

# A morphological study on the reproductive organs as a possible cause of developmental abnormalities in diabetic NOD mice

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**Summary.** The reproductive organs in non-obese diabetic (NOD) mice were histopathologically studied, in order to elucidate the relationships between developmental abnormalities, such as diminished rates of implantation and viable embryos, and structural changes in the reproductive organs. NOD mice with (NOD-DM) and without (NOD-N) diabetes mellitus and ICR mice were compared. The severity of histopathological changes in the pancreas and in the liver were used as parameters which indicated the severity of diabetes itself and of the secondary metabolic disorder. NOD-DM mice exhibited uterine weight loss, accumulation of lipids in luminal and glandular epithelium, atrophies of the endometrium and myometrium and a decrease in the number of muscle cell layers. They also showed a high concentration of lipid droplets in ovarian granulosa cells, atretic follicles and atrophy and lack of lipids in ovarian stroma cells. The severity of these structural changes in the reproductive organs corresponded to those of the changes in the pancreas and the liver. The structural alterations in the ovary suggested disorder in oocyte maturation. The structural changes in the uterus appeared to be related to the decrease in the ratios of implantation and of viable embryos at post-implantation stage. The present studies suggest that the impaired structural environment together with the metabolic environment caused the abnormal development seen, for example, in the oocyte maturation, and at the implantation and post-implantation stage of diabetic mice. It also caused alterations in their hormonal environment.

**Key words:** NOD mouse, Uterus, Oviduct, Ovary, Histopathology

## Introduction

The diabetic condition is well recognized to have

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deleterious effects on pregnancy (Shipley and Danley, 1947; Koller, 1953; Driscoll, 1960; Kyle, 1963; Pedersen et al., 1964). Since preconceptional diabetic control was tried, mortality has greatly decreased in the infants of diabetic mothers (Pedersen et al., 1974; Drury et al., 1977; Mills et al., 1979; Mills, 1982; Tchobrowsky, 1983; Lavin et al., 1983; Fuhrmann et al., 1984; Mølsted Pedersen and Pedersen, 1985; Omori et al., 1986; Goldman et al., 1986; Nelson, 1986; Jensen et al., 1986). The frequency of congenital abnormalities, however, does not appear to have declined (Steel et al., 1982; Smorenberg-Schoorl and Heringa, 1983; Ballard et al., 1984). The reasons for the increased rate of these congenital anomalies could be to do with environmental factors such as hyperglycemia and hyperketonemia (Takano and Nishimura, 1967; Sadler, 1980; Shambaugh et al., 1984; Horton et al., 1985), and could also be because of genetic predisposition (Stanley et al., 1985; Eriksson et al., 1987; Eriksson, 1988). Furthermore many studies have been done using spontaneous (Funaki and Mikamo, 1983; Garris and Smith, 1983; Garris et al., 1985) and drug-induced diabetic animals (Lawrence and Contopoulos, 1960; Endo, 1966; Eriksson et al., 1980), but to date, the causal mechanism of congenital anomalies is in fact unclear.

We have investigated the causal mechanism of abnormalities in diabetic pregnancies using non-obese diabetic (NOD) and ICR mice (Otani et al., 1987; Tatewaki et al., 1987, 1988). The results suggest that environment, including structural abnormalities in reproductive organs, influenced the development of diabetic mice, although the effects of genetic predisposition were also considered. Morphological changes in the uterus and the ovary of diabetic mice were observed in our preliminary study. Therefore it is necessary to check in detail the existence of diabetes-associated alterations in uterine structure in relation to development.

There are some morphological studies on the reproductive organs in diabetic Chinese hamsters (Garris et al., 1982, 1984; Garris and Smith, 1983; Garris,

1984) and in the C57BL/KsJ mouse (Garris, 1985; Garris et al., 1985, 1986). No systematic study describing the relationships between developmental abnormalities, such as diminished rates of implantation and viable embryos, and structural changes in the reproductive organs in diabetic NOD mice has been reported. The present paper investigates these relative changes in the uterus, the oviduct and the ovary on the metabolic disorder of diabetes mellitus in this species.

## Materials and methods

### 1. Animals

The NOD mice used in this study were originally obtained from Shionogi Research Laboratory and have been maintained in the Institute of Experimental Animals in Shimane Medical University. Air temperature and humidity in the animal room were kept at  $24 \pm 2^\circ\text{C}$  and 60 to 70%, respectively, and the room was artificially illuminated from 8AM to 8PM. NOD mice were tested for urinary glucose content using Glucose-prettest (Wako, Japan) weekly from 10 weeks of age onward, female NOD mice with (NOD-DM) and without (NOD-N) diabetes mellitus being confirmed. Furthermore ICR (Slc:ICR) mice were used for comparison with NOD mice; we could not use Jcl/ICR mice, from which the NOD mice were derived (Makino et al., 1980), because the supply ceased during the present study. Incidences of spontaneous external malformations are not significantly different between Jcl:ICR and Slc:ICR mice (Morita et al., 1987). The ICR mice were purchased and kept for at least 2 weeks under the experimental conditions described above.

