CHAPTER I

INTRODUCTION

Golden hamster (*Cricetus auratus*) is a small rodent which has been widely adopted as a laboratory animal. It has a regular 4-day estrous cycle, and the gestation period is only 16 days (Bruce & Hindle, 1934), which is quite short comparing to the 21-day period of gestation in mice and rats. Therefore, the hamster is the most ideal animal for research on problems of reproduction. Several workers have studied the estrous cycle of the hamster. It has a 4-day duration similar to that observed in the mouse and rat. Deanesly (1938) reported that the cyclic changes were difficult to trace by means of vaginal smears because of a constant process of growth, corification and sloughing in the epithelium of the lower vaginal wall. Yet she stated that an external examination of the daily vaginal discharge of female hamster made every morning can reveal a cyclic, opaque and sticky discharge, referred to as the post-estrus discharge, which could be squeezed out of the vagina by gentle pressure around the vulva every 4 days. This discharge coincides with the ruptured follicular stage in the ovary.

The receptive stage has been found to last 4 or 5 hours after sunset (Hamilton & Samuel, 1956). If a female is placed with a male during this receptive stage, copulation occurs within a few minutes. The approximate ovulation time is between 0100 to 0200 hr of the day post-estrus discharge is found (Grave, 1945; Ward, 1946). After ovulation, the eggs pass through the bursa and infundibulum and enter

the ampulla of the oviduct where fertilization takes place. Embryos remain in the oviduct for approximately 2½ days during which undergo a few cleavage divisions. They enter the uterus between the 4- and 8- cell stages at some time in the afternoon of the third day after ovulation (Graves, 1945; Venable, 1946; Ward, 1948). Development in the uterine environment results in embryos reaching the blastocyst stage. Subsequently the embryos shed their zonae during the fourth day, and implantation take place in the morning of the fifth day after ovulation (Grave, 1945; Wards, 1948; Hamilton & Samuel, 1956) (Table 1.1).

Table 1.1 Development schedule of hamster embryo in vivo

Day of pregnancy	Stage of embryo	Location
1 (0900-1800)	1-cell	oviduct
(1900-)	2-cell	oviduct
2	2-cell	oviduct
3 (-1000)	4-cell	oviduct
(1000-1300)	4-, 8-cell	oviduct/uterus
(1300-1800)	4-, 8-cell	uterus
(1800-1900)	8-cell, morula early blastocyst	uterus
4 (-0900)	blastocyst*	uterus

* Beginning of attachment after 0900 on Day 4

Data from Austin (1955); Orsini (1964); Sato and Yanagimachi (1972); and Yodyingyuad (1982).

Hamster eggs are widely employed for the assessment of fertilizing capability of human spermatozoa (Yanagimachi, Yanagimachi & Rogers, 1976; Binor, Sokoloski & Wolf, 1980). These eggs were routinely obtained from superovulated females. Superovulation in hamsters can be achieved by injection of 20-30 i.u. pregnant mare's serum gonadotrophin (PMSG) followed by the injection of 20-30 i.u. human chorionic gonadotrophin (hCG) 46-72 hrs after the PMSG administration (Sato, 1962; Yanagimachi & Chang, 1964; Ahuja & Tzartos, 1981). Hamster embryos resulted from superovulated eggs have been shown to develop normally to term (Greenwald, 1976; Fleming & Yanagimachi, 1980).

Unlike several other mammalian species such as mouse and rabbit, a study on the continuous development of hamster embryos *in vitro* has not yet been achieved to date. This is probably due to the lack of information on suitable culture medium condition.

1.1 General ultrastructural appearance of the egg

During the preimplantation period the fertilized egg progresses by a series of cleavages to blastocyst stage. There is no net increase in mass during this period, while synthetic processes are activated. A process termed compaction which occurs during the 8-cell stage is marked by flattening as well as increasing adhesion between apposed blastomeres. The blastocyst itself consists of a spherical outer epithelial layer of trophectoderm cells encompassing the fluid-filled blastocoel cavity and a group of internal cells, the inner cell mass (ICM), which is localized at one end of the blastocyst (Johnson & Calarco, 1980a).

Scanning electron microscopic investigations have revealed that the egg and cleavage stage embryo have microvilli on external surface (Calarco, 1975; Calarco & Epstein, 1973). In mouse ovum, the microvilli are present on about 96% of the egg surface, and this region of the egg is referred to as the microvillus area, while the remaining surface is referred to as microvillus-free area (Longo & Chen, 1984 a, b). Phillips and Shalgi (1980) have indicated that morphology of the microvillus-free bulge overlying the second meiotic apparatus varied among individual mouse ova. By contrast, the surface of the hamster egg is characterized by numerous microvilli; a bulge typical of mature mouse ova is not present (Phillips & Shalgi, 1980; Longo, 1975). Microvilli have been proposed as sites of initial contact and fusion with sperm (Austin, 1968; Barres & Herrera, 1977; Kochler, De Curtis & Stenchever, 1982). With advancing developmental stage the surface of the rat embryo during preimplantation period reveals an increase in the surface area and an increase in the complexity of projections (Burgos, Segal & Passantino, 1976). By contrast, the surface of the preimplantation mouse embryo shows a decrease in both number and length of microvilli during its development (Bergstrom & Nilsson, 1974; Shalgi & Sherman, 1979). There has not yet been complete reports on the surface changes of preimplantation hamster embryo from 1-cell to blastocyst stages.

