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SUPEROXIDE DISMUTASE AND GLUTATHIONE PEROXIDASE ACTIVITY IN PORCINE FOLLICULAR FLUID IN RELATION TO FOLLICLE SIZE, BIRTH STATUS OF GILTS, OVARIAN LOCATION AND YEAR SEASON

Bogdan Lasota, Barbara Błaszczyk, Tomasz Stankiewicz, Jan Udała, Monika Szewczyk, Danuta Matusiak-Bielska

West Pomeranian University of Technology, Szczecin

Abstract. Oxidative metabolism is essential for the gamete and the embryo energy production and is unavoidably associated with generation of reactive oxygen species (ROS). Enzymatic antioxidant defenses are present in the mammalian oocytes, embryos and follicular fluid (FF). The protection of porcine oocytes against the oxidation stress during IVM enhanced the developmental competence after fertilization. An addition of porcine FF to maturation media have beneficial effects on the IVM and IVF results. The aim of this study was to investigate the total SOD and GSH-Px activity in the FF of gilts, considering birth status, size of follicle, season and site of the body where the ovary for pFF was collected (left or right). The ovaries were collected from a total of 263 gilts (127 nulliparae at age 6–8 month and 136 multiparae gilts at age up to 12 month). To determine SOD activity, Ransod kits were used. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with I.N.T. to form a red formazan dye. GSH-Px activity in pFF was measured using Ransel kits with hydroxycumene as substrate. In all the analyzed samples of FF SOD and GSH-Px activity was found. The activity of SOD in different seasons varied from 0.656 to 0.886 $U \cdot ml^{-1}$, and that of GSH-Px from 1277 to 2372 $U \cdot l^{-1}$. Negative correlation between SOD and GSH-Px activity in pFF was generally slender to medium. The size of follicles, birth status and site from which the ovary was taken seems to play secondary role in the GSH-Px activity, however the birth status is more important for SOD activity. The GSH-Px activity was significantly lower in winter than in the other seasons. The differences in SOD activity between the seasons were not significant. It suggest that seasonally differences in activity of antioxidant enzymes should be taken into account while collecting pFF for IVM/IVF media.

Key words: birth status, glutathione peroxidase, porcine follicular fluid, season, superoxide dismutase

INTRODUCTION

Oxidative metabolism is essential for gamete and embryo energy production and is unavoidably associated with the generation of reactive oxygen species (ROS). An increase

Corresponding author – Adres do korespondencji: Dr hab. Bogdan Lasota, Department of Animal Reproduction, West Pomeranian University of Technology Szczecin, Doktora Judyma 6, 71-466 Szczecin, Poland, e-mail: Bogdan.Lasota@zut.edu.pl

in their concentration may lead to an oxidative stress. Under such conditions, ROS are responsible for damages to molecular and cell structures, with deleterious effects on cellular functions. Apart from non-enzymatic mechanisms, all organisms have an enzymatic mechanism to scavenge the oxidants, or repair damages caused by ROS.

Enzymatic antioxidant defences are present in mammalian oocytes and embryos [Moautassim et al. 1999, Cetica et al. 2000, Tarin et al. 2000, Guérin et al. 2001], as well as in human follicular fluid [Paszkowsky et al. 1995, Bisseling et al. 1997].

Superoxide dismutase (SOD) removes the superoxide anion in the dismutation reaction producing hydrogen peroxide and molecular oxygen. The removal of hydrogen peroxide is catalysed by either catalase or GSH-Px. SOD activity is localized in developing follicles and in postovulatory follicles [Laloraya et al. 1989]. The studies on rat and human ovaries suggest that anion superoxide and SOD may play a role in the ovulation process and in the development of oocytes [Shiotani et al. 1991, Sato et al. 1992].

Investigations led to determine optimal conditions for livestock animal follicular oocytes *in vitro* maturation have not brought satisfactory results so far. Porcine oocytes can develop to the blastocyst stage following maturation and fertilization in vitro, but their developmental potential is lower than that of oocytes which matured *in vivo* [Beckmann et al. 1993, Petters and Wells 1993, Dobrinsky et al. 1996, Abeydeera and Day 1997, Wang et al. 1997, Kano et al. 1998, Marchal et al. 2003].

