CHAPTER IV

DISCUSSION

4.1 Changes in the surface characteristic of preimplantation embryos

Scanning electron microscopic investigations had revealed that the preimplantation hamster embryos had rod-shaped microvilli on the entire external surface. These rod-shape microvilli were reduced in number and length during development. These morphological changes were similar to mouse embryo, and in both types of embryos the dramatic reduction of microvilli was observed in blastocyst stage (Calarco & Epstein, 1973; Phillips & Shalgi, 1980). In contrast, the surface of rat preimplantation embryos revealed an increase of the surface area and the complexity of projections with advancing developmental stages (Burgos et al., 1976). The surface changes in preimplantation period may play significant roles in the increase of surface area in relation to the increased demand for transferring of nutrient materials between the uterine epithelium and trophoblast during the early stages of implantation and subsequence rapid cell division. The presence of numerous microvilli in earlier stages may represent in the means whereby embryos can increase surface area to cope with transport of nutrient molecules and for increasing as well as for storage of the presynthesized membrane for subsequence utilization in the rapid cell division during cleavage (e.g., 4- to 8-cell stages). As the rate of cell division and rapid macromolecular synthesis are curtailed in later stage embryo (beyond 8-cell stages), the surface becomes relatively smaller in relation to total volume

due to the rapid increase in the number of cells and the embryo's volume, the cell will partition their own cell membrane from the already presynthesized membrane in earlier stage. Hence the surface area per volume of each cell may be increased, and as the result, the overall surface appears smoother.

4.2 Ultrastructural characteristic of preimplantation embryos

Transmission electron microscopic studies have shown that the cytoplasm of 1- to 4-cell hamster embryos showed three distinctive zones. No similar feature was reported in other rodents. This characteristic may be unique to hamster embryo; perhaps because hamster is not closely related to rodents. This cytoplasmic pattern was changed in 8-cell stage, at which time the zonation of cytoplasm begins to disappear and the polarity of each blastomere was first observed. The pattern of polarization of cytoplasmic organelles was similar that observed in mouse embryo as described by Handyside (1980); the polarity is elaborated and stabilized at the 16- and 32-cell stages to generate the definitive trophectoderm (Fleming, Warren, Chisholm & Johnson, 1984; Fleming & Pickering, 1985). The process of polarization of blastomeres in the early mouse embryo is of central important to the generation of cell diversity in the blastocyst (Johnson, 1985). The polarization process is orientated by contact signals from other cells (Ziomek & Johnson, 1980), and is initiated at a specific stage of development. The acquisition of polarized features by the cell occurs progressively; new polar features are acquired, and established at successive 8-, 16- and 32-cell stages (Johnson, 1985; Fleming et al., 1984; Fleming & Pickering, 1985). During the phenomena of zonation and polarization,each of the cytoplasmic organelles will be organized and distributed in different

patterns as discussed below :

(i) Lamellar structures

Lamellar structures (LSs) visible only with electron microscopy in the cytoplasm of preimplantation hamster embryos were similar to those described in several species of myomorph rodents. Previously they were referred to as fibrous material (Enders & Schlafke, 1965), cytoplasmic lamellae (Weakley, 1967, 1968), cytoplasmic whorls (Hadex, 1966), yolk plateles (Szollosi, 1972), plaques (Schlafke & Enders, 1967; Tachi & Lindner, 1970) and paracrystalline inclusions (Parkening, Payer & Given, 1985). In mouse the LSs have characteristic linear periodicity, with two basic patterns of periodicities associated in the bilaminar lamellae. The smaller interval is approximately 22 nm whereas the larger is approximately 49 nm, and the ratio of these two periodicities is about 2 (Green, 1985). Some investigators (Szollosi, 1972; Nilson, 1980) have referred to the LSs present in myomorph rodents as yolk or yolk platelets; however, there is no proof that these bodies are composed of phospholipids or lipoproteins. The LSs have apparently been designated as yolk because there is little ultrastructural evidence for the presence of lipid in the early, yet biochemically it is unequivocally present. Another consideration has been that the LSs decrease as the embryo matures; hence they may serve as a nutritive source for the developing embryos. The LSs begin to disappear in late rat blastocysts (Schlafke and Enders, 1967), are absent in rat blastocysts after 15 days of delayed implantation (Enders and Schlafke, 1965), and disappear in Syrian hamster blastocysts following implantation (Parkening, 1976). By contrast, in this study, LSs were still present in golden hamster blastocysts, and decreased in

trophectoderm but still appear quite abundant in ICM.

Zamboni (1970) speculated, on the basis of morphology, that LSs in mouse oocytes represented lattices of ribosomal RNA. Studies utilizing enzyme digestion techniques (Burkholder, Coming & Okada, 1971; Garcia, Bareyra & Alfonso, 1979) and CsCl density-gradient centrifugation (Bachvarova et al., 1981) on mouse oocytes also implied that the LSs consisted of ribosome-like particles embedded in a crystalline lattice pattern. Piko' and Clegg (1982) have argued against the idea that the fibrillar material present in mouse oocytes represents ribosomes. This is based on electron microscopic studies in which they found that the fibrous units had a low electron density when selectively stained to visualize nucleic acids. In addition, alkali-treated sections that abolish the staining of ribosomes previously visualized in the cytoplasm, has little if any effect on the staining of the fibrillar arrays. In this study, the dense dots in the LSs were measured (Figure 5E) and found that the diameter of particles embedded in LSs is approximately 195 x 285 Å, which is similar to the dimension that of normal ribosomes whose size is about 150 x 250 Å in diameter (Fawcett, 1986). However, up to now there is no direct histochemical or autoradiographic evidence showing that the LSs in golden hamster embryos contained RNA. The only way that will provide a clearcut proof that the LSs contain ribosomes is to use the ribosomal RNA probe that can hybridize with ribosomes; this could be performed by *in situ* hybridization combined with electron microscopic autoradiography.

(ii) <u>Lysosomes</u>

In this study, the presence of secondary lysosome system was observed at 1-cell embryo to blastocyst. They increased in number

and variety of morphologies during developmental process. Most secondary lysosomes appear as multivesicular bodies, MVB, and another which is characterized by associated intracellular membrane or organelles is autophagic vacuole, AV (Alberts et al., 1983). In 1- and 2-cell embryo, most secondary lysosomes were found in the inner zone, while a few secondary lysosomes were found in the cortical zone began to increase when embryos were reaching 4-cell and 8-cell stages. These observations were similar to those of Fleming and Pickering(1985) who reported that blastomeres of mouse embryos up to the early 8-cell stage contained clusters of prelysosomal endosomes distributed randomly in the cortical cytoplasm. In addition, during the 8-cell stage and continuing into the early 16-cell stage, endosomes become progressively localized in the peripheral cytoplasm. The morphological and tracer observations indicate that the secondary lysosomes are derived from heterogenous processes including : (a) autolytic digestion of specific cytoplasmic components, and (b) endocytotic processing, probably via the transformation of endosomes as described in the maturation model proposed by Helenius et al. (1983). Cytochemical analyses in early mouse embryos demonstrate a minimal reactivity for acid hydrolase prior to the 16-cell stage (Mulnard, 1965; Solter, Damjanov & Skreb, 1973); and in an ultrastructural study, hydrolase was first detectable at a low level in the 8-cell embryo, and increased thereafter up to the blastocyst stage (Vobrodt, Konwinski, Solter & Koprowski, 1976). These results were consistent with the present study since autophagic vacuoles started to increase in number in 8-cell embryo, and they became most abundant in trophectoderm as the dense bodies, while in ICM they decreased in number. The maturation and autolytic processes observed during the 8-cell stage represent the

earliest occurrence of lysosomal activity in the developing embryo (Fleming & Pickering, 1985).