The feature of preimplantation embryo development is mostly studied in mouse. Polar organization of cells develops first early in embryogenesis with the formation of the primary epithelial germ layers and the delamination of extraembryonic epithelia (Maro et al., 1985). The earliest evidence of this process in the mouse embryo has been detected at the 8-cell stage, during which elements of the

cell surface (Handyside, 1980) and endocytotic processing pathways (Reeve, 1981; Fleming & Pickering, 1985) undergo a radial reorganization to convert a non-polar cell to a highly polarized cell over a period of 8-10 hrs (Ziomek & Johnson, 1980), and the polarity is elaborated and stabilized at the 16- and 32-cell stages to generate the formation of polar outside and apolar inside cell populations (Johnson & Ziomek, 1981; Reeve, 1981). The polar and apolar phenotypes thus derived tend to divide to yield trophectoderm and ICM cell types respectively at the blastocyst stage (Ziomek & Johnson, 1982; Ziomek, Johnson & Handyside, 1982).

By contrast there have been limited studies on hamster embryo. In transmission electron microscopic (TEM), investigation the most striking organelle in hamster egg is the presence of lamellar structures (LSs). These lamellae, first described by Enders and Schlafke (1965) as fibrous material, have also been referred to as paracrystalline lattices (Szollosi, 1965), fibrillar ribosome aggregated (Mazanek, 1965), cytoplasmic whorls (Hadek, 1966), cytoplasmic lamellae (Weakley, 1967, 1968; King and Tibbitts, 1977), yolk-plates (Szollosi, 1972), and plaques (Schlafke & Enders, 1967; Tachi, Tachi & Lindner, 1970). It has been postulated that the fibrous material is largely proteins (Enders & Schlafke, 1965; Weakley, 1967), lipoproteins (Szollosi, 1972; Nilsson, 1980), or RNA within a crystalline lattice (Zamboni, 1970; Burkholder, Coming, Okada, 1971; Bachvarova, De Leon & Spiegelman, 1981). In addition, Enders and Schlafke, (1965) reported that LSs disappeared in implanting blastocyst. Hitherto, there is no conclusion on what LSs really are and what role they perform in preimplantation hamster embryos. Another organelle, mitochondria, also have unique appearance. They appear

as oval or round shape with a few cristae (Parkening, 1976).

During cleavage stages, there are changes in cell shape possibly related to increasing cell migration and adhesion (Calarco & Epstein, 1973; Ducibella, Ukena & Karnovsky, 1977). Information concerning intercellular junctions which is related to the above phenomena is available in mouse embryo but not in hamster. The formation of intercellular junctions during mouse development has been described by several investigators using the techniques of TEM and freeze fracture (Enders & Schlafke, 1965; Calarco & Brown, 1969; Enders, 1971; Ducibella & Anderson, 1975; Ducibella, Albertini & Anderson, 1975; Magnuson, Demsey & Stackpole, 1977; Magnuson, Jacobson & Stackpole, 1978). Gap junctions are present between blastomeres beginning at the 8-cell stage and can be found between all cells of morulae and blastocysts, but zonular occludens do not begin to form between presumptive trophectodermal cells until the morula stage, probably establishing the permeability barrier required for blastocoel formation. The point in development at which this barrier is completed may be as early as the morula stage (Magnuson et al., 1978) or as late as the mid to late blastocyst stage (McLaren & Smith,1977).

Because of limited information on the morphology of hamster embryos during preimplantation, TEM has been employed to investigate the general ultrastructural features of hamster embryos during the whole preimplantation period, and the SEM to investigate in detail the surface changes.

1.2 The cytoskeletons

It is likely that the basic cytoskeletal elements in the egg cell are similar to those found in other somatic cells. The differences should be concerned with the relative organization of

each component.

All eucaryotic cells have distinct shapes and high degree of internal organization. Moreover, they are capable of changing their shapes, repositioning their internal organelles, and in many cases, migrating from one place to another. These properties depend on complex networks of protein filaments in the cytoplasm that serve as the "bone and muscle" of the eucaryotic cell, called cell's cytoskeleton (Fawcett, 1986). There are four types of the cytoskeleton : microtubules, microfilaments, intermediate filaments and a microtrabecular lattice.

1.2.1 Microtubules

Microtubules (MTs) are thought to maintain cell shape, by their orientation and distribution in the cytoplasm. They are also capable of sliding relative to one another, thus generating the motive force for ciliary, flagellar, sperm tail movement, and chromosome movement during mitosis (McIntoch, Hepler & Van Wie, 1969). In addition, MTs seem to be involved in certain kinds of intercellular transport phenomena e.g., cyclosis and exonal transport. Finally, astral rays of MTs apparently stimulate the cell cortex to begin cytokinesis in animal cells (Rappaport, 1974). The drug colchicine has served as a useful tool to explore MT-dependent processes because it disrupts intact MTs (Bryan, 1974). In electron microscope, MTs appear as cylindrical pipe-like structures with average 240 to 250 Å in diameter (Fawcett, 1986). Biochemically, MTs consist of molecules of tubulin, a globular polypeptide of 50,000 daltons. They are dimer composed of two polypeptides, α - tubulin and β - tubulin, which have closely related amino acid sequence (Alberts, Bray, Lewis, Raff, Roberts & Watson, 1983).