In vitro cultures are performed under higher concentrations of oxygen as compared to the conditions of *in vivo* cultures, which results in enhanced production of ROS. According to Tatemoto et al. [2000], following IVM, cumulus-oocyte-complexes significantly increased the concentration of intracellular glutathione (GSH), whereas the GSH content in cumulus-denuded oocytes decreased. Also, exposure of denuded oocytes to ROS resulted in an increased frequency of apoptotic cell death. The protection of porcine oocytes against oxidation stress during IVM enhanced the post-fertilization developmental competence [Tatemoto et al. 2004].

The effects of adding FF to maturation media in relation to the developmental competence after IVF have been investigated in domestic animals [Naito et al. 1988, Elmileik et al. 1995, Ikeda et al. 1999, Vatzias et al. 1999, Gallardo et al. 2001, Ito et al. 2008], and the findings show that an addition of porcine FF to IVM medium have beneficial effects on IVM and IVF results.

The aim of this study was to investigate into the total SOD and GSH-Px activity in the porcine follicle fluid of gilts, considering the birth status of the gilt, the size of the follicle, the season of the year, as well as the site of the body the ovary was collected from (left or right).

MATERIAL AND METHODS

Animals and collection of pFF samples

The ovaries were collected from a total of 263 gilts (127 nulliparae at the age of 6–8 month and 136 multiparae at the age of up to 14 month) at the slaughter house and transported to the laboratory in 0.9% NaCl at 30°C. Within 2–3 h post slaughter, the pFF was

aspirated separately from non cystic follicles of diameters <5 mm and >5 mm, and from the left and the right ovary as well, and centrifuged at 3000 G for 10 minutes to remove debris, blood and granulosa cells. Thereafter, FF supernatant was transferred to Eppendorf tubes and stored at –20°C until assayed. Follicular fluid that was contaminated with significant quantities of blood were not used for analyses. The collection of pFF was performed during each of the four seasons of the year (winter, spring, summer and autumn).

Determination of SOD activity

To determine SOD activity the Ransod kits (RANDOX Laboratories Ltd., London, UK) were used. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The SOD activity is measured by the degree of inhibition of this reaction. The absorbance was monitored continuously at 504 nm and 37ºC. The SOD activity of each sample was determined based on the standard curve made as recommended in the manual of the kit. All standards rates and diluted sample rates were converted into the percentage sample dilution rate and subtracted from 100% to produce the percentage of inhibition. One unit of SOD activity expresses 50% inhibition of I.N.T. reduction. The SOD activity is presented in Tables as units in 1 ml of pFF.

Determination of GSH-Px activity

GSH-Px activity in pFF was measured using RANSEL kits (RANDOX Laboratories Ltd., London, UK), based on the method of Paglia and Valentine [1967], with hydroxycumene as substrate. The reaction was carried out in a spectrophotometer (EPOLL 20, Poll Ltd., Warsaw, Poland) at 37ºC. The method was based on an NADPH-coupled reaction, whereby oxidised glutathione (GSSG) produced by GSH-Px and hydroxyperoxide was reduced by exogenous glutathione reductase and NADPH. Enzyme activity was measured at 340 nm and expressed in units, each representing the oxidation of 1 micromole NADPH per minute per ml pFF. In Tables the GSH-Px activity is given in $U \cdot l^{-1}$.

Statistical analysis

The results were statistically analysed by means of the Statistica software package [StatSoft®Inc. Tulsa, OK, USA), using one-way analysis of variance (ANOVA), multivariate analysis of variance (MANOVA), Wilks-Rosenbaum tests, the Tukey statistic and the correlation matrix.

RESULTS

In all the analyzed samples of pFF, activity of SOD and GSH-Px was found. The activity of SOD in different seasons varied from 0.656 to 0.886, but the differences (up to 35%) were not significant (Table 1). The differences of GSH-Px activity between spring, summer and autumn reached maximum 14%; in winter, however, the GSH-Px activity represented only 53%, 60% and 61% of that found in spring, autumn and summer, respectively (Table 1).

Table 1. GSH and SOD activity in pFF in seasons
Tabela 1. Aktywność GSH-Px i SOD w płynie pęcherzykowym w poszczególnych sezonach

Season Sezon	n	SOD activity, U·ml^{-1} Aktywność SOD, U·ml^{-1}	n	GSH-Px activity, U·l^{-1} Aktywność GSH-Px, U·l^{-1}
Spring – Wiosna	46	0.656 ± 0.209	49	2080^{A} ± 582
Summer – Lato	58	0.832 ± 0.478	106	2372^{A} ± 1070
Autumn – Jesień	30	0.667 ± 0.355	46	2119^{A} ± 940
Winter – Zima	44	0.886 ± 0.568	62	1277^{B} ± 530
Total – Razem	178	0.772 ± 0.440	263	1277± 960

AB: $p \leq 0.01$.

