韓國家畜繁殖學會誌 25(4) : 327~337 (2001)
Korean J. Animal Reprod.

Lipid Peroxidation and Fertilizing Ability *In Vitro* by Superoxide Dismutase in Boar Spermatozoa Frozen-Thawed

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ABSTRACT

This study investigated the effects of superoxide dismutase (SOD) on lipid peroxidation and fertilizing ability *in vitro* of boar spermatozoa frozen-thawed. The percentages of motile sperm were highest when SOD of 10 units/ml was added to washing medium for spermatozoa. However, the rates of motile sperm were not significantly different in different concentrations of SOD. On the other hand, the motile rates of sperm washed with SOD were lower in sperm incubated for 120 min than 30 min regardless of the different concentrations of SOD. The percentage of spermatozoa that reached acrosome reaction were increased with incubation periods prolonged. No significant differences, however, were observed in acrosome reaction rates between sperm incubated with and without SOD supplementation for 0, 60 and 120 min. When oocytes inseminated with different concentrations of SOD, the penetration rates were significantly ($P<0.05$) higher in medium with 1 unit/ml than 0, 10 and 100 units/ml of SOD. However, the proportions of polyspermic oocytes were significantly ($P<0.05$) lower in medium with 10 and 100 units/ml than 0 unit/ml of SOD. In another experiment, the sperm suspension were also treated with different concentrations of SOD and were assayed for sulfhydryl(-SH) group content. In the groups treated with 100 units/ml of SOD, sperm-SH group were higher than another groups. However, sperm-SH group content were not significantly different in spermatozoa treated with different concentrations of SOD. Under the same conditions, the lipid peroxidation of sperm was evaluated on the basis of malondialdehyde production. The addition of SOD to sperm suspension decreased the formation of malondialdehyde. However, there were not significantly different in sperm treated with different concentrations of SOD. The activity of sperm binding to zona pellucida was also evaluated through binding to salt-stored porcine oocytes. The sperm binding to zona pellucida were gradually increased with SOD concentrations added. The number of spermatozoa binded to zona pellucida were significantly ($P<0.05$) higher in medium with 100 units/ml than 0 units/ml of SOD. These findings

This work was supported by grant No. 2000-1-22200-001-3 from the Basic Research Program of the Korea Science & Engineering Foundation.

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suggested that SOD cause an enhancement penetrarion ability and sperm zona binding in boar spermatozoa frozen-thawed.

(Key words: Boar, Frozen-thawed spermatozoa, Superoxide dismutase, Lipid peroxidation, Fertilizing ability)

Ⅰ. INTRODUCTION

It is well known that superoxide radicals cause tissue damage, whereas superoxide dismutase (SOD) works protectively by scavenging superoxide radicals. Recent evidence has shown that the superoxide radical and its scavenging system play important roles in reproductive function (Kato et al., 1997; Suzuki et al., 1999). Oxygen free radicals have been implicated in a variety of circumstances relevant to the function of spermatozoa. Spermatozoa have been shown to release hydrogen peroxide and superoxide radical (Griveau et al., 1995; Zhang and Zheng, 1996). There is a relationship between loss of motility and peroxidation of spermatozoa lipids (MacLeod, 1995; Storey, 1997). Also, the superoxide radical has recently been implicated in the capacitation reaction (de Lamirande and Gagnon, 1995; Zhang and Zheng, 1996).

Before oocyte fertilization becomes possible, mammalian spermatozoa must complete capacitation, a process that globally includes the series of membrane and metabolic changes that occur in the female genital tract (Roldan and Harrison, 1990). These changes are temporally associated with a specific, high-velocity, and whiplash-like type of motility called hyperactivation that, *in vitro*, peaks after 1 to 3 h of incubation for human spermatozoa (Burkman, 1990).

