Hydrolase content of the dorsal and ventral pancreatic regions

Hydrolase (U/mg protein)	n	Dorsal region	Ventral region	Dorsal/ventral paired ratio
Amylase	18	213.1± 7.1	173.1± 7.4[a]	1.271±0.076[b]
Lipase	10	287.1±12.9	300.5±16.4	0.967±0.045
Chymotrypsinogen	10	37.7± 2.5	36.3± 2.4	1.045±0.039

Mean values (±SEM) are shown together with the number of individual determinations (n) and significance of differences between the dorsal and ventral regions. [a] $p<0.001$ by group comparison; [b] $p<0.005$ by paired comparison.

the pancreas was not significantly different in the dorsal and ventral regions, respectively, with a mean value of 154±4 µg protein/mg wet wt (n=36). In a 1st set of 10 rats, the lipase and chymotrypsinogen contents were also similar in the dorsal and ventral regions, respectively; the amylase content, however was significantly higher in the dorsal than ventral area ($t=2.223$; $p<0.05$). The latter finding was confirmed in a 2nd set of 8 rats ($t=3.354$; $p<0.005$). The pooled results of these 2 sets of experiments are illustrated in the table (1st line), which indicates that the difference in amylase content between the dorsal and ventral areas was highly significant, whether judged by group or paired comparison. The amylase/lipase or amylase/chymotrypsinogen ratio was also significantly higher in the dorsal than in the ventral region (data not shown).

In several mammalian species, including man, the dorsal and ventral regions of the pancreas can be distinguished from one another by their embryogenesis, topography, vascularization, exocrine drainage and relative richness in 1 or 2 islet types[2,5]. The present work reveals that these 2 regions also differ in their hydrolase content. The amylase content was higher in the dorsal or splenic region containing glucagon-rich islets than in the ventral or duodenal region containing pancreatic polypeptide-rich islets. Since the amylase/lipase or amylase/chymotrypsinogen ratios are not identical in the teleinsular and periinsular acinar tissue[3], it is conceivable, but remains to be proved, that the difference between the dorsal and ventral regions reflects mainly a difference in the hydrolase content of the periinsular halos surrounding glucagon- and pancreatic polypeptide-rich islets, respectively. It would be interesting to investigate whether acinar cells of the dorsal and ventral regions differ from one another in their response to secretagogues. The existence of a dual functional compartmentalization of the acinar tissue - periinsular vs teleinsular and dorsal vs ventral - may help to reconcile the finding of a preferential and rapid release of certain hydrolases[6,7] with the knowledge that pancreatic enzyme secretion results from an 'en masse' discharge of secretory granules[8].

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Fate of rabbit eggs transferred asynchronously to the oviducts or uteri of oestradiol-treated recipients after ovulation[1]

M.L. Norris, C.E. Adams and M.C. Chang[2]

A.R.C. Institute of Animal Physiology, Animal Research Station, 307 Huntingdon Road, Cambridge CB3 0JQ (England), March 14, 1983

Summary. The effectiveness of 200 µg oestradiol benzoate (ODB) given at various times following ovulation, to overcome a 3-4-day difference in ovulation times between donor and recipient was examined. In approximately half of the recipients in which the interval from the administration of human chorionic gonadotrophin (hCG) to ODB was 2 days, some of the eggs implanted. With a 4-day interval, however, neither implantations nor degenerate blastocysts were found at autopsy on days 11 or 12 in recipients of either tubal or uterine eggs.

Synchronization of donor and recipient animals is a prerequisite for successful egg transfer. However, the characteristic pattern of changes in the protein components of rabbit uterine secretions can be altered by giving oestrogen systemically in early pregnancy, resulting in so called 'delayed secretion' and retardation of blastocyst development[3,4]. Under such conditions, following the 'asynchronous' transfer of 4-day eggs to 8-day uteri, 38%[5] and 33% (M.C. Chang, unpublished observations) of the transferred eggs developed into foetuses. The use of oestradiol benzoate (ODB) to permit asynchronous egg transfer was further explored by Adams[6], who showed that exogenous ODB acts directly on the endometrium rather than via the corpora lutea and that the early conceptus plays an important role in maintaining the corpora lutea.

The present study examines the survival and continued development of rabbit eggs transferred asynchronously to the oviducts or uteri of recipients, relative to the time of treatment with ODB.

Materials and methods. A total of 45 sexually mature female rabbits was used. They were purchased locally and then caged separately and maintained under conditions described elsewhere[7] for at least 3 weeks prior to use.

Donors. 12 of the does were treated s.c. with a horse anterior pituitary preparation, HPI[8]. At 15-20 h after the last priming injecting 35 IU hCG was injected i.v. followed

Table 1. Asynchronous transfer of rabbit eggs to recipients given 200 µg ODB 2, 3, or 4 days after hCG

Experiment	Stage of donor and recipient	Interval hCG to ODB (days)	No. recipients	No. recipients* with Implants Only	+ degenerate blastocysts	Degenerate blastocysts Tubal	Tubal + uterine	Uterine	Neither implants nor degenerate blastocysts
a	1-day egg to 4-day oviduct	2	4	2	0	1	0	0	1
		3	5	1	1	0	1	1	1
		4	3	0	0	0	0	0	3
		Control	4	0	0	1	2	1	0
b	4-day egg to 8-day uterus	2	5	1	1	-	-	1	2
		3	5	0	0	-	-	1	4
		4	4	0	0	-	-	0	4
		Control	3	0	0	-	-	3	0

* Autopsy on day 11 (experiment a) or day 12 (experiment b).