The differences in relation to the other seasons were significant ($p \leq 0.01$). Table 2 presents SOD and GSH-Px activity in the follicular fluid taken from either the right or the left ovary in different seasons. The GSH-Px activity was about 11 to 28 percent higher in the pFF from the right ovary if taken in summer, autumn and winter, but lower in spring (~14%); however, the differences were non-significant. Reverse relations were noted in the SOD activity: in spring was the activity in pFF from the left ovary higher, while in summer, autumn and winter it was lower than in the follicles from the right ovary. The observed differences were not significant.

Table 2. GSH and SOD activity in pFF from right or left ovary in seasons
Tabela 2. Aktywność GSH-Px i SOD w płynie pęcherzykowym z prawego lub lewego jajnika w poszczególnych sezonach

Season/ovary Sezon/jajnik	n	SOD activity, U·ml^{-1} Aktywność SOD, U·ml^{-1}	n	GSH-Px activity, U·l^{-1} aktywność GSH-Px, U·l^{-1}
Spring – Wiosna				
Right – prawy	28	0.564 ± 0.203	28	2022 ± 425
Left – lewy	16	0.779 ± 0.127	13	2325 ± 680
Summer – Lato				
Right – prawy	23	0.802 ± 0.213	43	2509 ± 1240
Left – lewy	20	0.854 ± 0,602	50	2259 ± 991
Autumn – Jesień				
Right – prawy	20	0.643 ± 0.308	19	2367 ± 936
Left – lewy	19	0.690 ± 0.406	16	1848 ± 888
Winter – Zima				
Right – prawy	13	0.718 ± 0.294	26	1379 ± 670
Left – lewy	16	0.992 ± 0.670	26	1193 ± 369

The effect of birth status (nullipara or multipara) on GSH-Px or SOD activity was also investigated (Table 3). The activity of both enzymes in nulliparae or multiparae varied in different seasons. The SOD activity was significantly higher in pFF from nulliparae in each season, but only in autumn and winter were the differences significant ($p \leq 0.01$ and $p \leq 0.05$ respectively). The GSH-Px activity in nulliparae, however, was higher in spring, summer and autumn, and lower in winter; but the noted differences were not significant. The size of the follicle from which FF was taken seems to affect either GSH-Px nor SOD activity (Table 4). The highest differences between both GSH-Px and SOD activity were noted in autumn (19% and 27% respectively), though all resulting differences were not significant.

Table 3. GSH and SOD activity in pFF taken from either nulliparae or multiparae in seasons
Tabela 3. Aktywność GSH-Px i SOD w płynie pęcherzykowym pierwiastek lub wieloródek w poszczególnych sezonach

Season Sezon Birth status – status porodowy	n	SOD activity, $U \cdot ml^{-1}$ Aktywność SOD, $U \cdot ml^{-1}$	n	GSH-Px activity, $U \cdot l^{-1}$ Aktywność GSH-Px, $U \cdot l^{-1}$
Spring – Wiosna				
nullipara – pierwiastki	35	0.665 ± 0.197	38	2054 ± 625
multipara – wieloródki	11	0.626 ± 0.252	11	2169 ± 409
Summer – Lato				
nullipara – pierwiastki	40	0.903 ± 0.560	68	2253 ± 974
multipara – wieloródki	18	0.676 ± 0.098	38	2584 ± 1209
Autumn – Jesień				
nullipara – pierwiastki	11	$1{,}047^{A}$ ± 0.246	11	1571 ± 390
multipara – wieloródki	19	0.447^{B} ± 0.174	35	2291 ± 999
Winter – Zima				
nullipara – pierwiastki	10	1.314^{a} ± 0.915	10	1492 ± 259
multipara – wieloródki	34	0.761^{b} ± 0.346	52	1236 ± 560

AB: $p \leq 0.01$; ab: $p \leq 0.05$.

