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Studies on In Vitro Capacitation by Lysolecithin and In Vitro Fertilizing Ability of Ejaculated Rabbit Sperm

Kim, C. K[1], K. S. Im[2], X. Zheng[3] and R. H. Foote
Department of Animal Science, Cornell University, Ithaca,
New York 14853, USA

Summary

This study was conducted to define the effect of addition of lysolecithin (LC) and 20% v/v rabbit serum to sperm preincubation medium on the induction of acrosome reaction (AR) an fertilizing ability in vitro of LC-added sperm. Ejaculated rabbit sperm from New Zealand White buck was washed once by centrifugation, then preincubated for 2 or 4 hrs in a chemically defined medium (DM), DM plus 20% rabbit serum or BSA-free DM plus 20% rabbit serum at 37°C water bath or CO_2 incubator. At the end of preincubation LC was added to the preincubated sperm, which was stained at 0.5 to 4 hr later and examined for AR and sperm motility. For in vitro fertilization, gametes were coincubated in DM up to 24 hrs and thereafter fertilized embryos were incubated in BSM-II up to 48 hrs.

Addition of LC to 4-hr preincubated sperm was more effective for the AR and sperm motility than that to 2-hr preincubated sperm and optimal concentration of LC for AR was about 80μg/ml. A significant increase in AR occurred from 20 to 30 min. after addition of 80 to 100μg/ml in 4-hr preincubated sperm. BSA-free DM plus 20% rabbit serum showed a higher AR and sperm motility than those of DM plus 20% rabbit serum in LC-added sperm after 4-hr preincubation. The incidence of AR after 4-hr preincubation and at 30 min after 60μg/ml LC addition varied greatly among individual bucks. Sixty μg/ml LC-added sperm showed a slight high cleavage rate over control levels, but 100μg/ml LC-added sperm showed lower cleavage rate rather than 60μg/ml LC. It is concluded that optimal concentration of LC for high AR induction and sperm motility in 4-hr preincubated sperm was about 80μg/ml, but 60μg/ml level was more useful for in vitro fertilization.

Present address:

1. Dept. of Animal Science, Chung-Ang University, Seoul, Korea.
2. Dept. of Animal Science, Seoul National University, Suwon, Korea.
3. Beijing Agricultural University, China.

Introduction

Despite considerable interest in sperm capacitation, the exact nature of capacitation and/or acrosome reaction is still unclear. Many studies in the rabbit have been directed toward eliminating the requirement of female tract in capacitation process or reducing the required time for in vitro capacitation (Kirton and Hafs, 1965; Ericsson et al., 1971; Ogawa, 1972; Rosado et al., 1974; Dandekar and Gorden, 1975). However, these attempts did not achieved complete capacitation process but the results varied widely among investigators. The first successful capacitation and fertilization in vitro in a single system was reported by Brackett and Oliphant (1975). Unfortunately, their method has not been adequately confirmed. Akruk et al. (1979), Brackett (1979), Hosoi et al. (1981), Viriyapanichi and Bedford (1981) and Brackett et al. (1982) reported that one of conditions favoring in vitro sperm capacitation was additional preincubation of 10 to 12 hrs in artificial media. The time and rate of sperm capacitation in mammals seemed to be greatly influenced by various factors, such as physiological state of male (Bedford, 1970) and composition and condition of media (Rogers and Yanagimachi, 1975).

More recently, several investigators have reported interesting experimental results in which lysolecithin, a highly fusogenic sustance, markedly reduced the time necessary to obtain the acrosome reaction in vitro in the guinea pig sperm (Fleming and Yanagimachi, 1981; Yanagimachi and Suzuki, 1985) and in the hamster sperm (Ohzu and Yanagimachi, 1982). This possibility was observed already in a preliminary study of Brackett et al. (1972) in the rabbit.

The purpose of these experiments was to determine the effects of lysolecithin on the acrosome reaction and sperm motility in ejaculated rabbit sperm. Four serial experiments were designed:

1) to determine optimal concentration and time of lysolecithin (LC) addition in inducng the acrosome reaction (AR), and how rapidly sperm become acrosome-reacted after LC addition,
2) to determine whether AR and sperm motility will be improved further by addition of rabbit serum into preincubation medium,
3) to investigate individual variation in the AR among bucks according to LC addition, and
4) to confirm whether the results achieved from experiment 1, 2 and 3 induce effectively the AR under condition of in vitro fertilization.

