

Research Note

Effect of fatty acids on boar sperm motility, viability and acrosome reaction

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Aim: The present study was undertaken to determine which fatty acids improve motility, viability, and increase acrosome reaction (AR) in boar spermatozoa.

Methods: Boar spermatozoa were washed, swum-up and incubated at 37°C for 4 h in TALP medium supplemented with myristic, palmitic, stearic, lignoceric, oleic, linoleic, arachidonic, docosahexaenoic and palmitoleic acid. Sperm motility, viability and AR were evaluated during 4 h of incubation.

Results: Results show that oleic and linoleic acid significantly improved ($P < 0.05$) the motility and viability of boar spermatozoa. The AR was significantly improved ($P < 0.05$) by oleic and arachidonic acid in almost all incubation

periods. When combinations of oleic, linoleic and arachidonic acid were studied for motility, viability and AR, it was found that oleic plus linoleic acid significantly increased ($P < 0.05$) motility, whereas arachidonic plus oleic acid significantly increased ($P < 0.05$) AR.

Conclusion: Unsaturated fatty acids, especially arachidonic acid, can improve boar sperm motility and AR. A combination of arachidonic and oleic acid is important for inducing boar sperm AR. (Reprod Med Biol 2007; 6: 235–239)

Key words: acrosome reaction, boar spermatozoa, fatty acid, sperm motility, sperm viability.

INTRODUCTION

MOST SPERM INCUBATION media contain bovine serum albumin (BSA) for supporting sperm viability and motility or capacitation *in vitro*.¹ These media contain as much as 15 µg of fatty acid per milligram of BSA.² Boar seminal plasma contains many more saturated and unsaturated fatty acids than the BSA fraction V (BSA-V).³ In our previous study, when fatty acids were added to polyvinyl alcohol (PVA), the motility and acrosome reaction (AR) was almost the same as that with BSA-V.⁴ It was found from this study that fatty acids play an important role in sperm movement and AR. Motility of rabbit¹ and hamster⁵ spermatozoa was improved by fatty acids bound to BSA-V; increasing of motility might have resulted from fatty acids bound to BSA. There is little information about the effect of fatty acids on boar sperm AR. In our previous study, fatty acids bound to BSA-V enhanced boar sperm AR more than free fatty acids in the BSA in a time-dependent manner.⁴ With the addition of a fatty acids mixture to a fatty acid free BSA, the rate of AR again

became similar with fatty acids bound to BSA-V. This finding indicates the importance of fatty acids for boar sperm AR, but not all of the fatty acids in the fatty acid mixture may take part in the induction of AR. Exactly which fatty acids are responsible for boar sperm AR remains unclear. To date little attention has been given to the possible role of fatty acids on boar sperm AR.

Thus, the present study has been designed to investigate the effect of fatty acids on boar sperm AR, motility and viability.

MATERIALS AND METHODS

Chemicals

THE MYRISTIC, PALMITIC, stearic, lignoceric, oleic, linoleic, arachidonic, docosahexaenoic and palmitoleic acids, all approximately 99% pure, were obtained from Sigma Chemical Company (St Louis, MO, USA). All other chemicals were analytical grade and purchased from Nacalai Tesque (Kyoto, Japan).

Semen collection and preparation

Semen from Duroc boars aged between 2 and 3 years was collected using the gloved-hand technique at

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Received 27 March 2007; accepted 18 June 2007.

Nagano Animal Industry Experiment Station, Shiojiri, Nagano, Japan. The semen was diluted according to the method of Johnson *et al.*⁶ with a Modena extender giving a sperm concentration of 1×10^8 /mL at room temperature. The vial of semen was carried to the laboratory within 30 min using a cork box to keep it at 21°C temperature. A detailed description of the procedure of semen preparation was provided in our previous report.⁴ To evaluate motility and acrosome status, swim-up spermatozoa were resuspended separately at a concentration of 5×10^6 and 20×10^6 /mL, respectively, and incubated at 37°C temperature, 95% humidified air and 5% CO₂ at pH 7.4 for 0–4 h.

Semen treatment

To evaluate the effect of each fatty acid on motility, viability and acrosome reaction, 1.25 mmol/L of palmitic, myristic, stearic, lignoceric, oleic, linoleic, arachidonic, docosahexaenoic or palmitoleic acid was added to a basic TALP⁷ medium containing fatty acid free BSA according to our previous report.⁸

Progressive motility

After completion of the specified incubation periods, sperm samples were subjected to motility and movement quality evaluations. The evaluation method and categories were the same as those previously described.⁴ The percentage of spermatozoa with progressive motility was determined subjectively by scoring 400 individual spermatozoa using a phase-contrast microscope.