Age- and sexual cycle-matched NOD-DM, NOD-N and ICR mice from 10 to 30 weeks of age were used in these studies. Blood glucose level in diabetic mice was 180-550 mg/dl. The progress of the diabetic condition, however, was estimated individually by the severity of histopathological changes in the pancreas, the liver and the kidney. The animals were classified by age and by duration of diabetes; groups with duration of one week (NOD-DM(1D)), two weeks (NOD-DM(2D)) and long duration (NOD-DM(LD)), were compared with NOD-N and ICR mice.

### 2. Tissue collections and preparation

Following anesthetization with about 70mg/kg pentobarbital sodium (Nembutal, Abbott), some of the animals were perfused intracardially with a cold mixture of 2% glutaraldehyde (GLA) and 2% paraformaldehyde (PFA) in a 0.1M cacodylate buffer, pH 7.3, for 20 min. The liver, pancreas, kidney and reproductive organs (the uterus, ovary and oviduct) were removed and were fixed in the same solution for transmission electron microscopy (TEM) and in formaldehyde neutral buffer solution (pH 7.4) for light microscopic analysis. In the other animals, pieces of the tissues were immersed and fixed in formaldehyde neutral buffer solution.

After embedding in paraffin using conventional techniques, sections of 5 $\mu\text{m}$  thickness of these organs were obtained and were stained with hematoxylin-eosin staining for light microscopic analysis. Serial sections of the ovary were identically stained with hematoxylin-eosin staining and were studied morphometrically. Several sections of the uterus, oviduct and ovary were stained with azan staining in order to examine the existence of fibrosis in these organs. Changes in islet  $\beta$ -cells were checked using aldehyde-fuchsin staining.

Furthermore the uterus and ovary, for TEM analysis, were treated with propylene oxide, and were embedded in epoxy resin. Thin sections were examined under a JEM 1200 EX (JEOL, 80 kv) electron microscope.

The uterus and oviduct were examined by scanning electron microscopy (SEM) with regard to the surface uterine epithelium. These organs were sufficiently fixed in a mixture of 2% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer, postfixed with 1%  $\text{OsO}_4$  and stained with 2% tannic acid. After dehydration, the lumen were exposed, coated with gold and observed with a Hitachi S-540 (15kv or 20kv) scanning electron microscope.

### 3. Morphometric analysis of ovarian follicles and uterine myometrium

Each ovary sectioned serially was quantitatively analyzed. Two criteria (i.e., diameter size and granulosa layer number) were used for the analysis of follicular classification (Table 1). Only follicles having a nucleus of oocytes were counted, so as to avoid duplication in the counting procedure. A degenerating ovum and lipid accumulation in granulosa cell layers were both used as criteria for denoting atretic follicles. Half of the ovary was sectioned and analyzed systematically to determine follicular classifications. All data were shown by the respective percentages of the total number of follicles per size (diameter). Intergroup differences were compared by statistical analysis (multiple t-test).

In order to study the progress of atrophy in the myometrium, the numbers of the uterine smooth muscle (circular muscle) layer were counted at four typical places in a transverse section from inner to outer muscle fibers. These were analyzed using Wilcoxon's-rank test.

## Results

Histopathological studies of the uterus, the ovary and the oviduct were carried out and compared with the changes in the pancreas as a parameter of diabetes itself, and in the liver and the kidney as parameters of secondary metabolic disorder.

### 1. Histopathological studies of the pancreas, the liver and the kidney

#### 1. The pancreas

The morphological changes of islet  $\beta$  cells appeared to

be connected with the duration of diabetes mellitus in NOD mice. The process of lymphocyte infiltration and damage in pancreatic islets of NOD-N, NOD-DM mice progressed as described by Fujita et al. (1982), Fujita and Yui (1986) and Asamoto et al. (1986).

On the pancreas of NOD mice, no particular change in acinar tissue, ducts, blood vessels, lymphatics and nerves, except for islets of Langerhans, were recognized by light microscopic studies. Prior to the onset of diabetes mellitus, lymphocyte infiltrations were already observed in and around the pancreatic islets and in the neighboring connective tissue, blood vessels and lymphatic vessels in NOD-N mice, although these were not seen in ICR mice (Figs. 1a,b). The infiltrating lymphocytes gradually increased in number and more islets became involved in this lesion (Table 2). In NOD-DM(1D) mice severe lymphocyte infiltrations were observed in a number of islets. After destruction of  $\beta$  cells, infiltrating lymphocytes disappeared and they were replaced by fibrous connective tissue, therefore smaller, atrophic islets were observed in NOD-DM (2D) mice (Fig. 1c). NOD-DM(LD) mice had many reduced islets.

