ROLE OF ESTRADIOL IN THE CAPACITATION AND ACROSOME REACTION OF HAMSTER EPIDIDYMAL SPERMATOZOA IN THE ISOLATED UTERUS OF MICE INCUBATED *IN VITRO*

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Abstract : The site of sperm capacitation, the agents and mechanisms causing capacitation and acrosome reaction (AR) in vivo are not well understood. The female reproductive tract has been reported to play a key role during capacitation and AR. Some experiments were carried out on the capacitation and AR of hamster epididymal spermatozoa in the estrogen and progesterone dominated uterus (estrous and diestrous respectively) albino mice, incubated in TALP without calcium and BSA. Also the effect of estradiol (200 µg/ml) supplemented to TALP, on capacitation and AR was examined. Capacitation and AR of hamster spermatozoa incubated in the isolated uterus of both estrous and diestrous mice were significantly (P<0.05) higher in the presence of exogenous estradiol than that in its absence. Acrosome shedding occurred earlier i.e. at 3rd hour as compared to the *in vitro* studies where it occurred at 5th hour. The present study thus reveals that uterus of both estrogen and progesterone dominated mice play an important role in the induction of capacitation and AR. The addition of estradiol might have the influence on the synthesis of uterine proteins of mice which might be important for capacitation and AR.

Key words : capacitation   acrosome reaction   hamster
    estradiol   female reproductive tract

INTRODUCTION

The mamalian sperm surface is stabilized during maturation in the epididymis and during ejaculation by selective coating with secretions from male accessory glands (1, 2), the reverse occurs in the female reproductive tract, where the sperm surface is remodified to destabilize the membrame for the occurrence of capacitation and acrosome reaction (3). The uterine factors secreted by the endometrium into the uterine fluid are apparently advantageous *in vitro* in inducing human sperm capacitation and hence may play a significant role in promoting maturation of ejaculated sperm in the female reproductive tract by enhancing calcium uptake into sperm (4). The molecular mechanisms and signal transduction pathways mediating processes of capacitation and AR are only partly defined and appear to involve

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modification of intracellular calcium and other ions, lipid transfer and phosphorylation remodification in sperm plasma membrane as well as changes in protein phosphorylation (5). The site of this pathway, endocrine status and the various factors responsible for inducing sperm capacitation and acrosome reaction are still not well understood (6).

The main objectives of this study are to standardize xenogenic site of both estrogen and progesterons dominated isolated reproductive tract (uterus) of female mice, for induction of capacitation and acrosome reaction in hamster spermatozoa as well as to see the effect of calcium and estradiol in inducing capacitation and acrosome reaction (AR) in the isolated uterus.

METHODS

Hamster cauda epididymal spermatozoa were used for studies on capacitation and acrosome reaction. The medium used for in vitro culturing of cells was TALP i.e. Tyrode's modified solution adjusted to pH 7.4 and consisting of sodium chloride (114.0 mM), potassium chloride (3.16 mM), calcium chloride dihydrate (2.0 mM), magnesium chloride hexahydrate (0.5 mM), sodium lactate (10.0 mM), sodium dihydrogen orthophosphate (0.35 mM), sodium bicardonate (25.0 mM), glucose (5.0 mM), penicillin (8 mg/100 ml, sodium pyruvate (0.10 mM) and BSA (15 mg/mL). supplemented with motility factors, taurine (100.0 µM) and epiephrine (1 µM) in a proper ratio (7:1) (7).

Sperm collection and washing procedures: Male hamster (120–150 mg), 3–6 months old

were used for sperm collection after killing either by cervical dislocation or by etherization. The cauda epididymides were removed, cleared off all the fat and blood by clotting and placed in a polypropylene petridish containing previously saturated liquid paraffin with media. The viscous cauda epididymal contents (CEC) were released into the paraffin oil by puncturing with sharp meedle. The CEC were checked for motile spermatozoa by resuspending them in TALP. Samples with motility >80% were considered as good. 0.5 ml of these prepared CEC were dispersed in 3 ml of PBS in a plastic tube by gentle shaking for 5 minutes, then centrifuged at 200g for 5 minutes.

