Effect of *Lepidium meyenii* on *in vitro* fertilization via improvement in acrosome reaction and motility of mouse and human sperm

Yusuke Aoki[1,2], Akira Tsujimura[1], Yuki Nagashima[1], Ippei Hiramatsu[1,2], Yuka Uesaka[1], Taiji Nozaki[1], Tatsuya Ogishima[1], Masato Shirai[1], Yukihiro Shoyama[3], Hiromitsu Tanaka[3], Shigeo Horie[2]*

[1]Department of Urology, Juntendo University Urayasu Hospital, 2-1-1 Tomioka, Urayasu, Chiba 279-0021, Japan

[2]Department of Urology, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

[3]Faculty of Pharmaceutical Sciences, Nagasaki International University, 2825-7 Huis Ten Bosch, Sasebo, Nagasaki 859-3298, Japan

***Correspondence:** Shigeo Horie, Department of Urology, Juntendo University Graduate School of Medicine, 2-1-1 Hongo Bunkyo-ku Tokyo, 113-8431 Japan. Tel: +81-3-5802-1227; Fax: +81-3-5802-1227; E-mail: shorie@juntendo.ac.jp

ABSTRACT

Purpose: The direct effects of *Lepidium meyenii* (Maca) on sperm remain unclear. Herein, we examined the direct effect of Maca on *in vitro* fertilization.

Methods: We examined the fertilization rate in a mouse model and the rate of acrosome reaction in sperm from transgenic mice expressing enhanced green fluorescent protein (EGFP) in a Maca extract-containing human tubal fluid (HTF) medium. Using human sperm, we assessed acrosome status via fluorescein isothiocyanate-conjugated peanut agglutinin (FITC-PNA) staining and performed detailed analysis using a sperm motility analysis system (SMAS).

Results: In the mouse model, the fertilization rate in the Maca extract-containing HTF was significantly higher than that in the control medium. The acrosome reaction rate in sperm from transgenic mice expressing EGFP was also significantly higher in the Maca extract-containing HTF than that in the control medium. Similarly, a high acrosome reaction rate, identified via FITC-PNA staining of human sperm samples, was found in the Maca extract-containing HTF compared with that in the control medium. Human sperm motility in the Maca extract-containing HTF was also increased compared with that in the control medium as measured using an SMAS.

Conclusions: Maca improved *in vitro* fertilization rates by inducing an acrosome reaction and increasing sperm motility.

Keywords: acrosome reaction, in vitro fertilization, *Lepidium meyenii*, Maca, sperm motility

INTRODUCTION

Human fertility rates are declining worldwide, and infertility is a serious and rapidly increasing problem in reproductive medicine and public health. Infertility is defined as failure to achieve clinical pregnancy after ≥12 months of regular unprotected sexual intercourse [1], and its prevalence is approximately 9% [2]. It is known that the cause of infertility exists in the male partner in approximately half of all infertile couples. There are several causes of male infertility, including production of defective spermatozoa, obstruction of the reproductive tract, inflammation, and sexual disorders such as erectile dysfunction and retrograde ejaculation [3]. A recent review revealed that the prevalence of male infertility, defined as men reporting an experience of infertility (generally >12 months in duration), varied from 9.0% to 15.8% in surveys of general populations [4]. Treatment of male infertility has become a significant issue for urologists and physicians in reproductive medicine. Therefore, several technical innovations in surgical treatment, including microdissection testicular sperm extraction for patients with azoospermia and microsurgery for patients with varicoceles, have been reported [5, 6]. However, very few treatment options are available for idiopathic oligoasthenoteratozoospermia (OAT), which is the most common phenotype observed in male infertility. Although more than half of all infertile couples seek medical care, no effective and reliable medicines have been developed or approved for patients with OAT so far [2]. To address this problem, several supplements, including vitamins B12, C, and E, and herbal medicines have been used clinically as OAT treatment options and have shown relatively favorable effects on spermatogenesis. Antioxidants such as coenzyme Q10 and L-carnitine have also been used to reverse oxidative stress-induced sperm dysfunction, and small clinical trials have shown their efficacy, particularly with

57 respect to sperm motility [7–9]. However, randomized control studies with larger numbers of participants have
58 not yet been performed to show clinical evidence for the recommendation of these antioxidants as OAT
59 treatment options. Thus, in practice, several antioxidants and other substances have been used for treating male
60 infertility based on the clinical experience of the attending physicians.