Aldehyde-fuchsin staining showed that  $\beta$  cells were damaged by lymphocyte infiltrations and that they gradually decreased in NOD-N mice (Fig. 1d). More destruction of  $\beta$  cells was observed in NOD-DM, and the rates of severely destroyed  $\beta$  cells were 91.7% in NOD-DM (2D) and 95.7% in NOD-DM (1D) and 95.7% in NOD-DM (LD) as shown in Table 2. These percentages in NOD-DM (1D), NOD-DM (2D) and NOD-DM (LD) were more than those of lymphocyte infiltration, and they showed that lymphocyte infiltration disappeared from islets where  $\beta$  cells had been destroyed.

## 2. The liver

As for the liver of diabetic mice, there were vacuoles in liver cells stained by hematoxylin-eosin (Fig. 2a). From observation by fat staining using osmium tetroxide, accumulations of fat in these vacuoles were not found, and it was recognized to be a vacuolar degeneration (Fig. 2b). The longer the duration of diabetes mellitus became, the more severe the change in the liver cells appeared: a large vacuolar degeneration was confirmed in the liver cells of NOD-DM (2D) mice, compared with a small degeneration in NOD-DM (1D) mice. In the other tissues of the liver, such as capillary, bile duct, central vein and Glisson's sheath, there was no pathological change in NOD or ICR mice.

## 3. The kidney

No change in the kidney in NOD-DM (1D), NOD-DM (2D), NOD-N and ICR mice was observed, although a slight increase in the parietal layer of Bowman's capsule was found in NOD-DM (LD). It seems to be related to the long duration of diabetes mellitus.

## II. Histopathological studies of the reproductive organs

### 1. The uterus

In the uterus of estrus NOD-DM mice the following changes were found: significant decreases in the numbers of coiled artery and uterine gland, decline in the proliferative growth of the endometrium and a reduced column in the luminal and glandular epithelium. While these alterations in NOD-DM (1D) mice were mild, those in NOD-DM (2D) mice were very marked. It was confirmed that the uterus of NOD-DM mice had shifted from the histological pattern in estrus to that in diestrus, in proportion to the progress of the diabetic condition.

#### a. Macroscopic changes

There was a large difference in size between the uterus of NOD-DM (2D) and that of NOD-N mice. The uterus of NOD-DM (2D) mice was lower in weight than that of NOD-N mice. The uterus of lower weight was found in NOD-DM mice with severely damaged pancreas and liver. Transverse sections of uterus in NOD-DM (2D) mice were smaller than those of NOD-N mice in proportion to weight of the uterus and of the body.

#### b. Histopathological changes

##### 1) Uterine epithelium

Vacuoles in the uterine epithelium of NOD-DM mice were found on observation by hematoxylin-eosin staining. It was confirmed by fat staining that the change was a fatty degeneration. In ICR and NOD-N mice, the luminal and glandular epithelium showed a similar layer of columnar epithelium, and each cell had little lipid accumulation in the cytoplasm (Figs. 3a, b). In contrast, the luminal and glandular epithelium of match-paired NOD-DM mice appeared as lower columns than NOD-N or ICR mice, and exhibited an obvious accumulation of cytoplasmic lipid. The lipid was located at the basal pole of the epithelial cells, and increased temporally with the lesion of diabetes mellitus: NOD-DM (1D), which has little degeneration of islet  $\beta$  cells and liver cells, showed a low accumulation of the lipid (Fig. 3c), and NOD-DM (2D), in which islet  $\beta$  cells and liver cells were severely destroyed, exhibited a high accumulation of the lipid in the epithelium (Fig. 3d). The cytoplasmic lipids were also confirmed from the TEM observation, and many lipid deposits were located at the basal pole of the epithelium in NOD-DM (2D) and NOD-DM (1D) mice (Fig. 4). Many abundant accumulations were found especially in NOD-DM (2D). In spite of the accumulation of cytoplasmic lipid, the luminal surface of the epithelium showed no change on the SEM observation.

##### 2) Endometrium

The endometrium was atrophic and the coiled arteries decreased in NOD-DM (2D) mice, having a high

accumulation of cytoplasmic lipid. However, in NOD-DM (1D) mice these changes were almost never observed. No fibrosis was found in the endometrium of NOD-DM (2D) mice from observation by azan staining.

### 3) Myometrium

Myometrium in ICR mice was larger than that of NOD mice from observation by light microscopy. Alteration of myometrium in NOD-DM (1D), NOD-N and ICR mice was almost never found (Figs. 5a-c), whereas in NOD-DM (2D) mice it was observed as atrophy (Fig. 5d). But no fibrosis in the atrophic myometrium was found by azan staining. In comparison with NOD-N mice, the numbers of circular muscle layers in NOD-DM (2D) were markedly fewer, although those of NOD-DM (1D) showed no change (Fig. 6). In ultrastructural observation of smooth muscle of NOD-DM (2D) mice, lipid deposits were found.

### 2. The oviduct

In the histopathological study of the oviduct, there

was no change in the diabetic conditions in these animals. Results observed by SEM also showed no alteration in the surface of the oviductal epithelium in NOD mice.

