Upsala J Med Sci 86: 225–232, 1981

# Electron Microscopy of Particles Similar to Type-C Virus in Human Oocytes

B. Ove Nilsson,[1] Erik Larsson,[2] Per Sundström[3] and Sibylle Widéhn[1]

*From the Departments of Human Anatomy[1] and Microbiology[2], Biomedical Centre, Uppsala and Department of Obstetrics[3], Malmö, Sweden*

ABSTRACT

Human oocytes in different stages of maturation were obtained by follicular aspiration from women given Clomovid and Gonadex. Particles similar to type-C virus were observed in 3 out of 16 oocytes. The particles were irregularily distributed along the oocyte membrane. They were seen both in a state of budding and as free lying in the perivitelline space. Reverse transcriptase activity was detected in 3 out of 9 samples of follicular fluid obtained from women other than those yielding the oocytes. It is assumed that these findings indicate the expression of endogeneous retroviruses in human oocytes.

## INTRODUCTION

Two classes of type-C RNA viruses, exogenous and endogenous, are defined. The exogenous virus are horizontally transmitted which implies that the individual is infected via contact with an exogenous virus. The proviral genome is not present in the DNA of all cells and is not transmitted by inheretance to the offspring. The endogenous viruses are vertically transmitted to the offspring as Mendelian genes and proviral genes are present in all DNA of these offsprings. Exogenous viruses can induce different types of tumors in vivo by transformation of appropriate target cells, while the role for endogenous viruses is still unclear. It has been speculated, however, that this type of virus is active during normal embryological development and also in the differentiation process of organ systems, like the hematopoietic tissues (4, 9).

The endogenous viruses have been found in various tissues of most animals including non-human primates (for a review see 18), but the possibility of the presence of a human endogenous virus is still debated. There are, however, reports on observations of viral particles and/or detection of viral footprints in components involved in human reproduction and embryogenesis, like placenta and cultured embryonic fibroblasts (2, 3, 6, 11, 14, 19, 20, for review see 1).

As previously communicated, we have detected particles similar to type-C virus in human oocytes (7, 8, 12, 13). Herein we report these electron microscopical findings in greater detail and give further results from analyses of reverse transcriptase of follicular fluids.

## MATERIAL AND METHODS

### Oocytes and Follicular Fluids

The oocytes were obtained from women admitted to hospital for infertility due mainly to Fallopian tube damage. The women were given 50 mg/day of Clomovid$^{R}$ for 5 days from the 5th day of the menstrual cycle and 6 000 I.U. Gonadex$^{R}$ 34-36 hrs before operation (17). The oocytes were recovered by laparascopy and classified as non-preovulatory or preovulatory according to Lopata (10).

Seven non-preovulatory and 9 preovulatory oocytes were processed for electron microscopical observations. On a few occasions, both non-preovulatory and preovulatory oocytes were obtained from the same woman. Follicular fluids were recovered from 9 women other than those yielding oocytes. The samples were froozen to -70°C within a few hours.

### Electron Microscopy

The fixation was made either directly or after about 3 hrs in follicular fluid or Thyrode's buffer solution. The fixative was 2.5 per cent glutaraldehyde in Ham F 10 tissue culture medium (Flow Chemicals), pH 7.2-7.4. After a fixation for some days the specimens were washed in phosphate buffer, postfixed for 15 min in 1% osmium tetroxide in phosphate buffer, dehydrated for about 20 min in increasing concentrations of ethanol and passed through a few changes of Epon before being embedded in Epon. The eggs were sectioned at two or three levels separated by a distance of about 10 µm. The sections were stained in uranyl acetate and lead citrate.