Table 4. GSH and SOD activity in pFF from follicles with different size (diameter < 5 mm and > 5 mm) in each seasons

Tabela 4. Aktywność GSH-Px i SOD w płynie pęcherzykowym z pęcherzyków róznej wielkości (średnica < 5 mm i > 5 mm) w poszczególnych sezonach

Season Sezon Size Wielkość	n	SOD activity, U·ml^{-1} Aktywność SOD, U·ml^{-1}	n	GSH-Px activity, U·l^{-1} Aktywność GSH-Px, U·l^{-1}
Spring – wiosna				
<5	15	0.723 ± 0.187	15	1961 ± 1255
>5	41	0.648 ± 0.212	44	2093 ± 479
Summer – lato				
<5	20	0.784 ± 0.179	40	2535 ± 1183
>5	38	0.858 ± 0.578	66	2273 ± 992
Autumn – jesień				
<5	16	0.804 ± 0.284	18	1828 ± 910
>5	24	0.632 ± 0.368	38	2180 ± 947
Winter – zima				
<5	23	0.847 ± 0.475	28	1139 ± 500
>5	26	0.898 ± 0.598	34	1314 ± 536

Correlation between SOD and GSH-Px activity in pFF was generally slender to medium. A significant correlation was noted between SOD and GSH-Px activity in all samples totally ($r = –0.23$, $p \leq 0.05$). In summer and autumn, the correlation coefficients were also significant ($r = –0.32$ and $r = –0.29$, respectively), but not in spring or winter ($r = –0.06$ and $r = 0.05$, respectively). The MANOVA analysis showed that the season had the most important effect on the activity of both enzymes (Wilks-Rosenbaum test, $F = 10.415$; $p \leq 0.0001$).

DISCUSSION

Both tested enzymes showed their activity in all the samples of porcine follicular fluid. The GSH-Px activity in pFF was about twice lower than that in the blood plasma (5300 U·l^{-1}) of boars (measured in the same laboratory using the same method like in pFF [Lasota et al. 2004]). In a study of Paszkowsky et al. [1995], the mean GSH-Px activity in human follicular fluid was found to be about 70% of its serum activity. The comparison of SOD activity obtained in this paper against the results reported by other authors is difficult due to the diversity of used methods and their modifications. Many authors ascertained SOD activity in follicular fluid of pigs [Tatemoto et al. 2004], ruminants [Singh et al. 1998], rats [Tilly and Tilly 1995], as well as women [Carbone et al. 2003]. The relations between the

analyed factors (season, follicle size, site from which the ovary was taken, and birth status) and GSH-Px activity appeared clear only in the case of seasons. The GSH-Px activity in winter was significantly lower than in the remaining seasons. Interestingly, the GSH-Px activity in pFF –low in winter, high in summer – have formed like in the seminal plasma of boars [Lasota et al. 2004]. A clear difference in the SOD activity was ascertained only between pFF taken in summer and winter (the highest activity) and in spring and autumn (when the activity was lowest). But the occurred differences were not significant. A the comparison of the activity of both enzymes in different seasons reveals that in summer the SOD and GSH-Px activity was high. This can suggest that the processes leading to production of ROS intensify during this season, and a high levels of SOD as well as GSH-Px are need to neutralize them. Sabatini et al. [1999] found that a high SOD activity can inhibit the rupture of follicles, a process which based on mechanism used ROS. Therefore, it is not groundless if we suppose that it has an association with the so called "summer infertility" in swine, especially if the GSH-Px activity in the blood and the semen of boars in summer be included. But why the high SOD activity in winter has been accompanied by low GSH-Px activity is not clear. Further studies are need to clarify it.

The comparison of activity of both tested enzymes in pFF from nulliparae or multipara pigs showed that in younger sows SOD activity was higher than in older ones, but the differences were significant only in autumn and winter. On the contrary, Carbone et al. [2003] reported, that in women of advanced reproductive age (39–45) the SOD activity in follicular fluid increased significantly. The mentioned authors did not report the birth status of the patients, though. The investigated sows, however, were young (6–12 month) and in this case probably the birth status and season differentiated the SOD activity in follicular fluid rather than age. In contrast, the GSH-Px activity in pFF was higher in spring, summer and autumn in the multiparae. This corresponds with the report of Carbone et al. [2003], who observed a trend of increased GSH-Px activity in older women. In contrast, the GSH-Px activity in winter was lower in pFF of the multiparae. It seems that GSH-Px activity in pFF is not affected by birth status or age.