Materials and Methods

1. Collection of semen

Semen was collected twice weekly from New Zealand White bucks with an artificial vagina and teaser. Sperm motility and concentration of each ejaculate were examined prior to using samples. The semen with above 50% level of sperm motility after 2-hr preincubation in vitro was used.

2. Treatment and preincubation of sperm

Pooled semen from 3 to 4 bucks and individual ejaculate was first diluted to 8 to 10 ml in standard defined medium (DM) (Brackett and Oliphant, 1975) and centrifuged at 350 x g for 5 min at room temperature. After the supernatant was discarded, sperm pellet was resuspended in fresh capacitation medium. At that time, sperm concentration was adjusted to about 1.0×10^7/ml. A 0.4ml portion of the adjusted sperm suspension was transferred into

12 x 55mm air-tight capped plastic tubes and preincubated for 2 or 4 hrs in a 30°C water bath or CO_2 incubator.

3. Preparation and addition of lysolecithin (LC)

LC (lysophosphatidyl choline, Type I from egg yolk, Sigma) was dissolved directly in DM at a concentration of 2mg/ml immediately before use. At the end of 2 or 4 hr preincubation, this solution was added into each tube containing sperm suspension to obtain a final concentration of LC.

4. Evaluation of sperm motility and acrosome reaction (AR)

Sperm motility was expressed as the percentage of sperm that are motile (0 to 85%) and estimated to the nearest 5%. Speed of sperm movement at which they move on a 0 to 5 rating was expressed as motility score, where 5 was the best speed. A acrosome-reacted sperm expressed as the loss of acrosomal caps was counted under phase-contrast microscopy according to the staining procedure of Bryan and Akruk (1977), in which the acrosome caps were stained cherry-red (apical ridge) to pink (dorsal and ventral aspects) and the acrosome-reacted sperm had no acrosomal cap at all

5. Medium for capacitation fertilization and ova culture in vitro

DM used for sperm capacitation was prepared according to the procedure of Brackett and Oliphant (1975) and DM plus 20% rabbit serum or BSA-free DM plus 20% rabbit serum medium was made according to the procedure of Akruk et al. (1979), respectively. Rabbit serum was collected near the time of ovulation (approximately 11 hr post-HCG injection), sterilized by filteration through 0.45μm pore size and heated at 56°C for 30 min before use. Medium for in vitro fertilization and ova culture was DM and a modified Ham's F10 medium (BSM-II) developed by Kane and Foote (1970), respectively.

6. Fertilization and ova culture in vitro

Ova were collected from oviduct of New Zealand White and Dutch-belted superovulated donor rabbits with DM at 12 to 15 hrs after LH injection, then washed three times with DM, and distributed randomly to each plastic dish with 4 to 6 ova per 0.25ml medium droplet. A DM-droplet in 35 x 10mm sterile plastic dishes was covered with 4ml of Dow Corning 360 medical fluid and equilibrated for 3 to 4 hr in an incubator at 38°C with 5% CO_2 in air. For in vitro fertilization about 0.05ml (1.0 x 10^6 sperm/ml in total 0.3ml droplet) of pooled sperm suspension treated with LC for 30 to 40 min. after 4 hr preincubation was added into each droplet.

Sperm and ova were coincubated for 24 hr in a CO_2 incubator. After that, normal ova were transferred to BSM-II droplets for further development and cultured again for 24 hr until 48 hr postinsemination. Egg cleavage was taken as evidence for in vitro fertilization.

7. Experimental designs for AR induction and fertilization

Experiment 1. Experiment 1 was designed to determine optimal concentration and time of LC addition for AR induction of ejaculated sperm and how quickly sperm become acrosome-reacted by LC. Washed sperm was preincubated for 2 or 4 hr in DM in a 37°C water bath. Several concentrations from 40 to 100μg/ml LC were added to each plastic tube containing 0.4ml sperm suspension at the end of 2 or 4 hr pre-

incubation and sperm motility and percent AR were then examined at 0, 0.5, 2 and 4 hr after addition of LC, respectively. In order to determine optimal time required for the AR, samples were collected at 5-minute intervals up to 60 min. after LC addition.