Rabbit, mouse, and human sperm generate superoxide and hydrogen peroxide (H_2O_2) during aerobic incubation (Alvarez et al., 1987). Most of the H_2O_2 appears to be generated by the action of sperm superoxide dismutase on the superoxide radical produced by the sperm (Alvarez and Storey, 1989). Under nomal conditions, the H_2O_2 generated by nonphagocytic cells represents only 1~2% of the total oxygen consumption in animal tissues; in most tissues, the concentrations of catalase and peroxidases are well in excess to provent the toxic effects of H_2O_2 (Chance et al., 1979). Although correlations have been reported in effects of SOD on sperm fertility in many species, the action of SOD for boar spermatozoa frozen-thawed has not been elucidated. Therefore, the present study was designed to evaluate effects of superoxide dismutase on *in vitro* fertility and lipid peroxidation in boar spermatozoa frozen-thawed.

Ⅱ. MATERIALS AND METHODS

1. Sperm Motility Analysis

Pooled ejaculate from boar were frozen, and the straws were thawed by immersion in a 37℃ waterbath for about 30 sec. Spermatozoa were washed twice in TC-199 medium containing 2 mM-caffeine (caffeine-sodium benzoate, Sigma Chemical Co., St Louis, MO, USA) by centrifugation at 833×g each for 10 min. At 0 and 120 min after the final washing with 0, 1, 10 and 100 units/ml SOD, the percent of motile sperm was evaluated by eosine staining method.

2. Spermatozoa Assessment

The functional state of the spermatozoa was assessed using the chlortetracycline (CTC) fluorescence assay method described by DasGupta et al. (1993). CTC solution was prepared on the day of use and contained 750 μM CTC (Sigma) in a buffer

of 130 mM NaCl, 5 mM cysteine, 20 mM Tris-HCl; the pH was adjusted to 7.8. This solution was kept wrapped in foil at 4℃ until just before use. Hoechst -treated sperm suspension (45 μl) was added to 45 μl of CTC solution at room temperature in a foil -wrapped centrifuge tube and mixed thoroughly. Spermatozoa incubated for 0, 60 and 120 min with concentrations of 10 units/ml of SOD were then fixed by adding 8 μl 12.5% w/v paraformaldehyde in 0.5 M Tris-HCl (pH 7.4). Sample slide was prepared by placing 10 μl of the stained, fixed suspension on a slide. One drop of 0.22 M 1,4-di-azabicyclo(2.2.2)octane dissolved glycerol : PBS (9 : 1) was mixed in carefully to retard fading of the fluorescence. A coverslip was placed on top. The slide was compressed firmly between tissues to remove any excess fluid and to maximize the number of spermatozoa lying flat on the slide. The coverslip was then sealed and stored wrapped in foil in the cold.

An assessment was carried out on either the same or the following day using an Olympus BHS microscope (BX50F4, Olympus Optical Co. Ltd. Japan) equipped with phase-contrast and epifluorescent optics. Cells were assessed for CTC staining using violet light. The exicitation beam was passed through a 405 nm bandpass filter and fluorescence emission was observed through a DM 455 dichroic mirror. There are three main patterns of CTC fluorescence that can be identified: F, with uniform fluorescence over the entire head, characteristic of uncapacitated, acrosome-intact cells; B, with a fluorescence-free band in the post-acrosomal region, characteristic of capacitated, acrosome-intact cells; and AR, with dull or absent fluorescence over the sperm head, characteristic of capacitated, acrosome -reacted cells. At all three stages bright fluorescence on the midpiece could be seen.

