Original Article

Cryopreservation Effect on Expression of Sex Steroid Receptors of Boar Spermatozoa

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Abstract

This study aimed to investigate the expressions of estrogen receptor (ER) alpha, ER beta, and progesterone receptor (PR) during semen cryopreservation, to evaluate the correlation among ERα, ERβ, PR, capacitation, acrosome reaction, and semen quality parameters, and to investigate the effect of different concentrations of L-cysteine on the expressions of ERα, ERβ, and PR. Semen samples were collected from 12 boars [Duroc (n = 4), Landrace (n=4), and Large white (n=4)]. The controlled rate-freezing method was used to cryopreserve the semen samples. The samples were collected at different times: fresh semen, after adding Modena™, after adding extender II (TE2), after adding extender III (TE3), and post-thaw (PT). For extender II and III, the samples were divided into 4 groups: negative control (group 1), 5 (group 2), 10 (group 3), and 15 (group 4) mM/100ml L-cysteine supplementation. Sperm parameters including motility, intact plasma membrane, acrosome integrity, non-capacitated sperm, ERα-, ERβ-, and PR-positive spermatozoa were evaluated. Correlations among all parameters were also investigated. Results demonstrated that the acrosome integrity of group 3 was higher than that of group 1 and 4. Moreover, the other parameters of group 3 tended to be higher than those of the other groups. Regarding time during cryopreservation, all parameters in TE2 and TE3 were higher than those in PT. The expression of sex steroid receptors (ERα, ERβ, and PR) positively correlated with motility, intact plasma membrane, acrosome integrity, and non-capacitated spermatozoa. In conclusion, cryopreservation and thawing resulted in decreased expressions of ERα, ERβ, PR, and sperm qualities. Moreover, L-cysteine supplementation in different concentrations did not affect the expressions of ERα, ERβ and PR.

Keywords: boar, cryopreservation, ERα, ERβ, PR, spermatozoa

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Introduction

Nowadays artificial insemination (AI) with fresh semen is a preferable technique used for swine production. However, swine AI with fresh semen has been limited due to the short storage time. In order to conserve the superior boar genetics, semen cryopreservation technique is applied. However, frozen boar semen are not widely used because the farrowing rate and number of total pigs born/litter are 10-25% lower than fresh semen insemination (Waterhouse et al., 2006). These might be caused by the effect of cryopreservation process which induces changes in spermatozoa quality after thawing, including sperm morphology, acrosome integrity, and survival rate (Cerolini et al., 2001). The effect of cold shock on spermatozoa during cryopreservation occurs during the rapid temperature decreasing process, resulting in ice crystals in spermatozoa and water reflux from osmotic changes, contributing to abrupt alteration of plasma membrane integrity (Hammerstedt et al., 1990). The increased free radicals, oxidants, and reactive oxygen species affect the fertilizability of spermatozoa by decreasing membrane fluidity and inducing DNA fragmentation (Aitken and Clarkson, 1988). Moreover, spermatozoa capacitation is induced by cryopreservation, causing short survival time after thawing (Maxwell and Johnson, 1997; Vishwanath and Shannon, 1997).

Previous studies demonstrated the role of sex steroid hormones and their receptors to sperm quality, for example, a study in human spermatozoa showed that the capacitation, hyperactivation, and acrosome reaction took place via progesterone stimulation by binding with its receptor located on sperm membrane (Contreras and Llanos, 2001). Moreover, the presence of progesterone receptor (PR) positively correlated with spermatozoa membrane integrity and normal morphology (Meizel and Turner, 1991; Yang et al., 1994). The role of estrogen in human spermatozoa, including the reduction of calcium reflux and the inhibition of acrosome reaction, is induced by progesterone (Yang et al., 1994). Rago et al. (2007) demonstrated the expression of estrogen receptor subtype alpha (ERα) and beta (ERβ) on different locations of boar spermatozoa and suggested different roles of estrogens on boar sperm physiology.

However, the study of ER and PR expressions on boar spermatozoa has been scant, especially in cryopreserved spermatozoa. The expressions of sex steroid receptors may relate to the quality and fertilizability of boar spermatozoa. Therefore, the study of the expression of ER and PR during the cryopreservation process was performed to elucidate the effect of sex steroid hormones on spermatozoa quality.

