Effects of Oxytocin on Sperm Motility and *In Vitro* Fertilization in the Mouse

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(Received December 20, 1993)

Abstract Motility and the acrosome-reacted rate of mouse spermatozoa, and the *in vitro* fertilization rate in oxytocin-supplemented media were investigated. Spermatozoa collected from the vas deferens and cauda epididymis were incubated in a medium containing 10^{-10} M oxytocin for 5 h. Although the percentage of motile spermatozoa decreased in the course of time, it remained significantly high except for 1 h after incubation when compared to the control. The percentage of hyperactivated spermatozoa was higher than in the control throughout the incubation period. There was also an increase in the percentage of acrosome-reacted spermatozoa at 1, 3 and 5 h after incubation. The fertilization rate in cumulus-free eggs (46%) was lower than that of cumulus-intact eggs (83%). When 10^{-12} , 10^{-10} and 10^{-8} M oxytocin were supplemented into the fertilization medium, the fertilization rates increased significantly to 63, 73, and 57%, respectively. The present results suggest that oxytocin in the medium stimulates sperm motility and is effective for *in vitro* fertilization.

Anim. Sci. Technol. (Jpn.) 65 (8) : 695-700, 1994

Key words : sperm motility, *in vitro* fertilization, oxytocin, mouse

It is known that oxytocin is secreted from neurohypophysis in mammals. Oxytocin also exists in the ovary^{4,13,14,32}, adrenal tissue²¹, testis²¹ and central nervous system other than in the hypothalamus¹⁹. The sources of oxytocin in the ovary are granulosa cells^{12,24} and the luteal cells derived from the granulosa cells²⁷. It has been proposed that ovarian oxytocin supports luteolysis^{5,7} and is involved in steroidogenesis¹¹. MAKIMURA *et al.*¹⁷ have suggested that oxytocin has a beneficial effect on the *in vitro* development of early stage embryos in the mouse. In addition, it seems likely that oxytocin may affect eggs and spermatozoa before fertilization if oxytocin is present in the lumen of the female genital tract during the periovulatory period. This study was carried out in order to clarify the effects of oxytocin on the motility of spermatozoa and

the *in vitro* fertilization rate.

Materials and Methods

Motility and acrosome reaction of spermatozoa : Mature male mice of the ddY strain were killed by cervical dislocation and the vas deferens and cauda epididymis were excised. After washing and cutting them into small pieces, they were incubated in modified Tyrode's solution, T₆ medium²⁵, containing 4 mg/ml bovine serum albumin (BSA) for the dispersion of spermatozoa at 37°C for 25 min. Then the upper part of the sperm suspension was gently sucked up.

The sperm suspension was added to each 0.4 ml of T₆ medium supplemented with 10^{-10} M oxytocin (Bachem Inc. Torrance CA) and without oxytocin (control) to give a final concentration of approximately 1×10^6 cells/ml. The

spermatozoa were incubated at 37°C for 5 h under paraffin oil in a humidified atmosphere of 5% CO₂ in air. The motility and incidence of acrosome reaction of spermatozoa were evaluated at 1 h intervals. More than 200 spermatozoa were examined for the percentage of active motile spermatozoa and for the percentage of hyperactivated spermatozoa by phase-contrast microscopy. Spermatozoa showing a star-spin trajectory or a transitional trajectory with vigorous activity were identified as hyperactivated spermatozoa²⁶⁾. For determining acrosome-reacted spermatozoa, more than 100 motile spermatozoa were examined under a phase-contrast microscope (400×). The percentages of hyperactivated spermatozoa and acrosome-reacted spermatozoa in the entire sperm population were calculated, and data were analyzed using the paired t-test after arcsine-transformation.

In vitro fertilization: Immature mice of the ddY strain (3~4 weeks of age) were super-ovulated with an intraperitoneal injection of 5 iu pregnant mare serum gonadotrophin (Serotropin, Teikoku Zouki Co., Tokyo), followed 48 h later by 5 iu human chorionic gonadotrophin (hCG; Gonatropin, Teikoku Zouki Co., Tokyo). The eggs were collected from the ampulla of the uterine tube 15 h after hCG injection. Some of the eggs obtained were transferred to the T₆ medium containing 0.1% hyaluronidase (Type V, Sigma Chemical Co., St. Louis MO) so as to remove the cumulus cells.