3. Oocyte Preparation

Porcine ovaries were collected from a local slaughter-house and kept in saline (NaCl, 0.9% W/V; Penicillin 100,000 IU/L; Streptomycin 100 mg/L and Amphotericin B 250 μg/L; Sigma Chemical, St-Louis, MO, USA) at 30 to 32℃. Cumulus -oocyte complexes were aspirated from 2 to 6 mm follicles with a 10-ml syringe with an 18-G needle. The collected oocytes were washed three times in Hepes-buffered Tyrode's medium (TLH) and once in maturation medium. Oocytes with compact and complete cumulus cells were introduced to droplets of maturation medium (10 oocytes/50 μl droplet), covered with mineral oil and cultured under an atmosphere of 5% CO_2 in air at 39℃ for 42～44 h. The maturation medium consisted of TCM-199 with Earle's salts (Gibco Lab., NY, USA) supplemented with 3.05 mM glucose, 0.32 mM Ca-lactate, 2.5 mM Hepes (Sigma), 10% fetal bovine serum (FBS; Gibco, Life Technologies, Inc. NY 14072 USA), 0.2 mM Na-pyruvate (Sigma), 50 μg /ml gentamycin (Sigma), 1 μg/ml FSH (from porcine pituitary; Sigma), 5 μg/ml LH (from equine pituitary; Sigma), 1 μg/ml estradiol 17β (Sigma) and 10% (v/v) porcine follicular fluid (PFF). The PFF was aspirated from follicles (2 to 5mm in diameter) at estrus with a syringe and 18-gauge needle, and centrifuged at 3850×g for 15 min. The supernatant fluid was frozen at −20℃ until used.

4. Oocyte Penetration Test

Thawed spermatozoa were diluted with 2 ml of BTS (Beltsville Thawing Solution) and equilibrated in air-tight tubes at 37℃ in a waterbath for 10 minutes. After equilibration, 2 ml semen was placed over 2 layers of percoll (65 and 70%) and centrifuged at 2000×g for 15 minutes. The spermatozoa in the 65% percoll layer were carefully collected, washed in preincubation medium (TCM-199) with Earle's salts (Gibco), supplemented with 3.05 mM glucose, 2.92 mM Ca-lactate (Sigma), 10% FCS,

0.2 mM Na-pyruvate, and 50 μg/ml gentamycin (Sigma) by suspension and centrifugation two times at 250×g for 10 minutes and resuspended in *preincubation medium*.

The fertilization medium with 0, 1, 10 and 100 units/ml SOD was the same as the preincubation medium, enriched with 2 mM caffeine (Sigma) and adjusted to a pH of 7.2 to 7.4. The final concentration of spermatozoa was adjusted to 1×10^6 cells/ml motile sperm during fertilization *in vitro*.

5. Determination of Lipid Peroxidation

The levels of lipid peroxidation induced in porcine spermatozoa were assessed by the determination of malondialdehyde (MDA). For this assay, approximately 1×10^6 sperm/ml were treated with 0, 1, 10 and 100 units/ml SOD in fertilization medium for 30 min at 39℃. Subsequently, 0.6 ml of reaction solution was mixed with 0.2 ml of 15.2 % trichloroacetic acid (Sigma) and centrifuged at 8000 × g for 10 min. Then, 0.6 ml of the supernatant was mixed with 2 ml of 0.6% 2-thiobarbituric acid (Sigma), and incubation was conducted at 95 ℃ for 40 min. The absorbance of the mixture, measured with a spectrophotometer at 534 nm, was used to determine the MDA concentration.

6. Measurement of Sperm Sulfhydryl(-SH) Groups

After washing of frozen-thawed spermatozoa in fertilization medium containing 0, 1, 10 and 100 units/ml SOD, spermatozoa were centrifuged on a 40% and 80% Percoll gradient in fertilization medium devoid of FCS. The soft sperm pellet was resuspended into 50 volumes of fertilization medium without FCS and centrifuged for 5 min at 5,000 × g. The pellet was resuspended and assayed for -SH group content. The assay mixture contained 180 μl sperm suspension, 10 μl 10% sodium dodecyl sulfate (SDS, Sigma), and 10 μl of 4 mM 5,5' -dithio-bis (2-nitrobenzoic acid), and the absorbance was measured at 405 nm. A standard curve was generated using different concentrations of dithiothreitol.