Experiment 2. In experiment 2, effects of addition of 20% rabbit serum to capacitation medium on sperm motility and percent AR were studied. Sperm was preincubated for 4 hrs in either DM plus 20% rabbit serum or BSA-free DM plus 20% rabbit serum. Sperm motility and percent AR were determine at 30 and 60 min. after LC addition.

Experiment 3. Experiment 3 was conducted to observe individual variation in percent AR after LC addition. A total of 16 bucks was classified into 4 groups according to the degrees of percent AR at the end of 4-hr preincubation. Sperm motility and percent AR were studied at 30 min. after 60μg/ml LC addition.

Experiment 4. Finally, experiment 4 was tested the fertilizing ability in vitro of acrosome-reacted sperm by LC treatment, based on the effective results obtained in the preceding experiments. The conditions of this experiment were as follows: 4 hr preincubation in DM or BSA-free DM plus 20% rabbit serum, addition of 60 or 80μg/ml LC at the end of 4 hr preincubation, and ova culture in DM by 24 hrs postinsemination and in BSM-II until 48 hr postinsemination.

Results

The results from experiment 1 are shown in Table 1 and 2, and Fig. 2, respectively. It can be seen from Table 1 that when LC were added to medium at the end of 2-hr preincubation, the average percentages of acrosome-reacted sperm, noted after staining, during the first 30 min. after addition of 40 to 100μg/ml LC ranged from 15 to 26%, but an accelerated percent AR was observed when sperm were continuously exposed to 100μg/ml LC for 2 hrs. On the other had, when LC were added to medium after 4 hr preincubation, 60 to 100μg/ml LC added sperm did exhibit a highly synchronous AR at 30 min. after LC addition compared with unadded controls. Continuous exposure of sperm in the presence of 100μg/ml LC for 2 to 4 hr resulted in AR induction in about 60% of sperm and the percent AR were significiantly higher than those of controls or 40 to 80μg/ml LC added sperm.

Fig. 1 indicates that when 40 to 80μg/ml LC were added to 2 or 4 hr preincubated sperm, the decline in percentage of motile sperm up to 4 hrs after LC addition did not differ with those of controls but a high concentration of 100μg/ml showed a very rapid decline in sperm motility. As compare with the percent motile sperm by 4 to 6 hr after the start of preincubation, addition of LC to sperm which had previously been preincubated for 4 hr had a more high motility than that of LC addition to 2-hr preincubated sperm. Motility scores showed also a trend towards a decrease with time after LC addition except for the addition of 100μg/ml LC, where the scores were significantly decreased immediately after LC addition. Although not significant 80μg/ml LC showed slightly higher motility than others throughout all the periods. It is clear from the above results that addition of LC to 4 hr preincubated sperm was more beenficial to induction of AR in vitro than the addition of LC to 2 hr preincubated sperm and a high concentration of 100μg/ml LC was detrimental to the sperm.

Changes in percent AR at 5 minute intervals after LC addition to 4 hr preincubated sperm are given in Table 2. Percent AR of sperm

Table 1. Percentage of acrosome-reacted sperm after addition of LC following 2-hr or 4-hr preincubation in DM

Preincubation time (hr)			Concentration of LC (μg/ml)				
Before LC	After LC	Total	0	40	60	80	100
2	0	2	15.0±2.7				
	0.5	2.5	16.5±4.4	15.0±3.1	19.1±4.0	17.2±5.5	26.3±5.7
	2	4	20.2±5.3^{a}	19.8±4.2^{a}	24.6±5.8^{a}	31.9±3.8^{ab}	44.0±5.6^{b}
	4	6	23.9±5.3	22.6±6.2	32.2±7.9	34.0±5.8	45.1±4.5
4	0	4	16.5±3.7				
	0.5	4.5	15.4±5.0^{a}	17.7±3.1^{a}	28.0±6.4^{ab}	30.4±6.1^{ab}	45.5±10.9^{b}
	2	6	25.1±5.7^{a}	29.9±7.0^{a}	32.0±6.6^{a}	36.2±6.7^{a}	63.6±5.4^{b}
	4	8	35.3±3.7^{a}	38.5±3.8^{a}	38.0±6.4^{a}	37.8±3.9^{a}	73.1±3.3^{b}

Tabular values are expressed as means ±SE from 6 replicates.

$^{a\ b}$ Means within a row with different superscripts differ significantly ($P<0.05$).