Lysosome-like structures are common in the blastocysts of many mammalian species (Enders, 1971), they are particularly abundant in the late blastocysts of sheep (Wintenberger, Torres & Flechon, 1974) and cow (Mohr & Trouson, 1981). In addition, the human and primate blastocysts have been observed to contain lysosome-like organelles, autophagic vacuoles in both the ICM and trophectoderm. It is suggested that cell death or organelles' turn over may be a normal feature of ICM and trophectoderm during development (Hurst, Jefferies, Eckstein & Wheeler, 1978), hence the progressive increase in number of lysosomes. Furthermore accumulation of lysosomes in the cytoplasm of trophectoderm may be in preparation for implantation process where the uterine epithelium is digested away by the released lysosomal enzyme to make way for implantating embryo (Carnegie, McCully & Robertson, 1985).

(iii) <u>Golgi complexes and vesicles</u>

It was shown in this study that Golgi complexes were localized throughout the cytoplasm in 1-cell embryo up to morula stage. This suggests that Golgi complexes can migrate up and down in the cytoplasm during development. Similar to this finding, Maro, Johnson and Pickering (1985) reported that Golgi antigens were distributed throughout the cytoplasm of early stage of mouse embryos up to 16-cell stages. Moreover, at 16-cell stages there appeared to be a greater concentration of Golgi antigen at the center of the blastomere. In these results showed the abundance of variety of cytoplasmic vesicles in asscciation with Golgi complexes. Since Golgi complex is known to be involved in the synthesis and packaging of glycoproteins this

indicates that a large amount of synthetic activities occur during early development, and one of the product could be membrane glycoproteins and other membrane components which needed to be synthesized before the rapid cell division during cleavage take place (Alberts et al., 1983). Later on in late stage embryo and morula, numerous vacuoles, endocytotic vesicles, autophagic vacuoles (including those that contained membrane whorls) were observed both in the cortical and inner zones. There are evidence indicating that new membrane is synthesized and assembled from 2- to 4-cell stage onwards (Izquierdo, 1977; Pratt, 1980, 1982), and probably inserted internally at sites of cell apposition (Izquierdo, Lopez & Marticorena, 1980; Izquierdo & Ebensperger, 1982) as occurs during cleavages of *Xenopus* egg (Tetteroo, Bluemink, Dictus, van Zoelen & de Laat, 1984). Alternatively, new membrane could be inserted throughout the cell surface (Pratt, 1985). However, direct evidence for the role of these vesicles in the synthesis of the membrane is still lacking. Mohr and Trouson (1982) suggested that some of the large vesicles could be involved in secretion and release of fluid into cavity of the rapidly expanding blastocysts.

(iv) Mitochondria

The present study showed that there were two types of mitochondria in preimplantation hamster embryos. The first type was round or oval containing few cristae and dense homogenous substance. They occur in 1-cell to morula stages. Another type found in blastocyst appeared smaller and had more conventional appearance in being globular, prossessing cristae. This is consistent with the observation of Grant, Nilsson and Bergstrom, (1977) who reported that the trophectoderm cells contained mitochondria appearing in two shapes:

one type consisting of spheroids measuring about 1 μm, while the other about 0.2 μm wide. Morphologically, the rapid transition of mitochondria within the blastocyst from primarily round or oval shape containing few cristae to cylindrical shape containing numerous expanded cristae, could be construed as representing an increase in metabolic activity. This seems plausible because the increase energy requirement is undoubtedly nescessary for the blastocyst invasion and the removal of uterine epithelium (Parkening, 1976). Similar changes had been described in ultrastructural studies of mitochondria in preimplantation mouse (Stern, Biggers & Anderson, 1971) and rabbit embryos (Anderson, Condon & Sharp, 1971). The decrease in density of the mitochondrial matrix and corresponding increase in the number of cristae had been interpreted as indicating that mitochondria in rabbit blastocysts were more metabolically active in term of energy production than mitochondria in rabbit morula (Anderson et al., 1971). It is therefore possible that the two forms of mitochondria may reflect different levels of energy production and could conceivably indicate early differences in energy-utilization between embryonic cells in different stages and between trophectoderm and ICM (Van Blerkom, Manes & Daniel Jr., 1973).

(v) <u>Rough endoplasmic reticulum</u> (RER)

Although RER is important organelle for protein synthesis in general cells. In this study, no RER with "classical" appearance was observed in preimplantation hamster embryos. In previous report , the paired cisternae of RER had been observed in the cytoplasm of small pre-antrum oocytes in golden hamster with a sharp increase of RER as the oocytes commences rapid growth (Weakley et al., 1986). However, by the time the oocytes had attained a third layer of follicle

cells, the amount of typical RER in the cytoplasm had sharply decreased. Once yolky materials had commenced to accumulate in the cytoplasm of the pre-antrum oocyte, the ER was composed almost entirely of the smooth or semi-smooth type and was largely confined to the immediate vicinity of mitochondrial clusters. The pair complexes might therefore be responsible for a rapid short-term increase in RER concerned in producing protein essential for the earliest growth phase. When these cisternae became redundant they might be broken down, or possibly might convert into the semi-smooth type which was observed in large oocytes (Weakley et al., 1986).

The possibility that may actually be a crystallization of ribosomes or a modified form of RER has already been discussed and this structure may be responsible for protein synthesis in the preimplantation embryos.