Electron and immunofluorescence microscopy revealed the existence of MTs in both fertilized (Longo & Anderson, 1969; Yanagimachi & Noda, 1970; Gondos, Bhiralons & Conner, 1972) and unfertilized oocytes during oogenesis (Wassarman & Fujiwara, 1978). MTs are ubiquitous structures in trophoblast and ICM (Paulin, Babinet, Weber & Osborn,1980). In addition, immunofluorescence methods also help to localize MTs in unfertilized oocyte, zygote (Schatten, Simerly & Schatten,1985) and all stages of preimplantation embryos (Paulin, et al. 1980).

1.2.2 Microfilaments

Microfilaments (MFs) are also ubiquitous organelles in all vetebrate cells. They appear in the electron microscope as filamentous structures averaging 40 to 60 Å in diameter. They are circumferentially arranged in the cortex of the cleavage plane in animal cells undergoing cytokinesis (Schroeder, 1968, 1972, 1973) and in bundles associated with the cytoplasmic streaming of the endoplasm in plant cells (Hepler & Palevitz, 1974). In addition, they are present as linear arrays and as networks in the cytoplasm of cells that are changing shapes and moving(Spooner, Yamada & Wessels, 1971; Wessels, Spooner, Ash, Bradley, Luduena, Tayler & Wrenn, 1971; Spooner, 1974, 1975). MFs are thought to generate changes in cell shape and to participate in cell locomotion by virtue of their contractile activity (Baker and Schroeder, 1967; Wessells et al., 1971) via a sliding interaction with myosin (Huxley, 1973; Polland & Weihing, 1974), another major protein present among bundles of MFs. Cytochalasin B presumably interferes with a number of MF-mediated cellular activity. Such interference often correlates with disappearance or morphological alteration of MFs

(Cloney 1972; Schroeder 1970; Spooner, 1974; Wessells et al., 1971).

In sea urchin, it was reported that the surface of unfertilized egg was adorned with short microvilli lacking MFs (Ito, 1969; Eddy, and Shapiro, 1976; Spiegel & Spiegel, 1977; Longo, 1980). At fertilization, microvilli were several folds longer than those of the unfertilized egg (Harris, 1968; Burgess & Schroder, 1977; Begg & Rebhun, 1979; Tilney & Jaffe, 1980). Microvillar elongation was accompanied by the appearance of MF bundles within the newly elongate microvilli, presumably to support and mediate their elongation (Harris, 1968; Burgess & Schroeder, 1977; Begg & Rebhun, 1979; Tilney & Jaffe, 1980). Furthermore, Moon, Nicosia, Olson, Hille & Jeffery (1983) reported that there was a correlation between the activation of protein synthesis and the association of polyribosomes with MFs in sea urchin eggs. In mouse, it has been demonstrated that MF and MT components of the cytoskeleton become localized asymetrically during cell polarization (Ducibella et al., 1977; Johnson and Maro, 1984), and that disruption of MFs (Ducibella & Anderson, 1975; Sutherland & Calarco-Gillam, 1983; Johnson & Maro, 1984), but not of MTs (Ducibella & Anderson, 1975; Ducibella, 1982; Maro & Pickering, 1984), inhibits the intercellar flattening that occurs at compaction. Antibodies to actin show that MFs are ubiquitous structures in both mouse trophoblast and ICM (Lehtonen & Badley, 1980).

1.2.3 Intermediate filaments

Early studies of the filamentous components of the cytoplasm were concentrated on MTs and MFs as these were previously considered to be principal components of the cytoskeleton. Later

it became apparent that there was another major category of filament, 70-110 Å in diameter, present in the cytoplasm of most eukaryotic cells. Because their cross-sectioned dimension fell between that of MTs and MFs, they came to be called intermediate filaments (IFs). Immunological and biochemical techniques allow the different classes of IFs to be distinguished by their specific polypeptides subunits,which are expressed in relation to programs of cell differentiation. At least five compositionally different classes are classified (Bennett, Fellini, Croop, Otto, Ryan & Holtzer, 1978; Franke, Weber, Osborn, Schmid & Freudenstein, 1978; Schonid, Tapscott, Bennet, Croop, Fellini, Holtzer & Franke, 1979): (i) "Cytokeratins" (α - keratins or prekeratin) are often expressed during the formation of desmosomes at the epithelial cell surface, i.e., intercellular junctions to which these cytokeration filaments are specifically attached (Franke et al., 1978; Campbell and Campbell, 1971; Staehelin, 1974; Drochmans, Freudenstein, Wanson, Laurent, Keenan, Stadler, Leloup & Franke, 1978); (ii) "Vimentin" is often distributed in non-epithelial cells, in particular cells of embryonic mesenchyme and their derivatives. This protein is characterized by a polypeptide with molecular weight of approximately 57,000 in mammals (Bennett et al., 1978; Franke et al., 1978; Hynes & Destree, 1978; Lazarides, 1980); (iii) "Desmin" or "skeletin" has been found in muscle cells, i.e., cross-striated myocardial and various smooth muscle cell types (Bennett et al., 1978; Lazarioles and Balzer, 1978; Schmid et al., 1979; Lazarides, 1980; Renner, Franke, Schmid, Geisler, Weber & Mandelkow, 1981), and certain vascular smooth muscle cells (Gabbiani, Schmid, Winter, Chaponnier, De Chastonay, Van de Kerckhove, Weber & Franke, 1981;