### 3. The ovary

#### a. Macroscopic changes

The ovary of NOD-DM (2D) mice was smaller than that of non-diabetic ones, and also weighed less, compared with that in NOD-DM (1D).

#### b. Histological changes

The degeneration of the ovary was milder in NOD-DM (1D) mice than in NOD-DM (2D), and seemed to be associated with the progress of the diabetic condition.

#### 1) Follicles

The histological analysis of the ovary revealed that large differences existed between diabetic and non-diabetic animals. The follicles in diabetic mice exhibited

**Table 1.** Classification of ovarian follicles in NOD and ICR mice.

Follicle Class	Follicle size ( $\mu$ m)	Number of granulosa layers	Comment
Primary	100 ~ 200	1 ~ 3	No antrum
Early secondary	200 ~ 300	> 4	Early antrum formation
Advanced secondary	300 ~ 450	4 ~ 7	Continued antrum formation
Tertiary	> 450	Multiple	Large antrum present
Atretic	None	None	Prominent pycnotic nuclei within granulosa cell layers

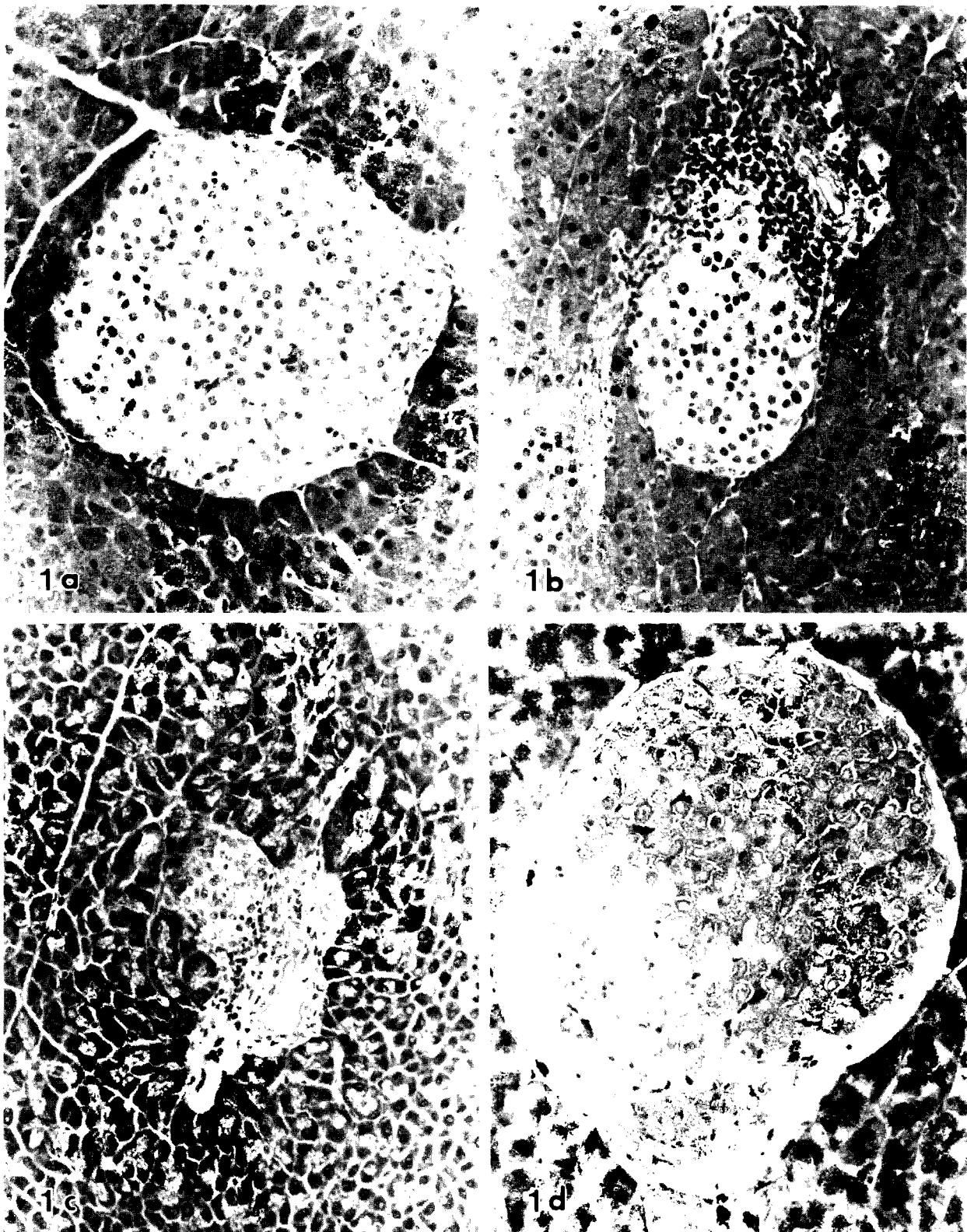
**Table 2.** Incidences of lymphocyte infiltration and islet  $\beta$ -cell destruction in NOD and ICR mice.