(vi) Intercellular junctions

It is interesting to consider the onset of junction-mediated communication in the early mouse embryo in the content of the development of the oocyte. Rat oocytes communicate with surrounding follicle cells (cumulus oophorus) prior to ovulation (Albertini and Anderson, 1974; Gilula, Epstein & Beers, 1978). As meiotic maturation resumes at the time of ovulation, the oocyte becomes progressively uncoupled from the surrounding cumulus cells (Gilula et al., 1978); the prefertilization oocyte is therefore a noncommucating, though it is previously communication-competent cell type. In previous studies of cell-to-cell communication in early embryonic cells of comparable stages in development, ionic coupling was usually observed; although it was never determined whether cytoplasmic bridges were responsible for the observed communication in those studies (Ito and

Hori, 1966; Ito and Loewenstein, 1969; Slack and Palmer, 1969). In addition, HRP injections had shown that the observed ionic coupling and dye transfer in the early 2-cell embryo were not mediated by a junctional pathway but rather via a cytoplasmic bridge (Lo & Giluta, 1979). Morphological studies had shown that mouse embryos do not have gap junctions until the 8-cell stage (Ducibella et al., 1975; Magnuson et al., 1978; Lo & Giluta, 1979; McLachlin, Caveney & Kidder, 1983). By contrast in this result gap junction was found as early as 4-cell stage of hamster embryos. The discrepancy between these results might reflect a strain difference in the timing of junction formation in relation to cleavage (McLachlin et al., 1983). The presence of gap junctions linking blastomeres could then provide a pathway for transferring and thus passively generating an intercellular gradient of ions, metabolites or other substances from the outside cells to the inside cells and vice versa (Lo & Giluta, 1979). It is possible that gap junction may play a role in embryogenesis (McLachlin et al., 1983). In addition gap junction formation was inferred by measuring ionic coupling as well as by observing the intercellular transfer of fluorescence dye. Inhibition of protein synthesis by cycloheximide treatment beginning as early as the late four-cell stage failed to block the acquisition of gap junctions, which demonstrated that the necessary proteinaceous components were present in advance of these events (McLachlin et al., 1983).

For other elements of the junctional complex, zonula occludens was firstly found between cells of morula stage. This is in agreement with the results of Ducibella et al., (1975) and Magnuson et al., (1977), in which the zonula occludens begin to form between presumtive trophoblast cells of mouse morulae and become more extensive

between trophoblast cells of blastocysts. In addition, Ducibella et al., (1975) found that diffusion of colloidal lanthanum hydroxide tracer into external intercellular spaces was prevented for the first time at the morula stage, at sites where zonula occludens were present. These junctions would appear, therefore, to be instrumental in creating an intercellular permeability barrier and thus establishing an extracellular compartment within the morula distinctive from the extraembryonic compartment. In this regard, Magnuson et al., (1978) found that the diffusion of horseradish peroxidase and microperoxidase into intercellular spaces of viable embryos was also impeded in some morulae and in all early blastocysts maintained on ice.

In the present study, it has observed that desmosomes began to form at the 2-cell embryo, which is the first report of such finding. In addition to desmosomes, in this study has also observed the presence of intermediate filaments at this embryonic stage; most of which could be associated with desmosomes. By contrast, rabbit embryos rarely contain specialized intercellular junctions in morula, and cell association were generally maintained through microvilli and membrane interdigitation. Only at the onset of blastulation do zonula occludens and desmosome began to form (Van Blerkom, et al., 1983).

In conclusion, the changes in ultrastructural features as observed in the preimplantation hamster embryo reflect the preparation of its constituent cells for subsequent events of development. The key changes consist of the zonation of cytoplasmic organelles in early embryonic cells, and later the polarization of these organelles beginning from 8-cell stage onwards (see Text Figures 1, 2, 3). The cytoplasmic zonation in early stage embryos probably represents the

Text Figure 1 Diagram of 1-4 cell embryos, showing the zonation of cytoplasmic organelles and the organization of cytoskeletons

Text figure 2 Diagram of 8-cell embryos onward, showing the beginning of polarazation of cytoplasmic organelle and the organization of cytoskeleton.

Text figure 3 Diagram of blastocyst, showing the distintive trophectoderm, ICM and the relative number and distribution of organelles in both type of cells.

Abbreviations : MTs = microtubules; IFs = intermediate filaments; MV = microvilli; MFs = microfilaments; Mi = mitochondria; V = vesicles; ML = microtrabecular lattice; LSs = lamellar structures; AV = autophagic vacuoles; DBs = dense bodies; pTr = polar trophectoderm; mTr = mural trophectoderm; ICM = inner cell mass; Bl = blastocoel ; Gol = Golgi complex.

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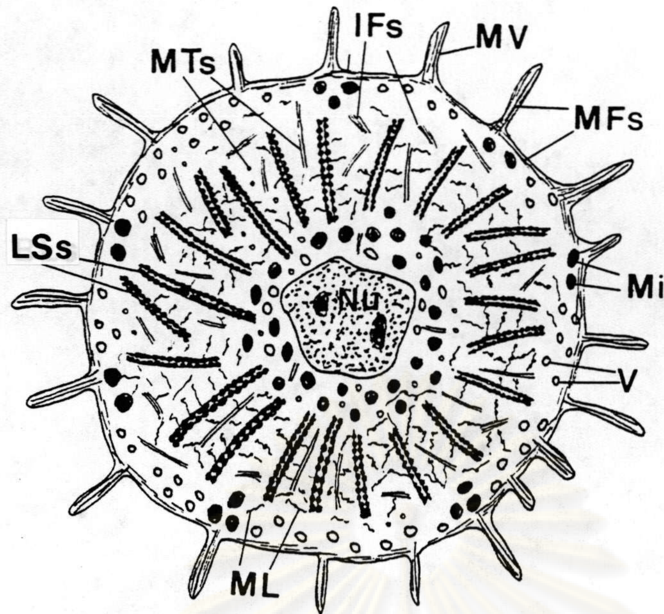
Text Figures



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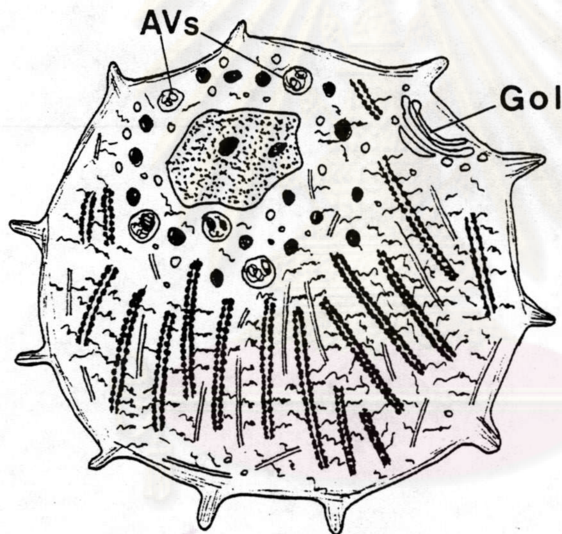

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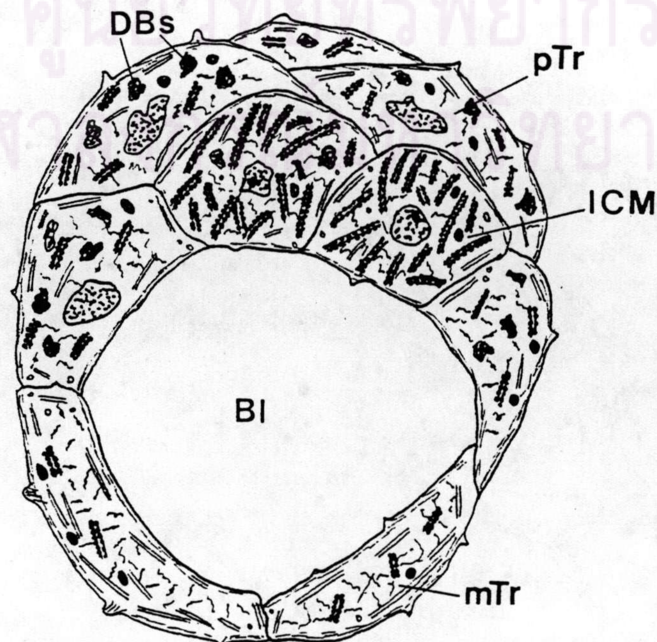