### Reverse Transcriptase

Assays for reverse transcriptase (RT) were done according to standard methods (15). Two ml of follicular fluid, which had been kept at -70°C, was diluted with 3 ml of TEN buffer (0.1 M Tris-HCl pH 7.8, 0.001 M ethylene diethylamine tetraacetic acid (EDTA), 0.1 M NaCl). The solution was clarified by low speed centrifugation at 9.000 g for 10 min and then centrifuged at 100.000 xg for two hours through a 15% sucrose cushion. The pelleted material was resuspended in 100 µl TEN and fractionated on a continuous 15-50% sucrose gradient 1000.000 xg for 2 hrs. One hundred µl fractions were collected, and 20 µl aliquots from fractions at the density between 1.15 and 1.20 were tested for reverse transcriptase activity.

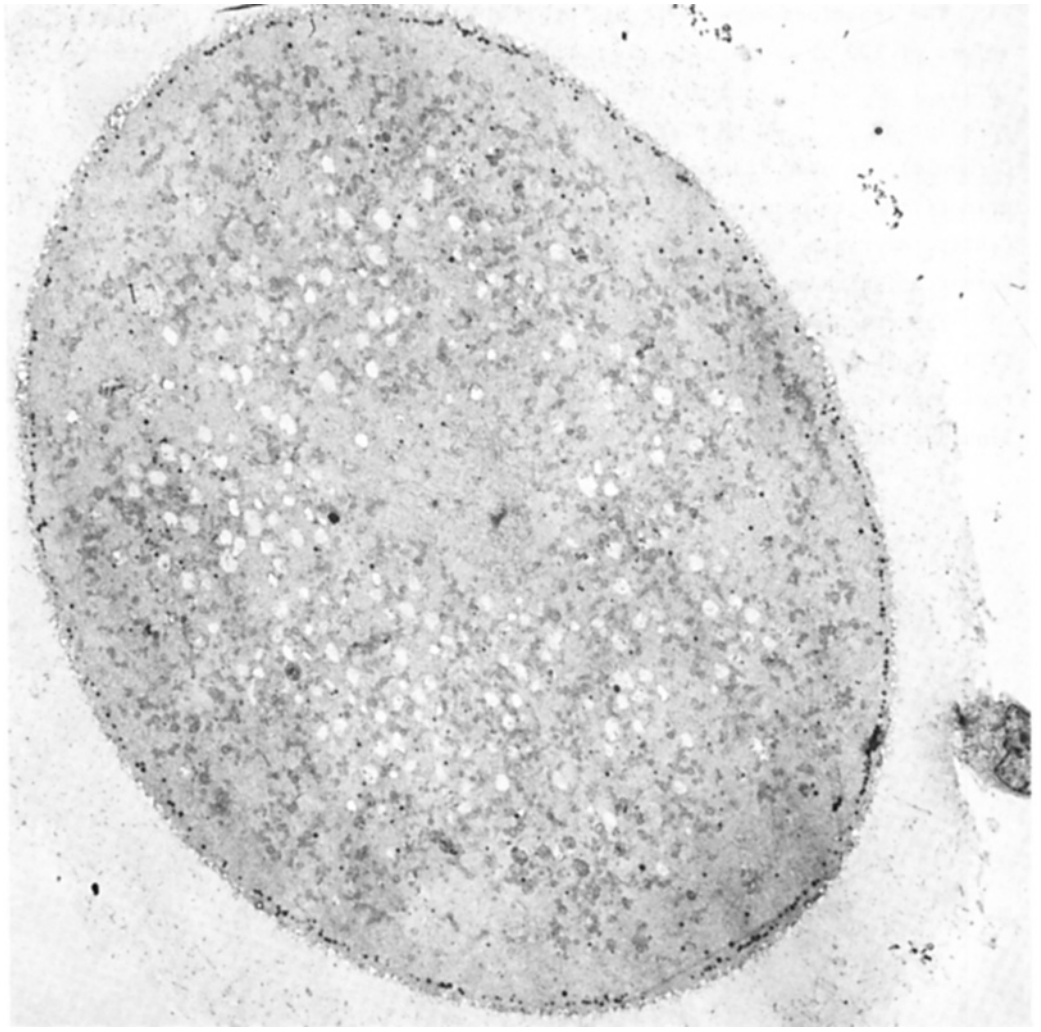

Fig. 1. Human oocyte surrounded by zona pellucida. Cortical granules are observed close to the surface membrane of the oocyte. A follicle cell is seen outside the zona pellucida in the lower right part of the picture. Mag. 1,300X.