Frank and Warren, 1981); (iv) "Neurofilaments" seem to be confined to neural cells (Bennett et al., 1978; Schachner, Smith & Schoonmaker, 1978; Liem, Yen, Solomon & Shelanski, 1978; Schmid et al., 1979; Franke et al., 1980; Yen & Field, 1981), although it is not clear whether all neuronal cells express these filament proteins; (v) "Glial filaments" contain only one major, probably exclusive, protein of an apparent molecular weight in the 50,000 - 55,000 range which has been described in astrocytes where it is often expressed relatively late in embryogenesis (Yen and Field, 1981; Liem et al., 1978; Rueger, Huston, Dahl & Bignami, 1979). The biological function of IFs found in epithelial cells is not known. It is suggested that these filaments may provide a mechanical framework contributing to intracellular stability and intercellular adhesion within the specific epithelium and may also serve in the stabilization of the orientated arrangements of contractile elements present in specific cells (Jackson, Grund, Schmid, Burki, Franke & Illmensee, 1980; Franke et al., 1980; Lazarides, 1980).

It has been suggested that the earliest preimplantation stage embryos lack IFs, and that IFs, reactive with antibodies to bovine hoof keratin, would appear concomitantly with cellular differentiation in the outer cells of late morulae, or in the trophectoderm cells of blastocysts (Jackson et al., 1980). In addition, the synthesis of two extraembryonic endodermal cytoskeleton proteins (Endo B, Mr = 50,000; Endo A, Mr = 55,000) is detected by immunoprecipitation at the 4- to 8-cell stages of preimplantation mouse development. It is confirmed that Endo B is an authentic IF protein. Endo B antibodies have also been shown to decorate trophoblast cytoskeleton (Oshima, Howe, Klier & Adamson, 1983). By

using indirect immunofluorescence microscopy, it was found that antibodies against prekeratin interacted with the outer trophoblastic cells but not the ICM in mouse blastocysts. Interestingly, vimentin filaments typical of mesenchymal cells as well as of cell growing in culture seem to be absent in both cell types of the blastocyst. In contrast to Lehtonen, Lehto, Vartio, Badley and Virtanen (1983), it is reported, by using immunoblotting and fluorescent techniques, that cytokeratin-like polypeptides are present in mouse oocytes and preimplantation embryos. Electron microscopy observations show that these early stages embryos also contain detergent-resistant 100-110 Å filaments (Lehtonen and Badley, 1980 ; Lehtonen et al., 1983).

Cytokeratins have also been identified in epithelial cells of amphibian (Franke et al., 1979). In addition, vitellogenic oocytes and eggs of the frog (*Xenopus laevis*) contain intermediate-size filaments that are resistant to extractions in high-salt buffers and Triton X-100 and are specifically stained with antibodies to cytokeratins (Franz, Gall, Williams, Picheral & Franke, 1983). Godsave, Anderson, Heasman & Wylie (1984) have reported that oocytes of *Xenopus laevis* contain IF from early previtellogenic stages to the fully grown state.

1.2.4 Microtrabecular lattice (ML)

There are different opinions about ML (Sheeler & Bianchi, 1987), the cytoplasm or ground substance of eukaryotic cell divided into the microtrabecular lattice (ML) and the intercellular spaces as observed in high-voltage EM. The lattice is not apparent in conventional thin sections because the sections lack the depth necessary to reveal the network. The microtrabeculae are rich in protein, whereas the intertrabecular space is aqueous and serves to

dissolve or suspend the great variety of small molecules involved in cellular metabolism. Among the specific proteins that are thought to be present are actin, myosin and tubulin. These proteins are also major constituents of MFs and MTs. A basic difference in the composition of microtrabeculae and the cytoskeletal elements is revealed when both are treated with organic detergents such as Triton X-100. Cells treated with Triton X-100 lose the ML along with membranous structures, but they retain their cytoskeleton. This behavior suggests that microtrabeculae have certain physical properties that are similar to those of membranes but unlike those of the cytoskeletal elements. In the present study, ML is grouped in the cytoskeletal components as described by Fawcett (1986).

When thinly spread cells in tissue culture are fixed, dried by the critical point method, and examined with the high-voltage electron microscope, a three-dimensional lattice of slender strands (100 - 120 Å) is seen throughout the ground substance of the cytoplasm. This provocative observation has led to the suggestion that the cytoplasmic matrix is not a homogeneous protein solution, but a gel with a highly structured "microtrabecular lattice" (ML) forming its solid phase, and linking together the other filamentous components and organelles into a single structural and functional unit called the "cytoplast". The molecular structure and composition of the ML have not been worked out in any detail and its existence in the living cell has not yet been accorded general acceptance (Fawcett, 1980). It is now clear that the microtrabeculae correspond well to the thin interconnecting strands in the freez fractured, and that all cells contain them (Kondo, 1985). Those who

accept its existence attribute the control of cell shape and cell movement to the integrated functioning of the cytoskeletal elements and the ML, and speculated that enzymes incorporated in ML may be spatially coordinated in such a way as to favor their sequential interactions with their substrates rather than relying on diffusion and random collision (Fawcett, 1986). So far there is no report about ML in mammalian oocytes or embryos.

From these previous reports about the cytoskeletons, it is shown that the knowledge of cytoskeletons in hamster embryos is still lacking. Therefore the present study is carried out to investigate the organization of cytoskeletons in these embryos by using selective extraction with non-ionic detergent, Triton X-100, as described for somatic cells by Small and Langanger (1981), and the organization of the residual cytoskeleton examined by TEM.