Incubation of spermatozoa in isolated uterus of mice cultured in vitro: Cycling albino mice (25 g body weight) were housed individually with 12 h light and 12 h dark light regime and with water and food ad libitum. The reproductive tracts of these mice were removed, cleared off the fat and washed twice with phosphate buffered saline, PBS (pH 7.4). The uteri were ligated at both the ends, separated and cultured in plastic vials containing TALP medium minus calcium and without BSA in sterile polypropylene petridishes at 37°C.

In the first set of experiments, uterus from estrogen and progesterone dominated mice (mice at estrus and proestrus respectively) were cultured separately as above. The loose pellet prepared in sperm samples were injected in isolated uteri with 20 gauge needle and incubated at 37°C. In the second set of experiments both the uteri of mice (estrogen and progesterone dominated) were cultured in TALP medium without BSA, but the addition of estradiol

(200 µg/ml) and 0.5 ml of washed CEC were injected into these incubated uteri. For control the washed CEC were incubated in TALP medium with BSA (15 mg/ml) in polypropylene tubes at 37°C for 5–6 hrs. In each experiment 10 µl of sperm samples were drawn after 0, 1, 2, 3, 4, 5, hrs of incubation and observed for per cent motility, hyperactive motility (sperm capacitation) which was characterized by the exhibition of whiplash sperm tail movements recorded by observations in the CCTV system and true acrosome reaction using dual staining technique of Sidhu et at (8). Approximately one hundred microlitres aliquotes of washed and incubated hamster epididymal spermatozoa were mixed with an equal volume or 0.2% trypan blue (in TALP without BSA) and incubated for 3–5 minutes on a clean glass slide at 37°C. After the incubation, smears of the semen were prepared on the glass slides and dried. Then the smeared slides were stained with Giemsa for 1 h at room temperature. Smears were dried between the folds of filter paper and stored. The dried smears were studied at 400xg under an inverted microscope. Four

types of spermatozoa could be identified after dual staining (a) live spermatozoa with intact acrosome in which the post-nuclear cap was clear and acrosome was pinkish violet (b) dead spermatozoa with intact acrosome which were blue at post-nuclear cap and pinkish violet at acrosome; (c) live spermatozoa without acrosome in which post-nuclear cap was clear and acrosome was colourless (true acrosome reaction (d) the dead spermatozoa in which post-nuclear cap was blue and acrosome was colourless (false acrosome reaction). The spermatozoa under categories c and d represent the total acrosome reaction. The data were analysed using computer programmes (CPCS1) for factorial analysis in Randomised Block Design (RBD).

RESULTS

The various parameters including per cent capacitation and acrosome reaction in the *in vitro* studies are given in Table I. The per cent motility gradually decreased with increasing time of incubation and the

TABLE I : Per cent motility, hyperactive motility, true acrosome reasction, total acrosome reaction in hamster, total acrosome reaction in hamster spermatozoa incubated *in vitro*.

Time of incuba- tion (h)	Per cent motility	Per cent hyperacti- vity	Per cent true acrosome reaction	Per cent total acrosome reaction
0 hr	82.5±2.50	9.50±2.02	1.25±0.25	5.25±0.94
1 hr	77.50±2.50	24.25±2.32	1.50±0.28	8.25±1.65
2 hr	70±4.08	37.00±5.80	12.00±0.40	20.25±1.70
3 hr	52.50±2.50	60.00±3.02	20.75±1.63	30.25±1.31
4 hr	30±4.08	32.57±3.14	52.25±3.32	80.00±3.18
5 hr	25±5.00	7.50±1.04	72.75±1.65	82.75±7.18

*Values are mean ± SE: n=4

TABLE II : Per cent motility hyperactive motility (HAM), true acrosome reaction (True AR), total acrosome reaction (Total AR) of hamster spermatozoa incubated in isolated uterus of estrogen dominated (Estrous) and progesterone dominated (Diestrus) mice.