Materials and Methods

The experimental plan was approved by the Ethical Committee for Experimentation with Animal at Faculty of Veterinary Science, Mahidol University.

***Animals*:** Twelve fertile boars (4 Landrace, 4 Duroc, and 4 Large white) aged >1 year old were used in the experiment. All were treated and fed on the same dietary and water management. Semen qualities were routinely evaluated.

***Freezing extenders*:** Freezing extenders used in this study were prepared as follows: extender II was composed of 80% (v/v) lactose solution and 20% (v/v) egg yolk; and extender III consisted of 89.5% of extender II, 9% (v/v) glycerol, and 1.5% (v/v) Equex-STM® (Nova chemical sales Inc, USA).

***Semen collection*:** Fresh semen samples were obtained by hand-gloved technique (Kaeoket et al., 2002). Only sperm rich fraction was collected for further analysis.

***Fresh semen preparation*:** The collected semen samples were evaluated for motility under phase-contrast microscope (Olympus, CX31), sperm viability using SYBR-14/Ethidium homodimer-1 staining, acrosome integrity using FITC/PNA (Chanapiwat et al., 2009), and capacitation with CTC staining (Maxwell and Johnson, 1997) under an inverted fluorescence microscope (Nikon, ECLIPSE, TE2000-U). Head morphology was evaluated using William's stain under a light microscope. Tail morphology was investigated under a phase-contrast microscope. Semen samples that had motility and sperm morphology more than 75% were included in the experiment. Thereafter, semen extender I (Modena™, Swine Genetics International, Ltd., USA) was added for transportation.

***Semen cryopreservation and thawing*:** Semen freezing and thawing protocols were performed according to Kaeoket and Tantiparinyakul (2008). Briefly, after the semen samples were diluted in extender I and equilibrated at 15°C for 2 h, they were centrifuged at 800x g for 10 min. The supernatants, afterwards, were removed and the sperm pellets were re-suspended with freezing extender II to a concentration of $1.5x10^6$ spz/ml. The semen was divided into 4 groups due to different concentrations of L-cysteine (Fluka Chemie GmbH, Sigma-Aldrich, Switzerland) [no L-cysteine (group 1), 5mM (group 2), 10 mM (group 3), and 15mM (group 4)] L-cysteine] in extender II. The sperm suspensions were equilibrated to 5°C for 90 min. Thereafter, extender III was added to the sperm suspensions to a final concentration of $1x10^6$ spz/ml. Then, the suspension was loaded into 0.5 ml straws which were placed 3 cm above liquid nitrogen vapor for 20 min and were plunged into liquid nitrogen for storage.

To thaw the semen samples, the straws were immersed in water at 50°C for 12 sec. The frozen-thawed spermatozoa were then diluted (1:4) into pre-warmed extender I (37°C). After incubation at 37°C for 5 min (0 h), 2 h, and 4 h, the sperm suspensions were taken to evaluate for sperm motility, plasma membrane integrity, acrosome integrity, and capacitated spermatozoa.

***Immunofluorescence for PR, ERa, and ERβ in boar spermatozoa*:** The **s**emen samples were collected during different processes of semen cryopreservation as follows: (1) Fresh semen, (2) after Modena was added at 15°C for 2 h, (3) after extender II was added

at 5°C for 1 h 30 min (TE2), (4) after extender III was added at 5°C for 30 min (TE3), and (5) post-thaw (PT).

Immunofluorescence was conducted according to the protocol of Rago et al. (2007). Briefly, after Percoll separation, the spermatozoa were rinsed in 0.5 mM Tris-HCl buffer, pH 7.5 for 3 times. Ten microliters of sperm suspension was mixed in 250 ml Tris buffer saline (TBS) at 37°C and dropped on a glass slide in a moist chamber. After the spermatozoa settled upon the glass slide, the supernatant was removed and replaced with absolute methanol. Then, the slides were incubated at -20°C for 7 min. The spermatozoa were washed with 0.1% (v/v) Triton X-100 (Acros Organics, USA) in TBS before performing immunofluorescence. The spermatozoa were overnight incubated with a primary antibody for PR (mouse monoclonal, Immunotech, clone 10A9, dilution 1:100), ERα (mouse monoclonal, Santa Cruz, clone F-10, dilution 1:100), and ERβ (rabbit polyclonal, Santa Cruz, clone H-150, dilution 1:100) at 4°C. Secondary antibodies which were anti-mouse IgG Texas red conjugated (Abcam, dilution 1:100) and anti-rabbit IgG FITC conjugated (Abcam, dilution 1:100) were applied with PR and ERα, and with ERβ, respectively, at room temperature for 1 h. The spermatozoa with omitted primary antibody served as negative control. Finally, 200 spermatozoa per slide were immediately evaluated under an epifluorescence microscope (Olympus, BX51) at 100x magnification. Results of steroid receptors expression were shown as percentage of steroid receptor-positive spermatozoa during cryopreservation and post-thawing.