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division of labor which made possible by specialized function of each cytoplasmic region. The cortical area is in immediate contact with the environment, and the early presence of numerous endosomes, lysosome and MVB may indicate its role in the absorption and digestion of nutrients. Furthermore, the presence of Golgi complexes together with many vesicles suggest that there may be a rapid recycling of surface membrane during endocytosis, and also Golgi complexes and some vesicles may participate in the rapid synthesis of membrane to increase the surface area, earlier this "excess" membrane may be stored in the form of membrane covering over microvilli. These structure will, of course, help to increase the surface area for absorbtion. This zone may also be involved in the control release of cortical granules that are well-established in many species (Nicosia, Wolf & Inoue, 1977). The middle zone contains very few other organelles except LSs complexes. It is possible that this zone specializes in the synthetic activity, and its products could be lipoproteins of yolk and/or of the components of rapidly increasing surface membrane (Szollosi, 1972). However, the direct proof that LSs are RER or its equivalent is still needed. The central zone contains mostly mitochondria, Golgi complexes and small vesicles some of which have flocculent materials insides that resemble cortical granules. This zone can be responsible for the production of cortical granules that may be transported to the periphery. The zonation of cytoplasmic organelles is generally maintained in the same pattern until the 8-cell stage where the polarization occur. This process may reflect the beginning of cell differentiation since it is clerly seen that the peripheral cells tend to take away the peripheral cytoplasm inclusive of its organelles, the most prominent of which are the

secondary lysosomes and the Golgi complexes-vesicles groups, while the inner cells tend to take away the middle and central cytoplasm that is rich in LSs and mitochondria. In blastocyst, peripheral cells differentiate to become trophoblastic cells. It is remarkably but not entirely unexpected that these cells are rich in secondary lysosomes, and therefore could carry out efficient hydrolytic procedures. The lysosomal enzymes released from these cells could be responsible for partial digestion of endometrium during implantation process. The inner cells differentiate to inner cell mass that conceivably still maintain high synthetic activity through the presence of massive LSs. These cells will differentiate further to form different cell types of the embryo proper.

4.3 The organization of cytoskeleton in preimplantation embryos

The present study showed that suitable time for the extraction of preimplantation hamster embryos was by using glytaraldehyde-Triton X-100 for 1 hr. Most of cytoskeleton was still intact by this procedure. In earlier studies using Triton X-100 alone before glutaraldehyde fixation (Small & Celis, 1978; Small et al., 1980) in Hela cells extracted and leached out 50% of the total actin, about 70% of α and β tubulin, and 60% of α-actinin (Bravo, Small, Fey, Larsen & Celis, 1981). According to other studies (Blikslad, Markey, Carlsson, Persson & Lindberg, 1978; Bray & Thomas, 1976), a high proportion of the released actin is likely to be in the unpolymerized form. The addition of glutaraldehyde to the extraction buffer, a modification introduced by Hoglund, Karlsson, Arro, Fredriksson & Lindberg (1980) and Small & Langanger, (1981), resulted in the retention of cytoskeleton; and the denser packing of MFs in

microvilli had been maintained. In the present studies following the extraction four types of cytoskeletons were observed. Their organization and roles will be discussed as follows :

(i) Microfilaments

By using antibodies against smooth muscle actin in preimplantation mouse embryos, it was found that actin appeared to be located immediately beneath the cell membrane of 1-, 2- and 8-cell stages. In blastocyst stage, the ICM showed diffuse actin-specific fluorescence, while the trophectoderm showed actin concentrated at the cell borders. (Lehtonen & Badley, 1980). This ultrastructural studies confirm that similar organization of MFs existed in microvilli and underneath plasma membrane of all stages of preimplantation hamster embryos. In mouse embryo, Johnson and Maro (1984) also found that actin was localized principally in the cortical region especially in microvilli. The microvillous actin appears to be more cytochalasin D- resistant, presumable because of its much lower turnover rate (Pratt et al., 1981). Furthermore, in 8-cell blastomere in culture, immunofluorescence observation by Johnson and Maro (1984) showed that cytoplasmic actin appeared in non-homogenous pattern and distribution that could be characterized as a focal, zonal, and polar distributions, were all observed. Newly formed blastomeres showed an actin distribution in association with the remnant of previous mitotic spindles (Johnson & Maro, 1984). In the present study, it was found that cytoplasmic MFs in hamster embryos were organized in loosely fashion. Consistent with the finding using immunofluorescence method by Lehtonen and Badley (1980) which reported that in the cells of cleavage-stage mouse embryos, actin was dispersed chiefly in the cortical layer, while relative weak fluorescence was seen in the

cytoplasmic background.

There is compelling evidence to suggest that MFs not only redistribute during the 8-cell stage at the time of cell flattening and polarization (Lehtonen & Badley, 1980; Johnson & Maro, 1984), but also that they are involved actively in the process of cell flattening itself (Ducibella et al., 1977; Surani, Barton & Burling, 1980; Pratt et al., 1981; Johnson & Maro, 1984). This study supported the above notions because in blastocyst the flattened cells, trophectoderm, showed packed MFs arranged parallel to both the outer and inner membranes. MF layer possibly provides the cell cortex with mechanical rigidity. During cytokinesis cell contacts appear to restrict the direction of cell elongation (Graham & Lehtonen, 1979) and the maintenance of these contacts may well be mediated by the stiffness of cytoskeletal elements (Lehtonen & Badley, 1980).

(ii) Microtubules

MTs have been studied within fertilized mammalian eggs by electron microscopy (Longo & Anderson, 1969; Yanagimachi & Noda, 1970; Condos et al., 1972; Schatten et al., 1985), and during oogenesis by immunofluorescence microscope (Wassarman & Fujiwara, 1978). It has been shown that actin and tubulin are actively synthesized in the cleavage-stage mouse embryos (Abren & Brinster, 1975). Recent electron microspical observations had suggested that both 2-cell and 8-cell (Ducibella et al., 1977) stage mouse embryos had a cortical MF layer, whereas the MTs were arranged more orthogonally to the cell surface. In 8-cell stage embryos, however, the MTs appeared to be arrayed parallel to the membrane at the cell contact areas (Ducibella & Anderson, 1975; Ducibella et al., 1977). In addition, Lehtonen &

