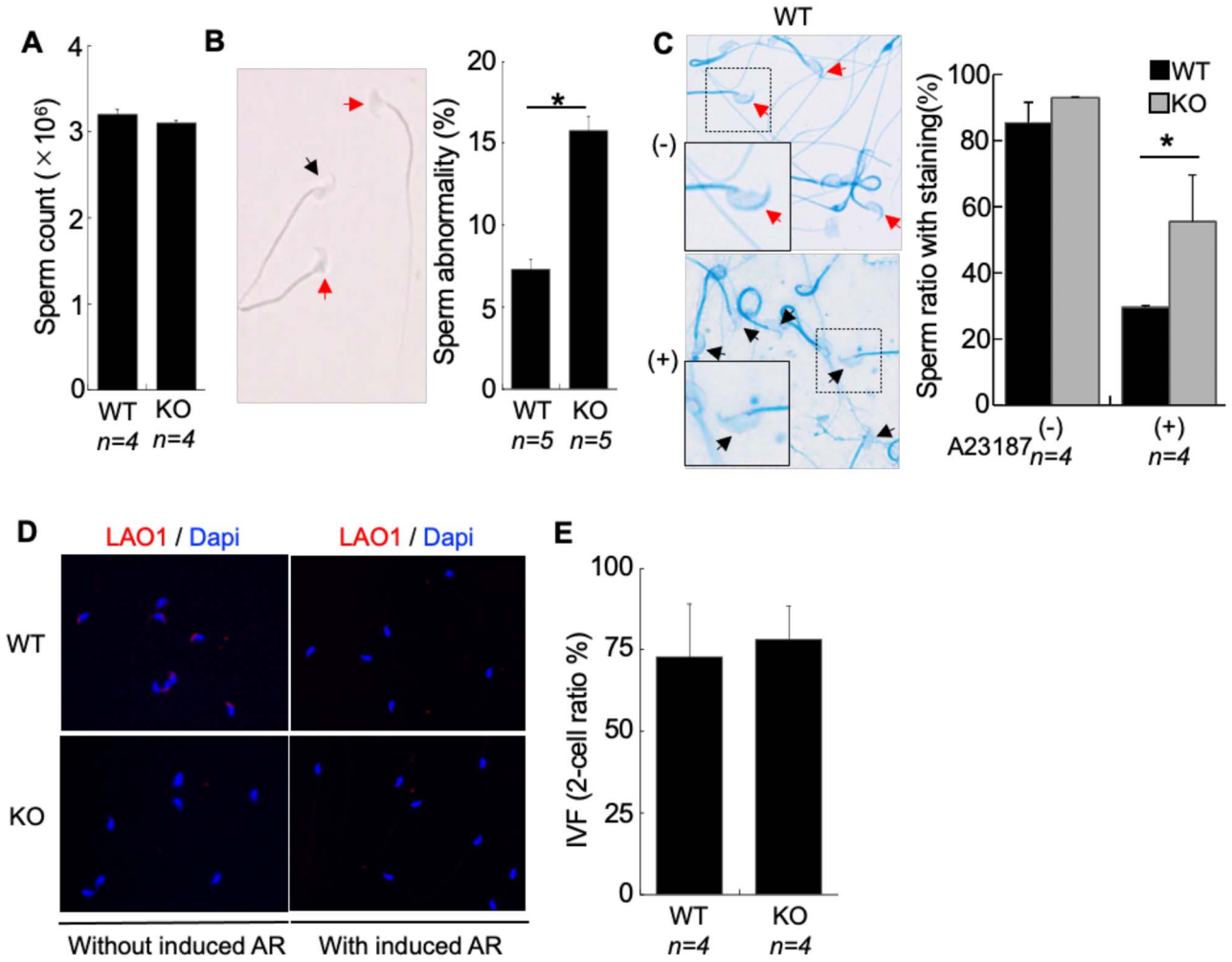


Figure 3. Comparisons of (A) sperm count from cauda epididymis, (B) ratios of sperm abnormalities (black and red arrows, sperm with normal and abnormal morphologies, respectively), (C) ratios of sperm with Coomassie blue staining without or with A23187 treatment (red and black arrows, sperm with or without Coomassie blue staining, respectively), (D) LAO1 localization (red) in sperm without or with induced acrosome reaction (AR), and (E) in vitro fertilization ratio (two-cell stage) between WT and *Lao1*$^{-/-}$ male mice; $*P < 0.05$.