***Statistical analysis*:** Data were handled and statistically analyzed using the SAS statistical package (SAS Inst. V.9.0, Cary, NC USA). Normal distribution of residuals from the statistical models was tested using UNIVARIATE procedure option NORMAL PLOT. Statistical differences in percentages of sperm motility, plasma membrane integrity, acrosome integrity, non-capacitated spermatozoa, positive ERα, ERβ, and PR among groups were tested by General linear mixed model (MIXED). All data were expressed as means ± SD. Values with $p < 0.05$ were considered statistically significant.

Results

***Expressions of estrogen and progesterone receptors*:** The positive immunofluorescence staining was demonstrated as follows: positive red fluorescence staining of PR was observed at the spermatozoa mid-piece (Fig 1), positive red immunofluorescence staining of ERα was observed at mid-piece and tail of the spermatozoa (Fig 2), and positive green immunofluorescence staining of ERβ was presented at acrosomal head of the spermatozoa (Fig 3). The negative control showed no immunoreaction after being counterstained with DAPI (Fig 4).

***Qualities of fresh semen and Modena™-added semen*:** The parameters of fresh and Modena™-added semen are presented in Table 1. The percentage of sperm motility, plasma membrane integrity, acrosome integrity, and non-capacitated sperm between these 2 groups were not significantly different.

Table 1 Semen parameters of fresh semen and Modena™-added semen (mean ± SD)

Semen parameters	Fresh semen (n=13)	Modena (n=13)	*p*-value
Motility (%)	77.7 ± 5.3	76.0 ± 6.5	NS
Intact plasma membrane (%)	78.5 ± 9.3	79.3 ± 11.9	NS
Intact acrosome in live spermatozoa (%)	79.2 ± 8.3	78.4 ± 7.7	NS
Capacitation F-pattern (%)	79.7 ± 4.4	73.3 ± 12.5	NS
ERα	62.6 ± 8.2	64.2 ± 7.7	NS
ERβ	74.6 ± 12.1	67.0 ± 10.2	NS
PR	65.5 ± 3.3	67.5 ± 3.7	NS

NS = no significant difference ($P > 0.05$)

Table 2 Percentage of sperm motility during cryopreservation process and post-thaw (mean ± SD)

Groups	TE2	TE3	Post-thaw
1 (n=12)	$70.6 \pm 10.8^{A, a}$	$58.8 \pm 11.3^{A, a}$	$30.0 \pm 15.7^{B, a}$
2 (n=12)	$68.8 \pm 9.9^{A, a}$	$61.7 \pm 16.2^{A, a}$	$28.1 \pm 13.9^{B, a}$
3 (n=12)	$76.3 \pm 5.2^{A, a}$	$66.7 \pm 16.6^{A, a}$	$36.9 \pm 16.1^{B, a}$
4 (n=12)	$72.5 \pm 7.1^{A, a}$	$55.6 \pm 18.1^{A, a}$	$23.5 \pm 12.5^{B, a}$

Means (± SD) within the same column followed by different small superscript letters, and within the same row followed by different capital superscript letters are significantly different ($p < 0.05$).