61 Among several candidate medications for male infertility, *Lepidium meyenii* (Maca) has been one of the
62 most popular supplements gaining increased attention as a treatment option. Maca is known as an antioxidant
63 supplement [10] with efficacy in several diseases and conditions. It has been reported that Maca may improve
64 hypertension, diabetes mellitus, dyslipidemia, and depression. With respect to sexual and reproductive function
65 in rats, Maca has favorable effects on sexual behavior [11], testicular weight [12], and spermatogenesis,
66 particularly at the initial stages [13]. In humans, treatment with Maca is reported to improve sexual desire
67 without a change in the state of mind in healthy adult men [14]. A systematic review of three randomized
68 clinical trials and two uncontrolled observational studies has already provided suggestive evidence for the
69 effectiveness of Maca in improving semen quality, although the total number of trials and the total sample size
70 of the included studies prevented concrete conclusions [15]. To date, the direct effect of Maca on sperm has not
71 been investigated.

72 In the present study, we evaluated the direct effect of Maca on the rate of successful *in vitro* fertilization
73 (IVF) in mice. We also investigated the direct effect of Maca on acrosome reaction in mice and humans and its
74 efficacy in improving human sperm motility.

75

MATERIALS AND METHODS

Animals

Female ICR mice (10 weeks old) and male BALB/cA mice were purchased from Japan SLC (Shizuoka, Japan). C57BL/6 mice expressing enhanced green fluorescent protein (EGFP) in their acrosomes were bred in the animal experimentation facility of Nagasaki International University [16]. The animals were euthanized via cervical dislocation immediately prior to the experiments. All animal experiments conformed to the Guide for the Care and Use of Laboratory Animals (Guide for the Care and Use of Laboratory Animals. 2011) and were approved by the Institutional Committee of Laboratory Animal Experimentation (Nagasaki International University, Nagasaki, Japan). The mice were maintained under controlled temperature (22°C ± 3°C), humidity (60% ± 20%), and light conditions throughout the experiments and were provided with food and water ad libitum.

Preparation of Maca (Lepidium meyenii)

Maca was collected, dried, and powdered in Japan and was obtained from Shokubunka Co. Ltd., Saitama, Japan. Twenty milligrams of the air-dried Maca powder was added to 1 mL of dimethyl sulfoxide (DMSO) (D2650; Sigma–Aldrich Japan, Tokyo, Japan), and the supernatant of this Maca solution was added to human tubal fluid (HTF) medium (LifeGlobal Group, Guilford, CT, USA).

IVF in mice

95 Female mice were superovulated via intraperitoneal injection of 5 IU pregnant mare serum gonadotropin (Asuka
96 Inc., Tokyo, Japan), followed by a 5 IU human chorionic gonadotropin (Asuka Inc.) injection 46–48 h later, and
97 then euthanized via cervical dislocation 14–16 h later, immediately prior to the start of the experiment. Ovaries
98 with oviducts were transferred to 30-mm-diameter dishes filled with paraffin oil (Nacalai Tesque, Kyoto, Japan).
99 Cumulus–oocyte complexes were obtained from the ampullae of uterine tubes and transferred under a
100 stereomicroscope to the dishes, each containing a 200-μL drop of HTF medium covered with paraffin oil. Two
101 to four cumulus–oocyte masses were transferred to each 200-μL drop of HTF medium covered with paraffin oil
102 for insemination. Approximately 10-month-old Balb/c mice were euthanized via cervical dislocation
103 immediately prior to the start of the experiments. Mature caudal epididymal sperm cells were collected for this
104 examination. Mature caudal epididymal sperm cells (approximately 8×10^6) from each mouse were incubated in
105 200 μL of HTF medium without bovine serum albumin (BSA), covered with paraffin oil. After 5 min, each
106 sperm suspension was transferred to conditioned medium for preincubation. The control conditioned medium
107 for sperm preincubation was HTF medium containing 0.4% BSA. Subsequently, 25-μL aliquots of the sperm
108 suspensions in HTF without BSA were transferred to 25 μL of conditioned medium samples containing twice
109 the concentration of Maca extract and were placed at 37°C in a humidified incubator under 5% CO_2/95% air
110 (motile sperm concentration, approximately 10,000 per mL). After 50 min, 2–4 μL aliquots of sperm from each
111 conditioned medium sample were used for insemination (final motile sperm concentration, 150 per mL). Motile
112 sperm swimming at the periphery of each drop were used for insemination, as described previously [17]. A
113 sperm suspension cultured in conditioned medium was transferred to the insemination drop. At 24 h after

