after transferring 4-day blastocysts to 8-day uteri. Presumably this is a reflection of the smaller difference in the level of donor-recipient asynchrony, i.e. 3 as against 4 days respectively. However, the absence of any implantations and the failure to recover any blastocysts from any of the recipients when the interval from hCG to ODB treatment was 4 days indicates that this interval cannot be extended much beyond 3 days. It may be noted that some degenerate blastocysts were found in all of the control females at autopsy.

An implantation rate of 21% was recorded from the transfer of 4-day eggs to the uteri of day 8 recipients having an interval of 2 days from hCG to ODB. This result, which is much lower than that (74%) recorded by Beier et al.[5], is probably due to the later time relative to ovulation of administering ODB to the recipients in the present study. It is concluded that the level of success from asynchronous egg transfer, to either the oviducts or uteri, is strictly related to the interval from ovulation to exogenous ODB treatment of the recipient, with 4 days marking the limit of any expectation of success using this technique.

1 Acknowledgments. This work was supported by grant No. HD 15291 from NICHD made to M.C. Chang. We thank Mrs Wendy Turner for technical assistance.

2 Present address: Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury (Massachusetts 01545, USA).

3 Beier, H.M., Acta endocr. *63* (1970) 141.
4 Beier, H.M., Kühnel, W., and Petry, G., Adv. Biosci. *6* (1970) 165.
5 Beier, H.M., Mootz, U., and Kühnel, W., 7th Int. Congr. Anim. Reprod., Munich 1972, p. 1891.
6 Adams, C.E., J. Reprod. Fert. *35* (1973) 613.
7 Adams, C.E., J. Reprod. Fert. *60* (1980) 309.
8 Adams, C.E., J. Reprod. Fert. *26* (1971) 99.
9 Adams, C.E., J. Reprod. Fert. *24* (1962) 471.

0014-4754/83/091046-03$1.50 + 0.20/0

Stimulation of in vitro fertilization in mice with 17β-estradiol

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Summary. The effect of various concentrations of estradiol on in vitro fertilization frequency was studied. Fertilization was stimulated by 5×10^{-9} M 17β-estradiol but not by 5×10^{-9} M 17α-estradiol. At 5×10^{-6}, 5×10^{-7}, 5×10^{-8}, and 5×10^{-10} M 17β-estradiol there was no difference in the fertilization frequency between test and control samples. It is suggested that stimulation of the acrosome reaction is instrumental in increasing the fertilization rate.

Lindahl and coworkers have studied head-to-head association (HHA) between bovine spermatozoa and have elucidated some mechanisms involved in its induction[1,2]. The present study concerns a mechanism by which the HHA is elicited by hormones. Although the HHA is an expression of reactions under artificial circumstances and may have no biological significance per se, some similarities with the acrosome reaction can be discerned as regards the mechanisms. In view of these similarities, a study was made of the possible role in prefertilization events of 17β-estradiol, a hormone which induces HHA and is present in ova and their investments[3].

Methods. All experiments were performed with mature C57/bl mice. One male and 12 females were used for 4 experiments, each experiment using the ova of 3 females divided between control and test sample. Spermatozoa were obtained from caudae epididymides minced in 1 ml BMOC-2 (in vitro fertilization medium[4]) and kept for 2 h in an incubator set at 37 °C (the temperature of all media) and 5% CO_2 in air until insemination. Superovulation was induced by i.p. injections of 10 IU PMSG (Sigma) followed by 10 IU HCG 48–52 h later. Between 13 and 16 h after the injection of HCG the animals were sacrified and the Fallopian tubes were excised and put into saline. From each animal one tube, alternately from the left or right side, was taken to the control pool, the other to the test pool. When the oviducts had been collected they were transferred to 3 ml BMOC-2 where the ampullae were punctured and the egg clots extruded. The egg clots were then transferred into 1 ml BMOC-2 (containing the relevant test substance, e.g. 17β-estradiol) in a Nunclon Multidish and incubated until the addition of 50 µl sperm suspension. The

Effect on fertilization frequency of substances added to the in vitro fertilization medium

Substance, concentration M	No. of experiments	No. of eggs	Percent fertilization ± SD	Significance of difference between test and control samples			
				t*	p	U**	p
Control	6	247	38.4 ± 27.8	0.28	NS	15	NS
17β-estradiol, 5×10^{-6}		266	42.6 ± 23.7				
Control	10	337	59.8 ± 38.5	0.63	NS	42	NS
17β-estradiol, 5×10^{-7}		392	54.9 ± 38.8				
Control	7	147	49.0 ± 17.1	0.09	NS	24	NS
17β-estradiol, 5×10^{-8}		194	47.9 ± 28.1				
Control	11	195	49.0 ± 13.8	3.09	< 0.02	22	< 0.02
17β-estradiol, 5×10^{-9}		208	67.4 ± 14.9				
Control	10	457	44.4 ± 17.4	0.48	NS	39	NS
17β-estradiol, 5×10^{-10}		547	40.0 ± 22.6				
Control	6	208	32.3 ± 13.3	0.49	NS	14	NS
17α-estradiol, 5×10^{-9}		228	28.7 ± 12.0				

* Student's t-test; ** Mann-Whitney U-test. NS, not significant.

sperm-egg-mixture, containing $0.6–1.2 \times 10^6$ sperm/ml, was incubated for 4.5 h and then fixed with 2 ml Türk's solution at 5 °C over night. The ova were then stained with 0.5% lacmoid in 40% acetic acid, mounted on slides and examined for presence of enlarged sperm head(s) or pronucleus(ei), indicating fertilization. The significance of the difference in fertilization frequency between the control and the treated eggs was estimated with the t-test and the Mann-Whitney U-test.