Group 1 = control group (no L-cysteine supplement)
Group 2 = 5 mMol/100 ml L-cysteine supplement
Group 3 = 10 mMol/100 ml L-cysteine supplement
Group 4 = 15 mMol/100 ml L-cysteine supplement
TE2 = 1.5 h after the addition of freezing extender II
TE3 = 30 min after the addition of freezing extender III

Table 3 Percentage of intact sperm plasma membrane during cryopreservation process and post-thaw (mean ± SD)

Groups	TE2	TE3	Post-thaw
1 (n=12)	75.1 ± 9.2$^{A, a}$	76.7 ± 6.6$^{A, a}$	34.0 ± 13.0$^{B, a}$
2 (n=12)	73.5 ± 9.2$^{A, a}$	75.9 ± 9.0$^{A, a}$	31.0 ± 18.2$^{B, a}$
3 (n=12)	70.4 ± 22.3$^{A, a}$	77.9 ± 5.6$^{A, a}$	45.1 ± 21.5$^{B, a}$
4 (n=12)	73.2 ± 6.6$^{A, a}$	76.4 ± 6.0$^{A, a}$	29.0 ± 14.9$^{B, a}$

Means (± SD) within the same column followed by different small superscript letters, and within the same row followed by different capital superscript letters are significantly different (p <0.05).

Group 1 = control group (no L-cysteine supplement)
Group 2 = 5 mMol/100 ml L-cysteine supplement
Group 3 = 10 mMol/100 ml L-cysteine supplement
Group 4 = 15 mMol/100 ml L-cysteine supplement
TE2 = 1.5 h after the addition of freezing extender II
TE3 = 30 min after the addition of freezing extender III

Table 4 Percentage of intact acrosome during cryopreservation process and post-thaw (mean ± SD)

Groups	TE2	TE3	Post-thaw
1 (n=12)	70.5 ± 9.5$^{A, a}$	66.8 ± 8.6$^{A, a}$	44.1 ± 7.7$^{B, a}$
2 (n=12)	69.3 ± 9.1$^{A, a}$	65.1 ± 10.0$^{A, a}$	47.7 ± 8.5$^{B, ab}$
3 (n=12)	69.7 ± 11.0$^{A, a}$	69.8 ± 10.6$^{A, a}$	54.0 ± 10.8$^{B, b}$
4 (n=12)	67.3 ± 10.7$^{A, a}$	63.3 ± 11.0$^{A, a}$	41.8 ± 9.0$^{B, a}$

Means (± SD) within the same column followed by different small superscript letters, and within the same row followed by different capital superscript letters are significantly different (p <0.05).

Group 1 = control group (no L-cysteine supplement)
Group 2 = 5 mMol/100 ml L-cysteine supplement
Group 3 = 10 mMol/100 ml L-cysteine supplement
Group 4 = 15 mMol/100 ml L-cysteine supplement
TE2 = 1.5 h after the addition of freezing extender II
TE3 = 30 min after the addition of freezing extender III

Table 5 Percentage of non-capacitated spermatozoa during cryopreservation process and post-thaw (mean ± SD)

Groups	TE2	TE3	Post-thaw
1 (n=12)	70.5 ± 7.8$^{A, a}$	64.3 ± 13.3$^{A, a}$	22.7 ± 12.5$^{B, a}$
2 (n=12)	69.7 ± 11.1 $^{A, a}$	61.2 ± 13.2$^{A, a}$	27.5 ± 7.4$^{B, a}$
3 (n=12)	72.3 ± 9.1$^{A, a}$	67.3 ± 14.4$^{A, a}$	30.5 ± 13.3$^{B, a}$
4 (n=12)	68.2 ± 7.5$^{A, a}$	61.8 ± 12.7$^{A, a}$	23.4 ± 7.9$^{B, a}$

Means (± SD) within the same column followed by different small superscript letters, and within the same row followed by different capital superscript letters are significantly different (p <0.05).

Group 1 = control group (no L-cysteine supplement)
Group 2 = 5 mMol/100 ml L-cysteine supplement
Group 3 = 10 mMol/100 ml L-cysteine supplement
Group 4 = 15 mMol/100 ml L-cysteine supplement
TE2 = 1.5 h after the addition of freezing extender II
TE3 = 30 min after the addition of freezing extender III

Table 6 Percentage of ERα-positive spermatozoa during cryopreservation and post-thaw (mean ± SD)

Groups	TE2	TE3	Post-thaw
1 (n=12)	57.8 ± 10.2$^{A, a}$	55.8 ± 9.2$^{A, a}$	47.4 ± 3.3$^{B, a}$
2 (n=12)	57.5 ± 6.9$^{A, a}$	58.8 ± 7.6$^{A, a}$	47.8 ± 2.6$^{B, a}$
3 (n=12)	61.1 ± 7.4$^{A, a}$	58.2 ± 8.5$^{A, a}$	49.0 ± 4.0$^{B, a}$
4 (n=12)	55.8 ± 8.1$^{A, a}$	55.6 ± 6.3$^{A, a}$	44.3 ± 2.8$^{B, a}$

Means (± SD) within the same column followed by different small superscript letters, and within the same row followed by different capital superscript letters are significantly different (p <0.05).