T₆ media with 15 mg BSA/ml containing 10⁻⁶, 10⁻⁸, 10⁻¹⁰M and 10⁻¹²M oxytocin were prepared as the fertilization medium for cumulus-free eggs. The media without oxytocin for cumulus-intact eggs and cumulus-free eggs served as controls. The sperm suspension was prepared by the method described above and then spermatozoa were pre-incubated for capacitation at 37°C for 2 h. A small amount of preincubated sperm suspension was added to 0.4 ml of each fertilization

medium to give a final concentration of approximately 1.5×10⁶ cells/ml. Then 4~10 eggs were introduced into the sperm suspension under paraffin oil. The mixtures of spermatozoa and eggs were incubated at 37°C under 5% CO₂ in air. After 6 h of incubation, the eggs were examined for the presence of two pronuclei. For further confirmation of sperm penetration, the eggs were cultured in HOPPE and PITTS's medium⁸⁾ for 24 h to examine their development to a 2-cell stage. Data were analyzed by the χ^2 -test.

Results

In the 10⁻¹⁰M oxytocin-supplemented group, the percentage of motile spermatozoa decreased in the course of time after the start of incubation. However, the degree of decrease was significantly less than that of the control except for 1 h after incubation (Fig. 1). The percentage of hyperactivated spermatozoa was significantly higher than in the control throughout the incubation period. There was also an increase in the percentage of acrosome-reacted spermatozoa at 1, 3 and 5 h after incubation.

The fertilization rate in cumulus-free eggs (46%) was lower than that in cumulus-intact eggs (83%). When media with various concentrations of oxytocin were tested as a fertilization medium for cumulus-free eggs, fertilization rates in the 10⁻¹², 10⁻¹⁰ and 10⁻⁸M groups increased significantly, although the rates were significantly lower than those for cumulus-intact eggs. Among the three concentrations of oxytocin, 10⁻¹⁰M oxytocin supported the maximal level of fertilization. Oxytocin at the concentration of 10⁻⁶M was not effective in supporting the fertilization of cumulus-free eggs (Table 1).

Discussion

It has been suggested that ovarian oxytocin in ruminants is involved in luteolysis via the positive feedback loop in which oxytocin and

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Fig. 1. Effect of oxytocin on the motility and acrosome reaction of spermatozoa. The percentages of hyperactivated spermatozoa and acrosome-reacted spermatozoa show the values in the entire population. Each value represents the mean $\pm$ SE. ●—● 10^{-10} M Oxytocin-supplemented group, ○—○ control. * $P < 0.05$, ** $P < 0.01$.

Table 1. Effect of oxytocin on fertilization *in vitro*

Condition of egg	Concentration of oxytocin (M)	No. of eggs examined	No. (%) of eggs fertilized
Cumulus-intact	—	246	204(83) ^a
Cumulus-free	—	246	112(46) ^e
	10^{-12}	63	40(63) ^{bc}
	10^{-10}	182	133(73) ^b
	10^{-8}	149	85(57) ^{cd}
	10^{-6}	110	50(45) ^{de}

^{a-e} Values with different superscripts are significantly different ($p < 0.05$).

prostaglandin $F_{2\alpha}$ are mutually reinforced^{15,7)}. Oxytocin has also been detected in human follicular fluid²⁸⁾. In the mouse, the secretion of oxytocin from cumulus cells during culture has been reported¹⁸⁾. This information suggests the presence of oxytocin in the female genital tract in the periovulatory period.

Mouse spermatozoa are stored in the lower isthmus before ovulation and are transported to the ampulla at the time of ovulation²²⁾, in the

same way as spermatozoa in other species³⁾. SMITH and YANAGIMACHI²⁹⁾ have reported that capacitation in hamster spermatozoa proceeds at a faster rate when mating occurs immediately after ovulation rather than shortly after the onset of estrus. Iro *et al.*¹⁰⁾ have suggested that ovulatory products stimulate the sperm transport from the caudal isthmus to the ampulla. Furthermore BRADLEY and GARBERS²⁾ have shown that a factor(s) released from

bovine cumulus cells stimulates sperm motility *in vitro*. The results of this study indicate that oxytocin in the medium supports the survival of spermatozoa, as well as the induction of sperm hyperactivation and the acrosome reaction. Therefore, oxytocin seems to be one of factors involved in the storage of spermatozoa in the lower isthmus and the transport of spermatozoa from the isthmus.

The present study shows that the removal of cumulus oophorus from the egg decreases the fertilization rate. This result supports many reports on other species^{6,15,16)} including the mouse²³⁾. As possible causes of the decreased fertilization rate, we can presume the zona pellucida hardening during culture and/or the modification of sperm receptors of the zona pellucida during the process of cumulus denudation as suggested by MOCHIZUKI *et al.*²⁰⁾, and ITAGAKI and TOYODA⁹⁾. In addition, cumulus cells stimulate the acrosome reaction of spermatozoa⁶⁾, and the secretions from the cumulus cells increase sperm motility²⁾, supporting that cumulus cells have a beneficial effect on *in vitro* fertilization. In this study the oxytocin supplement increased the percentage of hyperactivation and acrosome reaction, and improved the fertilization rate of cumulus-free eggs. MAKIMURA *et al.*¹⁸⁾ found that mouse cumulus cells secreted oxytocin. These findings suggest the possibility that cumulus cells may secrete oxytocin which stimulates spermatozoa to support fertilization.