Time of Incubation		Estrogen-Dominated				Progesterone Dominated			
		% Motility	HAM	True AR	Total AR	% Motility	HAM	True AR	Total AR
½h	+	87.50a±2.50	17.25a±1.11	32.25a±1.65	35.75±2.02	67.50b±2.50	9.00b±1.47	29.00ab±2.27	30.75±2.78
	–	77.50ab±4.79	9.75b±1.31	29.50±1.32	55.00c±7.07	4.25c±0.85	36.00b±1.22	34.75±4.53	52.75±6.93
1h	+	67.50a±2.50	43.25a±1.89	53.75±7.69	55.50±7.56	32.50c±2.50	16.75c±3.25	43.50±6.66	59.00±6.45
	–	60.00b±4.08	31.50b±3.23	56.25±6.61	59.25±6.79	22.50d±2.50	9.00d±2.38	37.25±6.10	54.75±4.01
2h	+	57.50a±2.50	5.50a±1.71	64.75a±1.49	69.50a±1.04	30.00c±4.01	1.00c±0	44.25b±3.09	52.00±1.47
	–	37.50b±2.50	6.50b±1.85	52.50b±1.44	57.25b±1.38	11.25d±3.15	0	39.75c±2.35	
3h	+	22.50a±2.50	1.67a±0.19	86.75a±3.04	92.25a±2.59	10.00d±3.54	0	51.75c±3.47	72.25b±4.01
	–	7.50b±1.44	2.33b±0.51	75.00b±5.15	81.75b±6.71	0	0	72.00d±3.09	72.50b±5.39

Figures with same superscripts do not differ significantly (P<0.05); n=4
Values are mean ± S.E (n=4)
(+) : with Estradiol
(–) : without

sample did not run upto 6th hour of incubation since only 2–5 per cent motile sperm could be seen after the occurrence of maximum true AR at 5th hour of incubation. There was a progressive increase in the hyperactive motile spermatozoa (capacitated) where it occurred maximum at 3rd hour of incubation (60.00%).

The effect of estradiol on per cent capacitation and acrosome reaction of hamster spermatozoa incubated in isolated and *in vivo* cultured uterus of estrogen and progesterone dominated mice are shown in Table II.

As observed from the table, the per cent motility of hamster spermatozoa almost decreases to less than 30% in the uteri of progesterone dominated mice (both with and without estradiol) during 2nd hour of incubation in the media. During incubation of hamster spermatozoa in the isolated and *in vitro* cultured uterus of estrogen dominated mice, both with and without estradiol, the maximum per cent capacitation was 43.25% and 31.50% respectively and in uterus of progesterone dominated mice it was 16.75% and 9.00% respectively, Fig.2, occurring at 1 hr of incubation.