Group 1 = control group (no L-cysteine supplement)
Group 2 = 5 mMol/100 ml L-cysteine supplement
Group 3 = 10 mMol/100 ml L-cysteine supplement
Group 4 = 15 mMol/100 ml L-cysteine supplement
TE2 = 1.5 h after the addition of freezing extender II
TE3 = 30 min after the addition of freezing extender III

Table 7 Percentage of ERβ-positive spermatozoa during cryopreservation and post-thaw (mean ± SD)

Groups	TE2	TE3	Post-thaw
1 (n=12)	$61.7 \pm 8.2^{A, a}$	$60.7 \pm 8.7^{A, a}$	$55.5 \pm 6.8^{A, a}$
2 (n=12)	$66.5 \pm 7.3^{A, a}$	$60.9 \pm 8.6^{AB, a}$	$54.7 \pm 6.0^{B, a}$
3 (n=12)	$64.8 \pm 9.0^{A, a}$	$61.3 \pm 7.4^{A, a}$	$57.7 \pm 9.1^{A, a}$
4 (n=12)	$64.2 \pm 10.2^{A, a}$	$60.8 \pm 7.1^{A, a}$	$50.2 \pm 6.1^{B, a}$

Means (± SD) within the same column followed by different small superscript letters, and within the same row followed by different capital superscript letters are significantly different (p <0.05).

Group 1 = control group (no L-cysteine supplement)
Group 2 = 5 mMol/100 ml L-cysteine supplement
Group 3 = 10 mMol/100 ml L-cysteine supplement
Group 4 = 15 mMol/100 ml L-cysteine supplement
TE2 = 1.5 h after the addition of freezing extender II
TE3 = 30 min after the addition of freezing extender III

Table 8 Percentage of PR-positive spermatozoa during cryopreservation and post-thaw (mean ± SD)

Groups	TE2	TE3	Post thaw
1 (n=12)	$62.4 \pm 6.6^{A, a}$	$61.4 \pm 7.6^{A, a}$	$50.4 \pm 3.9^{B, a}$
2 (n=12)	$62.6 \pm 6.4^{A, a}$	$60.9 \pm 6.5^{A, a}$	$51.4 \pm 6.5^{B, a}$
3 (n=12)	$63.2 \pm 5.8^{A, a}$	$62.1 \pm 4.9^{A, a}$	$52.8 \pm 5.0^{B, a}$
4 (n=12)	$58.5 \pm 9.2^{A, a}$	$59.5 \pm 5.3^{A, a}$	$49.4 \pm 4.5^{B, a}$

Means (± SD) within the same column followed by different small superscript letters, and within the same row followed by different capital superscript letters are significantly different (p <0.05).

Group 1 = control group (no L-cysteine supplement)
Group 2 = 5 mMol/100 ml L-cysteine supplement
Group 3 = 10 mMol/100 ml L-cysteine supplement
Group 4 = 15 mMol/100 ml L-cysteine supplement
TE2 = 1.5 h after the addition of freezing extender II
TE3 = 30 min after the addition of freezing extender III

Table 9 Correlation coefficients of sex steroid receptors and spermatozoa parameters

Sperm parameter	Correlation coefficient			*p*-value
	ERα	ERβ	PR	
Motility	0.62	0.47	0.59	< 0.01
Intact plasma membrane	0.49	0.46	0.54	< 0.01
Intact acrosome	0.61	0.56	0.53	< 0.01
Capacitation	0.57	0.51	0.54	< 0.01

Semen parameters during cryopreservation processes and post-thawing

Sperm motility: The percentage of progressive motility in TE2 and TE3 was significantly higher than that of the post-thawed group (p<0.05) as shown in Table 2.

Plasma membrane integrity: The percentage of intact plasma membrane spermatozoa (Table 3) in the TE2 and TE3 groups were significantly higher than that in the post-thawed group. Among the different concentrations of L-cysteine, the percentage of intact-plasma membrane spermatozoa was not significantly different.