Group	Lymphocyte infiltration**				Destroyed islet $\beta$ -cells***		
	Intact(%)	Mild(%)	Moderate(%)	Severe(%)	Intact(%)	Moderate(%)	Severe(%)
ICR	100.0	0	0	0	100.0	0	0
NOD-N	60.0	13.9	12.5	13.9	65.2	26.1	8.7
NOD-DM (1D)*	27.8	25.0	11.1	36.1	3.6	14.3	82.1
NOD-DM (2D)*	7.9	15.8	5.3	71.1	0	8.3	91.7
NOD-DM (LD)*	16.7	16.7	3.3	63.3	0	4.4	95.7

\* 1D, 2D and LD: See the item of materials and methods.

\*\* Intact: An islet has no lymphocytes attached. Mild: Lymphocytes attached on the side of islet. Moderate: Half of islet is eclipsed by lymphocytes. Severe: The majority of islets are infiltrated by lymphocytes.

\*\*\* Intact: All  $\beta$ -cell is intact. Moderate: Half of islet  $\beta$ -cells are destroyed. Severe: The majority of islets are destroyed and reduced.



**Fig. 1.** Pancreatic islets in ICR, NOD-N, NOD-DM (1D) and NOD-DM (2D) mice.  $\times 530$ . **a.** An intact islet in an ICR mouse by hematoxylin-eosin staining (H.E. staining). **b.** An islet surrounded by lymphocytes in NOD-N mice (H.E. staining). **c.** An islet reduced after lymphocyte infiltration in NOD-DM (2D) mice. (H.E. staining). **d.** Islet  $\beta$ -cells destroyed by lymphocytes in NOD-N mice (aldehyde-fuchsin staining).