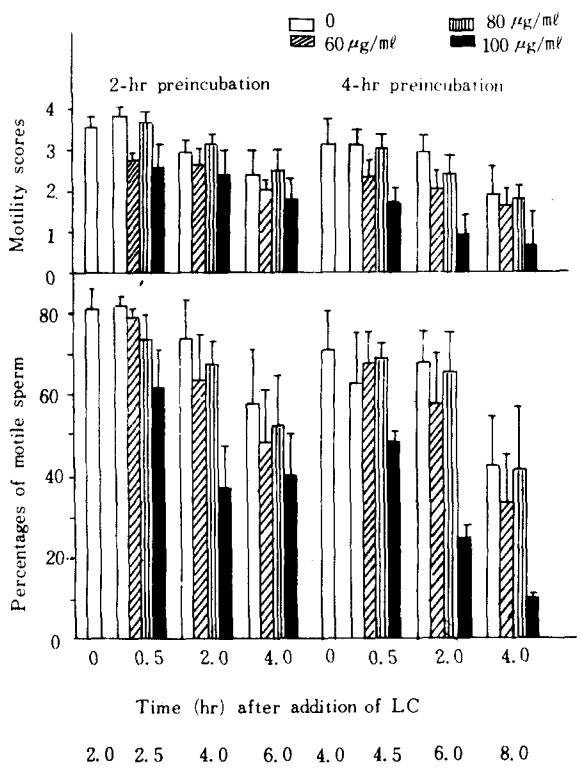

Fig. 1. Percentages of motile sperm and motility scores following addition of LC in 2-or 4-hr preincubated sperm. Mean ± SE was calculated from 6 replicated.

Table 2. Rate of acrosome reaction in 4-hr preincubated sperm investigated at 5-minute intervals after LC addition

Time after	Concentration of LC (μg/ml)		
LC (min)	0	80	100
0	19.4±2.1	20.3±2.1^{a}	24.0±3.1^{a}
5	20.5±1.6	26.6±1.1^{a}	29.1±5.4^{a}
10	19.1±4.4	33.6±5.6^{a}	40.9±2.2^{ab}
15	20.6±1.7	34.5±8.0^{ab}	46.0±2.2^{b}
20	18.8±4.0	34.8±8.7^{ab}	55.4±6.3^{c}
25	26.2±3.6	48.1±6.7^{bc}	65.8±6.0^{cd}
30	23.8±1.6	55.7±7.1^{c}	67.2±6.7^{cd}
60	25.7±5.1	61.7±6.7^{c}	77.7±4.9^{d}

Tabular values are expressed as means±SE from 5 repicates.

^{a,b,c,d} Means within a column with different superscripts differ significantly ($P<0.05$).

exposed to 80μg/ml LC increased significantly from 25 min. after LC addition and showed, thereafter, a continuous increase in percent AR with the lapse of time, but the percentages between 25 and 60 min did not greatly differ. On the other hand, percent AR of sperm exposed to 100=g/ml LC increased significan
to 100μg/ml LC increased significantly from 15 min after LC addition and percent AR from 25 to 60 min in 80 and 100μg/ml LC added sperm showed very similar pattern of a steady increase with time.

Table 3 is the results of experiment 2 in which the effects of addition of 20% rabbit serum to preincubation medium on percent AR dency to lower percent AR for 2 to 4 hr preincubation, compared with that of DM containing 20% rabbit serum. The addition of LC to 2 hr and sperm motility were studied. BSA-free DM plus 20% rabbit serum generally showed a tenpreincubated sperm in BSA-free DM plus 20% rabbit serum increased greatly percent AR over controls at 60 min. after LC addition without any decrease in the percentage of motile sperm. On the other hand, the addition of LC to 4 hr preincubated sperm showed a significant increase in percent AR at 60 min. after LC addition in both media compared with control levels and at that time percentage of motile sperm in DM plus 20% rabbit serum greatly decreased than

Table 3. Ef ect of addition of 20% rabbit serum (RS) to preincubation medium on percent AR and sperm motility