114 insemination, the fertilization rate was determined as the proportion of two-cell-stage embryos among all
115 oocytes.
116
117 ***Acrosome reaction in mouse sperm***
118 To examine the acrosome reaction rate, sperm from transgenic mice expressing EGFP [16] were incubated in
119 each conditioned medium sample for 3 h and spotted onto glass slides with 5% glycerol. The acrosome status
120 was observed under a fluorescence microscope following propidium iodide staining.
121
122 ***Acrosome reaction in human sperm***
123 Human semen samples were obtained from fertile male volunteers via masturbation after 2–3 days of
124 abstinence, after they provided their informed consent. The semen was covered with 2 mL of HTF and incubated
125 at 37°C for 1 h. Then, 25-μL aliquots of swim-up sperm from the semen in the HTF were transferred to 25 μL of
126 the control conditioned medium or Maca extract-containing conditioned medium and placed at 37°C in a
127 humidified incubator under 5% CO_2/95% air for 4 h. The acrosome status was observed as previously described
128 [18]. The human sperm samples were spotted onto silane-coated Superfrost glass microslides (Matsunami Glass
129 Ind. Ltd., Osaka, Japan), treated with 70% methanol on ice for 10 min, and stained with fluorescein
130 isothiocyanate-conjugated peanut agglutinin (FITC-PNA) (L7381; Sigma–Aldrich, Japan).
131
132 ***Human sperm motility***

Sperm motility was analyzed using a sperm motility analysis system (SMAS) (SOLNET, Tokyo, Japan) [19]. Sperm in conditioned medium were spotted onto a 20-μm Leja counting chamber (standard Count Analysis Chamber 20 micron; Nieuw-Vennep, The Netherlands) for the analysis of sperm motility. The SMAS consists of a high-resolution digital scanning camera, personal computer with a digital frame grabber and image-processing software, and computer monitor. The system records images at a rate of 1 frame per second (60 Hz) and can analyze approximately 200 spermatozoa simultaneously in real time. A previous study showed that the results obtained using the SMAS strongly correlated with those obtained from manual microscopic sperm analysis based on the World Health Organization Laboratory Manual [19]. The procedures were approved by the regional ethics committee of Juntendo University Urayasu Hospital.

Statistical analysis

Data were expressed as average ± SEM and compared between the groups using the Mann–Whitney *U* test. The threshold for significance was $p < 0.05$. All statistical analyses were conducted using IBM SPSS version 24.0.

RESULTS

Success rate of IVF in mice

The fertilization ability of sperm from wild-type C57BL/6 mice was higher when using the standard medium. When sperm from aged BALB/cA mice (age > 48 weeks) were used [20], IVF was difficult and the fertilization ability of the sperm varied between mice. We tested whether the fertilization rate increased because of the

addition of Maca extract. The fertilization rates in HTF medium and Maca extract-containing medium are shown in Fig. 1. The fertilization rate in HTF medium containing Maca extract at a concentration of 4% (w/v) with 1% DMSO was significantly higher (33.4% ± 7.6%) than that in HTF medium without Maca extract (14.3% ± 4.6%; $p < 0.05$). The fertilization rates in HTF medium with Maca extract at concentrations of 2% and 8% (w/v) were also higher (23.3% ± 5.9% and 20.7% ± 9.6%, respectively) than that in HTF medium without Maca extract. However, these differences were not statistically significant.

Insert Fig. 1 here

Acrosome reaction rate in mouse sperm

We examined the sperm acrosome reaction rate in Maca extract-containing HTF medium using mouse sperm expressing GFP in their acrosomes. The rates of acrosome-reacted sperm in HTF medium containing Maca extract at concentrations of 4.0%, 8.0%, and 16.0% (w/v) with 1% DMSO were significantly higher (68% ± 3.1%, 71% ± 1.2%, and 71% ± 2.9%, respectively) than those in medium without Maca (44% ± 5.1%; $p < 0.05$, Fig. 2). The rate of acrosome-reacted sperm in HTF medium containing Maca extract at a concentration of 2.0% (w/v) with 1% DMSO was also higher (59% ± 5.5%) than that in HTF medium without Maca extract. However, this difference was not statistically significant.