The occurrence of acrosome shedding in hamster spermatozoa was maximum 5th hour of incubation in TALP with BSA i.e. *in vitro* whereas it occurred at 3rd hour in the spermatozoa incubated in isolated uteri (Table I, II). The per cent true AR in hamster spermatozoa was significantly higher during incubation in uterus of estrogen dominated mice both with and without estradiol (Table II: Fig. 2).
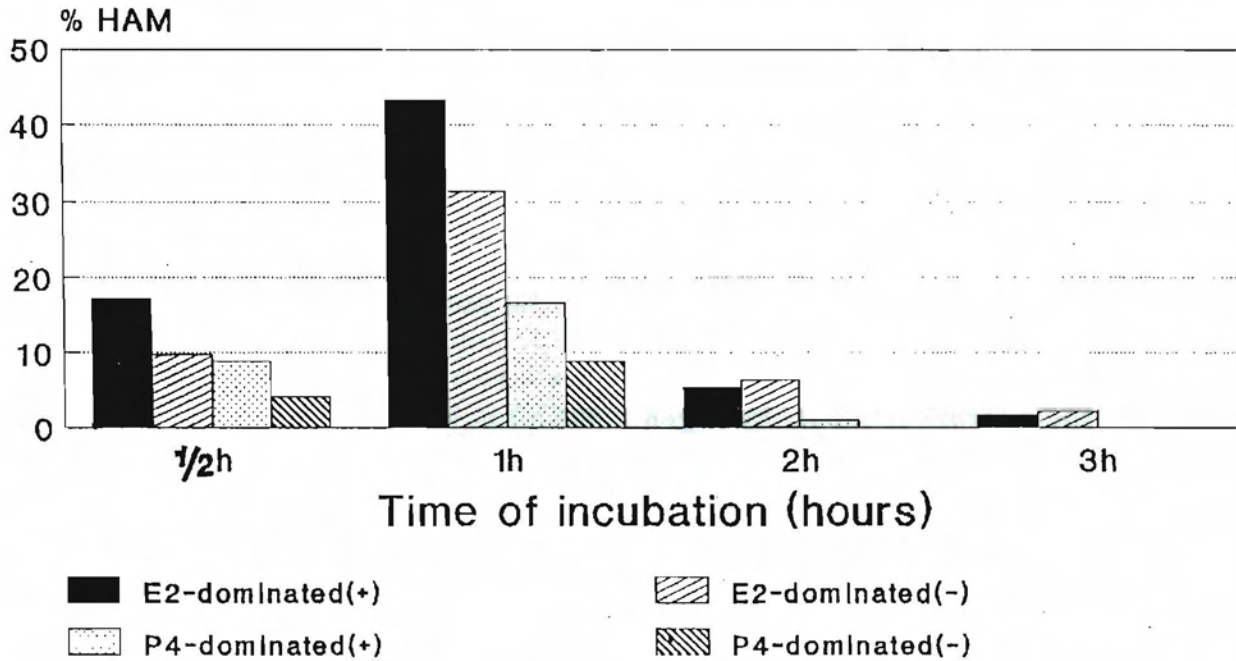
% HAM

Time of incubation (hours)

■ E2-dominated(+)   ▨ E2-dominated(-)

▨ P4-dominated(+)   ▧ P4-dominated(-)

Fig. 1 : Percent hyperactive motility (HAM) of hamster spermatozoa incubated in isolated uterus.

% AR

Time of incubation (hours)

■ E2-dominated(+)   ▨ E2-dominated(-)

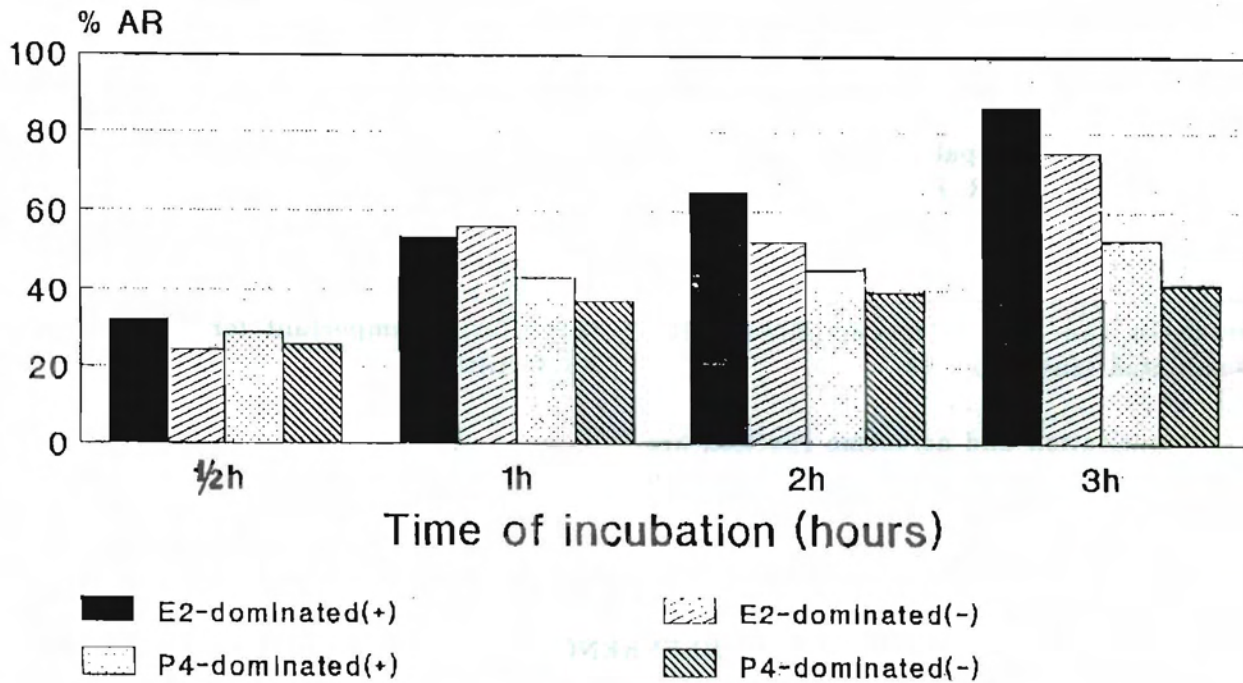▨ P4-dominated(+)   ▧ P4-dominated(-)