Not uniformly was SOD and GSH-Px activity in pFF from follicle with different size. Singh et al. [1998] estimated SOD activity of goats and sheep in the follicles < 3 mm, 3–6 mm, and > 6 mm in diameter. Small follicles of both species domstrated higher activity, whereas large follicles showed the lowest SOD activity. Further investigations are needed to find out if the SOD activity in pFF is affected by the size of the follicle and whether the observations by Singh et al. [1998] are characteristic only for the species.

Further research is also needed to ascertain that the SOD activity is higher in follicle fluid from the left ovary. In woman, the right ovarian artery is slightly shorter than the left one [Krechowiecki and Czerwiński 1991], what can lead to uneven supply of oxygen to the left and the right ovary and in different production of ROS. Probably, the vascularization of the right and the left ovary in gilts is different from that in woman. Further investigations are need to clarify that as well.

The correlations between the SOD and GSH-Px activities were generally negative, slender to medium depending on the season negatively. It is well known that SOD belongs to the first enzymatic step that protect cells against toxic oxygen radicals. The H_2O_2, the

product of SOD activity, is eliminated either by catalase or by GSH-Px. The latter enzyme response for catabolism of majority amount of H_2O_2 in cells, if its concentration is low. However, if H_2O_2 concentration is high, catalase takes over the role of GSH-Px [Comhair and Erzurum 2005]. The possible explanation for the negative correlation between GSH-Px and SOD activity is the assumption that catalase has taken over the catabolism of H_2O_2. The assumption may be true for spring and autumn, when SOD activity was lowest of all the seasons (consequently, low production of H_2O_2); GSH-Px activity was high, however, and such high activity of both enzymes in summer can not be explained with the above presented hypothesis. A possible explanation can lie in the theory that catalase is effective only against H_2O_2, while GSH-Px reduces lipid hydroxyperoxides and H_2O_2 as well [Guérin et al. 2001]. The high activity of both enzymes can suggest that an increased lipid peroxidation in follicles occurs in summer.

The following conclusions can be drawn from the presented results: the size of the follicles, birth status and the site from which the ovary was taken seem to play secondary role in the GSH-Px activity, although the birth status is more important for the SOD activity. The differences in the SOD activity between the seasons were not significant. The GSH-Px activity was significantly lower in winter as compared to the other seasons ($p < 0.01$). Consequently, the seasonally differences in activity of antioxidant enzymes should be taken into account while collecting pFF for IVM/IMF media.

REFERENCES

Abeydeera L.R., Day B.N., 1997. Fertilization and subsequent development *in vitro* of pig oocytes inseminated in a modified tris-buffered medium with frozen-thawed ejaculated spermatozoa. Biol. Reprod. 57, 729–734.

Beckmann L.S., Day B.N., 1993. Effects of media NaCl concentration and osmolarity on the culture of early-stage pig embryos and the viability of embryos cultured in a selected superior medium. Theriogenology 39, 611–622.

Bisseling J.G.A., Knapen M.F.C.M., Goverde H.J.M., Mulder T.P.J., Peters W.H.M., Willemsen W.N.P., Thomas C.M.G., Steegers E.A.P., 1997. Glutathione S-transferaze in human ovarian follicular fluid. Fertil. Steril. 68, 907–911.

Carbone M.C., Tatone C., Delle Monach S., Marci R., Caserta D., Colonna R., Amiracelli F., 2003. Antioxidant enzymatic defences in human follicular fluid: characterization and age-dependent changes. Mol. Hum. Reprod. 9 (11), 639–643.

Cetica P.D., Pintos L.N., Dalvit G.C., Becon, M.T., 2000. Antioxidant enzyme activity and oxidant stress in bovine oocyte *in vitro* maturation. IUBMB Life 51, 57–64.

Comhair S.A., Erzurum S.C., 2005. The regulation and role of extracellular glutathione peroxidase. Antioxid. Redox, Signal. 7, 72–79.

Dobrinsky J.R., Johnson L.A., Rath D., 1996. Development of a culture medium (BECM3) for pig embryos: effects of bovine serum albumin and fetal bovine serum on embryo development. Biol. Reprod. 55, 1069–1074.