Insert Fig. 2 here

171

172 ***Acrosome reaction rate in human sperm***

173 To investigate the effect of Maca on human sperm, the acrosome reaction in Maca extract-containing HTF

174 medium was assessed using FITC-PNA staining. The rate of acrosome-reacted sperm in HTF medium

175 containing Maca extract at a concentration of 1.0% (w/v) with 1% DMSO was significantly higher

176 (69% ± 7.3%) than that in medium without Maca (23% ± 5.4%; $p < 0.01$, Fig. 3). The rates of acrosome-reacted

177 sperm in HTF medium containing Maca extract at concentrations of 0.5% and 2.0% (w/v) with 1% DMSO were

178 also higher (39.6% ± 3.2% and 39.8% ± 5.8%, respectively) than those in HTF medium without Maca extract,

179 but these differences were not statistically significant.

180

181 Insert Fig. 3 here

182

183 ***Human sperm motility and amplitude of lateral head displacement (ALH)***

184 The motility of human sperm in Maca extract-containing HTF medium was analyzed in detail using SMAS

185 (Table.1). The percentage of motile sperm in the medium containing Maca extract was significantly higher

186 (82.1% ± 5.1%) than that in the control medium containing 1% DMSO (53.9% ± 9.5%; $p < 0.05$, Fig. 4). ALH

187 in the medium containing Maca extract was also higher (2.9 ± 0.3 μm) than that in the control medium

188 (2.4 ± 0.2 μm). However, this difference was not statistically significant.

189

190 Insert Table1 and Fig. 4 here

191

192 **DISCUSSION**

193 Maca belongs to the plant family Brassicaceae and is native to Peru. It has traditionally been used as folk
194 medicine and is considered a food supplement. It was first cultivated at least 2000 years ago in the Andes
195 Mountains of Peru at an altitude of 4000–4500 m. There are numerous substances in the tubers of Maca; several
196 typical components include amino acids, alkaloids (macaines), fatty acids (linoleic, palmitic, oleic acid, etc.),
197 tannins, saponins, and several microelements (Cu, Su, Mn, Al, etc.) [10]. Because Maca is rich in these
198 substances, it has significant potential to treat several diseases and disorders. Maca has been thought to improve
199 sexual dysfunction and male infertility since long, even before it was reported that consumption of Maca extract
200 increased serum testosterone concentration by enhancing the steroidogenic ability of Leydig cells and improving
201 sexual performance parameters in an animal model [11, 21]. Furthermore, a previous study in humans showed
202 that the administration of Maca improves seminal volume, sperm count per ejaculum sample, the number of
203 motile sperms, and sperm motility despite the fact that it does not affect the levels of relevant serum hormones,
204 including serum luteinizing hormone, follicle-stimulating hormone (FSH), prolactin, testosterone, or estradiol
205 [12]. This indicates that improved semen quality observed because of the administration of Maca might be
206 caused by enhanced bioavailable testosterone or testosterone receptors and an improved response of Sertoli cells
207 to FSH [22, 23]. A systematic review recently supported the effectiveness of Maca in improving semen quality
208 in humans [15]. Maca has currently gained increased attention in reproductive medicine, particularly in male

infertility, because no reliable and effective medical treatment has been established for this condition so far. However, the underlying mechanism of Maca in male infertility, particularly the direct effect of Maca on sperm, has not been elucidated.

In the present study, we first investigated whether the addition of Maca to culture medium improved the rate of successful IVF. As expected, in mice, the rate of fertilization in medium containing Maca was significantly increased compared with that in the control medium. Our results indicated that HTF medium containing Maca extract at a concentration of 4% (w/v) was the most suitable for IVF in mice. Second, we evaluated the influence of Maca on acrosome reaction as another direct effect. In mouse samples, we showed that the rate of acrosome-reacted sperm in HTF medium containing Maca extract was significantly higher than that in medium without Maca extract. Furthermore, this tendency was also found in human sperm, although the effective concentration of Maca was different from that in mice. Finally, we showed that Maca was clearly beneficial for sperm motility in humans. On the basis of our findings, we speculate that adding Maca to the medium used during IVF may increase its success rate by improving acrosome reaction and sperm motility.