7. Determination of Zona-binding Properties of Spermatozoa

The activity of sperm binding to zona pellucida was evaluated through binding to salt-stored homologous zona pellucida. Oocytes matured *in vitro* were treated with 0.1% hyaluronidase to dissociate the cumulus oophorus and equilibrated with 1.5 M magnesium chloride containing 1% dextran. The oocytes were stored at 4℃ for 3 ~4 days until examined. The oocytes were washed and re-equilibrated in fertilization medium with FCS for 1 hour prior to experimentation. Spermatozoa (1×10^6 sperm /ml) were incubated for 1 hour at 39℃ with oocytes in 50 μl of fertilization medium with 0, 1, 10 and 100 units/ml SOD covered with mineral oil. The number of spermatozoa attached to the zona pellucida was then evaluated using an inverted microscope.

8. Statistics

Data were evaluated by ANOVA and differences between individual means were assessed by the least significant difference test or Turkey's test for unequal sample sizes, as appropriate.

Ⅲ. RESULTS

Fig. 1 shows effects of SOD concentrations on *in vitro* motility of frozen-thawed boar spermatozoa. When spermatozoa were washed with different concentrations of SOD, the percentage of motile sperm were 37, 38, 39 and 33% for 0, 1, 10 and 100 units/ml of SOD. At 120 min of sper- matozoa incubation after washing with SOD, the percentage of motile sperm were 14, 22, 24 and 23% for 0, 1, 10 and 100 units/ml of SOD, and were lower than

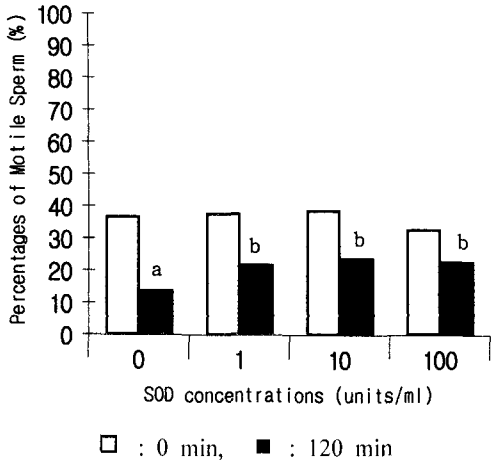

Fig. 1. Effects of concentrations of superoxide dismutase (SOD) on percentage of motile sperm in boar spermatozoa frozen-thawed. a,b : P<0.05.

in spermatozoa non-incubated regardless of the different concentrations of SOD.

The percentage of spermatozoa that reached acrosome reaction were increased (P<0.05) with incubation periods prolonged (Table 1). No significant differences, however, were observed between acrosome reaction rates of sperm incubated with and without SOD supplementation for 0, 60 and 120 min.

When spermatozoa were inseminated with different concentrations of SOD in oocytes matured *in vitro*, the penetration rates were significantly (P< 0.05) higher in medium with 1 units/ml(72%) than 0 (64%), 10 (55%) and 100 (47%) units/ml of SOD, respectively (Table 2). However, the proportions of polyspermic oocytes were significantly lower in medium with 1 (39%), 10 (34%, P<0.05) and 100 (34 %, P<0.05) units/ml than 0 (46%) units/ml of SOD.

The sperm suspension were also treated with different concentrations of SOD and assayed for sulfhydryl(-SH) group content. In the groups treated with 100 units/ml of SOD, sperm-SH group were higher than another groups (Table 3). However, sperm-SH group content were not significantly different in spermatozoa treated with different concentrations of SOD.

Under the same conditions, the lipid peroxidation of sperm was evaluated on the basis of malondialdehyde production (Table 4). SOD added to sperm suspension decreased the formation of malondialdehyde. However, there was not significant difference in sperm treated with different concentrations of SOD.