Badley (1980) showed that within the blastomeres and polar body of cleavage-stage mouse embryos is intensely stained with antibodies against tubulin. No clear cortical concentration of fluorescence comparable to those in the case of MF-associated protein could be observed. These reports are concistent with the present study in which the majority of MTs were observed in the region where polar body was being protuded, and a few of MTs were found at the periphery of 1-, 2-cell blastomeres. In addition, this study also showed numerous MTs in the area of intercellular junction up to 8-cell stages. This is in agreement with results reported by Lehtonen and Badley (1980), Ducibella & Anderson (1975), Ducibella et al., (1977), that the staining pattern of antibodies to tubulin was found at intercellular contacts at each cleavage stage. In addition, apparent perinuclear concentration of the label was frequently seen. In the present study, MTs were also observed to distribute ubiquitously in the cytoplasm of preimplantation embryos and they are arranged in all directions. In blastocyst stage, both trophectoderm and ICM showed tubulin-specific fluorescence (Lehtonen & Badley, 1980) which is more intense in the flattened trophectoderm than in round ICM. Nevertheless the presence of MTs in ICM fits the general concept that these structures are a feature of all cells (Paulin et al., 1980). The present study agrees with these reports, because ultrastructurally MTs were also observed in trophectoderm and ICM with cross and longitudinal sections but with higher concentration in the former. The use of drugs or the injection of antibodies reacting with tubulin (Wehland & Willinghan, 1983) had demonstrated that MTs control cell shape (Wells & Mallucci, 1978; Solomon, 1980; Solomon & Magendanz, 1981) and the intracellular location of various organelles (Freed & Leibewitz, 1976; Toyoma,

Forry-Schaudies, Hoffman & Holtzer, 1982; Summerhayes, Wong & Chen, 1983; Maro et al., 1983). In the present study MTs were observed in all stages of preimplantation embryos and with their characteristic distribution. It is possible that MTs play regulatory role in modulating cell shape and specific distribution of intracellular organelles.

(iii) Intermediate filaments

IFs seem to be present in all nucleated mammalian cells and also in preimplantation embryos. However, the reports of IFs in these embryos are still controversial. Jackson et al. (1980) reported that the first IFs detected during the preimplantation stages of mouse embryogenesis were bundles of filaments of cytokeratin type which appeared in the outer cells of morulae and in the trophectoderm of blastocysts. Their appearances were concomitant with the first formation of desmosome-like junctions, and intimate associations of these filaments with nascent desmosomes; thus it was likely that the antibodies used by Jackson et al. (1980) might detect preferentially desmosome-associated proteins not present in the earliest stages. By contrast Lehtonen and Badley (1980), Lehtonen et al. (1983) and Oshima et al. (1983) reported that IFs can be detected in oocyte, 1- to 8-cell embryos and blastocyst stage with immunofluorescence antibodies and electron microscopy. The results of these studies suggested that cleavage-stage and blastocyst-stage embryos as well as unfertilized oocytes contain cytokeratin-like polypeptides. The present study is consistent with that of Lehtonen and Badley (1980) and Lehtonen et al. (1983). By electron microscopic investigation, IF was observed to scatter in bundles of 80-100 Å fibrils in detergent-extracted 1- and 2-cell hamster embryos. In other stages of

preimplantation hamster embryos, these IFs were not observed. Since the organization of IFs in hamster embryos were localized in the cortical zone in small bundles, its whole course is impossible to observe in thin sections. Alternatively, IF may exist at a very low level in the cytoplasm after 2-cell embryos, since desmosomes begin to appear at 2-cell stage, and most IFs may be relocalized towards desmosome regions.

Previous reports showed that oocytes of *Xenopus laevis* contain IFs that were specifically stained with antibodies to cytokeratins (Franz et al., 1983). In addition, vimentin-containing filaments were also observed in frog oocytes and early embryo (Godsave et al., 1984). However in mouse oocytes and preimplantation embryos, IF proteins are reactive with cytokeratin antibodies but not with antibodies to vimentin (Lehtonen et al., 1983). From these previous data it is likely that the major type of IFs in hamster embryos may be cytokeratin since this protein is found in association with desmosome as tonofilaments. At this stage, it is not certain as to what role cytokeratin IFs play in preimplantation embryos. Since cytokeratin IFs appear to be preferentially distributed in epithelial cells, they may provide a mechanical framework contributing to intracellular stability and intercellular cohesion within specific epithelium (Franke et al., 1980; Lazarides, 1980). Similarly Godsave et al., (1984) suggested that IFs play a role in maintaining the oocyte cytoarchitecture and in later stage embryo they might also be involved in the cell to cell adhesion through desmosomes. Other functional role of IFs in hamster embryos should be studied further.

(iv) Microtrabecular network (MN)

There was controversy when a network or lattice like scaffold which is later referred to as microtrabecular lattice

is first observed in somatic cells with TEM by means of conventional method. Previous studies suggested that MLs might be an artifact of cytoplasmic condensation during fixation or dehydration from a homogeneous, protein-rich cytosol (Buckley & Porter, 1967; Bunge, 1973; Spooner et al., 1971; Yamada et al., 1971). By contrast, Wolosewick and Porter (1979) reported that thin sections of conventionally prepared, plastic-embedded material reveals varying amount of flocculent materials associated with MTs, MFs, free ribosomes, membranes of ER. None of these structures has clean or smooth surfaces. This flocculent material is thought to be equivalent to ML in stereo images of whole cells prepared by critical point drying (Wolosewick & Porter, 1976,1979). In the present study, the network-like materials were also observed in all stages of preimplantation hamster embryos similar to those report in somatic cells by Wolosewick and Porter(1979) and was designated as MN. MN filled up the space and served to connect all organelles of preimplantation embryos. It was estimated that approximately 80% of the cytoplasmic volume comprised intertrabecular space, but this varies with cell type (Wolosewick & Porter, 1979). In addition, ML may measure more than 100 Å thick at their ends where they fused with other trabeculae, MTs and MFs, etc, but only 20-30 Å thick at their midpoint (Wolosewick & Porter, 1979). By contrast, this result showed that in hamster embryonic cells the diameter of individual filament in MN was about 165(at the beads) and 92Å (at the strings), respectively. Furthermore, the structure of MN in hamster embryos was different from ML observed in cultured cells (Wolosewick & Porter, 1976, 1979). This, however may be species as well as cell specific or preparation process. It is believed that this study is the first report of MN in mammalian preimplantation embryo. The beads appearance may represent the highly coiled state of most of MN, and beads of adjacent MN may be cross-linked to form the network.

The roles of MN in embryo still remains in doubt, however, it is tempting to suggest that uneven distribution of ML relative to other structures makes it an integral part of a totally organized cytoplasm. Functionally the cell requires a structural framework for the non-random distribution of organelles and membranous system such as the Golgi complex,LSs,mitochondria and ribosomes which are kept in place during cytoplasmic polarization and organelle zonation. These components of the cytoplasm were not free flowing in the living cell as they would be in an unstructured solution, as was believed earlier by Buckley (1964). Additionally, the MN would appear to provide a suitable system for the cohesiveness of the cell cytoplasm during the distortion or change in shape by highly asymmetric MTs. It is therefore highly possible that the two are integral components that play synergistic roles in controlling cell volume and cell shape during embryogenesis.