Acrosome integrity: The percentage of intact-acrosome spermatozoa (Table 4) in the TE2 and TE3 groups were higher than that in the post-thawed group (p <0.05). For the post-thawed group, the percentage of intact-acrosome spermatozoa in the 10mM L-cysteine group were significantly higher than that in the control and 15mM L-cysteine groups (p<0.05).

Spermatozoa capacitation: The percentage of non-capacitated spermatozoa (Table 5) in the TE2 and TE3 groups was significantly higher than that in the post-thawed group (p<0.05); however, they were not significantly different among different L-cysteine concentrations.

Expression of PR, ERα and ERβ on boar spermatozoa: The percentage of PR- (Table 6), ERα- (Table 7), and ERβ-positive spermatozoa (Table 8) in the TE2 and TE3 groups were significantly higher than that in the post-thawed group (p <0.05).

***Correlations among expressions of PR, ERα, ERβ, and spermatozoa qualities*:** The positive percentage of each steroid receptors positively correlated with motility, plasma membrane integrity, acrosome integrity, and non-capacitated spermatozoa (Table 9). Besides, the positive correlations were found between ERα and ERβ expressions (r = 0.41, p<0.01), together with ERα and PR expressions (r = 0.48, p<0.01). The correlations among spermatozoa quality parameters are shown in table 9.

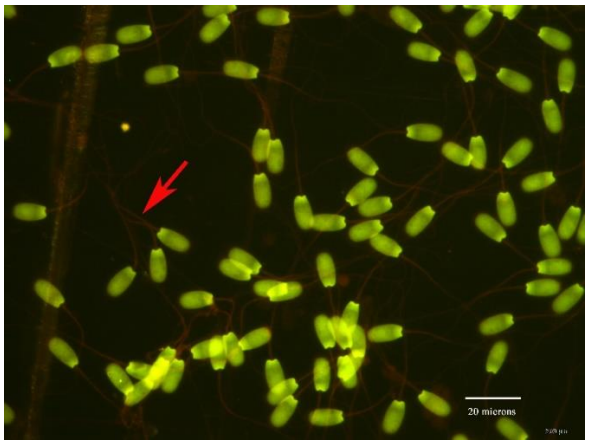

Figure 1 Expression of ERα on mid-piece and tail of boar spermatozoa (arrow) (40x)

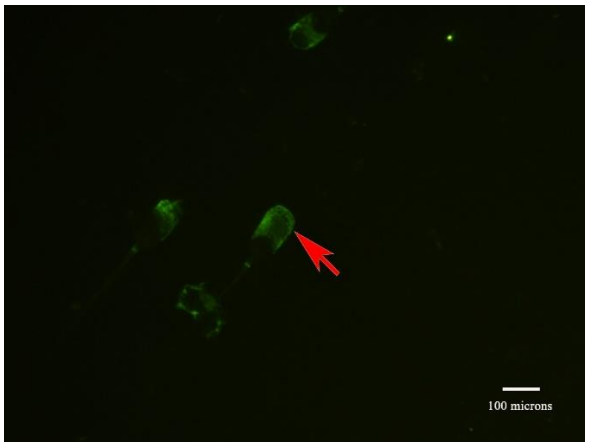

Figure 2 Expression of ERβ on acrosomal region of boar spermatozoa (arrow) (100x)

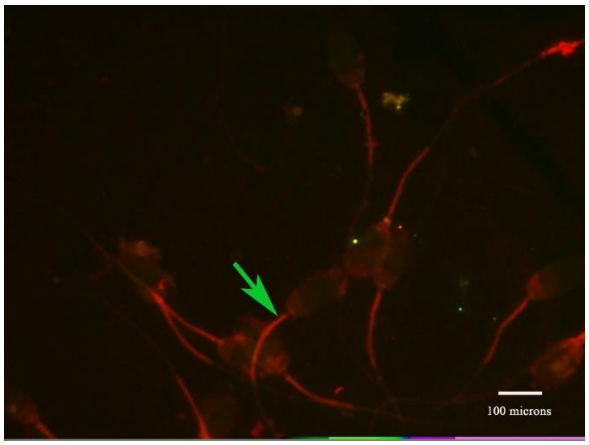

Figure 3 Expression of PR on mid-piece of boar spermatozoa (arrow) (100x)