Table 1. Effect of superoxide dismutase (SOD) on CTC analysis at various times of preincubation in boar spermatozoa frozen-thawed

Periods of preincubation (min)	Presence of SOD (10 units/ml)	No. of spermatozoa examined	No. of spermatozoa with (%)		
			F	B	AR
0	+	600	279(47)	218(36)	103(17)[a]
	−	600	348(58)	167(28)	86(14)[a]
60	+	600	182(30)	255(43)	163(27)[b]
	−	600	168(28)	283(47)	149(25)[b]
120	+	600	134(22)	271(45)	195(33)[c]
	−	600	134(22)	271(45)	195(33)[c]

F: non-capacitated and dead, B: capacitated, AR: acrosome-reacted.

[a,b,c]: Values with different superscripts are significantly different (P<0.05).

Table 2. Effect of superoxide dismutase (SOD) on *in vitro* fertilization by frozen-thawed spermatozoa in porcine follicular oocytes

Concentrations of SOD (units/ml)	No. of oocytes examined	No. of oocytes penetrated (%)			No. of polyspermic oocytes(%)
		Total	ESH*	BPN**	
0	118	76(64)[a]	53	23(30)	35(46)[a]
1	100	72(72)[b]	49	23(32)	28(39)[ab]
10	122	67(55)[c]	36	31(46)	23(34)[b]
100	120	56(47)[c]	30	26(46)	19(34)[b]

* ESH: enlarged sperm head, ** BPN: both pronuclei

[a,b,c] Within a column, values with different superscripts are significantly different ($P<0.05$ at least).

Table 3. Effect of superoxide dismutase (SOD) on sulfhydryl(-SH) grups of frozen -thawed boar spermatozoa

Concentrations of SOD (units/ml)	Sperm-SH groups (nmole/10^6 spermatozoa) (Average ±SD)*
0	2.05 ± 0.09
1	2.07 ± 0.08
10	2.08 ± 0.07
100	2.13 ± 0.05

* 3 replicates.

Table 4. Lipid peroxidation of boar spermatozoa treated with different concentrations of superoxide dismutase (SOD)

Concentrations of SOD (units/ml)	Density of MDA (10^{-15} mole $sperm^{-1}$) (Average ±SD)*
0	0.741 ± 0.005
1	0.739 ± 0.005
10	0.736 ± 0.005
100	0.727 ± 0.010

* MDA : malondialdehyde, 3 replicates.

In another experiment, the activity of sperm binding to zona pellucida was evaluated through binding to salt-stored porcine oocytes. The sperm binding to zona pellucida were gradually increased with SOD concentrations (Table 5). The number of spermatozoa binded to zona pellucida were significantly ($P<0.05$) higher in medium with 100 units/ml than 0 units/ml of SOD.

Table 5. Effect of superoxide dismutase (SOD) on zona pellucida binding of frozen-thawed boar spermatozoa

Concentrations of SOD (units/ml)	No. of oocytes examined	Average no. of sperm binded to zona pellucida
0	25	105 ± 29[a]
1	25	111 ± 21[ab]
10	25	116 ± 25[ab]
100	25	120 ± 77[b]

[a,b] Different letters are significantly different ($P<0.05$).

Ⅳ. DISCUSSION

Even though many investigators have focused heavily on understanding the effects of free radicals on various cells and organs, the exact mechanism(s) of action of many of the antioxidant enzymes, including SOD, in spermatozoa of pig remains unknown. In this study, we have investigated with different concentrations of SOD for *in vitro* fertilizing ability in boar spermatozoa frozen-thawed.