4.4 The binding of lectins to embryos and uterine epithelia

4.4.1 The binding of lectins to embryos

There are more than a hundred different monosaccharides found in nature, but only a few occur in membrane glycoproteins and glycolipids. They are mainly glucose, mannose, fucose, galactose, galactosamine, glucosamine and sialic acid (Alberts et al., 1983). The binding of lectins on the surface of preimplantation hamster embryos suggests the presence of certain saccharide residues for which lectins are known to bind specifically. Usually, binding occurs in the terminal nonreducing end of the oligosaccharide moieties of cell surface components, or in case of Con A, in subterminal position as well. In cases of WGA and PNA, binding indicates the presence of specific disaccharide moieties at the nonreducing terminus (Chavez and Enders, 1981; Nicolson, 1974).

Peroxidase visualization utilizing DAB (Graham & Karnovsky, 1966) was first used by Avrameas (1970) and Bernhard and Avrameas (1971) to develop an electron-dense marker system for lectins. Since HRP contains approximately 18% carbohydrate including lectin-accessible α-D mannoside residues, it can bind to the unoccupied valencies of cell surface-bound lectins such as Con A and LCA (Nicolson, 1978). Therefore, in the present study, a two step Con A-peroxidase method was employed to localize α-D mannose or α-D glucose on the plasma membrane of preimplantation embryos and uterine epithelia. In case of WGA and RCA_1 which do not react with HRP (Torpier & Capron, 1980), a one-step lectins-peroxidase method, using WGA-HRP and RCA_1-HRP complexes, was employed to localize N-acetyl glucosamine and/or sialic acid and D-galactose. It had been reported that Con A bound to both zona pellcida (Nicolson et al., 1975; Oikawa, Nicolson & Yanagimachi, 1974) and unfertilized egg plasma membrane (Nicolson et. al., 1975) of hamster, mouse and rat as well as to hamster and mouse preimplantation embryos (Yanagimachi and Nicolson, 1976; Brownell, 1977). Results obtained in this study was consistent to that of Yanagimachi and Nicolson (1976) who found that FITC-Con A bound to the entire surface of hamster unfertilize eggs and embryos, and that the binding was maximum in 1- to 8-cell embryos and decreased in blastocysts. Rowinski et al. (1976) also observed that mouse embryos during early cleavage (Zygote to 8-cell stages) were agglutinable with low concentration of Con A (10 μg/ml),while a high concentration of Con A (100 μg/ml) was required for morula but not for blastocyst. In this study Con A bound to the surface of all stages of preimplantation embryos and was gradually reduced from 2-cell embryo to blastocyst. However these results are in contrast to the studies by Wu and Chang (1978), Carollo and Weitlauf (1979) and Wu (1980), all of which reported significant increase of

Con A binding during the preimplantation development. Con A bound, albeit in varying degree, to the surface of all stages of preimplantation hamster embryo indicated the presence of α-D glucose or mannose on the surface plasma membrane of these embryos. And it appears that Con A receptor sites, or corresponding sugars, may decrease in number as embryo becomes more developed.

Since WGA showed preferential binding for both the dimer of N-acetyl glucosamine and sialic acid (Burger & Goldberg, 1967; Goldstein, Hammarstrom & Sundblad, 1975), the strong intensity of WGA binding on the surface of 1-, 2-, 4-cell embryos and blastocysts in this study suggest that there is an abundance of N-acetyl glucosamine and/or sialic acid on the cell coat of preimplantation hamster embryos. Jenkinson and Searle (1977) reported the loss of sialic acid before implantation of mouse blastocyst. In addition, using FITC-WGA, Yanagimachi and Nicolson (1976) noticed that WGA binding was maximum when eggs attained full maturity, but decreased as the embryo develop through later stages of preimplantation. Nilsson, Lindquist & Ronquist (1973) observed that the negative surface charges on rabbit blastocyst (probably sialic acid or its derivatives) decreased prior to implantation of the embryo to the uterus. By contrast, there was no reduction of WGA binding at later stages of preimplantation hamster embryos in this study, and the binding of WGA to these embryos at the 2-, 4-cell and blastocyst stages was not different, and that the binding was found on the entire surface of these embryos. This result is similar to that of Chavez and Enders (1981), who found no diminution in the binding of WGA on the surface of mouse preimplantation and postimplantation blastocyst.

The present observation of the strong RCA_1 binding on the surface of all stages of preimplantation hamster embryo indicates the

presence of abundant oligosaccharide containing β-D galactose (Nicolson et al., 1974) on the glycocalyx component. Data obtained in this study were in agreement with that of Chavez and Enders (1981) who found that ferritin-conjugated RCA_1 bound heavily and uniformly to the surface of D_5 mouse blastocyst and an apparent diminution of RCA_1 binding was noted on D_6 blastocyst (adhesive stage). By contrast, Yanagimachi and Nicolson (1976), using FITC-RCA_1, had noticed that the binding of RCA_1 decreased during the latter stages of preimplantation hamster embryo. This may due to the different in lectin markers and the limited resolution of flurorescent microscopy (Anderson et al., 1986). The abundance of D-galactose on blastocyst prior to implantation possibly suggest that this oligosaccharide might be involved in the process of implantation. Since incorporation of D-galactose is characteristics of mural trophectoderm in preimplantation mouse embryos (Chavez, Enders & Schlafke, 1984), it was postulated that blastocyst surface receptors for D-galactose may interact with terminal residues of this saccharide in the glycocalyx of endometrial epithelial cells, promoting adhesion of these surfaces (Anderson, et al. 1986). Similarly, Cook, Zalik, Milos & Scott (1979) and Milos and Zalik (1982) have demonstrated that both galactose-containing glycoproteins and endogenous galactose-binding lectins are components of chick extraembryonic blastodermal membranes, and are intimately involved in adhesion among these cells.

An important point to bear in mind from these results and others' is that there are changes of glycoproteins or glycolipids, at least in terms of carbohydrate moieties, during the preimplantation development. The changes in the number of binding sites for lectins could reflect either a change in the total number of sites synthesized,

or the masking of specific sites by addition of other sugars to the oligosaccharide chains. Alternatively, degradation of binding sites by developmentally regulated glycosidases could also account for the observed decrease in lectin receptors (Brownell, 1977). Chavez and Enders (1981) also concluded that the observed changes in the glycocalyx of the trophoblast were parts of the developmental program that resulted in an altered surface exposed to the uterine environment, but not necessarily directly involved with embryonic adherence to the uterine epithelium during implantation.

4.4.2 The binding of lectins to uterine epithelia

Previous studies by Nicolson et al. (1976) and Yanagimachi and Nicolson (1976) indicated that there were receptors of Con A, RCA_1, and WGA on surface of the plasma membrane of hamster embryos. Since implanting embryos enter into a close contact with the maternal endometrial cells, and this contact entails cell to cell recognition and interaction (Schlafke & Enders, 1975), a certain complementarity of the surface oligosaccharides between blastocyst and uterine epithelium could play a role in implantation process. Data on lectins bindings obtained in this study indicated that both embryos and uterine epithelia posses receptors of Con A, WGA and RCA_1 on their surface coats.