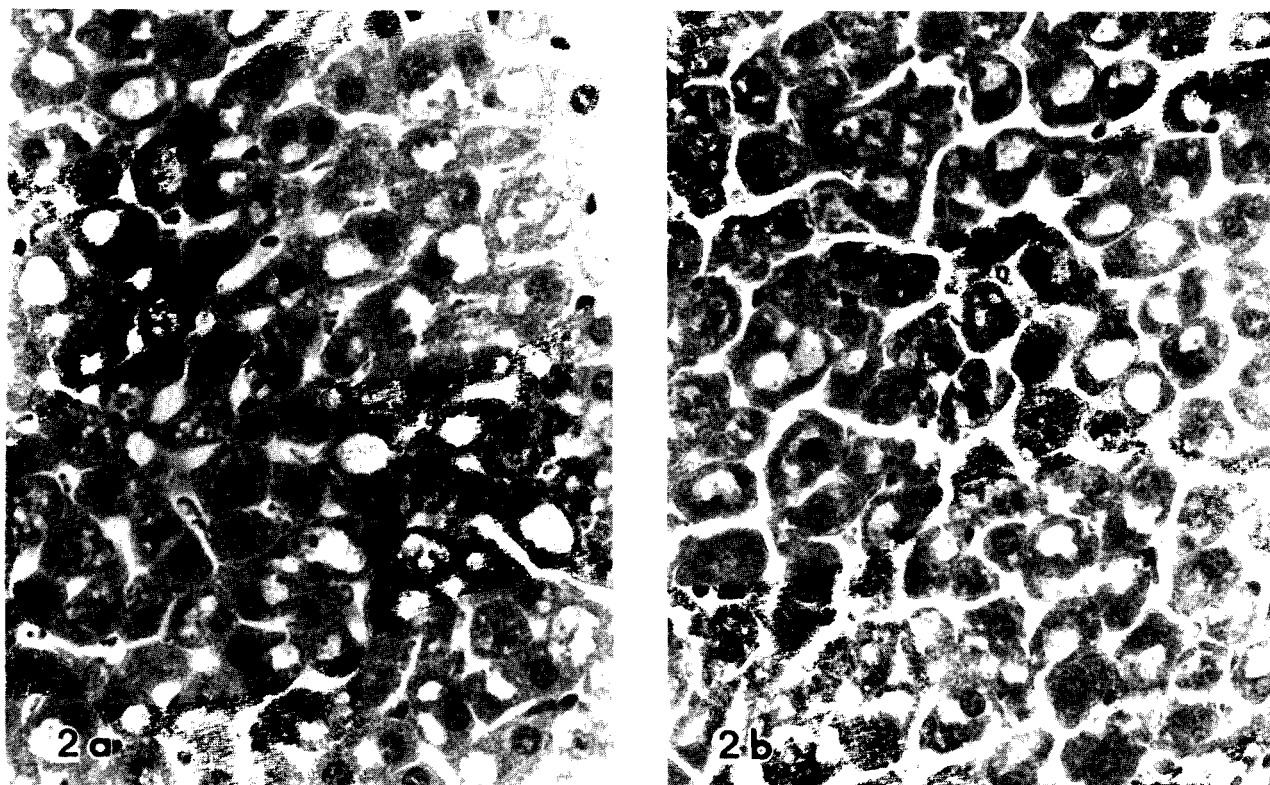
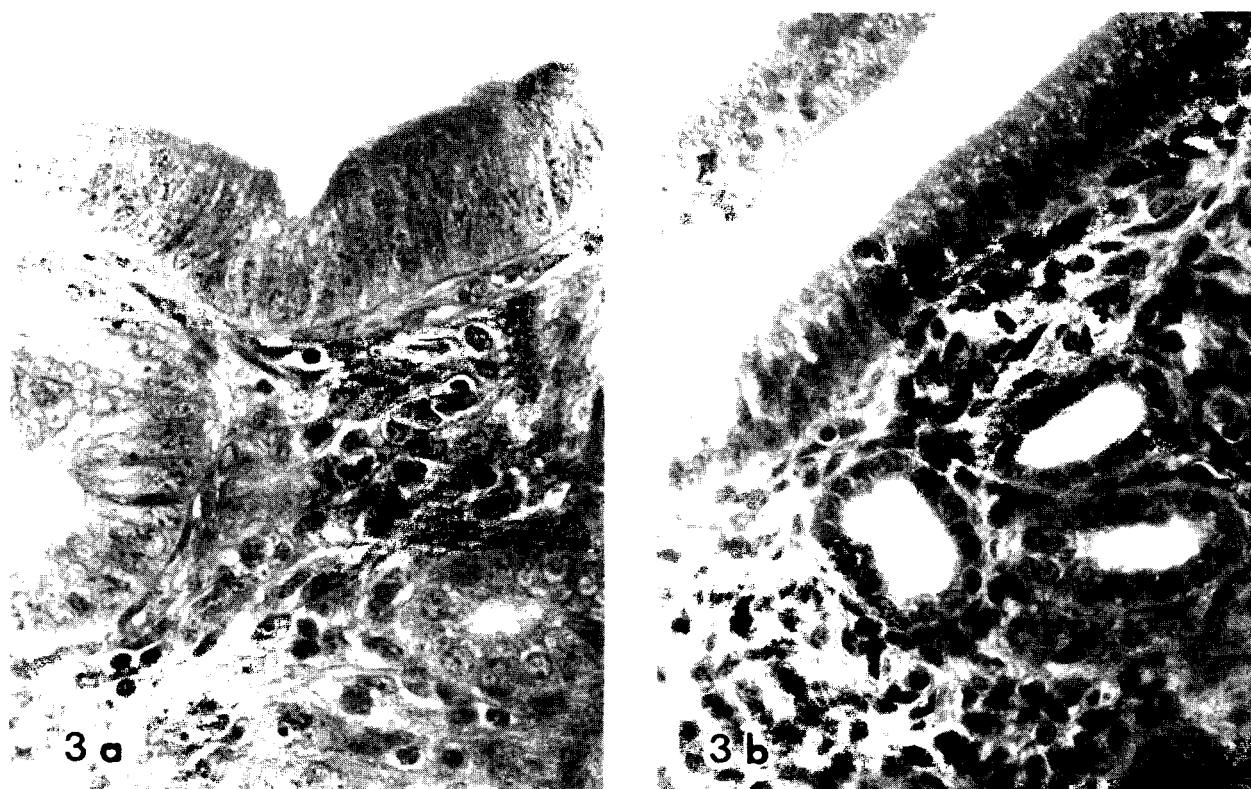