With reference to the CTC analysis of spermatozoa, a recent study (Perry et al., 1995) reported a range of CTC patterns. This method has been used to assess the functional status of mouse (Ward and Storey, 1984), human (DasGupta et al., 1993), bull (Green et al., 1996), and boar spermatozoa (Lim et al., 1997). In the present study, 10 units/ml SOD had a stimulatory effect on sperm motility at 120 min after sperm incubation in fertilization medium. This was shown by a shift from the F pattern of CTC staining, characteristic of capacitated, acrosome -intact cells. The response was observed early, within 1～3 h of the start of incubation, and did not appear to be concentration dependent; there was no significant stimulation of the acrosome reaction in human spermatozoa (Green et al., 1996). In this study, however, SOD may be able to stimulate the motility and acrosome reaction of frozen-thawed spermatozoa.

The results of this study show that supplementation of media with SOD for *in vitro* fertilization of porcine oocytes affects the ability of sperm penetration. Addition of 1 unit/ml SOD to fertilization medium was effective in promoting this ability. The limited capacity of male pronucleus formation in porcine oocytes matured and penetrated *in vitro* has been described by several investigators (Mattioli et al., 1988; Zheng and Sirard, 1992). In the present study, we show that it is possible to maintain the rate of pronucleus formation after insemination in oocytes matured *in vitro*. It has been previously shown that the pronucleus formation capacity of porcine oocytes can be maintained by the composition of the maturation medium and by physical interaction with oviductal cells (Dubuc and Sirard, 1995). Umaoka et al. (1991) demonstrated the presence of SOD in the oviduct fluid or oviduct epithelium by biochemical (Noda et al., 1991) or immunohistochemical (Liu et al., 1995) analysis. Taking these results into consideration, it is suggested that pronucleus formation is protected from the oxidative stress under SOD-rich conditions in the oviduct fluid.

In mammals, all cells are exposed to the risk of injury by active oxygen, which is formed when molecular oxygen is utilized as an electron acceptor during oxidoreductive reactions in the cells. Superoxide anion is a major species of active oxygen and is formed by enzymes such as monomine oxidase, xanthine oxidase, and aldehyde oxidase and by auto-oxidation reactions such as those of ubiquinone, catechols, ferredoxins and hemoproteins (Noda et al., 1991; Puglia and Powell, 1984). Superoxide anions react with proteins or lipids located in the vicinity of the O^{2-} generated. Inactivation of enzymes and lipid peroxidation in the membrane would be expected without adequate defense mechanism against active oxygen. Superoxide dismutase catalyzes the dismutation of superoxide free radical anion as follow : $2H^{+} + 2O_2 \rightarrow H_2O_2 + O_2$. The enzymes has also been found to be ubiquitous among oxygen-metabolizing organism (McCord et al., 1971) and is the major radical scavenger in the defense against oxygen toxicity. Thus the biological effects of SOD on boar spermatozoa appears to be due to catalytic activity of this enzyme, scavenging the superoxide free radicals generated within the culture medium.

The present study shows that exposure of frozen -thawed boar spermatozoa to SOD lead to an increase of lipid hydroperoxide content and stimulated acrosome reaction and fertilizing ability. These experimental results obtained were very similar to those of human (Kodama et al., 1996), and suggested that SOD should also stimulate the capacitation and fertilizing ability of boar spermatozoa *in vitro*. Alvarez and Storey (1984) established that lipoperoxidative lethal endpoint for mouse spermatozoa, defind as the amount of malondialdehyde produced by a cell population at the point where high percen-

tages of the cells have lost their motility, corresponds to 0.6% thiobarbituric acid-reactive substances per 10^6 cells. In the present study, the value of malondialdehyde obtained with SOD corresponds to a leval decreased of mild peroxidation.

In this study, in addition to its effects on sperm motility, lipid peroxidation has also shown to increase the sperm-oocyte interaction, in contrary to measured by a decreased penetration of zona-free hamster oocytes by a human spermatozoa (Aitken et al., 1989). Because the sperm-zona free hamster oocyte penetration assay is used by some investigators to predict the fertilizing potential of human spermatozoa (Aitken et al., 1991), it was suggested that lipid peroxidation, even at a level that those not affect motility, may decrease the fertilizing potential of spermatozoa (Aitken et al., 1989). Thus the results of the present study differ those obtained with zona-free hamster oocytes, because a percentage increased of fertilization following 1 unit/ml SOD treatment is obtained in the porcine *in vitro* fertilization assay. This discrepancy may emphasize differences in sperm species and in the mechanism by which oocytes with and without zona pellucida are penetrated by spermatozoa.