Results and discussion. It appears from the table that at 5×10^{-9} M 17β-estradiol fertilization is stimulated, an effect not shown by 17α-estradiol at the same level. However, no effect on fertilization frequency was exerted by 17β-estradiol at concentrations of 5×10^{-6}, 5×10^{-7}, 5×10^{-8} and 5×10^{-10} M.

Despite efforts to eliminate sources of systematical error the number of ova was always greater in the test pool than in the control pool. This could possibly be explained by an unintentional inclination to choose the larger of the 2 pools of eggs to be used as the test pool.

Earlier it has been reported[5,6] that 10^{-5} M 17β-estradiol inhibits fertilization. The same authors found, too, that as the concentration was lowered to 10^{-6} M the inhibition was abolished, a finding which is in conformity with the present results.

Since stimulation of fertilization appears at 5×10^{-9} M 17β-estradiol it may be assumed that at the higher concentrations tested, the stimulating and the inhibiting effects balance each other to the degree that no significant change in fertilization frequency can be observed against the background of variation in the present data.

As the concentration of added steroid is lowered the relative contribution from the naturally-occurring estrogens increases. An estimate of the latter is of necessity uncertain, since several factors, e.g. rate of synthesis may influence the result. It can, however, be said that if endogenous estrogens are necessary for fertilization they should have a concentration not very different from that at which exogenous 17β-estradiol stimulates fertilization.

The stimulation is probably mediated by a structure-recognizing mechanism, since 5×10^{-9} M 17α-estradiol fails to influence the frequency of fertilization. Furthermore, binding sites for which 17β- but not 17α-estradiol compete have been demonstrated by Briggs[7] and these binding sites appear to be located near to the plasma membrane[8]. On the basis of results reported by Szego[9], Lindahl[10] suggested that HHA induced by 17β-estradiol involves activation of adenylate cyclase. Indeed, Cheng and Boettcher[11] recently reported that 17β-estradiol stimulates spermatozoal adenylate cyclase, an enzyme which is present immediately inside the sperm head plasma membrane[12]. The ability of cAMP and catecholamines to induce HHA also suggest the involvement of adenylate cyclase; besides, the effect of the latter is inhibited by adrenergic receptor blockers[13]. The catecholamines are, moreover, capable of inducing the acrosome reaction[14] and, as pointed out by Lindahl and Sjöblom[2], it is likely that HHA and the acrosome reaction have reaction links in common. Thus, it is possible that the increase in fertilization rate caused by 17β-estradiol depends on the stimulation of certain steps in the acrosome reaction.

1 Lindahl, P.E., and Kihlström, J.E., in: Report of the 2nd International Congress on the Physiology and Pathology of Animal Reproduction and of Artificial Insemination, p. 70. Copenhagen 1952.
2 Lindahl, P.E., and Sjöblom, P., Biol. Reprod. *25* (1981) 29.
3 Lindahl, P.E., Örberg, J., Torsberg, S., and Sjöblom, P., J. Reprod. Fert. *52* (1978) 123.
4 Cross, P.C., and Brinster, R.L., Biol. Reprod. *3* (1970) 298.
5 Gwatkin, R.B.L., and Williams, D.T., Nature *227* (1970) 182.
6 Briggs, M.H., Steroids *22* (1973) 547.
7 Briggs, M.H., Acta endocr. *75* (1974) 785.
8 Cheng, C.Y., Rose, R.J., and Boettcher, B., Int. J. Androl. *4* (1981) 304.
9 Szego, C.M., and Davis, J.S., Proc. natl Acad. Sci. USA *58* (1967) 1711.
10 Lindahl, P.E., Exp. Cell Res. *111* (1978) 73.
11 Cheng, C.Y., and Boettcher, B., Int. J. Androl. *5* (1982) 253.
12 Peterson, R.N., Russel, L., Hook, L., Bundman, D., and Freund, M., J. Cell Sci. *43* (1980) 93.
13 Lindahl, P.E., Exp. Cell Res. *113* (1978) 421.
14 Cornett, L.E., and Meizel, S., Proc. natl Acad. Sci. USA *75* (1978) 4954.

0014-4754/83/091048-02$1.50 + 0.20/0

Diurnal changes of cone mosaic in a teleost retina

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Summary. In *Poecilia reticulata*, a surface-living fish, the square mosaic changes into a row mosaic in the dark.

Fernald[1] reports that he did not find any mosaic pattern changes in the retina of *Haplochromis burtoni* during dark adaptation. He, therefore, questions my results on changes from square to row mosaic observed in the guppy *Poecilia reticulata*[2]. Fernald suggests that my findings were due to our method of preparing the tissue, i.e. the separation of retina from pigment epithelium. I would like to draw his attention to the 'Material and methods' section in our paper, where we refer to a previous publication[3] which describes in great detail the preparation of the specimens from which our electronmicrographs (figs 2, A and B) were obtained. A reading of the above-mentioned publication would have made it clear that our electronmicrographs of light and dark adapted photoreceptors were obtained from intact hemisected eye-cups, fixed immediately after sacrifice of the fish, and that neither Ca-free Ringer nor removal of pigment epithelium was involved. Moreover, I would like to stress that the figures shown in my publication[2] were taken at the level of the inner segment (ellipsoid), and not outer segment, as Fernald states. We have since shown that the rotation of the inner segments of twin cones during retinomotor activity develops already in the guppy embryo and is established in the neonate. Therefore, at the onset of vision the change from square to row pattern in the dark is already present as an intrinsic property of the retina[4,5].

Fernald is correct when he states that the preparation of isolating the retina in Ca-free Ringer solution greatly