Several studies have reported improvement in sperm quality using pharmaceutical agents such as caffeine, pentoxifylline, theophylline, Chinese herbal medicine, and myo-inositol, *in vivo* and/or *in vitro*. Caffeine, pentoxifylline, and theophylline are considered to be inhibitors of phosphodiesterase and have been primarily used *in vitro* in humans. Caffeine is a methylxanthine alkaloid that causes an increase in intracellular cyclic adenosine monophosphate (cAMP) and is reported to induce the acrosome reaction in boar and human sperm *in vitro* [24, 25]. Furthermore, caffeine may induce sperm hyperactivation in the course of promoting activation of

228 calcium ion-permeable cation channels in the plasma membrane of sperm [26]. Pentoxifylline, a methylxanthine
229 derivative, decreases blood viscosity because of platelet inhibition and is primarily used for symptomatic relief
230 of intermittent thrombosis [27]. In contrast to caffeine, pentoxifylline significantly increases sperm viability in
231 infertile men without improving sperm motility [28]. Apart from its lack of effect on increasing the acrosome
232 reaction rate and sperm motility, pentoxifylline treatment also does not increase sperm responsiveness to the
233 acrosome reaction induced by stimulation with follicular fluid and the ionophore A23187 [23]. However,
234 pentoxifylline significantly enhances hyperactivated sperm motility and tight binding to the homologous zona
235 pellucida [29]. Although caffeine and pentoxifylline have equal inhibitory effects on sperm phosphodiesterase,
236 their effects are different. Myo-inositol is one of the most biologically important agents and acts in concert with
237 group B vitamins. Administration of myo-inositol improves spermatozoa concentration in patients with
238 oligoasthenospermia but does not significantly improve sperm motility [30]. Myo-inositol increases the number
239 of spermatozoa with high mitochondrial membrane potential (MMP) *in vitro* and decreases the number of those
240 with low MMP in patients with oligoasthenospermia [31]. MMP is considered a marker of apoptosis because
241 viable cells show high MMP and apoptotic cells show low MMP. Fertile men have spermatozoa with high MMP,
242 whereas infertile men have spermatozoa with low MMP. In herbal medicine, the preparation “Hochuekkito” has
243 also been used clinically to treat male infertility in Japan. Although common oral consumption of Hochuekkito
244 may be expected in the future, it has already been suggested to have a direct protective effect on sperm in
245 infertile men [32].

246 The exact mechanisms of fertilization processes, including those of the acrosome reaction and

247 hyperactivation, remain unknown. The acrosome reaction (i.e., exocytosis of the sperm vesicle) is a prerequisite
248 for fertilization. We showed that culture medium containing Maca extract improved the fertilization rate in mice
249 and that it was not dose related (Fig. 1). There are no reports about the Maca specific toxicities; however, it is expected
250 that excess amounts of chemical substances can cause negative effects. We also showed that the medium containing
251 Maca extract increased the rate of acrosome-reacted sperm in mouse and human samples (Figs. 2 and 3). These
252 results support the improvement in infertility due to Maca extract via induction of the acrosome reaction. There
253 is difference in suitable concentration of Maca for acrosome reaction between mouse and human sperm. Optimal medium
254 varies among species. It is possible that the quantity of the molecules inside the sperm on which Maca acts and the influence
255 of Maca on the molecules may vary depending on species. In fact, it is known from an IVF experimental model that albumin
256 has an influence on acrosome reaction, and that albumin draws fat from the membrane of sperm and by doing so gradually
257 facilitates the induction of acrosome reaction and promotes the sperm to mature and become active. The amounts of albumin
258 to be added to the medium for conducting efficient IVF are slightly different between mice and humans. Furthermore, the
259 albumin from different lots can cause a large change on the acrosome reactions and the fertility rate. In this experiment as
260 well, the complicated conditions, as stated above, are considered to be the reasons why the suitable Maca concentration was
261 different between mice and humans.

262 Hyperactivation may support sperm in detachment from the endosalpingeal epithelium, enhancing their ability
263 to easily swim through viscoelastic substances. In addition, hyperactivation assists sperm in penetrating the zona
264 pellucida [33]. An association between ALH and sperm hyperactivation has been reported [34]. ALH in the
265 present study tended to be higher in the Maca extract-containing medium than in the control medium, although