1.3 Cell surface or surface coats

The cell surface is known to participate directly in several developmental processes, including cell recognition, adhesion, communication, and motility (Moscona, 1974; Poste & Nicolson, 1976). Reactions at the cell surface also affect development indirectly by inducing intracellular changes in metabolism, the cell cycle, and gene expression (Johnson & Calarco, 1980a). These intracellular changes, in turn, can alter the composition and/or organization of surface components. Sequences of such reciprocal interactions mediated by the cell surface probably function to guide embryonic cells along specific pathways of differentiation and development (Johnson & Calarco, 1980a).

Previous studies of the surface coats of the mouse blastocyst using either whole mounts or extraction techniques. Holmes

and Dickson (1973) used Prussian Blue staining whole mounts of mouse blastocysts in an attempt to demonstrate surface coats. Since the blastocyst did not stain heavily with this method until after the estrogen surge, they concluded that there was a change in the amount or functional activity of the surface glycoproteins of the blastocyst at this stage. Pinsker and Mintz (1973) exposed preimplantation mouse embryos *in vitro* to trypsin after previous incubation with radioactive glucosamine. Since the cleavage embryos and blastocysts were still viable after exposure to trypsin, they concluded that anything removed by the trypsin was from the surface. The trypsinate thus removed showed an increase in incorporation of glucosamine and its derivatives, and also an increase in molecular weight of the polysaccharide units in morula and early blastocysts as compare to 2- to 4-cell stages. Although these authors suggest that this change may be related to the ability of trophoblast cells to attach to the uterine wall and become invasive, many other interpretations are possible. Surface proteins of preimplantation mouse embryos labelled by lactoperoxidase-catalyzed iodination and analyzed by SDS-polyacrylamide gel electrophoresis do not show this enrichment in higher molecular weight forms (Johnson & Calarco, 1980b). The greatest qualitative changes in surface protein pattern, in fact, occur subsequently to fertilization and concomitant with blastulation. The electrophoretic patterns of surface proteins of 2-cell to morula stages are very similar (Johnson & Calarco, 1980b).

In addition, charged surface groups on preimplantation mammalian embryos have been examined histochemically in several species. Cooper and Bedford (1971) have shown an increase in negatively-charged residues on the rabbit egg surface following fertilization. Analysis following neuraminidase treatment and KOH saponification suggest that

the negative surface charges are due to carboxyl residues of sialic acid. However, the hamster plasma membrane shows no apparent change in the density or distribution of negatively-charged residues following fertilization (Yanagimachi, Nicolson, Noda & Fujimoto, 1973). A decrease in surface negative charge was reported at the blastocyst stage in the mouse, associated with implantation (Jenkinson & Searle, 1977; Nilsson, Lindquist & Ronquist, 1973, 1975). The surface charge may be due to protein-bound sialic acid residues, since the charge can be reduced by treatment with neuraminidase, pronase, or trypsin (Holmes & Dickson, 1973; Jenkinson & Searle, 1977). In contrast, Enders and Schlafke (1974) have shown an increase in negative charges on the mouse blastocyst and uterine epithelium on day 4 and 5 of normal pregnancy, and on day 7 of delayed implantation. Consistent with the results in mice, the rat blastocyst behaves as a negatively-charged component on electrophoresis (Clemetson, Kim, Mallikerjuneswara & Wilds, 1970).

Of the current hypothesis regarding cell-to-cell interactions, many investigators consider that the complex carbohydrate moieties of the glycoproteins, glycolipids and glycosaminoglycans located on the cell surface may be involved in cell recognition and adhesion. It would be of interest to examine some of the carbohydrates which are normally composed of cell surface of the embryo prior to implantation. Since carbohydrate groups on the cell surface can be revealed by means of some well-known substances-lectins, investigations were made in the present study to find out the changes in surface carbohydrates of preimplantation hamster embryo in concurrent with uterine epithelium during preimplantation period by TEM, using three types of lectins as probes (Con A, WGA, RCA_1).

1.4 Lectins

Lectins are proteins or glycoproteins that can bind noncovalently to specific carbohydrate groups without modifying them chemically. Binding is reversible and all lectins have more than one specific carbohydrate-binding site. The presence of more than one carbohydrate binding site allows individual lectin molecules to serve as cross-linking agents (Brown and Hunt, 1978). The classical use of lectins is in cell agglutination; and agglutinability has been found to vary with different antigenic types, states of infection, growth, cell cycle, embryonic development, and oncogenic transformation, etc., (Makela, 1957; Lis & Sharon, 1973; Nicolson, 1974). Quantitative lectin binding using isotopic labelling has been successfully used to measure the number of lectin receptors. In addition, fluorescent lectins have been utilized for low-resolution lectin receptor localization at the level of light microscopy.

Many lectins possess important biological properties. Lectins can affect cell transport system (Isselbacher, 1972; Czech & Lynn, 1973), mimic hormone action (Cuatrecasas & Tell, 1973; Czech & Lynn, 1973), stimulate mitosis (Moller, 1970; Powell & Leon, 1970; Janossy & Greaves, 1971; Anderson, Sjoberg, & Moller, 1972) as well as other processes (Nicolson, 1974). They have been found to inhibit cell growth (Dent, 1971; Inbar, Huet, Oseroff, Ben-Bassat & Sach, 1973; Ralph & Nakoinz, 1973), movement (Friberg, Golub, Lilliehook & Cochran, 1972), phagocytosis (Berlin, 1972), delayed hypersensitivity (Leon & Schwartz, 1969) and allograft rejection (Markowitz, Person, Gitnick & Ritto, 1969).