The mode of action of oxytocin on spermatozoon is unclear. Both hyperactivation and the acrosome reaction of spermatozoa appear to be initiated by the influx of calcium through the plasma membrane^{30,31)}. Furthermore, according to BATRA¹⁾, oxytocin-induced contraction of the myometrium is largely dependent on the influx of extracellular calcium. Therefore, it is probable that oxytocin action on the induction of sperm hyperactivation and acrosome reaction may occur via the process of calcium influx.

References

- 1) BATRA, S., Effect of oxytocin on calcium influx and efflux in the rat myometrium. *Eur. J. Pharmacol.*, **120** : 57-61. 1986.
- 2) BRADLEY, M.P. and D.L. GARBERS, The stimulation of bovine caudal epididymal sperm forward motility by bovine cumulus-egg complexes *in vitro*. *Biochem. Biophys. Res. Commun.*, **115** : 777-787. 1983.
- 3) EISENBACH, M. and D. RALT, Precontact mammalian sperm-egg communication and role in fertilization. *Am. J. Physiol.*, **262** : C1095-C1101. 1992.
- 4) FLINT, A.P.F. and E.L. SHELDRIK, Ovarian secretion of oxytocin is stimulated by prostaglandin. *Nature*, **297** : 587-588. 1982.
- 5) FLINT, A.P.F. and E.L. SHELDRIK, Evidence for a systemic role for ovarian oxytocin in luteal regression in sheep. *J. Reprod. Fertil.*, **67** : 215-225. 1983.
- 6) FUKUI, Y., Effect of follicle cells on the acrosome reaction, fertilization, and developmental competence of bovine oocytes matured *in vitro*. *Mol. Reprod. Dev.*, **26** : 40-46. 1990.
- 7) HOOPER, S.B., W.B. WATKINS and G.D. THORBURN, Oxytocin, oxytocin-associated neurophysin, and prostaglandin $F_{2\alpha}$ concentrations in the utero-ovarian vein of pregnant and non-pregnant sheep. *Endocrinology*, **119** : 2590-2597. 1986.
- 8) HOPPE, P.C. and S. PITTS, Fertilization *in vitro* and development of mouse ova. *Biol. Reprod.*, **8** : 420-426. 1973.
- 9) ITAGAKI, Y. and Y. TOYODA, Effects of prolonged sperm preincubation and elevated calcium concentration on fertilization of cumulus-free mouse eggs *in vitro*. *J. Reprod. Dev.*, **38** : 219-224. 1992.
- 10) ITO, M., T.T. SMITH and R. YANAGIMACHI, Effect of ovulation on sperm transport in the hamster oviduct. *J. Reprod. Fertil.*, **93** : 157-163. 1991.
- 11) JARRY, H., A. EINSPIANIER, L. KANNGIEßER, M. DIETRICH, L. PITZEL, W. HOLTZ and W. WUTTKE, Release and effects of oxytocin on estradiol and progesterone secretion in porcine corpora lutea as measured by an *in vivo* microdialysis system. *Endocrinology*, **126** : 2350-2358. 1990.
- 12) JUNGCLAUS, B. and M.R. LUCK, Evidence for granulosa-theca interaction in the secretion of oxytocin by bovine ovarian tissue. *J.*