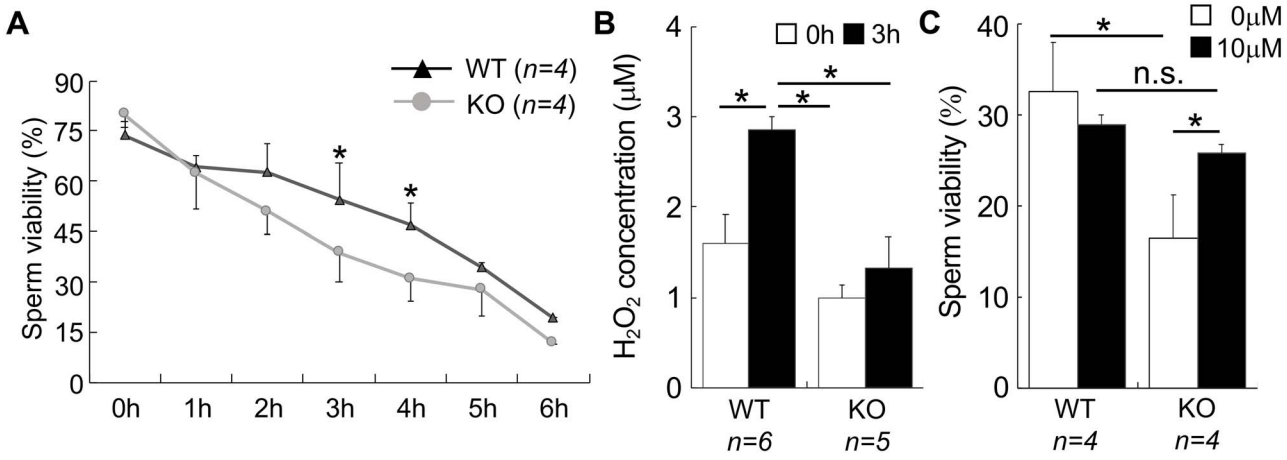


Figure 4. Comparisons of (A) sperm viability at various time points during culture in KSOM medium in vitro, (B) H_2O_2 concentrations in culture medium at 0 or 3 h, and (C) sperm viability after incubation with 10 μM H_2O_2 between WT and *Lao1*$^{-/-}$ sperm. $*P < 0.05$; n.s., no significant difference.

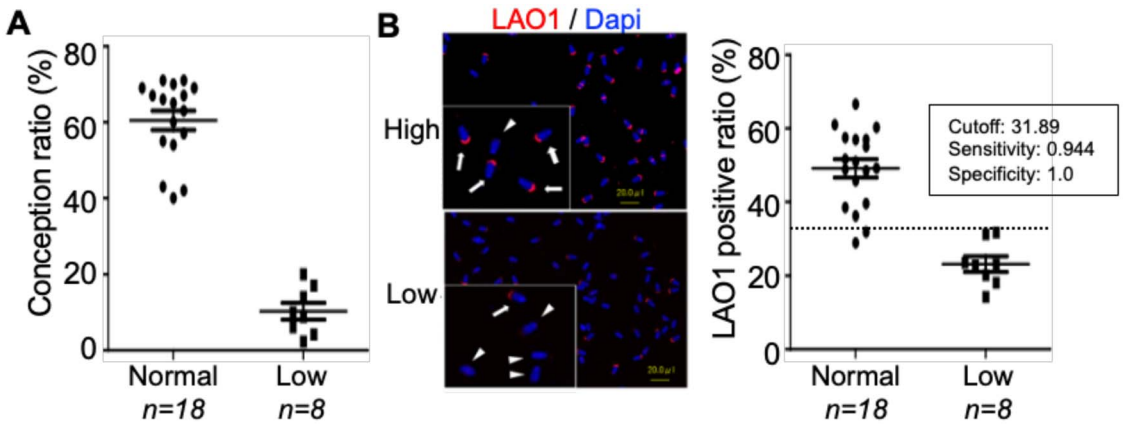


Figure 5. Comparison of bovine sperm with normal or low fertility to (A) conception ratios and (B) LAO1 immunofluorescence staining (red) in sperm and ratios of LAO1-positive sperm. Bold arrows and arrowheads indicate LAO1 positive and negative staining, respectively.