The reactions were performed at 37°C for 30 min in a final reaction volume of 100 µl containing 50 mM Tris HCl pH 7.5, 80 mM KCl, 1 mM Dithiotritol (DTT), 1 mM $MnCl_2$, 4 µg of the prehydridized template primer complex poly rA/oligo $dT_{12-18}$, 20 µM dTTP and 1 µCi of $^3H$ dTTP (40-50 Ci/mmole). $^3H$-dGTP (20 Ci/mmole) was used instead of $^3H$ dTTP, when poly rC/oligo dG was used as primer/template complex. The radioactive trinucleotides were from Radiochemical Center, Amersham, England and the primer/template complexes from Collaborative Research (Waltham, Mass., USA).

The reactions were stopped by adding 1 ml of cold 10% trichloroacetic acid (TCA), 0.5% pyrophosphate and 50 µg yeast RNA to each tube. The acid insoluble radioactivity was collected on a millipore filter, dried and counted in a liquid scintillation counter.

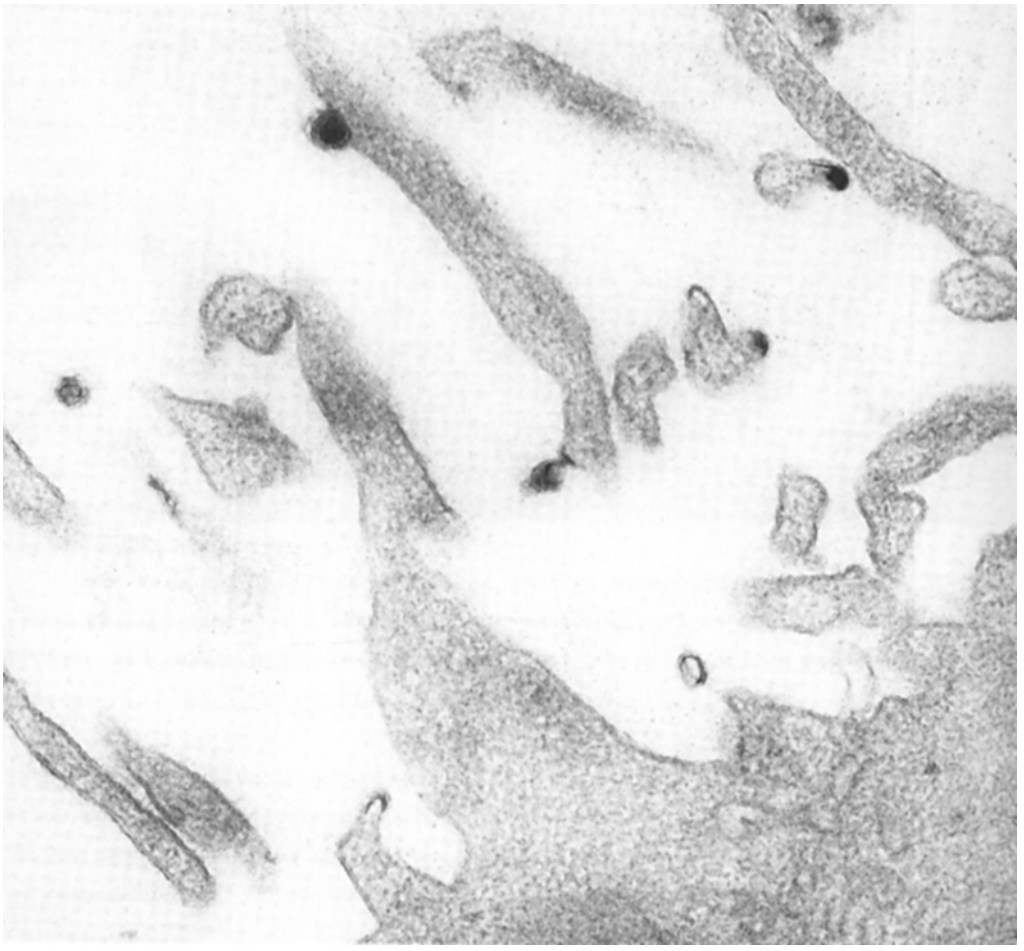

Fig. 2. Microvilli projecting into the perivitelline space between the oocyte and the zona pellucida. Several virus-like particles in various stages of budding is noticed at the cell membrane. Mag. 90,000X.

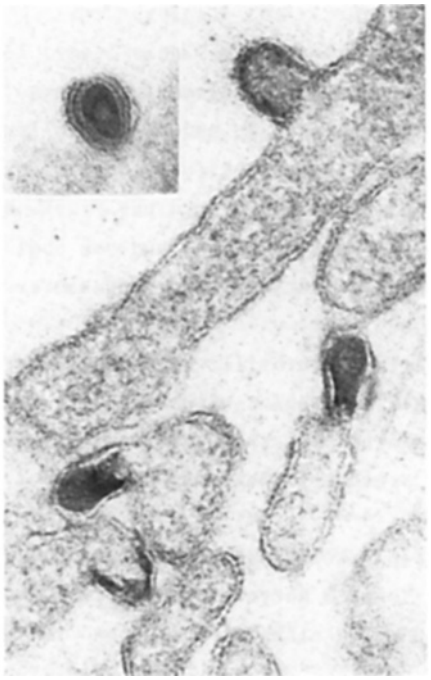

Fig. 3. Budding virus-like particles. The budding usually takes place at the surface of the microvilli and is noticed as a cell membrane elevation with an underlying dense condensation of the cytoplasm. Mag. 160,000X. Insert: Free-lying virus-like particle. The particle is bounded by a triple-layered membrane and contains a dark core with a less dark central part. Mag. 160,000X.

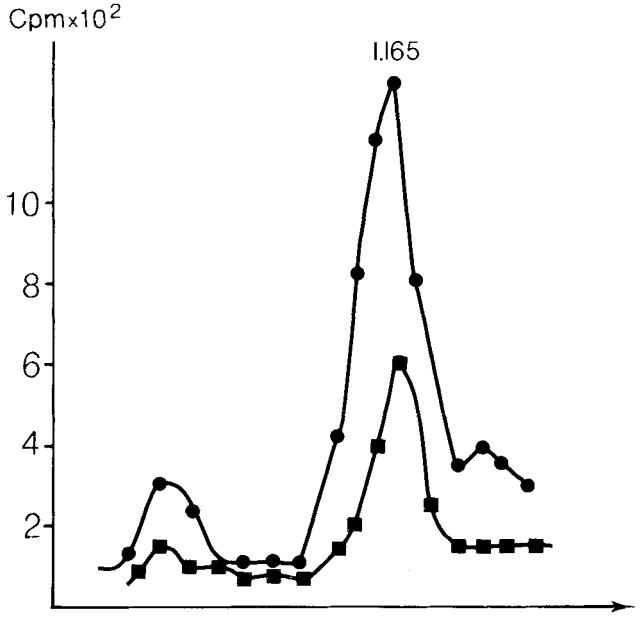


Fig. 4. Activity of reverse transcriptase from particulate material of follicular fluid fractionated on a continuous sucrose gradient (20-50%).