Table 2. The fate of 1-day eggs transferred to 4-day oviducts (experiment a) and 4-day eggs transferred to 8-day uteri (experiment b) of pseudopregnant recipients given 200 µg ODB 2-4 days after hCG (percentages in parentheses)

Experiment	Interval hCG to ODB (days)	No. recipients Observed	Pregnant	No. eggs transferred	No. transferred eggs recovered or implants observed* Total	Implants	Degenerate blastocysts Tubal	Uterine
a	2	4	2	34	9 (26)	5 (15)	4	0
	3	5	2	41	19 (47)	9 (22)	3	7
	4	3	0	21	0	0	0	0
	Control	4	0	38	21 (55)	0	14	7
b	2	5	2	29	11 (38)	6 (21)	-	5
	3	5	0	30	3 (10)	0	-	3
	4	4	0	22	0	0	-	0
	Control	3	0	18	9 (50)	0	-	9

* Autopsy on day 11 (experiment a) or day 12 (experiment b).

by mating with 2 fertile males. In experiment a) egg recovery was performed 26 h after hCG by flushing the oviducts with phosphate-buffered saline (PBS), pH 7.4 (Dulbecco 'A': Oxoid Ltd, London), and in experiment b) the uterine horns were flushed with PBS 96 h after hCG.

Recipients. 33 does were induced to ovulate by i.v. injection of 35 IU hCG. After 2, 3 or 4 days, 26 of these does were treated s.c. with 200 µg ODB (β-estradiol-3-benzoate, Sigma Chemical Co. Ltd, Kingston-upon-Thames, Surrey). Subsequently, eggs were transferred as described elsewhere[9]. In experiment a) 1-day eggs were transferred to the oviducts of 4-day does (n = 16), and in experiment b) 4-day eggs were transferred to the uterine horns of 8-day does (n = 17).

The performance of recipients was evaluated at autopsy either 7 days (experiment a) or 4 days (experiment b) after transfer. At autopsy the entire reproductive tract was removed; the number of corpora lutea and implantation sites were noted. Implantation sites were measured across their maximum diameter using dial calipers (Mitutoya, Tokyo, Japan). The oviducts and uteri from the does in experiment a) and the uteri from does in experiment b) were flushed; all flushings were immediately searched for eggs using a stereomicroscope.

Results. Transfers of 1-day eggs to 4-day oviducts (experiment a) resulted in implantations only when the interval from ovulation to exogenous ODB treatment was no more than 3 days (table 1). In the 4 does with implantations, the number of implantations relative to the number of eggs transferred was very variable; viz 4/7 (57%) and 1/9 (11%) when the interval from hCG to ODB was 2 days, and 7/8 (87%) and 2/6 (33%) when the interval was 3 days. However, the diameters of the swellings were similar, the mean implantation diameters per female being 9.0 (7.9-10.3) mm and 8.7 mm when the interval from hCG to ODB was 2 days, and 9.4 (8.5-10.7) mm and 8.9 (8.5-9.2) mm when the interval was 3 days. Transfers of 4-day eggs to 8-day uteri (experiment b) resulted in implantations only when the interval from ovulation to ODB treatment was 2 days. In the 2 does with implantations, the number of implantations relative to the number of eggs transferred was 5/6 (86%) and 1/6 (17%), and the mean implantation diameters were 9.1 (7.9-10.0) mm and 10.0 mm respectively. Neither implantations nor degenerate blastocysts were recovered from recipients in the 2 groups in which the interval from ovulation to ODB treatment was 4 days, whereas all of the control recipients yielded some degenerate blastocysts (table 1). In experiment a, blastocysts were recovered from the oviducts of 3/4 control recipients.

A total of 134 2-cell eggs was transferred to the oviducts of the 16 recipients in experiment a, and 99 late morulae or early blastocysts were transferred to the uteri of the 17 recipients in experiment b. Only 19% of the 1-day eggs transferred to the oviducts of females given ODB 2 or 3 days after ovulation, and 21% of the 4-day eggs transferred to the uteri of females given ODB 2 days after ovulation implanted (table 2). None of the 43 eggs transferred to the 7 recipients from experiments a and b, given ODB 4 days after ovulation, was recovered at autopsy. The most successful recoveries, amounting to about 50% of the transferred eggs, were obtained from control recipients in both experiments a and b. Most of the eggs recovered were degenerate blastocysts. Typically, they showed evidence of having undergone earlier expansion followed by collapse, with a very large irregular, shrunken zona pellucida surrounding dark, granular, irregular blastomeres. The zonae pellucidae of these blastocysts were easily detached, and when this occurred zona-free forms became very difficult to identify.

Discussion. The present results in recipients having an interval from hCG to ODB of 2 or 3 days were generally better after transferring 1-day eggs to 4-day oviducts than

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.

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

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

P. Sjöblom

Institute of Zoophysiology, University of Uppsala, P.O. Box 560, S-751 22 Uppsala (Sweden), December 22, 1982

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.