Fig. 2 : Percent true acrosome reaction (AR) of hamster spermatozoa incubated in isolated uterus.

DISCUSSION

The present study has revealed that true AR in hamster is accelerated by the exposure of spermatozoa to the isolated uterus of mice. Similarly, the xenogenic site for induction of sperm capacitation and acrosome reaction have been reported in other mammalian species (9, 10, 11, 12). Both capacitation and AR in hamster spermatozoa are affected by the endocrine status of the uterus of mice. Estrogen dominated uterus is more conducive than progesterone dominated one for sperm capacitation nad acrosome reaction. Higher palsma estrogen levels tend to promote the sperm capacitation uterus while progesterons levels suppress it (13, 14, 15, 16). Evidence indicates that spermatozoa during the transit in the female tract are involved in a complex interactive mechanisms with secretory glycoprotein of uterine epithelial origin (17). There might be a possible role of some uterine factors secreted by the glandular epithelium into the uterine fluid, capable of inducing sperm capacitation and AR *in vivo* (18, 19). The endocrine status also plays an important role in the elaboration of different proteins in the female reporductive tract which is more in the endometrium of estrogen dominated traçt (20, 16).

Capacitation and acrosome reaction are accelerated when estradiol at an appropriarte concentration is added to the media. The permeability of calcium increases during sperm passage through the female tract; some of the hormones are also known to regulate the calcium channel and hence efficient inducers of AR indirectly by increasing the intracellular calcium concentration, possibly by altering membrane fluidity through the cAMP dependent kinase pathway and consequently aiding in the production of a capacitated sperm and in the induction of membrane fusion; resutling in AR (21, 6). Recently, it has been reported that up regulation of ER mRNA in the endometriumof ovariectomized non-pregnant ewes is by estradiol (22). Thus the endometrial tissue possibly manifests its action through specific biochemical events commencing with the activated estrogen receptor, triggered by estradiol, followed by increase in the rates of RNA synthesis and ultimately by increase in the specific protein production.

Thus, these uterine proteins might contain some factors which probably seem to act through the calcium cAMP pathway in the induction of capacitation and AR; these factors being dependent on the calcium and estradiol as well as the endocrine status of the female reproductive tract. Further studies are being carried out to partially characterize these factors important for events crucial for fertilization.

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REFERENCES

1. Liu HW, Shang ST, Chao CF, Muller C. The scretion of two sperm maturation related glycoproteins in BALB/c mouse epididymis. *Cell & Tiss Res* 1991: 265: 409–414.

2. Eccleston ED, White TW, Howrd JB, Hamilton DW. Characterization of a cell surface glycoprotein associated with maturation of rat spermatozoa. *Molec Reprod Devel* 1994; 37: 110–119.

3. Sidhu KS, Guraya SS. Current concepts in gamete receptors for fertilization in mammals. *Int Rev Cytol* 1991; 181: 231–280.