Elmileik A.M.A., Maeda T., Terada T., 1995. Higher rates of development into blastocyst following the *in vitro* fertilization of bovine oocytes matured in a medium supplemented with the fluid from large bovine follicles. Anim. Reprod. Sci. 38, 85–96.

Gallardo O.L., Gonzalez M.H., Ducolomb Y., Casas E., Betancourt M., 2001. Influence of porcine follicular fluid protein fractions on oocyte maturation *in vitro.* Bioquimia 26, 59–63.

Guérin P., Mouatassim S.E.I., Ménézo Y., 2001. Oxidative stress and protection against reactive oxygene species in the pre-implantation embryo and its surroundings. Hum. Reprod. Update 7, 175–189.

Ikeda S., Azuma T., Hashimoto S., Yamada M., 1999. *In vitro* maturation of bovine oocytes with fractions of bovine follicular fluid separated by heparin affinity chromatography. J. Reprod. Dev. 45, 397–404.

Ito M., Iwata H., Kitagawa M., Kon Y., Kuwayama T., Monji Y., 2008. Effect of follicular fluid collected from various diameter follicles on the progression of nuclear maturation and developmental competence of pig oocytes. Anim. Reprod. Sci. 106, 421–430.

Kano K., Miyano T., Kato S., 1998. Effects of glycosaminoglycans on the development of *in vitro*-matured and -fertilized porcine oocytes to the blastocyst stage *in vitro*. Biol. Reprod. 58, 1226–1232.

Krechowiecki A., Czerwiński F., 1991. Zarys anatomii człowieka [Human anatomy in outline]. Wydawnictwo Lekarskie PZWL, Warszawa [in Polish].

Laloraya M., Kuma G.P., Laloray M.M., 1989. Histochemical study of superoxide dismutase in the ovary of the rat during the oestrus cycle. J. Reprod. Fertil. 86, 583–587.

Lasota B., Blaszczyk B., Seremak B., Udala J., 2004. Selenium status and GSH-Px activity in semen and blond of boars at different ages used for artificial insemination. Reprod. Dom. Anim. 39, 309–314.

Marchal R., Caillaud M., Martoriati A., Gerard N., Mermillod P., Goudet G., 2003. Effect of growth hormone (GH) on *in vitro* nuclear and cytoplasmic oocyte maturation, cumulus expansion, hyaluronan syntheses, and connexins 32 and 43 expression, and GH receptor messenger RNA expression in equine and porcine species. Biol. Reprod. 69, 1013–1022.

Moautassim S.E.I., Guérin P., Ménézo Y., 1999. Expression of genes encodingantioxidant enzymes in humen and mouse oocytes during the final stage of maturation. Mol. Hum. Reprod. 5, 720–725.

Naito K., Fukuda Y., Toyota Y., 1988. Effects of porcine follicular fluid on male pronucleus formation in porcine oocytes maturation *in vitro*. Gamete. Res. 21, 289–295.

Paszkowsky T., Traub A.I., Robinson S.Y., McMaster D., 1995. Selenium dependent glutathione peroxidase activity in human follicular fliud. Clin. Chim. Acta 236, 173–180.

Paglia D.E., Valentine W.N., 1967. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70, 158–169.

Petters R.M., Wells K.D., 1993. Culture of pig embryos. J. Reprod. Fertil. 48 (suppl.), 61–73.

Sabatini L., Wilson C., Lower A., Al-Shawaf T., Grudiznskaa J.G., 1999. Superoxide dismutase activity in human follicular fluid after controlled ovarian hyperstimulation in women undergoing *in vitro* fertilization. Fert. Steril. 72 (6), 1027–1034.

Sato E.F., Kobuchi H., Edashige K., Takahashi M., Yoshioka T., Utsumi K., Inoue M., 1992. Dynamic aspects of ovarian superoxide dismutase isoenzymes during the ovulatory process in the rat. FEBS Lett. 303, 121–125.

Singh D., Sharma M.K., Pandey R.S., 1998. Changes in superoxide dismutase activity and estradiol-17 beta content in follicles of different size from ruminants. Ind. J. Exp. Biol. 36 (4), 258–360.

Shiotani M., Noda Y., Narimoto K., Imai K., Mori T., Fujimoto K., Ogawa K., 1991. Immunohistochemical localization of superoxide dismutase in the human ovary. Hum. Reprod. 6, 1349–1351.

Tarin J.J., Pérez-Albalá S., Cano A., 2000. Consequences on offspring of abnormal function in ageing gametes. Hum. Reprod. Update 6, 532–549.