Acrosome reaction and viability

The evaluation of AR and viability was carried out after a specific incubation period of spermatozoa by applying the triple-staining technique.⁹ The live, dead, acrosome reacted and unreacted spermatozoa were evaluated based on the staining pattern. Viability and AR of the spermatozoa were evaluated from randomly selected fields of the triple-stained slides until 400 spermatozoa had been examined. Full details of the method and photography of triple-stained slides were shown in our previous report.⁴

Statistical analysis

The data were subjected to a Fisher's protected least significant difference test. The Number Cruncher Statistical System (NCSS statistical software; Kaysville,

UT, USA) version 5.01 computer software package was used for all statistical analyses. Each experiment was repeated four times from two boars and differences were considered significant at $P < 0.05$.

RESULTS

THE EFFECT OF each fatty acid on progressive motility, viability and AR in boar spermatozoa during 0–4 h of incubation is shown in Figure 1. The percentage of progressive motility was significantly increased ($P < 0.05$) by oleic and linoleic acids from 1 to 4 h of incubation periods; oleic acid was higher than linoleic acid (Fig. 1a). Unsaturated fatty acids, namely arachidonic, docosahexaenoic or palmitoleic, and saturated fatty acids, palmitic, myristic, stearic or lignoceric, also slightly increased motility. Viability was significantly improved ($P < 0.05$) by oleic and linoleic acids only at 1 and 2 h of incubation (Fig. 1b). The induction of AR was significantly increased ($P < 0.05$) by oleic and arachidonic acids from 1 to 4 h of incubation; arachidonic was a higher inducer than oleic acid (Fig. 1c). Linoleic, docosahexaenoic and palmitoleic acids were also effective inducers of boar sperm AR. All the saturated fatty acids, namely myristic, palmitic, stearic and lignoceric, failed to induce AR, but myristic acid had an adverse effect. The oleic, linoleic and arachidonic acids showed good results individually; for this reason these three fatty acid combinations were used to study sperm functions in the following experiment. The effects of a combination of fatty acids, such as oleic acid (OA) + linoleic acid (LA), OA + arachidonic acid (AA), AA + LA, and OA + LA + AA, on progressive motility, viability and AR of boar spermatozoa are shown in Figure 2. Compared to the control and the combination of fatty acids, the percentage of progressive motility was significantly higher ($P < 0.05$) with OA + LA only at 2 h of incubation. Viability was not increased significantly by any of the treatments, although OA + LA showed a slightly higher value. The AR was significantly increased ($P < 0.05$) by OA + AA over 1–4 h incubation periods.

DISCUSSION

IN THIS STUDY, fatty acids played an important role in enhancing sperm movements and AR. Compared with the control, motility was increased by almost every fatty acid, and performance of the unsaturated fatty acids was higher than the saturated fatty acids. Fleming and Yanagimachi¹⁰ observed that some unsaturated fatty acids were able to improve sperm motility in