Preincubation time (hr)			Preincubation	Percent AR			Percent motile sperm		
Before LC	After LC	Total	medium	0	60	80μg/ml	0	60	80μg/ml
2	0	2	DM+20% RS	11.7±1.7					
			BSA−free DM +20% RS	8.4±2.0					
	0.5	2.5	DM+20% RS	12.9±2.4	14.8±3.6	17.6±4.6	77.5±3.2	72.5±3.2	70.0±0.1
			BSA−free DM +20% RS	8.1±1.3	16.2±2.1	16.8±3.0	77.5±1.5	71.3±4.3	73.8±3.2
	1	3	DM+20% RS	11.3±1.6	20.9±4.7	18.2±3.1	75.0±2.9	66.7±6.0	63.3±6.0
			BSA−free DM +20% RS	8.6±0.8[a]	15.8±2.3[a]	15.9±2.1[b]	77.5±1.5	72.5±3.3	75.0±2.0
4	0	4	DM+20% RS	11.0±3.5					
			BSA−free DM +20% RS	9.5±2.9					
	0.5	4.5	DM+20% RS	10.2±0.7	13.6±1.1	19.1±2.2	70.0±3.6	63.8±4.3	62.5±6.0
			BSA−free DM +20% RS	10.4±1.1[a]	14.6±1.0[ab]	21.5±4.2[b]	68.8±3.2	66.3±4.3	70.0±3.6
	1	5	DM+20% RS	12.1±1.7[a]	21.9±2.8[b]	18.8±2.5[b]	68.7±7.5[a]	61.3±6.3[b]	56.3±4.8[b]
			BSA−free DM +20% RS	10.1±1.4[a]	17.2±2.6[b]	26.6±3.3[b]	71.3±4.5	66.3±8.5	63.8±7.5

Tabular values are expressed as means±SE from 4 replicates.

[a b] Means within a row with different superscripts differ signicantly ($P<0.05$).

that of control. When LC of 80μg/ml were added to 4-hr preincubated sperm, sperm preincubated in BSA-free DM containing 20% rabbit serum showed more high percent AR and motile sperm at 60 min. after LC addition than those sperm preincubated in DM plus 20% rabbit serum.

Table 4 shows the results of experiment 3. Percent AR of sperm before and after 4 hr preincubation without LC varied greatly among individual bucks and percent AR at 30 min. after addition of 60μg/ml LC also was significantly different among 4 buck groups. Especially,

Table 4. Individual variation in percent acrosome-reacted sperm among bucks following 4-hr preincubation and LC exposure (60 μg/ml) in DM

Buck group (%AR at end of 4-hr preincubation)	No. of bucks	Percentage of acrosome-reacted sperm			B/A	C/B
		Before 4-hr preincubation (A)	At end of 4-hr preincubation (B)	At 30 min after LC addition (C)		
I (Over 20%)	4	11.9 ± 2.6^a	33.3 ± 1.9^a	57.7 ± 3.4^a	2.8	1.7
		(77.0 ± 6.8)	(57.0 ± 2.3)	(48.0 ± 4.9)		
II (13-19%)	4	7.5 ± 0.3^a	14.5 ± 0.9^b	39.2 ± 6.2^b	1.9	2.7
		(84.1 ± 0.6)	(74.5 ± 4.0)	(66.1 ± 3.2)		
III (6-12%)	4	2.4 ± 0.5^b	8.5 ± 0.9^c	24.3 ± 2.3^c	3.5	2.8
		(85.1 ± 1.5)	(80.1 ± 1.5)	(73.3 ± 2.2)		
IV (Under 5%)	4	2.1 ± 0.5^b	2.9 ± 1.2^d	29.3 ± 4.8^c	1.4	10.1
		(88.3 ± 4.2)	(70.2 ± 4.6)	(62.6 ± 4.1)		
Total, Mean	16	6.0 ± 1.2	15.2 ± 2.9	37.0 ± 3.9	2.4	2.4
		(78.4 ± 3.0)	(66.1 ± 2.9)	(63.2 ± 3.7)		

Group differences in %AR were compared after 16 bucks were classified into 4 groups (I to IV) according to %AR at the end of 4-hr preincubation in DM.
Tabular values are expressed as means ± SE from 4 replicates per buck.
Numbers in parenthesis are percentage of motile sperm.
^{a,b,c,d} Means within a column with different superscripts differ significantly ($P<0.05$).

the increased percent AR by LC addition in group IV was 10.1 times larger than soon before LC addition compared with only 1.7 to 2.8 times larger in group I, II and III. These effects following addition of 60μg/ml LC were more considerable in which showed less percent AR before and after 4 hr preincubation. Also, percent AR at 30 min. after LC addition was highly correlated with those of before and after preincubation (r=0.85 and r=0.89).