In the present study, SOD increased sperm-SH groups, and its effect was more increased by concentrations of SOD. Mammoto et al. (1996) determined that treatment of sperm with various types of SH-depleting reagents, i.e., N-ethylmalemide, $Na_2S_4O_6$ and 5,5'-dithiobis, specifically blocked sperm-oocyte fusion in mice without affecting motility, the capacity to penetrate the zona pellucida, or sperm-oocyte binding, and that three proteins in mouse sperm were highly sensitive to these SH-depleting reagents. Sinha et al. (1991) showed that in human oligospermia there is a quantitative reduction in the SH-groups in the sperm head membrane compared to that of nomal spermatozoa. Thus, oxidation of fusion-related sperm SH-groups may be one mechanism responsible for the suppressive of oxygen stress on sperm function.

In summary, the present study suggests that SOD increase an sperm fertilizing ability that is associated with increaseing in penetration rates of boar spermatozoa. These increases are also associated with an improvement in sperm-binding properties to homologous zona pellucida.

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요 약

Superoxide Dismutase에 의한 돼지 동결-융해정자의 Lipid Peroxidation과 체외수정능력

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본 연구는 superoxide dismutase (SOD)가 동결-융해 돼지정자의 lipid peroxidation과 체외수정능력에 미치는 영향을 검토하였다. 그 결과 동결-융해 정자는 10 units/ml의 SOD가 첨가된 배양액에 의해 처리했을 때 가장 높은 정자생존율을 나타냈으나 서로 다른 농도에 의한 차이는 인정되지 않았다. 그러나 SOD농도에 관계없이 정자처리 직후의 생존율은 120분간 배양 후에 비해 유의적($P<0.05$)으로 높은 생존율을 나타냈다. 또한 정자처리 후 배양시간이 0, 60 및 120분으로 길어짐에 따라 정자의 첨체반응 유기율이 증가하였지만 SOD 첨가 또는 무첨가구 사이에서 유의적인 차이는 인정되지 않았다. 한편, 체외수정시 1 unit/ml의 SOD를 첨가한 경우 0, 10 및 100 units/ml 첨가시 보다 유의적($P<0.05$)으로 높은 정자침입율을 나타냈으며, 10과 100 units/ml의 SOD 첨가시 낮은 다정자 침입율을 나타냈다 ($P<0.05$). 정자의 peroxidation은 malondialdehyde의 생성에 기초를 두고 평가하였는데 SOD 무첨가에 비해 첨가농도가 높아질수록 malondialdehyde의 생성이 낮아졌지만 유의적인 차이는 인정되지 않았다. 또한 동결-융해된 정자는 sulfhydryl(-SH) group의 용량을 측정한 결과 SOD무첨가시 보다 첨가시 높은 용량이 측정되었지만 유의적인 차이는 인정되지 않았다. 한편, 동결-융해된 정자가 체외에서 성숙시킨 난자의 투명대에 접착하는 정도를 평가한 결과 SOD 무첨가보다는 첨가농도가 높아짐에 따라 접착정자수가 증가하였으며 100 units/ml 첨가시 유의적($P<0.05$)으로 높은 접착정자 수를 나타냈다. 본 연구의 결과로부터 SOD는 돼지 동결-융해정자에 있어서 난자의 투명대 접착능력의 증가와 함께 체외수정능력 향상에 영향을 미치는 것으로 나타났다.

(접수일자 : 2001. 10. 18. / 채택일자 : 2001. 11. 15.)