- Endocrinol., **109** : R1-R4. 1986.
- 13) KHAN-DAWOOD, F.S. and M.Y. DAWOOD, Human ovaries contain immunoreactive oxytocin. *J. Clin. Endocrinol. Metab.*, **57** : 1129-1132. 1983.
- 14) KHAN-DAWOOD, F.S., E.L. MARUT and M.Y. DAWOOD, Oxytocin in the corpus luteum of the cynomolgus monkey (*Macaca fascicularis*). *Endocrinology*, **115** : 570-574. 1984.
- 15) KIKUCHI, K., T. NAGAI, J. MOTLIK, Y. SHIOYA and Y. IZAIKE, Effect of follicle cells on in vitro fertilization of pig follicular oocytes. *Theriogenology*, **39** : 593-599. 1993.
- 16) KOBAYASHI, K., D.L. HILL, C.M. ROTHMAN, C.F. UNDERBERGER and S. OGAWA, Co-culture of subzonally microinseminated human oocytes with cumulus cells. *Theriogenology*, **39** : 247. 1993.
- 17) MAKIMURA, N., K. FURUYA, N. ISHIKAWA, T. HOSHIHARA, K. TSUBAMOTO, K. SEKI and I. NAGATA, The effect of oxytocin on the development of mouse embryo. *J. Fertil. Implant. (Tokyo)*, **9** : 194-196. 1992.
- 18) MAKIMURA, N., K. FURUYA, N. ISHIKAWA, T. HOSHIHARA, K. TUBAMOTO, K. SEKI and I. NAGATA, The secretion of oxytocin and progesterone by mouse cumulus cells. *J. Fertil. Implant. (Tokyo)*, **10** : 102-103. 1993.
- 19) MIASKOWSKI, C., G.L. ONG and J. HALDAR, Cyclic variations in spinal cord levels of oxytocin and vasopressin during the stages of the rat estrous cycle. *Endocrinology*, **120** : 1685-1687. 1987.
- 20) MOCHIZUKI, H., Y. FUKUI and H. ONO, Effect of the number of granulosa cells added to culture medium for in vitro maturation, fertilization and development of bovine oocytes. *Theriogenology*, **36** : 973-986. 1991.
- 21) NICHOLSON, H.D., R.W. SWANN, G.D. BURFORD, D. C. WATHES, D.G. PORTER and B.T. PICKERING, Identification of oxytocin and vasopressin in the testis and in adrenal tissue. *Regul. Pept.*, **8** : 141-146. 1984.
- 22) OLDS, P.J., Effect of the *T* locus on sperm distribution in the house mouse. *Biol. Reprod.*, **2** : 91-97. 1970.
- 23) PAVLOK, A. and A. McLAREN, The role of cumulus cells and the zona pellucida in fertilization of mouse eggs *in vitro*. *J. Reprod. Fertil.*, **29** : 91-97. 1972.
- 24) PLEVRAKIS, I., C. CLAMAGIRAND and G. PONTONNIER, Oxytocin biosynthesis in serum-free cultures of human granulosa cells. *J. Endocrinol.*, **124** : R5-R8. 1990.
- 25) QUINN, P., C. BARROS and D.G. WHITTINGHAM, Preservation of hamster oocytes to assay the fertilizing capacity of human spermatozoa. *J. Reprod. Fertil.*, **66** : 161-168. 1982.
- 26) ROBERTSON, L., D.P. WOLF and J.S. TASH, Temporal changes in motility parameters related to acrosomal status : Identification and characterization of populations of hyperactivated human sperm. *Biol. Reprod.*, **39** : 797-805. 1988.
- 27) RODGERS, R.J., J.D. O'SHEA, J.K. FINDLAY, A.P.F. FLINT and E.L. SHELDRICK, Large luteal cells the source of luteal oxytocin in the sheep. *Endocrinology*, **113** : 2302-2304. 1983.
- 28) SCHAEFFER, J.M., J. LIU, A.J.W. HSUEH and S.S.C. YEN, Presence of oxytocin and arginine vasopressin in human ovary, oviduct, and follicular fluid. *J. Clin. Endocrinol. Metab.*, **59** : 970-973. 1984.
- 29) SMITH, T.T. and R. YANAGIMACHI, Capacitation status of hamster spermatozoa in the oviduct at various times after mating. *J. Reprod. Fertil.*, **86** : 255-261. 1989.
- 30) TASH, J.S. and A.R. MEANS, Cyclic adenosine 3', 5' monophosphate, calcium and protein phosphorylation in flagellar motility. *Biol. Reprod.*, **28** : 75-104. 1983.
- 31) WASSARMAN, P.M., The biology and chemistry of fertilization. *Science*, **235** : 553-560. 1987.
- 32) WATHES, D.C., R.W. SWANN, S.D. BIRKETT, D.G. PORTER and B.T. PICKERING, Characterization of oxytocin, vasopressin, and neurophysin from the bovine corpus luteum. *Endocrinology*, **113** : 693-698. 1983.

In vitro におけるマウス精子の運動性および 受精率に及ぼすオキシトシンの効果

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培養液に添加したオキシトシンがマウス精子の運動性, 先体反応率および受精率に及ぼす効果を検討した。 10^{-10} M のオキシトシンを添加した培養液で精管・精巣上体尾部精子を 5 時間培養した結果, 運動精子の割合は時間の経過とともに低下したが, 培養開始後 1 時間目以降, 対照に比べて有意に高い値を維持した。 ハイパーアクチベーションを起した精子の割合は, 培養期間を通して有意に高く, 先体反応率は, 培養開始後 1, 3 および 5 時間に有意に高い値を示した。 卵丘除去卵子の受精率は (46%), 卵丘付着卵子 (83%) よりも低い値を示したが, 10^{-12} , 10^{-10} および 10^{-8} M のオキシトシンを添加した結果, 卵丘除去卵子の受精率はそれぞれ 64, 73, 57% に有意に増加した。 これらの結果から, オキシトシンは *in vitro* において精子の運動性を刺激し, 受精に有効な作用を及ぼすものと推察された。

日畜会報, 65 (8): 695-700, 1994