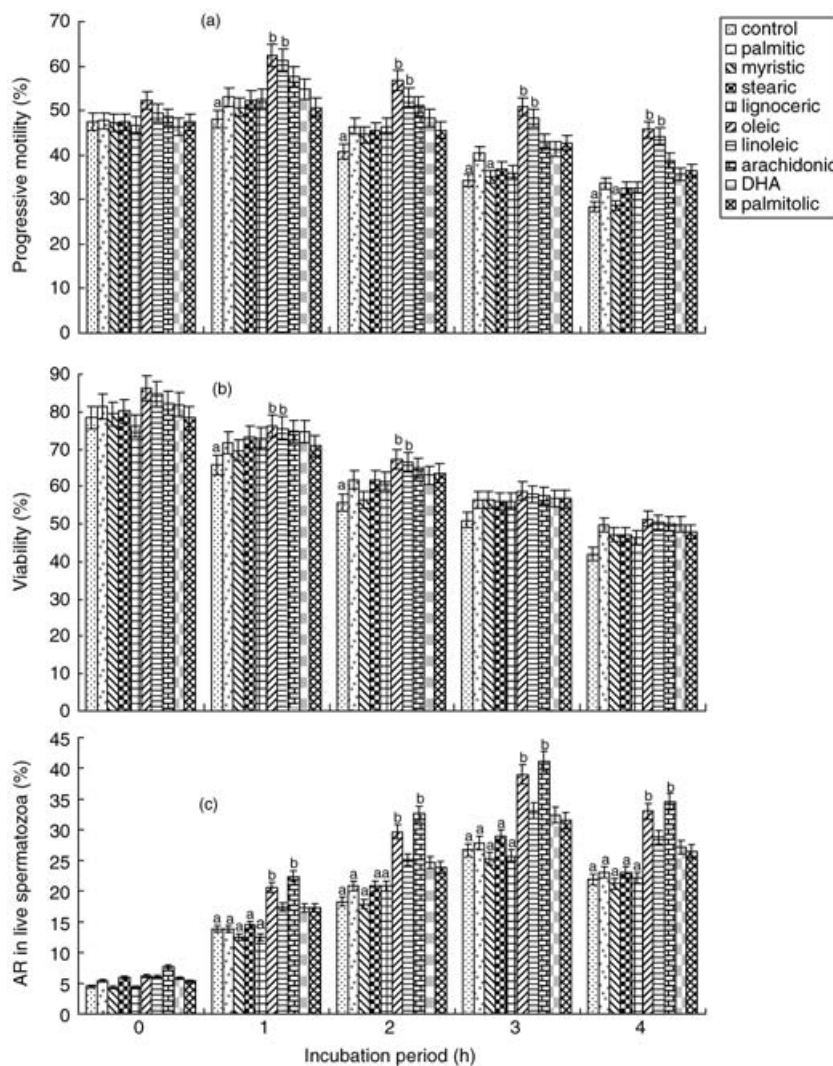


Figure 1 Mean effect of fatty acids on (a) the percentage of progressive motility, (b) viability and (c) acrosome-reacted (AR) live spermatozoa from 0 to 4 h of incubation. Error bars represent standard error ($n = 4$). Data points without a common lowercase letter indicate differences ($P < 0.05$) among the treatments.

guinea pig spermatozoa. The present result is in agreement with this result. To some extent, unsaturated oleic and linoleic acids improved boar sperm viability more than the saturated fatty acids. In the present study, the performances of oleic, linoleic and arachidonic acids were higher for motility, viability and AR. In mammalian cells, oleic, linoleic and arachidonic acids are considered to have metabolic significance and most of the long-chain, polyunsaturated fatty acids are derived from these fatty acids by chain elongation.¹¹ In the present study, nine fatty acids were used that are present in great amounts in BSA. These fatty acids resemble well the fatty acids of boar seminal plasma.³ Peter¹² and Meizel¹³ suggested that BSA releases a variety of small molecules, such as fatty acids and amines, which stimulate AR.

We postulate that fatty acids induce AR, an exocytotic event consisting of the fusion of the outer acrosomal membrane with the overlying plasma membrane, their fenestration, and the concomitant exposure, release or both of the acrosomal content. In general cell, when saturated fatty acids pass through the membrane they pack very tightly because saturated fatty acid chains have no gaps. In contrast, because of gaps in the chain, unsaturated fatty acids prevent the tight packing of fatty acids in the membrane and make it loose. The series of 18 and 20 carbon *cis*-unsaturated fatty acids, oleic, linoleic, linolenic and arachidonic acids, are effective inducers of exocytosis in mammalian cells.¹⁴ In this study, the highest induction of AR was carried out by arachidonic acid followed by oleic, linoleic, docosahexaenoic and