this difference was not statistically significant (Fig. 4). In Table 1, there is difference between sperm concentration with and without Maca extract though not statistically significant. We consider that this tendency was not due to Maca, but rather, it was because the substances from the sperm might have contaminated the medium and possibly influenced the results. These results suggest that Maca extract can induce hyperactivation in humans. Maca is a type of alkaloid similar to caffeine. It is possible that Maca induces the acrosome reaction and hyperactivation because of an increase in cAMP and the number of spermatozoa with high MMP. Furthermore, it has recently been suggested that some hormones, including progesterone, melatonin, and serotonin, enhance hyperactivation through specific membrane receptors and that 17β-estradiol suppresses such enhancement by progesterone and melatonin via a membrane estrogen receptor [35]. Because plant extracts can have several functions, including estrogenic actions, depending on the flavonoids present, it is possible that Maca also has a specific estrogenic action. If so, such an action may also be associated with our findings in the present study. It was recently shown that the rate of IVF in mice was improved by adding an aqueous extract of licorice to the artificial insemination culture medium. Furthermore, it was reported that isoliquiritigenin and formononetin were the active molecules in licorice that contributed to the improved IVF rate [36]. Similarly, the identification of molecules in Maca that contribute to the improvement in IVF has begun to attract increased attention.

There are many reports about past experiments regarding the effectiveness of oral intake of Maca. In this study, it became clear that some substances in Maca affect the ejaculated sperm. It is speculated that some of the substances, which can affect the ejaculated sperm, might have affected the activity of the ejaculated sperm through the body fluid even when Maca was taken orally. Additionally, because the quality of IVF medium was improved, there is a possibility that Maca can lead to

desirable results for women when it is taken orally.

We showed that *Lepidium meyenii* (Maca) added to culture medium may improve the IVF rate of mouse sperm. We also showed that Maca is beneficial in inducing an acrosome reaction in mouse and human sperm and an increase in motility in human sperm. Our study provides evidence that Maca has a direct effect on sperm in improving fertilization rates.

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DISCLOSURES

Conflict of interest: None of the authors declare competing financial interests.

Human and Animal Rights: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later revision. Informed consent or substitute for it was obtained from all patients for being included in the study. Additionally, the protocol for the research was approved by the Institutional Review Board of the Juntendo University Urayasu Hospital, Chiba, Japan. All institutional and national guidelines for the care and use of laboratory animals were followed.

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394

FIGURE LEGENDS

Fig. 1: Rate of successful fertilization determined as the proportion of two-cell-stage embryos among all mouse oocytes in the experiment

The fertilization rate in medium containing Maca extract at a concentration of 4% (w/v) with 1% DMSO was significantly higher (33.4% ± 7.6%) than that in HTF medium without Maca extract (14.3% ± 4.6%; $p < 0.05$). The fertilization rates in medium containing Maca extract at concentrations of 2% and 8% (w/v) were also higher (23.3% ± 5.9% and 20.7% ± 9.6%, respectively) than those in HTF medium without Maca extract, but these differences were not statistically significant. HTM, human tubal fluid; DMSO, dimethyl sulfoxide.

Fig. 2: The rate of acrosome reaction in mouse sperm

The rates of acrosome-reacted sperm in HTF medium containing Maca extract at concentrations of 4.0%, 8.0%, and 16.0% (w/v) with 1% DMSO were significantly higher (68% ± 3.1%, 71% ± 1.2%, and 71% ± 2.9%, respectively) than those in medium without Maca extract (44% ± 5.1%; $p < 0.05$). The rate of acrosome-reacted sperm in HTF medium containing Maca extract at a concentration of 2.0% (w/v) with 1% DMSO was also higher (59% ± 5.5%) than that in HTF medium without Maca extract, but this difference was not statistically significant. HTM, human tubal fluid; DMSO, dimethyl sulfoxide.

Fig. 3: The rate of acrosome reaction in human sperm

The rate of acrosome-reacted sperm in HTF medium containing Maca extract at a concentration of 1.0% (w/v)

414 with 1% DMSO was significantly higher (69% ± 7.3%) than that in medium without Maca extract (23% ± 5.4%;

415 $p < 0.01$). The rates of acrosome-reacted sperm in HTF medium containing Maca extract at concentrations of

416 0.5% and 2.0% (w/v) with 1% DMSO were also higher (39.6% ± 3.2% and 39.8% ± 5.8%, respectively) than

417 those in HTF medium without Maca extract, but these differences were not statistically significant. HTM,

418 human tubal fluid; DMSO, dimethyl sulfoxide.