## RESULTS

### Electron microscopy

The human oocyte had a diameter of about 100 µm and was surrounded by a 5-10 µm thick pellucida (Fig. 1). The zona pellucida was noticed as a loose fibrillar structure containing cytoplasmic processes of the follicular cells and many slightly dense particles with a diameter of about 100 nm. The perivitelline space, which was situated between the oocyte surface and the zona pellucida, was about 2-4 µm thick. Outside the zona pellucida, a varying number of follicular cells were present.

The surface of the oocyte possessed several thin microvilli which extended into the perivitelline space (Fig. 2). At the cell membrane C-virus like particles were observed. The particles were irregularly distributed along the cell membrane but at the places where they were present rather many were observed (Fig. 2).

The number of oocytes displaying virus-like particles was low - among the 16 oocytes examined, 3 possessed the particles. These 3 oocytes derived from different women. Two of these oocytes were clinically classified as non-preovulatory and one as preovulatory.

The particles were observed in various stages of budding from the cell surface (Fig. 3). Free-lying particles consisted of a dense core surrounded by a bilaminar membrane. The diameter of the particles were 60-80 nm. The core, which sometimes showed a pale central region, was separated by a thin space from the inner leaflet of the bilaminar membrane (Fig. 3).

### Reverse Transcriptase (RT)

Particle bound RT activity was detected in 3/9 tested follicular fluids at 1.17 in linear sucrose gradients. The RT activity in the follicular fluid, which gave the highest activity, is shown (Fig. 4). The activity was higher with poly rA/dT than poly rC/dG. The cation preference was not tested in this assay.

## DISCUSSION

Particles, indistinguishable from type-C virus particles by morphological criteria, have been observed in a few normal human tissues. The type C particles found in placenta might reflect an expression of a human endogenous virus, but they have not been possible to propagate and therefore no further classification of the virus has been possible. The virus isolated from human embryonic fibroblasts (3, 14) is related to the two primate virus, Baboon endogenous virus (BaEV) and Simian sarcoma virus (SSV) and it could represent an endogenous virus.

The particles described in this communication had the classical structure of type C virus and we are working according to the hypothesis that they represent a human endogenous virus. The particles were observed in all the well-known stages of virus replication from early phases of budding at the cell surface to a fully developed particle lying freely off the cell surface. However, the number of free particles with a type-C structure was rather low. Since free particles of a similar size but without any virus-like inner structure were frequently noticed in the perivitelline space and the zona pellucida, it could be that the structural organization of newly released particles is rapidly changed.

The particles were only observed in 3 out of 16 oocytes. This can be a concequence of the irregular distribution of the particles at the cell surface, since areas with particles could have been missed when sectioning at only 2-3 levels of each oocyte. However, there is also a further explanation for the presence of particles in only a few oocytes. It is known that the degree of maturation of the oocyte differs greatly at recovery from the follicles after a hormonal treatment (5, 16). Therefore, an irregular occurrence of virus-like particles could signify that the particles appear only at some specific stage(s) of maturation. The finding of RT activity in only 3 out of 9 samples of follicular fluids supports this suggest. Since the maturation has been initiated by hormone injections, the possibility that this treatment has induced an expression of virus must be considered.

## ACKNOWLEDGEMENT

This investigation has been supported by the Swedish Medical Research Council and the Swedish Cancer Society.

## REFERENCES

1. Deinhardt, F.: Biology of primate retroviruses. In: Viral Oncology (ed. G. Klein), pp. 357-398. Raven Press, New York, 1980.
2. Dirksen, E.R. & Levy, J.A.: Virus-like particles in placentas from normal individuals and patients with systemic lupus erythematosus. J Natl Cancer Inst 59:1187-1192, 1977.
3. Hefti, E., Reynolds, J.T., Panem, S. & Kirsten, W.H.: Characerization of unique nucleootide sequences and antigenic determinants of a type-C virus isolated from normal human cells HEL-12. Viruses in naturally occurring cancers. Cold Spring Harbor Conference on Cell Proliferation, 7:729-737, 1980.
4. Hellman, A., Fowler, A.K., Strickland, J.E. & Koutlab, N.H.: A possible physiological function for C-type RNA viruse. In: Comparative Leukemia Research (eds Clemmeson & Yohn), Biblthea haemat 43, pp. 161-165, Karger, Basel, 1976.
5. Jagiello, G., Karnicki, J. & Ryan, R.J.: Superovulation with pituitary gonadotrophins. Method for obtaining meiotic metaphase figures in human ova. Lancet 1:178-196, 1968.