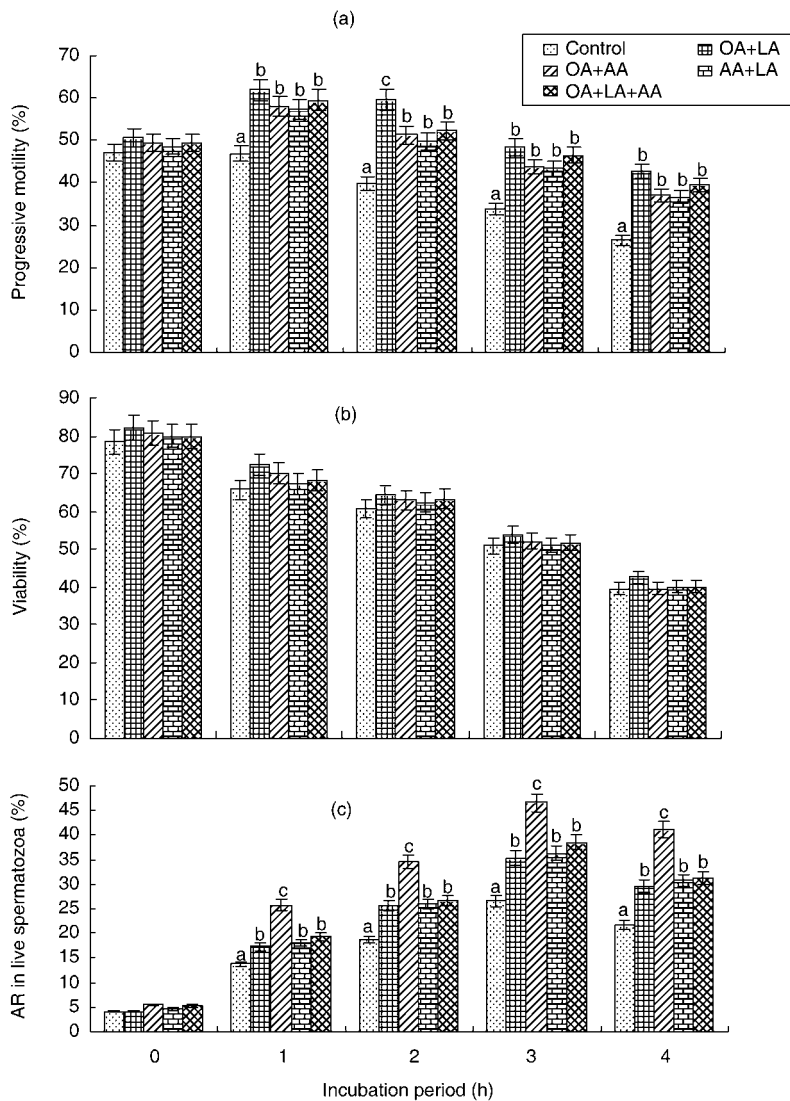


Figure 2 Mean effect of combinations of oleic acid (OA), linoleic acid (LA) and arachidonic acid (AA) on (a) the percentage of progressive motility, (b) viability and (c) acrosome-reacted (AR) live spermatozoa from 0 to 4 h of incubation. Error bars represent standard error ($n = 4$). Data points without a common lowercase letter indicate differences ($P < 0.05$) among the treatments.

palmitolic acids; whereas all of the saturated fatty acids, palmitic, stearic and lignoceric acids, failed to induce boar sperm AR. Myristic acid showed little adverse effect on AR. This result is in agreement with the result of Meizel and Turner,¹⁵ who showed that arachidonic and oleic acids induced AR in hamster spermatozoa *in vitro*, whereas lauric, myristic and stearic acids were not effective. The *cis*-unsaturated fatty acids enter a different lipid domain in sperm membranes than the *trans*-unsaturated or saturated fatty acids.¹⁶ In this study, compared to the control, the AR of live spermatozoa was increased twofold by arachidonic and oleic acids at 3 h of incubation. Dominguez *et al.*¹⁷ also reported that arachidonic acid is the best inducer of AR in

human spermatozoa. Together with oleic and arachidonic acids, linoleic acid is also an effective inducer of AR in guinea pig spermatozoa.¹⁰ Thus, the present result is in agreement with this result. In this study, four combinations of oleic, linoleic and arachidonic acids were used to study the induction of AR, and a combination of arachidonic and oleic acids showed significantly higher induction than the other combinations. This might result from the combined effect of the two highest inducing fatty acids. However, many components, including unsaturated fatty acids of intracellular signaling systems, have been implicated in the induction of the AR. Breitbart and Spungin¹⁸ found that arachidonic acid is converted to other metabolites to increase Ca^{2+} influx,

which in turn induces AR. We believe that the unsaturated fatty acids supplemented in the medium produce ATP and may increase intracellular Ca^{2+} and cAMP concentrations, which in turn could be responsible for inducing AR in boar spermatozoa. Thus, the intracellular concentration of cAMP, Ca^{+} influx and protein tyrosine phosphorylation by unsaturated fatty acids in boar spermatozoa needs to be studied further for a complete understanding of this mechanism.

In conclusion, unsaturated fatty acids, especially arachidonic acid, can improve boar sperm motility and AR. A combination of arachidonic and oleic acids is important for inducing boar sperm AR.

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