The most suitable lectins that can be used as labels for electron microscopy are listed in Table 1.2. (Brown & Hunt, 1978).

Table 1.2 Some lectins suitable for use in electron microscopy

Source	Common name or abbreviation	Carbohydrate specificities
1. *Canavalia ensiformis* (Jack bean)	Concanavalin A (Con A)	α-D-Mannose, α-D-Glucose
2. *Dolichos biflorus* (horse gram)	Dolichos biflorus (DBA)	α-D-N-Acetyl-galactosamine
3. *Arachis hypogaea*	Peanut agglutinin (PNA)	β-D-Galactose-D-N-Acetylgalactosamine
4. *Phaseolus vulgaris*	Phaseolus vulgaris (PHA-E, PHA-L)	D-N-Acetylgalactosamine
5. *Glycine max* (soy bean)	Soybean agglutinin (SBA)	α-D-N-Acetyl-galactosamine
6. *Lens culinaris* (lentil)	Lens culinaris agglutinin (LCA)	α-D-Mannose, α-D-Glucose
7. *Ricinus communis* (castor bean)	Ricinus communis agglutinin :	
	RCA_1	β-D-Galactose
	RCA_2	D-Galactose, D-N-Acetylgalactosamine
8. *Triticum vulgaris* (wheat germ)	Wheat germ agglutinin (WGA)	β-D-N-Acetylglucosamine, sialic acid
9. *Ulex europaeus*	Ulex europaeus agglutinin (UEA_1)	L-Fucose

Concanavalin A (Con A) from jack bean, has been most widely used in cell surface labelling studies. It has an approximate Mr of 102,000 dalton and specific binding for α-D-glucopyranose and α-D-mannopyranose sugars (Poretz & Goldstein, 1970). Binding of Con A to cell surfaces is effectively inhibited or reversed by addition of α-methyl mannoside in the concentration range of 0.01 - 0.1 M.

Wheat germ agglutinin (WGA) isolated from wheat germ lipase (Burger, 1969; Le Vine, Kaplan & Greenaway, 1972) has a Mr of 34,000 dalton and contains two carbohydrate binding sites specific for N-acetyl glucosamine and sialic acid (Burger & Goldberg, 1967; Uhlenbruck, Pardoe & Bird, 1968).

Ricinus communis agglutinins (RCA) derived from castor beans have been used as probes for the study of galactose-containing glycoproteins on cell surfaces. Two galactose-specific proteins have been isolated by Nicolson, Blaustein & Etzler (1974). The major agglutinin designated as RCA_{120} (RCA_1) is a tetramer of Mr of 120,000 dalton consisting of two sets of different glycopolypeptides of approximate Mr of 29,000 and 36,000 dalton (Nicolson, Laborbier & Hunter, 1975a). It has strong agglutinating activity against human erythrocytes. The second agglutinin designated as RCA_{60} (RCA_2) has a Mr of 60,000 dalton. This lectin species only weakly agglutinates erythrocytes (Nicolson & Blaustein, 1972).

In addition to their mitogenic and agglutinating properties, some lectins are found to be extremely toxic to animal cells. For example, it has long been known that seeds of the caster bean plant, *Ricinus communis*, are poisonous. This toxicity results from a lectin called ricin, and there has been considerable debate concerning

whether this toxin is RCA_2, which is isolated from castor beans by affinity chromatography (Nicolson & Blaustein, 1972; Kornfeld, Eiden & Gregory, 1974). Both ricin and RCA_2 bind to cell surface carbohydrates and to Sepharose (Tomita, Kurokawa, Onozaki, Ichiki, Osawa & Ukita, 1972; Kornfeld et al., 1974). Like ricin, RCA_2 is extremely toxic, and the toxicity of both RCA_2 and ricin is blocked by lactose. Ricin and a similar toxic protein called abrin, which can be isolated from the seeds of *Abrus pecatorius* (Olsnes, Saltvedt & Pihl, 1974), inhibit protein biosynthesis (Kornfeld et al., 1974; Onozaki, Tomita, Sakurai & Ukita, 1972).

Various ultrastructural markers have been used to identify lectins bound to their target sites. Some of these markers are covalently linked to lectins, while others are bound to target-attached lectins by virtue of the fact that they possess the appropriate carbohydrate sequences for lectin binding. Examples of the electron markers attached to lectins are hemocyanin, ferritin and horseradish peroxidase (HRP).

Concanavalin A has two active sites both of which can react with sugars or glycoproteins which contain non-reducing residues at the terminal branch (Goldstein, So, Young & Callies, 1969). However, Con A is fixed to the sugar on a cell surface only at one of its two active sites (see diagram on page 21). The free active site can then operate as an acceptor of another sugar secondarily added to the system. HRP which is a glycoprotein containing 18% carbohydrates can be used for this purpose. The catalytic activity of the peroxidase molecule can finally be revealed by the diaminobenzidine (DAB) method of Graham and Karnovsky (1966). The reaction can be inhibited by addition an excess of a sugar (e.g.