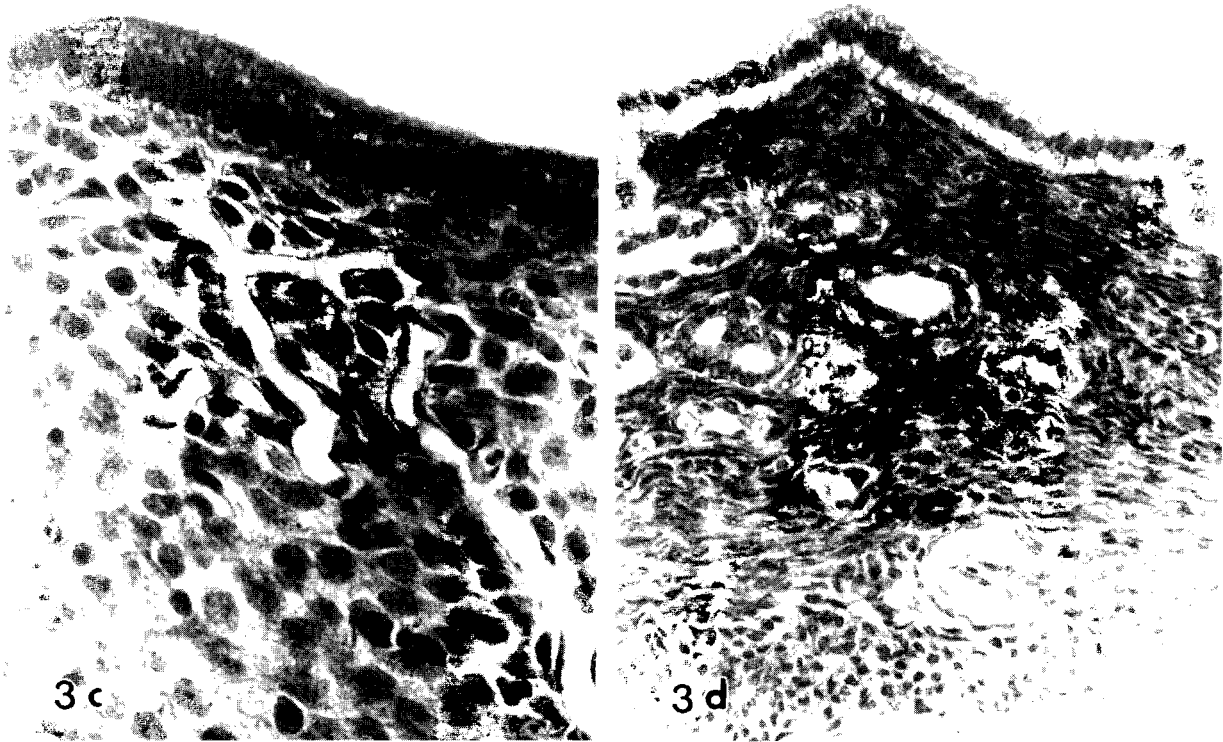
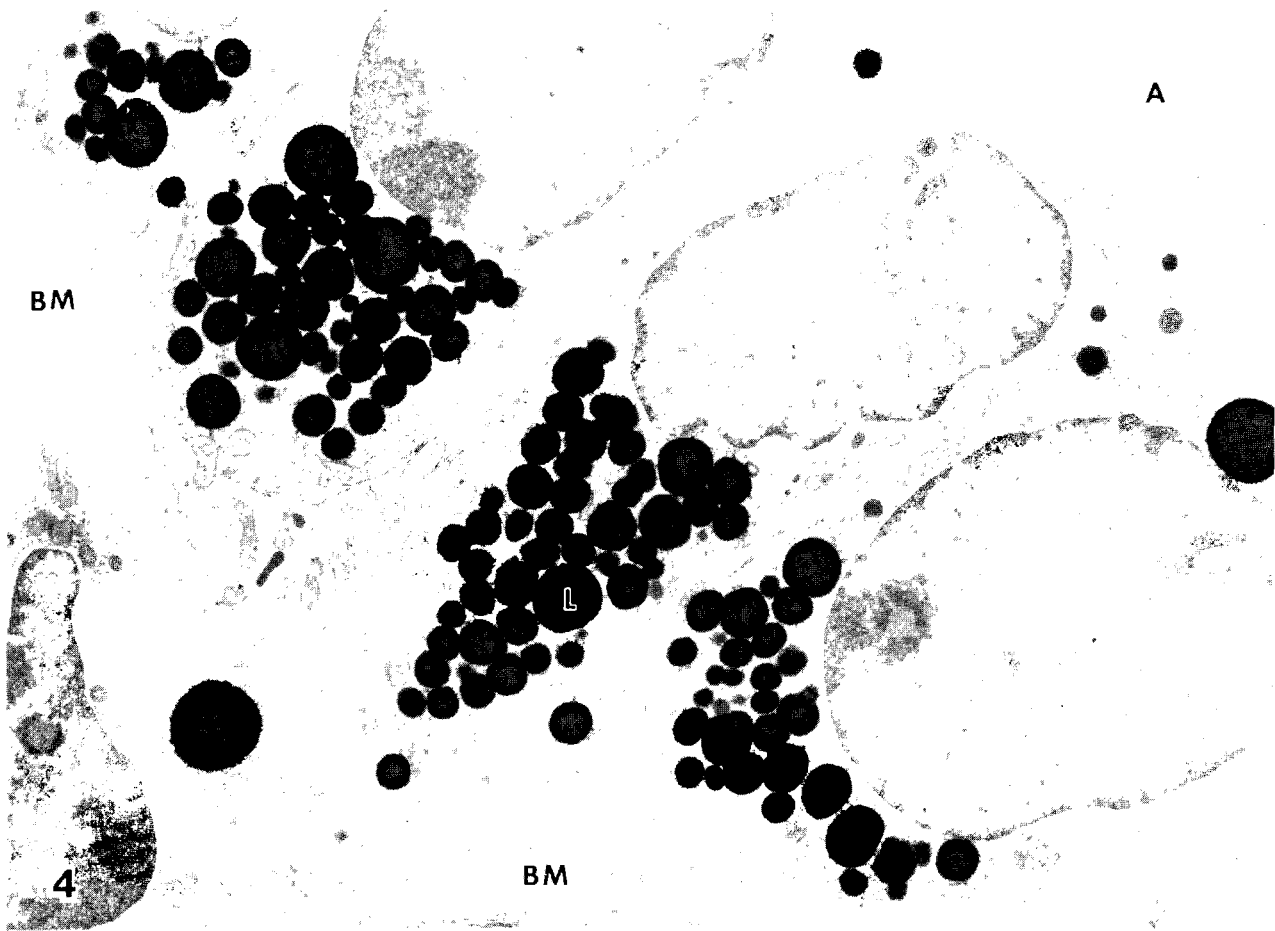