Light staining of Con A in D_1-uterine indicated low level of α-D mannose or glucose on the surface coat of D_1-uterine epithelium. The stain increased in D_2- to D_4-uteri. This result is similar to that of Enders and Schlafke (1974) which showed that the binding of Con A-peroxidase to D_5 mouse uterine epithelium (compare to D_4 hamster uterus) was rather thick. This possibly suggests that α-D mannose and/or glucose is gradually synthesized and added to the uterine

surface as pregnancy continue. Similarly, Anderson et al. (1986) reported the glycoprotein extracted from rabbit D_6 uteri could bind to Con A. They also found that α-D mannose or glucose was observed on rabbit uterine epithelium of both estrous and D_6- pseudopregnant animals by using FITC-Con A. Human uterine epithelium of both proliferative and pregnant stages showed FITC-Con A binding (Lee & Damjanov, 1985). Furthermore, FITC-Con A binding was also found in epithelial surface of mouse non-pregnant and pregnant uteri (Lee, We, Wan & Damjanov, 1983). These results suggest that α-D mannose or glucose is a general basic oligosaccharide component of the glycocalyx in mammalian uterine epithelia.

In this study, the patching phenomenon of Con A binding was observed in D_2- and D_4-uteri. It is possible that a change of configuration or the linear translocation of cell surface molecules to form an aggregate "patch" participating in adhesion of implanting embryo (Schlafke & Enders, 1975).

In the present study, the strong staining of WGA in D_1 to D_3-uterine epithelia implied the presence of abundant N-acetyl glycosamine and/or sialic acid on the surface coat during preimplantation period. The high quantity of N-acetyl glucosamine and/or sialic acid was observed on both embryos and uterine epithelia during preimplantation period. Abundance of these sugars in all stages of preimplantation embryos and uteri may also involve in the adhesion and recognition between blastocyst and the uterine epithelium. Anderson et al. (1986) had shown an extensive and uniform binding of FITC-WGA to luminal surface of uteri from estrous and D_6- pseudopregnant rabbits. In addition, no ferritin - sWGA binding was detected on the surface of uterine epithelium from estrous rabbit, while moderate binding

of this lectin was observed in D_7 pseudopregnant uterus. Since sWGA binds specifically to N-acetyl glucosamine, the binding of FITC-WGA in estrous uterine epithelium must due to the presence of sialic acid only. In rat, it was found that there was a reduction of anionic sites from the surface of uterine epithelium of D_2-to D_6- and completely disappeared from D_6 pregnant and pseudopregnant animals (Hewitt, Beer & Grinnell, 1979). This was also true in rabbits (Anderson & Hoffman, 1984). This may suggest the decrease of sialic acid content on the uterine surface since sialic acid is located at terminal residues of glycocalyx and causes negative charge at the surface coat (Jenkinson & Searl, 1977). On the other hand, Anderson et al. (1986) reported an increase in number of sWGA-binding glycoproteins on Western blots of membrane extracted from uteri of 7½ days pregnant rabbit, this suggests the increase of N-acetyl glucosamine during this time. However, in hamster, the abundance of N-acetylglucosamine still remain and possibly is the one that takes part in the adhesion of blastocysts to the uterus. Serious investigations have to be carried out to reveal this phenomenon in the future.

Strong binding of RCA_1 on D_1- to D_3-uterine epithelia indicated the abundance of D-galactose on the surface coat of these tissues. This was similar to the finding of Chavez and Anderson (1985), who demonstrated, using ferritin-conjugated RCA_1, the presence of D-galactose at terminal, nonreducing positions within the glycocalyx of mouse uterine epithelium during preimplantation period. The thickness of this glycocalyx was 0.1 μm. in D_3-uterus and reduced to 0.06-0.08 μm in D_4-uterus. Furthermore, during D_5 this thickness

was greatly reduced. The dramatic reduction of RCA_1 binding on hamster D_4-uterine epithelium suggests a decrease of D-galactose at the time of implantation.

The reduction of this sugar may be taken up by the blastocyst as Chavez et al. (1984) found that trophectoderms of mouse blastocyst could incorporate D-galactose from the uterine epithelium into cellular components. There might be receptors for this sugar at the apical membrane of the trophectoderm cells. Chavez and Anderson (1985) proposed an attractive hypothesis for the adhesion of blastocyst to the epithelial surface that adhesion might be the result of binding of uterine epithelial surface D-galactose to the receptors on the trophectoderm cells. However, Anderson et al. (1986) found that the binding of FITC-RCA_1 to the luminal surface of D_6 pseudopregnant rabbit uteri was strong with unknown reason.

The quantitative changes of D-mannose or D-glucose, N-acetyl glucosamine and/or sialic acid and D-galactose during preimplantation period found in this study perhaps to render the situation conductive to implantation and that the reduction of sialic acid and D-galactose might be essential for the initiation of the adhesion of blastocyst to uterine endometrium.

The mechanism of the changes in luminal glycocalyx morphology during the preimplantation period is not clear. It has been reported to be stimulated by progesterone (Chavez & Anderson, 1985), and in correlation, Murr, Stabenfelt, Bradford and Geschwind (1974) found that mouse serum progesterone level sharply increased from D_1 to D_3. Progestational changes in composition of the glycocalyx of endometrial epithelial cells could be attributed to the synthesis of new membrane glycoprotein and/or differential glycosylation of existing protein

and glycoproteins (Anderson et al., 1986). In addition, endocytotic activity of this epithelium is at its peak on D_4 (Parr & Parr, 1977), and may play a role in luminal glycocalyx alterations in order to wake the uterus receptive to blastocyst implantation.

4.5 Effects of lectins on implantation

Although implantation among groups of animal intrauterine injected with 200-800 μg Con A was not significantly different, more embryos survived in group treated with 200 μg Con A than those treated with 400 and 800 μg Con A. This might due to the effect of Con A on the histophysiology of the endometrium but not on the embryos themselves since embryo from 8-cell up to blastocyst stages preincubated with different concentrations of Con A and transferred to pseudopregnant recipients were able to implant as well as the controls treated with NS (Table 3.10). Wu and Gu (1981) had also found that Con A did not affect the embryonic development when injected intraluminally into pregnant D_4 mice and D_5 rats. The inhibition of implantation could be the effect of Con A on decidual formation, and its subsequent interference with implantation. Furthermore, Gordon and Dandekar (1976) had reported that zona-free rabbit ova, preincubated with Con A and WGA could be fertilized with capacitated sperm and the resulted embryos developed to 2- to 4-cell stages . Although implantation was not completely blocked with Con A, but some implanted embryos could not develop normally. This possibly due either to physical and physiological changes of endometrium (as expressed in edematous condition) and/or the rejection from maternal immune response. Beer & Billingham (1974) and Bernard (1977) suggested that decidualization of the uterine endometrium might be one

factor that help to suppress adverse maternal immune reaction-against otherwise genetically different embryos. As observed in the present study, endometrium of some Con A treated animals failed to decialize normally, instead it became edematous. This condition might be unfavorable for the continuation of development of the already implanted embryo.