419

420 **Fig. 4:** Sperm motility and amplitude of lateral head displacement in medium with or without Maca extract

421 Sperm motility was analyzed using a sperm motility analysis system. The percentage of sperm motility in the

422 Maca extract-containing medium was significantly higher (82.1% ± 5.1%) than that in the control medium

423 (53.9% ± 9.5%; $p < 0.05$). The amplitude of lateral head displacement in the Maca extract-containing medium

424 was also higher (2.9 ± 0.3 μm) than that in the control medium (2.4 ± 0.2 μm), but this difference was not

425 statistically significant.

426

Table 1 Results of sperm motility analysis using semen from healthy men in medium with or without Maca extract ($N = 5$).

	With Maca extract	Without Maca extract	*P* value
Concentration ($\times 10^6$)	95.5 ± 54.1	60.5 ± 20.6	NS
Total sperm count ($\times 10^6$)	190.9 ± 108.1	120.6 ± 41.5	NS
Sperm motility (%)	82.1 ± 5.1	53.9 ± 9.5	<0.05
Straight-line velocity (μm/s)	32.1 ± 6.4	34.7 ± 4.4	NS
Curvilinear velocity (μm/s)	86.3 ± 11.3	94.6 ± 8.6	NS
Linearity	0.36 ± 0.03	0.36 ± 0.02	NS
Amplitude of lateral head Displacement (μm)	2.9 ± 0.3	2.4 ± 0.2	NS
Beat-cross frequency (Hz)	13.4 ± 0.5	11.9 ± 0.9	NS