6. Kalter, S.S., Helmke, R.J. & Heberling, R.C.: C-type particles in normal human placentas. J Natl Cancer Inst 50:1081-1084, 1973.
7. Larsson, E., Nilsson, B.O., Sundström, P. & Widéhn, S.: Morphological and microbiological signs of endogeneous C-virus in human oocytes. Int J Cancer, 1981.
8. Larsson, E., Sundström, P. & Nilsson, B.O.: C-type virus like particles in human unfertilized oocytes. XIII European Tumor Virus Meeting, Bornholm, Abstract, 1981.
9. Lieberman, D., Hurrman-Lieberman, B. & Sachs, L.: Regulation of endogenous type C virus expression during normal myeloid cell differentiation. Evidence for a role in promoting myeloid cell proliferation and differentiation. Virology 107:121-135, 1980.
10. Lopata, A., Brown, I.B., Lecton, J.F. McTalbot, J. & Wood, C.: In vitro fertilization of preovulatory oocytes and embryo transfer in infertile patients treated with clomiphene and human chorionic gonadotropin. Fertil Steril 30:27-35, 1978.
11. Nelson, I., Leong, I.A., Levy, I.A.: Normal human placentas contain RNA-directed DNA polymerase like that in viruses. Proc Natl Acad Sci USA 75:6263-6267, 1978.
12. Nilsson, B.O., Larsson, E., Lindqvist, G. & Liedholm, P.: Virus-like particles in human oocytes. III. World Congress of Human Reproduction, Berlin, West Germany. Excerpta Medica, 1981. (In press).
13. Nilsson, B.O. & Sundström, P.: Ultrastructure of human oocytes and in vitro developed human embryos. World Conference, Kiel, West Germany, In: In Vitro Fertilization and Embryo Transfer (eds E.S.E. Hafez & K. Semm), MTP Press, Lancaster, U.K. 1980. (In press).
14. Panem, S.: Spontaneous type C virus expression in human embryonic lung cells (HEL-12). Academic Press, New York, 1976.
15. Sarngadharan, M.G., Sarin, P.S., Reitz, M.S. & Gallo, R.C.: Reverse transcriptase activity of human acute leukaemic cells: purification of the enzyme response to AMU 70S RNA and characterization of the DNA product. Nature New Biol 240:67-70, 1972.
16. Steptoe, P.C. & Edwards, R.G.: Laparascopic recovery of preovulatory human oocytes after priming of ovaries with gonadotrophins. Lancet 1:683-702, 1970.
17. Sundström, P., Nilsson, O. & Liedholm, P.: Cleavage rate and morphology of early human embryos obtained after artificial fertilization and culture. Acta Obstet Gynecol Scand 60:109-120, 1981.
18. Todero, G.J.: Interspecies transmission of mammalian retroviruses. In: Viral Oncology (ed. G. Klein), pp.291-309. Raven Press, New York, 1980.
19. Vernon, M.L., McMahon, J.M. & Hackett, J.J.: Additional evidence of type C particles in human placentas. J Natl Cancer Inst 52:987-992, 1974.
20. Witkin, S.S., Korngold, G.G. & Bendich, A.: Ribonuclease-sensitive DNA synthesizing complex in human sperm heads and seminal fluid. Proc Natl Acad Sci USA 72:3295-3299, 1978.

Received June 23, 1981

Address for reprints:

Professor Ove Nilsson
Department of Human Anatomy
Biomedical Centre
Box 571
S-751 23 Uppsala, Sweden