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70 Improving fertilization rate in vitro of male factor patients by Zona opening methods with microhooks. Y.Odawara, S.Chida, S.Iida, S.Mori, M.Suzuki. Dept.Obst.Gynec., Suzuki Hospital, Miyagi-ken.

Micromanipulator eas used to create small hall on zona pellucida to facilitate sperm entrance to PVS..42 couples with previous complete failure of fertilization due to poor semen(Group A,n=29),unex.failed fert.(Group B, n=9) and positive antisperm antibodies(Group C,n=4) were included this study. Eggs were collected by routine IVF procedures and treated by hyaluronidase to remove cumulus cells which was followed by zona opening methods with microhooks. Monospermic and polyspermic fert. rate were 20.5%(28/136) and 4.4%(6/136) in Group A,3.6%(1/28) and 32.1%(9/28) in Group B and 26.7% (4/15) and 6.7%(1/15) in Group C. Cleavage rate of the monospermic fert. embryo eas 74.0% (20/27) overall. Nosignificant differences of sperm count and motility was found between fertilized and unfertilized couples,however number of sperm in PVS and attached on zona after zona opening and sperm attachment of Hemi-zona assay were significantly higher in the fertilized couples. It was concluded that zona opening method was effective to improve fertilization rate in vitro of the patient with poor semen and positive antisperm antibodies.

71 Cleavage of rabbit eggs after microsurgical injection of testicular spermatozoa. H.Yazawa, K.Yanagida, H.Katayose, T.Sugano, K.Hoshi, A.Sato, Dept.Obst.and Gynec.Fukushima Medical College, Fukushima.

A rabbit testicular spermatozoon or sperm head was injected microsurgically into the cytoplasm of 80 mature rabbit oocytes and 71 control eggs were pricked similarly but without sperm injection. A significant proportion of injected eggs developed pronuclei by 9 hours and 21 eggs had undergone regular cleavage to two cells and 4 eggs to three cells by 22-24 hours. By contrast, only two of 71 control eggs had cleaved at that time, the others having fragmented or remained at the 1-cell stage. No pregnancies were obtained after surgical transfer of the cleaved injected eggs to the oviduct of synchronized recipients. The result nevertheless provide some preliminary support for efforts to obtain live young with testicular sperm nuclei.

72 The methods of preservation in mammalian's sperm nuclei. K.Yanagida, H.Yazawa, H.Katayose, T.Sugano, K.Hoshi, A.Sato, R.Yanagimachi*, Dept.Obst.and Gynec., Fukushima Medical College, Fukushima, *Dept.of Anatomy and Repro.Biol., Univ.of Hawaii, Sch.Med., USA

Various kind of storage methods of sperm nuclei were studied from a standpoint of the ability of male pronuclear formation. Hamster sperm nuclei collected from caudae epididymides were suspended in distilled water, and they were storaged under following four conditions,1)4 C, 2)-4 C, 3)freeze-dried and 4)air-dried. After each storage, one of sperm nuclei was injected into hamster unfertilized oocyte. We examined male pronucleal formation of injected oocytes after 5 hours incubation. Sperm nuclei stored in cold distilled water (4 C) for 7 weeks were capable of developing into pronuclei as same as control. The nuclei stored in frozen distilled water (-40 C), on the other hand, remained fully capable of developing into pronuclei even after 63 weeks of storage. The freeze-dried nuclei remained fully capable of developing after 12 weeks of storage. The air-dried nuclei might be recieved some damages. These observation suggest that fleeze-dry method ,is very easy for maintenance, of mammalian sperm nuclei may be utilized to experimental and clinical use.

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