Tatemoto H., Samurai N., Muto N., 2000. Protection of porcie oocytem against apoptotic cell Heath caused by oxidative stress during *In vitro* maturation: role of cumulus cells. Biol. Reprod. 63, 805–810.

Tatemoto H., Muto N., Sunagawa I., Shinjo A., Nakada T., 2004. Protection of porcine oocytes against cell damage caused by oxidative stress during *in vitro* maturation: role of superoxide dismutase activity in porcine follicular fluid. Biol. Reprod. 71, 1150–1157.

Tilly J.L., Till K.I., 1995. Inhibitors of oxidative stress mimic the ability of follicle-stimulating hormone to suppress apoptosis in cultured rat ovarian follicles. Endocrinology 136 (1), 242–252.

Vatzias G., Hagen D.R., 1999. Effects of porcine follicular fluid and oviduct-conditioned media on maturation and fertilization of porcine oocytes *in vitro*. Biol. Reprod. 60, 42–48.

Wang W.H., Abeydeera L.R., Cantley T.C., Day B.N., 1997. Effects of oocyte maturation media on development of pig embryos produced by *in vitro* fertilization. J. Reprod. Fertil. 111, 101–108.

AKTYWNOŚĆ DYSMUTAZY PONADTLENKOWEJ I PEROKSYDAZY GLUTATIONOWEJ W ŚWIŃSKIM PŁYNIE PĘCHERZYKOWYM W ODNIESIENIU DO WIELKOŚCI PĘCHERZYKA, STATUSU PORODOWEGO LOCH, POŁO

Streszczenie: Metabolizm oksydacyjny, niezbędny do wytwarzania energii dla gamet i zarodków, związany jest nieuchronnie z powstawaniem reaktywnych form tlenu (RFT). Stwierdzono, że u ssaków enzymatyczne mechanizmy obrony antyoksydacyjnej występują w oocytach, zarodkach oraz płynie pęcherzykowym. Na podstawie opublikowanych wyników badań można stwierdzić, że chroniąc świńskie komórki jajowe przed stresem oksydacyjnym podczas dojrzewania *in vitro* zwiększa się zdolność rozwojową oocytów po zapłodnieniu. Dodatek świńskiego płynu pęcherzykowego (pFF) do pożywek ma korzystny wpływ na wyniki dojrzewania i zapłodnienia *in vitro.* Celem niniejszej pracy było zbadanie całkowitej aktywności SOD i GSH-Px w płynie pęcherzykowym loch w różnych porach roku, z uwzględnieniem statusu porodowego, wielkości pęcherzyków i strony, z której pobierano płyn pęcherzykowy (prawa lub lewa). Jajniki pobrano od 127 pierwiastek w wieku 6–8 miesięcy i 136 wieloródek w wieku do 14 miesięcy. Aktywność SOD oznaczano za pomocą zestawów RANSOD, w których wykorzystuje się ksantynę i oksydazę ksantynową do wytworzenia rodników ponadtlenkowych. Aktywność GSH-Px oznaczana była zestawami RANSEL, w których substratem był nadtlenek kumenowy. Aktywność SOD wahała się w różnych sezonach od 0,656 do 0,886 $U \cdot ml^{-1}$, natomiast aktywność GSH-Px od 1277 do 2372 $U \cdot l^{-1}$. Stwierdzone ujemne korelacje między aktywnością SOD i GSH-Px w pFF były ogólnie przeciętne i nikłe. Wydaje się, że wielkość pęcherzyków, status porodowy i strona, z której pobierano jajnik, mają raczej niewielki związek z aktywnością GSH-Px, natomiast na aktywności SOD większy wpływ może mieć status porodowy loch. Aktywność GSH-Px była istotnie niższa w zimie niż w pozostałych sezonach. Różnice aktywności SOD w poszczególnych sezonach były statystycznie nieistotne. Uzyskane wyniki wskazują, że pobierając świński płyn pę-

cherzykowy z zamiarem stosowania go do dojrzewania i zapłodnienia *in vitro* można się liczyć z sezonowymi różnicami w aktywności enzymów antyoksydacyjnych.

Słowa kluczowe: dysmutaza ponadtlenkowa, peroksydaza glutationowa, sezon, status porodowy, świński płyn pęcherzykowy

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