[26], proliferation [27, 28], migration [29], and differentiation [30, 31]. The present study found that *Lao1*$^{-/-}$ sperm viability increased when H_2O_2 was added to sperm culture medium. Other evidence suggests that H_2O_2 promotes cell survival by activating AKT or the hypoxia-inducible factor-1alpha signaling pathways [26, 32]. Although details of H_2O_2 signaling in mice sperm are unclear, our findings suggest that H_2O_2 produced by LAO1 in mouse sperm is a cell survival signaling cue. In addition, morphological abnormalities were increased in *Lao1*$^{-/-}$ sperm. Although how an LAO1 deficiency could affect sperm morphology is unknown, the influence of H_2O_2 on the cytoskeleton might offer a clue [33, 34]. H_2O_2 might modulate the cell cytoskeleton by triggering phosphorylation of the actin-binding protein myristoylated alanine-rich C-kinase substrate [33] or by oxidizing the filamentous actin-binding mediator protein cofilin 1 [34]. Thus, H_2O_2 produced by LAO1 in sperm might also affect rearrangement of sperm cytoskeleton and the loss of H_2O_2 due to an LAO1 deficiency could lead to sperm morphological abnormality.

In addition to survival in female reproductive ducts, the acrosome reaction is a critical step that occurs during the journey of sperm to reach and interact with oocytes and it is necessary for successful fertilization. In the current study, the acrosome reaction induced by A23187 did not progress well in *Lao1*$^{-/-}$ sperm. Combined with the localization of LAO1 in the sperm acrosome, LAO1 in sperm might influence the acrosome reaction. Appropriate intrinsic or extrinsic ROS levels have positive physiological functions in sperm including capacitation and the acrosome reaction [35–39]. H_2O_2 benefits the boar sperm acrosome reaction [38, 39]. H_2O_2 enhances the A23187-induced acrosome reaction with human spermatozoa, and this process can be prevented by catalase or superoxide dismutase [4, 40]. Taken together, this evidence indicates that H_2O_2 produced by LAO1 could explain the increased acrosome reaction in WT sperm. With an insufficient acrosome reaction, decreased sperm viability, and increased atypical sperm morphology, breeding with *Lao1*$^{-/-}$ males resulted fewer fertilized oocytes and smaller litters. However, the number of two-cell in IVF did not differ, which seemed to contradict the findings in vivo. This could be explained by the different conditions involved in fertilization in vivo and in vitro. Sperm do not need to struggle for survival before they contact oocytes during IVF; thus, the fertilization process is relatively easier in IVF than in fertilization in vivo. The differences in viability and the ability to induce acrosome reactions between WT and *Lao1*$^{-/-}$ sperm led to distinct fertilization ratios in vivo. In addition, we could not exclude possible roles of LAO1 during early embryo development.

The prognosis and diagnosis of male infertility and subfertility in humans and other animals is a major global concern. About half of all human fertility problems are due to male-associated factors [41]. In animal husbandry, 50% of breeding system failures are attributed to the sire, leading to huge economic losses in the commercial sector [42]. Therefore, new methods or markers that could ensure more accurate prognoses and diagnoses of male infertility/subfertility would have significant value to society. We found that LAO1 was expressed in mouse and bovine sperm, and that high fecundity of bovine sperm was significantly associated with more abundant LAO1 expression. Furthermore, the abundant protein expression of LAAO in boar spermatozoa is linked with larger litters [17]. These results suggest that LAO1 expression is not confined to mouse sperm and might also be associated with sperm function in other animals. Therefore, we suggest that LAO1 expression level could be applied for prognoses and diagnoses of male infertility/subfertility in livestock.

In conclusion, a deficiency in *Lao1*$^{-/-}$ mouse sperm led to fewer pups, and LAO1 localized in acrosomes influences sperm viability as well as capacitation by metabolizing L-amino acids to produce H_2O_2. Moreover, LAO1 expression in bovine sperm correlated with sperm fertility. Therefore, LAO1 might serve as a marker to screen high-quality semen and/or spermatozoa for assisted reproduction, such as AI in animal husbandry.

Conflict of interest

The authors have declared that no conflict of interest exists.

Authors' Contributions

HZ, SK, HL, and JK conducted the mouse experiments and animal husbandry. HZ participated in manuscript preparation. MK and KU analyzed the bovine sperm data. GW and WJ discussed the results and revised the manuscript. KN conceived the study and wrote the manuscript.

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