The results of in vitro fertilization in experiment 4 are presented in Table 5. Cleavage rate from 60μg/ml LC added sperm after 4 hr preincubation was 28.9% at 24 hrs postinsemination, even though the rate was not greatly increased over control level 20.0%. When eggs were cultured further for 24 hrs in BSM-II medium, 60.3% of visually normal 24 hr ova were cleaved in 60μg/ml LC medium. However the control showed 72.0% cleavage for 24 hrs in BSM-II medium. In general, fertilization process and/or early cleavage development by 24 hrs postinsemination with control sperm seemed to be retarded than that with 60μg/ml LC added sperm. Cleavage rate from 4 hr preincubated sperm in BSA-free DM plus 20% rabbit serum was 13.4% for control, 24.6% for 60μg/ml LC and 11.1% for 80μg/ml LC. Sixty μg/ml LC

added sperm showed a higher cleavage rate than others at 24 hr postinsemination. The cleavage rate of 48 hr postinsemination was 51.5%, 46.9% and 28.5% in control, 60μg/ml LC and 80μg/ml LC added sperm, respectively. Eighty μg/ml LC added sperm showed a lower cleavage than others at both 24 and 48 hr postinsemination.

Table 5. In vitro fertilizing ability of acrosome-reacted sperm by LC addition

Preincubation medium	LC (μg/ml)	Normal cleavage of ova: 24 hrs postinsemination in DM					24 to 48 hrs postinsemination in BSM-II		
		No. of ova (trials)	Not cleaved or fragment	2-cell	4-cell	8-cell	%cleaved	No. of ova (trials)	Not cleaved or fragment	%cleaved
DM	0	40(2)	32	6	2	–	20.0	25(1)	7	72.0
	60	76(4)	55	12	8	2	28.9	58(3)	23	60.3
BSA-free DM 20% rabbit serum	0	67(4)	58	3	3	3	13.4	33(2)	16	51.5
	60	61(3)	46	7	6	2	24.6	32(2)	17	46.9
	80	54(3)	48	1	3	2	11.1	14(1)	10	28.5
Parthenogenetic activation in DM		18(2)	17	–	1	–	5.9	15(2)	14	6.7

Discussion

The results of experiment 1 indicate that more effective concentration and addition time of LC for induction of AR in vitro were from 60 to 80μg/ml and at the end of 4 hr preincubation in DM, respectively. According to Fleming and Yanagimachi (1981), most of lysophospholipids are known to be potent AR-promoting agents for the guinea pig sperm. Also, an acceleration of AR by LC has been confirmed by Ohzu and Yanagimachi (1982) with the hamster sperm and by Yanagimachi and Suzuki (1985) with guinea pig sperm. In this experiment, efficient synchronous AR was not similarly induced as those of Yanagimachi and his associates. The primary cause might be due to species variability in the response of sperm to LC. The optimal concentration and rate of AR after LC addition in the present experiment seem to be agree with those of other investigators. Ohzu and Yanagimachi (1982) reported that when hamster sperm was first preincubated for 1 or 2 hr in Ca^{2+} medium, then exposed to 50μg/ml LC, large proportions of sperm underwent AR within 15 min. Llanos and Meizel (1983) found that addition of 75μg/ml LC to 3.5 hr incubated hamster sperm stimulated 56% AR after 10 min. Yanagimachi and Suzuki (1985) again confirmed the previous results (Fleming and Yanagimachi, 1981), which demonstrated that the vast majority of 1 hr preincubated guinea pig sperm in Ca^{2+} free medium containing 85μg/ml LC underwent AR after exporsure to Ca^{2+} medium without LC. In this study, a considerable proportions of sperm exposed to 100μg/ml LC had a lower motility or sperm were rapidly rendered immobile in LC. These observations are in agreement with those found by Crose et al. (1971), who indicated that when rabbit sperm were treated with 120μg/ml LC for 1 min, more than 95% of sperm lost their motility. The reason of that immobilizing response seem to be due to cyto-

toxicity of LC as suggested by Koprowski and Crose (1973) and it seems likely that rabbit sperm is more susceptible to the toxic effect of LC than other species, since Ahkong et al. (1972) reported that a high concentration of LC had highly toxic and damaging effects and degrees of toxicity of LC varied with the cell type used. On the other hand, the reason of which 80μg/ml LC-added sperm increased slightly in motility scores than those of 40 to 60μg/ml LC addition is unknown but it seems to be related to an increased metabolic rate after LC as found by Brackett and Oliphant (1975). The results that percent AR of LC-added sperm in Table 2 was generally higher than those in Table 1 may probably be due to maximum exporsure to LC followed tube-shaking every 5 minute for sampling.