In this study, intraluminal injection of all dosages of WGA completely inhibited the implantation. The dose of 50 μg may be the threshold level in which critical number of receptors or the uterine glycoconjugates, that are specific for recognition and adhesion of blastocysts, may be bound, hence implantation is completely block. At a lower dosage (20 μg) a few embryos were able to implant (15.9 % implantation) (Sretarugsa, Sobhon, Bubpaniroj & Yodyingyuad, 1987). It appears that terminal sugars, composing of N-acetyl glycosamine and/or sialic acid, may be the most important determinant for implantation process in hamster. WGA may bind to these terminal sugars on the ZP of embryo and result in configuration changes of glycoconjugates of the ZP such that blastocysts can hardly hatch from ZP, or hatching is delayed to the time not in synchronization with the appropriate microenvironment within the uterus; as the result, blastocysts cannot implant. The present study, supports to the latter action of WGA, it was observed that embryos preincubated with 500 μg WGA upward could not implant in pseudopregnant recipients, while a few embryos preincubated in lower dose could still implant. Furthermore, hamster blastocysts with intact ZP could be recovered at 1300 hr from D_4-uteri of animals injected with 50 μg WGA on D_3 (Sretarugsa et al., 1987). The ZP of these embryos resisted to tryptic digestion (Sretarugsa, unpublished observation). Oikawa,

Yanagimachi & Nicolson (1973) had also found that after incubating hamster eggs with WGA, ZP was very resistant to trypsin or β-mercaptoethanol digestion. They suggested that WGA prevents the ZP matrix from dissolving by cross-linking oligosaccharide chains which are attached to adjacent polypeptides. These results indicate that WGA may not directly toxic to the process of embryonic development because after WGA administrations all blastocysts found on D_4 were basically normal (Sretarugsa et al., 1987).

In control NS-treated group, it was observed that the percent of implantation was reduced significantly when compared to intact group. The large volume of NS intraluminally injected could possibly flush out the blastocysts before implantation took place, hence reduced the number of implantations.

In this study, no significant difference in number of implantation from the transfer of embryos- preincubated with various dosages of Con A and NS was observed. This indicates that Con A is not toxic to the embryos and has nothing to do with their subsequent development. A few implantations from the transfer of embryos preincubated with 200 μg WGA possibly due to reduction of the masked N-acetyl glucosamine and/or sialic acid on the ZP through the washing process, therefore, embryos still liable to hatch and implant later.

Histology of D_4- and D_6-uteri showed no change in the structure of uterine epithelia and stroma layers from WGA-treated group. This suggested that WGA was not toxic to the uterus. In contrast to the treatment of Con A,edema in the stromal layer of endometrium was observed. Moreover, the high dose of Con A (400 μg) also caused vacualization in epithelial cells. Toxicity of Con A on

the endometrium may be on the temporary basis since both edema and vacuolization are reversible and normal histological feature is again observed on D_6. The real mechanism of Con A in causing edematous condition and failure for decidualization in the endometrium is still unknown.

4.6 Conclusions

In these studies the following conclusions have been reached.

I. The surface of early stages preimplantation hamster embryos were characterized by the presence of rod-shaped microvilli which were reduced in length and number during development, whereas the flat microridges progressively increased in number.

The cytoplasm of embryonic cells in 1- to 4-cell embryos exhibited three distinct regions : the outer region having numerous vesicles, a few mitochondria, few LSs and MVB; the middle region containing principally LSs; and the inner region that is characterized by the presence of numerous mitochondria and small vesicles, and Golgi complexes. Polarity of blastomeres began to be observed in the 8-cell embryos. From ultrastructural characteristics it appears that in blastocysts, trophectodermal cells are derived from the outer region of cytoplasm of cells in 1- to 4-cell embryos, while ICM are derived mainly from the middle region of the cytoplasm of cells 1- to 4-cell embryos.

II. With Triton X-100 extraction, four types of cytoskeletons were observed in the cytoplasmic scaffoldings of cells in preimplantation hamster embryos : (i) MFs which were arranged in longitudinal bundles of parallel and straight filaments that lie within the cores of microvilli and underneath the plasma membrane, however they were sparsely

observed in the interior of the cell cytoplasm; (ii) MTs which were distributed in all directions in the cytoplasm and became preferentially arranged in the peripheral cytoplasm in trophectoderm of blastocyst; (iii) IFs which were tightly packed together in principal cortical cytoplasm of 1- and 2-cell embryos; (iv) MNs which appeared as zig-zag fibers each with bead on string appearance; they were also cross-linked and formed major scaffolding of the cytoplasm and served to interconnect other cytoskeleton elements and all cytoplasmic organelles.

The surface oligosaccharides components of glycocalyx of hamster embryos and uterine epithelium during preimplantation period contained substantial amount of α-D mannose and/or α-D glucose, N-acetyl glucosamine and/or sialic acid β-D galactose. During embryogenesis α-D Mannose and/or α-D glucose on the surface decreased, while they increased on the uterine epithelia. N-acetyl glucosamine and/or sialic acid and β-D galactose on the surface of embryos and uterine epithelium were quite abundant. On the surface of embryos these oligosaccharides did not change during development, while they were slightly reduced on uterine epithelium at the time of attachment (D_4). β-D Galactose was abundant on the surface of embryos and uterine epithelium in all preimplantation stages, except on D_4 at which time the dramatic reduction of β-D galactose was observed on uterine epithelium. The abundance of carbohydrates on the surface of embryo, especially D-galactose, N-acetyl glucosamine and sialic acid, might be related to their important role in determining the success of implantation. Furthermore, the fact that increasing number of Golgi Complexes found in the peripheral region indirectly implied that these sugar residues may be added to the membrane via glycosylation in the Golgi complexes and being transported to the surface via membranous vesicles.

III. The importance of carbohydrates in determining the success of implantation was proven by experiments using lectins. It was found that WGA administrated intraluminally on D_3 following conceptus could completely inhibit implantation and did not have any apparent toxic effect towards endometrium structure, while Con A could reduce but not completely inhibit the number of implantation, and substantial portion of the implanted embryos were resorbed. Both uterine epithelium and stroma were affected by Con A, by exhibiting vacuolization in the epithelium and edema in the intercellular space. In supporting experiments, using embryo transfer technique, to confirm the effect of lectin, no implantation in surrogate mothers occurred if the transferred embryos were preincubated with WGA at the level beyond 500 μg/ml, whereas normal rate of implantation was observed if the transferred embryos were preincubated with Con A. WGA may bind to N-acetyl glucosamine or sialic acid, which may be the key oligosaccharides in determining the binding of blastocyst to endometrium, thus it can completely inhibit implantation process. In addition, WGA may bind permanently to ZP and results in the changing of ZP property such that blastocyst cannot hatch.

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