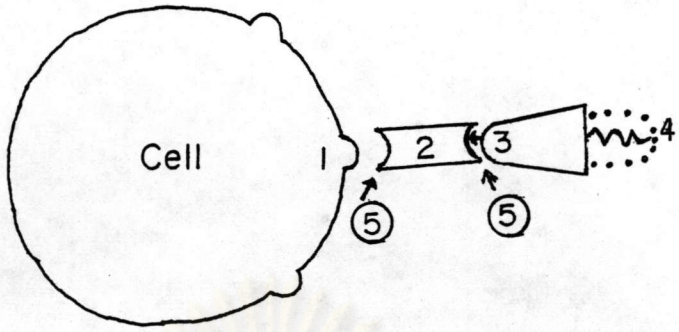

Diagram Mechanism of the Con A-peroxidase reaction. 1 = Cell with carbohydrate group; 2 = Con A; 3 = Peroxidase; 4 = Electron-dense reaction product after DAB; 5 = competitive sugar for control.

α-methyl-D-mannoside 0.1 to 0.2 M) which competes with the enzyme and the sugar present in the cell for the reactive group of Con A. However, this technique is limited since HRP can react only with Con A, but not with other lectins such as WGA and RCA_1.

1.4.1 Interaction of lectins with eggs and embryos

Eggs and embryonic cells of several organisms resemble erythrocytes and transformed cells in that they are strongly agglutinated by lectins. Fertilized eggs are frequently more agglutinable than unfertilized eggs but, as the embryo develops, it generally becomes less agglutinable. The molecular mechanism by which agglutinability is gained and lost during development has yet to be elucidated. It is reasonable to suppose that these changes may resemble those that take place after neoplastic transformation of normal cells. Since, transformed and embryonic cells have many properties in common, including a high rate of growth, the ability to migrate *in vivo*, and ability to invade surrounding tissues (Brown

Hunt, 1978).

Studies on the agglutination of chick embryonic cells in response to various lectins change significantly during embryonic development. At very early stages after fertilization, both Con A and WGA agglutinate chick embryonic cells. WGA agglutination is then lost rapidly,however, and 8-days after culture, chick embryonic liver or neural retina cells are agglutinated only by Con A or RCA_1 (Brow & Hunt, 1978).

Similar to chick embryonic cells, rodent embryos also exhibit changes in lectin agglutinability during development. After fertilization the eggs are strongly agglutinated (Pienkowski, 1974). The difference in agglutinability of the fertilized and unfertilized eggs may result from a difference in receptor distribution. With the use of fluorescent Con A, it has been demonstrated that lectin binds to both fertilized and unfertilized mouse eggs, but that the pattern of fluorescence is different. The lectin bound only to some parts of the unfertilized egg surface while in fertilized eggs, it bound uniformly over the whole surface (Johnson, Eager & Muggleton-Harris, 1975). As in chick embryos, agglutination is reduced as the embryo develops, so that by the blastocyst stage, high concentrations of Con A fail to agglutinate mouse embryo cells (Rewinski, Solter & Koprowski, 1976).

In hamster eggs, the change in lectin agglutinability after fertilization is not as pronounced as in the mouse. Fertilized zona pellucida-free eggs are only slightly more agglutinated by Con A, lentil (*Lens culinaris*) lectin, and WGA than unfertilized eggs. RCA_1 and D biflorus agglutinin (DBA) agglutinate the zona pellucida of both fertilized and unfertilized eggs, and binding of fluorescent lectins to the zona pellucida does not change during development.

In zona-free eggs the bindings of fluorescent Con A, RCA_1 and WGA decreases as the eggs develops(Yanagimachi & Nicolson, 1976).

Electron microscope analysis of unfertilized hamster, rat and mouse eggs after incubation with ferritin-labelled lectins has shown that there are receptors of RCA_1, Con A and WGA in the zona pellucida and the underlying plasma membrane. RCA_1 and WGA receptors are distributed asymmetrically throughout the zona pellucida, with the highest concentrations at the surface. Con A receptors are located sparsely throughout this layer (Nicolson, Yanagimachi & Yanagimachi, 1975b). Receptors of the three lectins in the plasma membrane are randomly distributed in fixed cells or in cells that have been incubated with the lectin at 4°C However, Con A and WGA receptors form clusters at 25° (Nicolson et al., 1975b).

1.4.2 Interaction of lectins with endometria

A common feature in the development of all eutherian mammals is the attachment of trophectoderm of the blastocyst to the luminal epithelium of uterus. The acquisition of adhesion between the two cell types is presumed to be an intrinsic property of the trophectoderm cells because they readily adhere to extrauterine sites (Kirby, 1965). The uterus is not always receptive to preimplantation stage embryos (McLaren & Michie, 1956; Finn, 1977; Psychoyos & Casimiri, 1980), and it has been assumed that there are specific properties of a gravid uterus that make it hospitable to the presence of an implantation-stage blastocyst, and receptive to the adhering trophectoderm. Receptivity of the uterus to preimplantation embryos seems to be cyclically modulated by steroid hormones; receptivity is followed by a period of nonreceptivity. Cellular and molecular mechanisms that underlie the trophectoderm-epithelial interaction remain