The results in experiment 2 have shown that BSA-free DM plus 20% rabbit serum had a relatively high AR and sperm motility than DM containing 20% rabbit serum. However, it is not know why the difference between two preincubation media occurred, even though rabbit serum might have a beneficial effect as reported by Akruk et al. (1979). Gabara et al. (1973) suggested that addition of serum would prevent the membrane labilizing action of LC. However, Jones (1976) reported that the only addition of serum did not prevent the loss of sperm motility followed after LC addition. More recently, Thompson and Cummins (1985) suggested that an increased sperm variability during preincubation with serum may be associated with greater numbers of sperm agglutination complexes. In relation to AR and serum, the necessity for BSA-supplemented media for inducing cpacitation in vitro is well documented (Rogers, 1978). Yanagimachi (1970) found that the factors in blood serum appeared to be a substance associated with the albumin fraction. Byrd (1981) reported that bovine serum was an causative agent in inducing the AR. Lui and Meizel (1977) reported that charcol-acid-treated BSA stimulated the AR in the hamsters. While Fleming and Yanagimachi (1981) indicated that the presence of albumin in capacitation medium was not absoluately necessary for the LC-mediated AR, but without albumin sperm survival was reduced. Thompson and Cummins (1985) also showed that no differences were seen in percent AR of ram sperm between the various protein supplementations, although serum promoted significantly better survival.

The present results from experiment 3 clearly showed that percent AR at the end of 4 hr preincubation and after LC addition varied greatly depending upon individual bucks tested. The present data are an interesting finding that support the assertion of Brackett and Oliphant (1975), Akruk et al. (1979) and Brackett et al. (1982), which demonstrated that individual variation in sperm quality used for in vitro fertilization is one of the most important factors affecting in vitro fertilization rate. Brackett and Oliphant (1975) reported that the individuality of fertilizing ability in individual bucks might be due to individual differences in metabolic characteristics of sperm or in the time required for the AR. Brackett et al. (1982) especially suggested that biologic variation in HIS-treated sperm was observed in semen quality within and between individual bucks. Akruk et al. (1979) showed that the broad range of fertilization rate was due to variation between individual bucks. While Byrd (1981) reported that the variation in limiting to induce the AR must be a result of physiological condition of the sperm in each of the jaculates used.

The results of in vitro fertilization in experi-

met 4 are comparable to that of broad ranges reported by several investigators who carried out in vitro fertilization with in vitro capacitated sperm based on Brackett and Oliphant's method. Cleavage rate for short-term incubated sperm were 30 to 40% (Brackeet and Oliphant, 1975) and 3 to 5% (Akruk et al., 1979), while for additional incubation of 10 to 17 hrs after short treatment, the rates were 49 to 62% (Brackett et al., 1982) and 67.3% (Akruk et al., 1979). Cleavage rate up to 24 hrs postinsemination in the present experiment was generally lower than the above results, but almost similar when compared with 48 hrs postinsemination in this experiment. The results that DM was much less favorable for fertilization and(or) development of one-cell ova than BSM-II are in conflict with those of Seidel et al. (1976), where Brackett's medium showed more fertilized ova than a modified Ham's F10 for fertilization. In this experiment, the reason that cleavage rates by 24 and 48 hrs postinsemination in BSA-free DM plus 20% rabbit serum were slightly lower than those in DM seems to be partly related to lower percent AR than DM as shown in experiment 2. Similar observations have been made by Quinn et al. (1982) with mouse ova, who demonstrated that fatty acid-free BSA inhibited in vitro fertilization. The results that 80μg/ml LC-added sperm showed a lower cleavage than sperm of 60μg/ml LC addition seem to be due to somewhat high level for sperm capacitation, even if the level caused a higher percent AR in vitro.

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