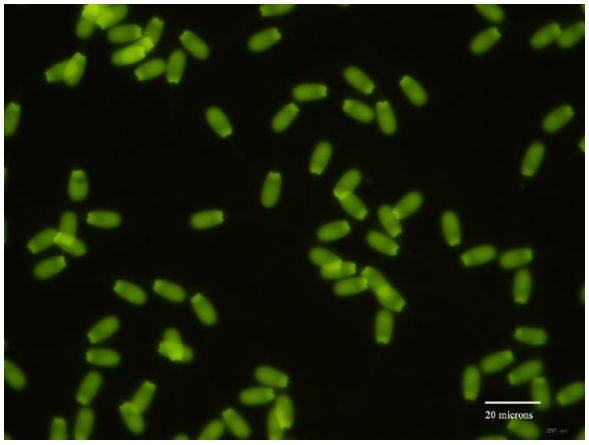

Figure 4 Negative control counterstained with DAPI (40x)

Discussion

Expression of PR and ER on boar spermatozoa: The pattern of immunofluorescence staining of ERα at mid-piece and tail of spermatozoa, in this study, presented in a similar pattern to a previous study conducted in boar spermatozoa (Rago et al., 2007). This finding implied that the localization of ERα at mid-piece and tail of spermatozoa might play a role in sperm survival and motility (Aquila et al., 2004). For ERβ, the localization at acrosomal region indicated that ERβ might be involved in the fertilization process since exocytosis occurred at acrosome before fertilization took place. Therefore, the result supported the hypothesis that estrogen played an important role in sperm capacitation and acrosome reaction in human (Aquila et al., 2003). Regarding PR, the expression of PR on spermatozoa mid-piece was different from that of human spermatozoa, which localized on acrosome (Gadkar et al., 2002). This finding indicated that progesterone might influence the motility of boar spermatozoa.

Correlation between sex steroid receptors and boar semen qualities: In the present study, we found the positive correlation of sex steroid receptors (ER and PR) with sperm motility, plasma membrane integrity, acrosome integrity, and non-capacitated spermatozoa. This finding supported the hypothesis that estrogen and progesterone are involved in sperm survival, motility, and processes of fertilization such as capacitation and acrosome reaction (Gadkar et al., 2002; Rago et al., 2007). Previous investigation in human spermatozoa demonstrated that the expression of PR positively correlated with sperm plasma membrane integrity and with normal morphology (Meizel and Turner, 1991). However, PR expression on abnormal morphology of human spermatozoa was lower than those with normal morphology (Gadkar et al., 2002). In addition, ERα might be important for sperm motility, as demonstrated in mice that ERα-knockout mice possessed decreased sperm motility (Korach, 2000).

Effect of changed temperature in cryopreservation on sex steroid hormone expression of boar spermatozoa: The results, in the present study, demonstrated the significant decline of ER and PR in post-thawed spermatozoa, suggesting that the rapid temperature decrease in cryopreservation could decrease the expression of sex steroid receptors and the quality of spermatozoa. The decrease in sperm qualities might have been caused by cold shock, loss of membrane fluidity, decrease in motility (Simpson and White, 1986; Robertson et al., 1990), and proliferation of free radicals (Aitken and Clarkson, 1988). As explained above, the expression of sex steroid receptors also decreased since it positively correlates with sperm qualities.

Effect of L-cysteine on sex steroid receptor expressions: The results showed the similar expressions of sex steroid receptors among different concentrations of L-cysteine. This might explain that L-cysteine has no effect on the expression of sex steroid receptors. However, 10 mM L-cysteine tended to give better semen qualities in cryopreservation than the others. This finding agrees with a previous study in boar semen cryopreservation which showed an improvement in post-thawed semen qualities when L-cysteine was added (Kaeoket et al., 2008).

In conclusion, the expression of sex steroid receptors correlates with sperm qualities. Cryopreservation could decrease the steroid receptor expressions and reduce sperm qualities. Thus, the fertilizability of frozen-thawed spermatozoa could be further investigated in order to understand the relationship between sex steroid receptors and fertilization process.