to be elucidated. Possible interactions between the surface coats of the trophoblast and uterine luminal epithelium have been speculated as there is discernable glycocalyx present on the luminal surface of the uterine epithelium (Enders & Schlafke, 1974; Schlafke & Enders, 1975) and on the trophectoderm (Pinsker & Mintz, 1973; Enders & Schlafke, 1974) of the mouse at the time of implanation. Enders and Schlafke (1974) noted that the surface coat of the uterine epithelium became much thinner at the time of blastocyst adhesion in the mouse. Quantitative and qualitative changes have also been noted to occur in the rabbit during pregnancy (Anderson,1982; Anderson & Hoffman, 1984; Anderson, Olson & Hoffman, 1986). In this regard, Wu, Wan and Damjanov (1983) reported that the surface coats of uterine epithelium in pregnant and non-pregnant female mice were different in *Bandeiraea simplicifolia* (BSA-I) lectin binding and strong BSA-I reactivity was noted in luminal epithelium of pregnant uterus while in non-pregnant uterus the reactivity was not observed. In addition, in human endometrium, Yen, Lee, Salzmann and Damjanov (1986) reported that there were receptors for succinyl ConA(s Con A), WGA, RCA_1, RCA_2, PNA, PHA-E and PHA-L. Lee, Wu, Wan and Damjanov (1983) treated various portions of the pregnant and non-pregnant murine oviducts and uteri with 20 fluorescein isothiocyanate (FITC) - labelled lectins. They found that five lectins (RCA_1, WGA, UEA-I, Maclura pornifer (MPA) and Banhinia purpurea (BPA) reacted differentially with the epithelium of pregnant as compared with the non-pregnant uterus. The reactivity of the remaining 15 lectins did not distinguish the pregnant and non-pregnant oviducts and uteri. In addition, Lee and Damjanov (1985) have studied the binding of 22 FITC-conjugated lectins to human proliferative phase and pregnant endometrium. They found that only the BPA lectin reacts exclusively with the epithelial cells. All

the others react to a certain extent with glandular and/or stromal cells. It is shown that FITC labelled lectins define specific carbohydrate moieties selectively expressed on either proliferative phase or pregnant endometrium. However, studies that correlate the concurrent changes in glycocalyx and lectin binding activities on the surface of uterine epithelium and fertilized eggs and embryo moving to adhere to that surface are still lacking.

1.4.3 The effect of lectin on implantation

It has been proposed that the contact between the blastocyst and endometrium requires specific change and specific glycoprotein components (Clemetson et al., 1972; Schlafke & Enders, 1975). If glycoproteins are changed, removed or bound, it may prevent implantation (Pinsker & Mintz, 1973). An important characteristic of lectins is the ability to bind with the specific carbohydrates on the glycoprotein, therefore some lectins may be used to inhibit implantation. Indeed, it has been reported that intrauterine injection of Con A during preimplantation in mice (Hicks & Guzman-Gonzalez, 1979; Wu & Gu, 1978) and rats (Wu & Gu, 1981) caused the reduction in implantation. Despite these reports, the mechanism to which implantation is prevented is still lacking and the effect of lectins on implantation needed to be studied further.

1.4.4 Embryo Transfer

Embryo transfer was first successfully performed in the last century (Heape, 1891) but the technique attracted relatively little attention prior to 1950. However, during the last decade, progress in embryo transfer has become increasingly dependent upon technical advances in a variety of ancillary fields. Nowadays, this technical know-how has been improved to a large extent. The first embryo

transfer was performed in rabbit (Heape, 1891) and it was later on applied to farm animals (Dziuk, 1969; Betteridge,1977; Murray, 1978), primates (Kraemer, Moore & Kramen, 1976; Marston, Penn & Sivelle, 1977) and human (Steptoe & Edwards, 1978; Edward, Steptoe & Purdy, 1980).

Many exciting opportunities have been created by the development of techniques associated with embryo transfer for example, storage of embryos for a prolong period by freezing and the production of identical offsprings and chimaeras. The possibility of obtaining increasing number of offsprings from selected farm animals has attracted many breeders, and the possibility of being able to specify the sex of the offspring has excited attention even further. Embryo transfer technique has been employed to solve a wide variety of problems in the literature.

Previous studies on embryo transfer in hamsters was designed to investigate various aspects of reproduction physiology. These include the effects of aging of donors and recipients on the development of transferred embryos (Blaha, 1964), the role of ovarian hormones in implantation (Orsini & Psychoyos, 1965), the effect of synchronous transplantation(Sato & Yanagimachi, 1972), the developmental potential of embryos derived from eggs fertilized *in vitro* and *in vivo* (Whittingham & Bavister, 1974) and testing the ability of cultured embryos to continue normal development (Yodyingyuad, 1982; Bavister, Leibfried & Lieberman, 1983).

In the present study, embryo transfer technique was employed to study the effect of lectins on embryos prior to transfer to pseudopregnant recipients in comparison with the *in utero* injection of lectins into the uterine cavity of pregnant hamsters.

1.5 Objectives

The purposes of this thesis consists of 2 aspects :

1. The investigation of ultrastructure of preimplantation hamster embryos which are composed of 4 aspects, namely :

1.1 The characterization of the surface of embryo by SEM.

1.2 The characterization of ultrastructure of embryo by TEM.

1.3 The characterization of cytoskeleton organization in embryo by TEM using conventional method and selective extraction with Triton X-100.

1.4 Identification and localization of some specific surface carbohydrate residues on the surfaces of hamster embryos and uterine epithelium during preimplantation period by using lectin probes that consist of Con A, WGA and RCA_1.

2. The investigation of the effects of lectins on implantation. The effects of Con A and WGA were studied by following methods (whereas RCA_1 was not employed because of its toxicity to cells):

2.1 The rate of implantation was determined following intraluminal administration of lectins.

2.2 The success of implantation was determined following incubation of embryos with lectins and transferring the treated embryos to surrogate mothers.

2.3 The effects of lectins on uterine morphology was observed.