4. Banerjee M, Chowdhury M. Induction of capacitation in human sperm in vitro by an endometrial sialic acid binding protein (SABP); in Young Scientists Symposium on Frontiers in Reproductive Biology Research, New Delhi, ICMR, Feb 1996; pp6.

5. Baldi E, Luconi M, Bonaccorsi L, Krausz C, Forti G. Human sperm activation during capacitation and acrosome reaction; Role of calcium, protein phosphorylation and lipid remodelling pathways. *Frontiers in Bioscience* 1996; 1: d189–205.

6. Yanagimachi R. Mammalian fertilization. In: Physiology of Reproduction. Knobil E, Neill JD (eds) 1994; pp 190–317.

7. Yanagimachi R. *In vitro* sperm capacitation and fertilization of golden hamster eggs in a chemically defined medium. In: *In Vitro* fertilization and embryo transfer. Hafez ESE, Semm, K (eds) Lancaster: MTP Press, 1982; pp65–76.

8. Sidhu KS, Dhindsa JS, Guraya SS. A simple staining procedure for detecting the true acrosome reaction in buffalo (Budalus bubalis) spermatozoa. *Biotechnic Histochem* 1992; 67: 35–39.

9. Hirst PJ, De Mayo FJ, Dukelow WR. Xenogenous fertilization of laboratory and domestic animals in the oviduct of the pseudopregnant rabbit. *Theriogenology* 1981; 15: 67–75.

10. Saling PM, Bedford JM. Absence of species specificity for mammalian sperm capacitation *in vivo*. *J Reprod Fertil* 1981; 63: 119–123.

11. Rao VH, Sarmah BC, Bhattacharyya NK. Xenogenous fertilization of goat ova in the rabbit oviduct. *J Reprod Fertil* 1984; 71: 377–379.

12. Kusunoki H, Sakaue M, Kato S, Kanda S. Induction of acrosome reaction in ejaculated goat and boar spermatozoa by preincubation in isolated rat uterus and elucidation of possible inducing factor(s). *Jpn J Anim Sci* 1988; 34: 223–233.

13. Bedford JM. Significance of the need for capacitation before fertilization in eutherian mammals. 1974; *Biol Reprod* 1974; 28: 108–120.

14. Parrish JJ, Susko-parrish JL, Handrow RR, Sims MM, First NL (1989) Capacitation of bovine spermatozoa by oviduct fluid. *Biol Reprod* 1989; 40: 1020–1026.

15. Demott RP, Suarez SS. Hyperactivated sperm progress in the mouse oviduct; Biol Reprod 1992; 46: 779–785.

16. Dhindsa JS, Sidhu KS, Guraya SS. Induction of buffalo (Bubalus bubalis) sperm capacitation and acrosome reaction in the reproductive tract of hamster cultured *in vitro*. *Theriogenology* 1995; 44: 599–608.

17. Sidhu KS, Bassi A, Gupta BC, Guraya SS. Indentification of the uterine factor (s) which induce the acrosome reaction in buffalo (Bubalus bubalis) spermatozoa. *Int J Androl* 1986; 9: 453–466.

18. Fazleabas AA. Female reproductive tract fluid and their influence on capacitation of bovine sperms. *Diss abstr Int* 1980; 418: 835.

19. Trifunac NP. Berstein GS. Effcct of human endometrial secretions on the metabolism of human spermatozoa. *Fertil Steril* 1981; 35: 58–63.

20. Buhi WC, Thatcher MJ, Shilla VM, Alverez IM, Lannon AP, Johnson J. Synthesis of uterine endometrial proteins during early diestrus in the cyclic and pregnant dog and after estrogen and progesterone treatment. *Biol Reprod* 1992; 47: 326–336.

21. DeJonge C. The cAMP-dependent kinase pathway and human sperm acrosomal exocytosis. *Frontiers in Bioscience* 1996; 1: d234–240.

22. Ing NH, Spencer TE, Bazer FW, Estradiol enhances endometrial estrogen receptor gene expression by a post-transcriptional mechanism in the overiectomized ewe. *Biol Reprod* 1996; 54: 591–599.