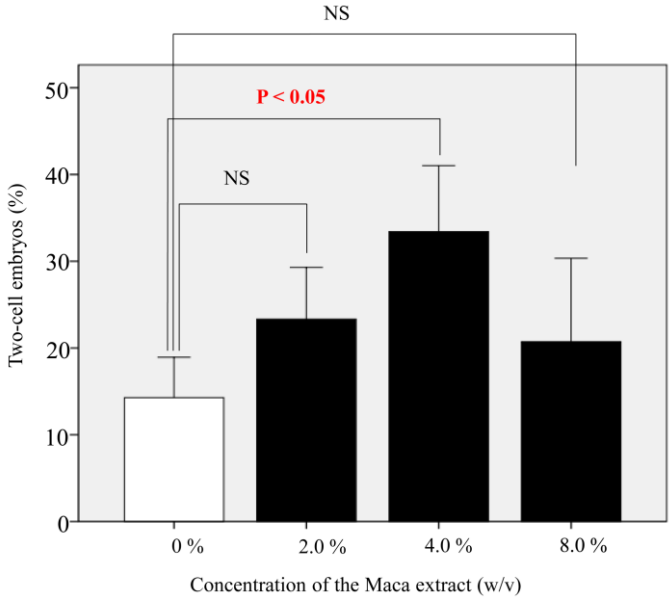

Fig 1

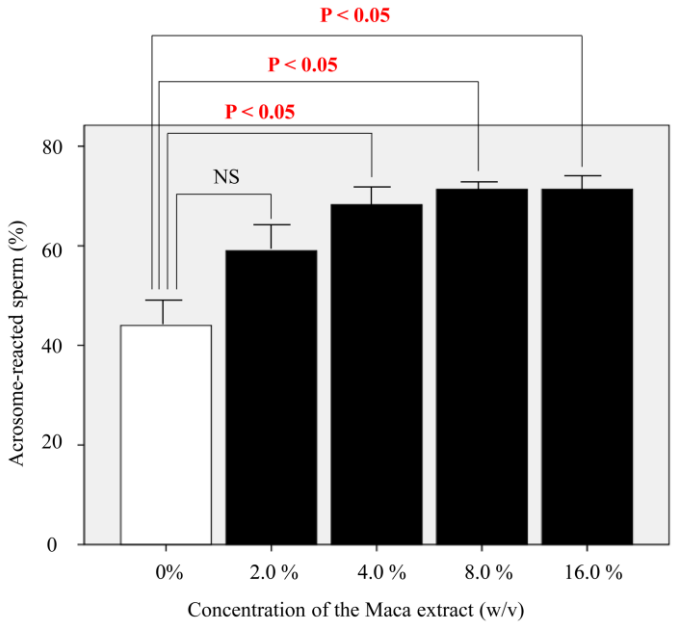

Fig 2

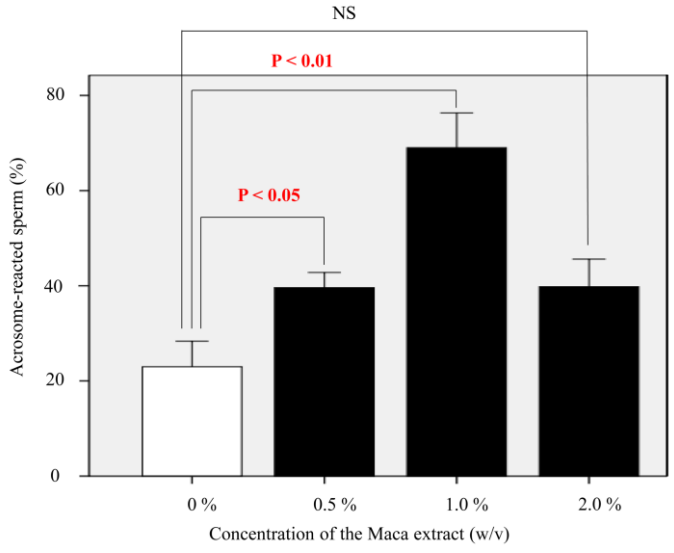

Fig 3

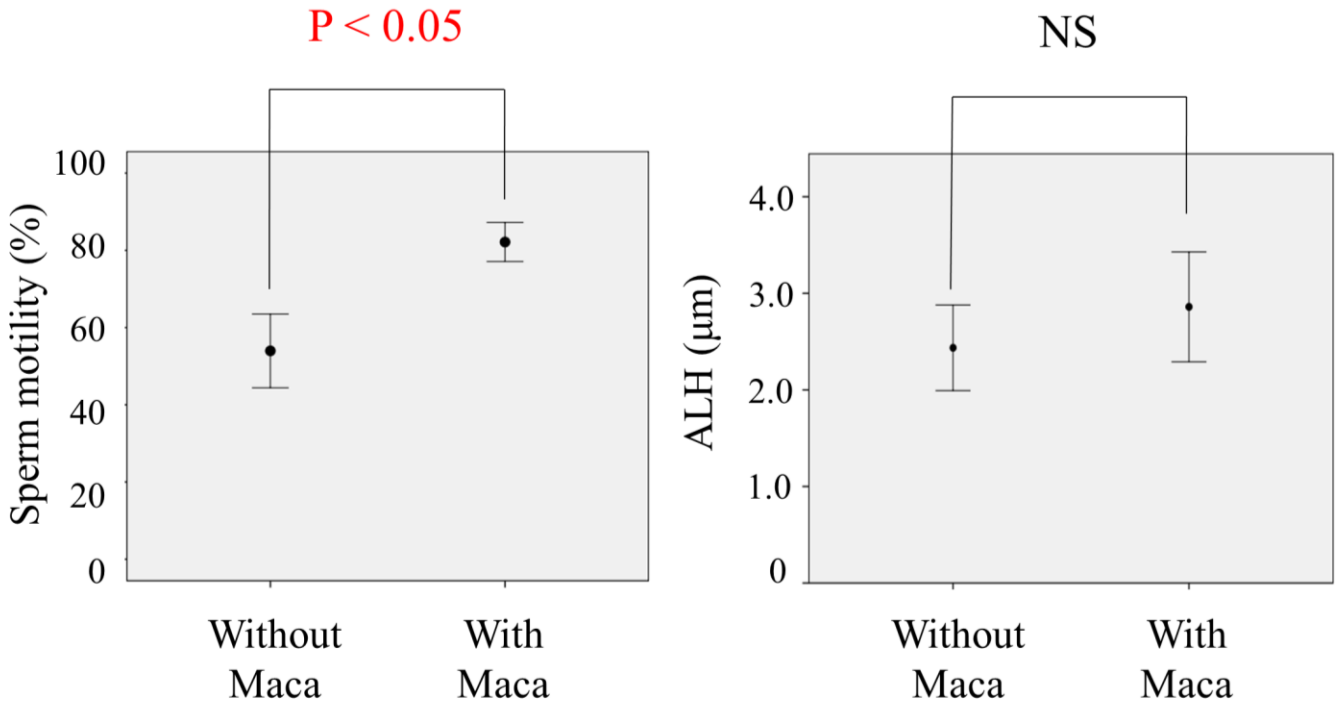
P < 0.05
Sperm motility (%)
100
80
60
40
20
0
Without Maca
With Maca
NS
ALH (µm)
4.0
3.0
2.0
1.0
0
Without Maca
With Maca

Fig 4