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บทคัดย่อ

ผลของการแช่แข็งต่อการแสดงออกของตัวรับสเตียรอยด์ฮอร์โมนเพศของตัวอสุจิสุกร

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การทดลองนี้มีวัตถุประสงค์เพื่อศึกษาการแสดงออกของตัวรับฮอร์โมนเอสโตรเจนชนิดอัลฟ่าและชนิดเบต้า และตัวรับฮอร์โมนโปรเจสเตอโรนบนตัวอสุจิสุกรในระหว่างกระบวนการแช่แข็ง เพื่อหาความสัมพันธ์ระหว่างตัวรับฮอร์โมนทั้งสามชนิดกับการเกิด capacitation, acrosome reaction และคุณภาพน้ำเชื้อสุกร และเพื่อศึกษาผลของ L-cysteine ที่ความเข้มข้นต่างกันต่อการแสดงออกของตัวรับฮอร์โมนทั้งสองชนิด ทำการเก็บตัวอย่างน้ำเชื้อจากพ่อสุกรจำนวน 12 ตัว ซึ่งเป็นพ่อสุกรพันธุ์ Duroc 4 ตัว Landrace 4 ตัว และ Large white 4 ตัว นำมาแช่แข็งด้วยการใช้คอมพิวเตอร์ควบคุมอุณหภูมิ และวัดผลจากพารามิเตอร์ดังต่อไปนี้ การเคลื่อนที่ไปข้างหน้า ความสมบูรณ์ของเยื่อหุ้มอสุจิ ความสมบูรณ์ของ acrosome ตัวอสุจิที่ยังไม่เกิด capacitation ตัวอสุจิที่มีการแสดงออกของตัวรับฮอร์โมนเอสโตรเจนชนิดอัลฟ่า ตัวรับฮอร์โมนเอสโตรเจนชนิดเบต้า และตัวรับฮอร์โมนโปรเจสเตอโรน และหาความสัมพันธ์ของพารามิเตอร์ต่างๆ โดยทำการเก็บน้ำเชื้อเพื่อตรวจพารามิเตอร์ดังที่กล่าวมาข้างต้นตามเวลาดังนี้ หลังจากเก็บตัวอย่างน้ำเชื้อทันที (fresh semen) หลังจากเติมสารละลายน้ำเชื้อ Modena™ หลังจากเติม extender II (TE2) หลังจากเติม extender III (TE3) และภายหลังการทำละลาย (PT) สำหรับ extender II และ III แบ่งตัวอย่างน้ำเชื้อออกเป็น 4 กลุ่ม คือ กลุ่มควบคุมที่ไม่มีการเสริม L-cysteine (กลุ่ม 1) กลุ่มที่มีการเสริม L-cysteine ที่ 5 (กลุ่ม 2) 10 (กลุ่ม 3) และ 15 (กลุ่ม 4) mM/100 ml จากการทดลองพบว่า กลุ่ม 3 มีความสมบูรณ์ของ acrosome สูงกว่ากลุ่ม 1 และกลุ่ม 4 และสำหรับพารามิเตอร์อื่นๆ กลุ่ม 3 มีแนวโน้มให้ผลการทดลองที่ดีกว่าในทุกพารามิเตอร์ ทุกพารามิเตอร์ในช่วงเวลา TE2 และ TE3 มีค่าสูงกว่าภายหลังการทำละลาย และพบความสัมพันธ์เชิงบวกของการแสดงออกของตัวรับฮอร์โมนทั้งสามชนิดกับการเคลื่อนที่ไปข้างหน้า ความสมบูรณ์ของเยื่อหุ้มอสุจิ ความสมบูรณ์ของ acrosome และตัวอสุจิที่ยังไม่เกิด capacitation จากผลการทดลองสรุปได้ว่า กระบวนการแช่แข็งและทำละลายน้ำเชื้อทำให้การแสดงออกของตัวรับฮอร์โมนเอสโตรเจนและโปรเจสเตอโรน รวมถึงคุณภาพน้ำเชื้อสุกร ลดต่ำลง ในขณะที่การเสริมสารต้านอนุมูลอิสระ (L-cysteine) ที่ความเข้มข้นต่างๆไม่มีผลต่อการแสดงออกของตัวรับฮอร์โมนเอสโตรเจนและโปรเจสเตอโรน

คำสำคัญ: พ่อสุกร กระบวนการแช่แข็ง ตัวรับฮอร์โมนเอสโตรเจน อัลฟ่า ตัวรับฮอร์โมนเอสโตรเจน เบต้า ตัวรับฮอร์โมนโปรเจสเตอโรน ตัวอสุจิ

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