Fig. 2. A vacuolar degeneration of liver cells in NOD-DM (2D) mice.  $\times 530$ . a. H.E. staining. b. Fat staining.

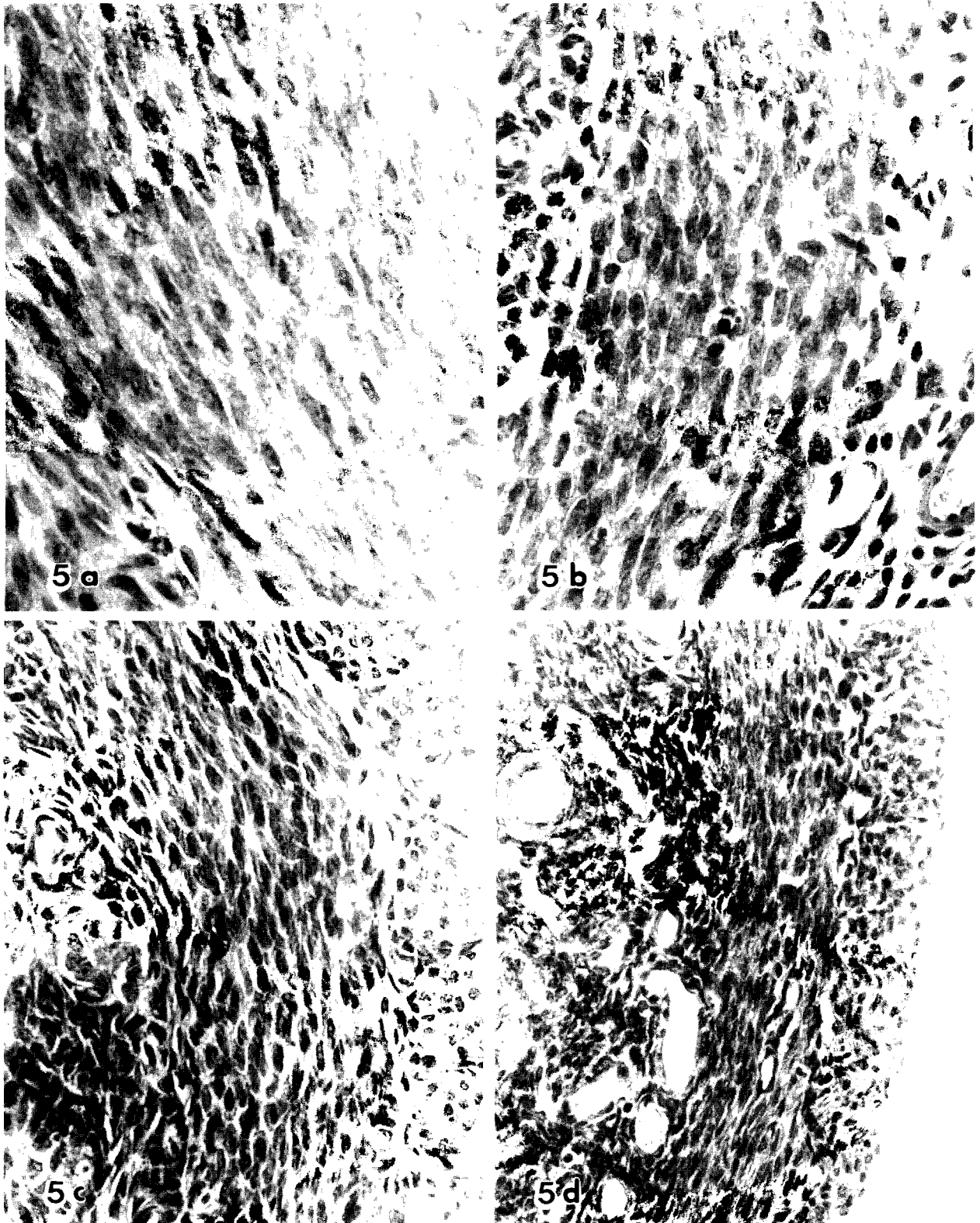




**Fig. 3.** The luminal and glandular epithelium and endometrium in ICR(a), NOD-N (b), NOD-DM (1D) (c) and NOD-DM (2D) mice (d).  $\times 530$ , H.E. staining. **a-b.** There is no abnormal lipid accumulation in the epithelium, and no change in the endometrium is observed. **c.** The epithelium shows a lower concentration of lipids, but the endometrium shows no change. **d.** The epithelium exhibits a high accumulation of lipids, and the endometrium is clearly reduced.



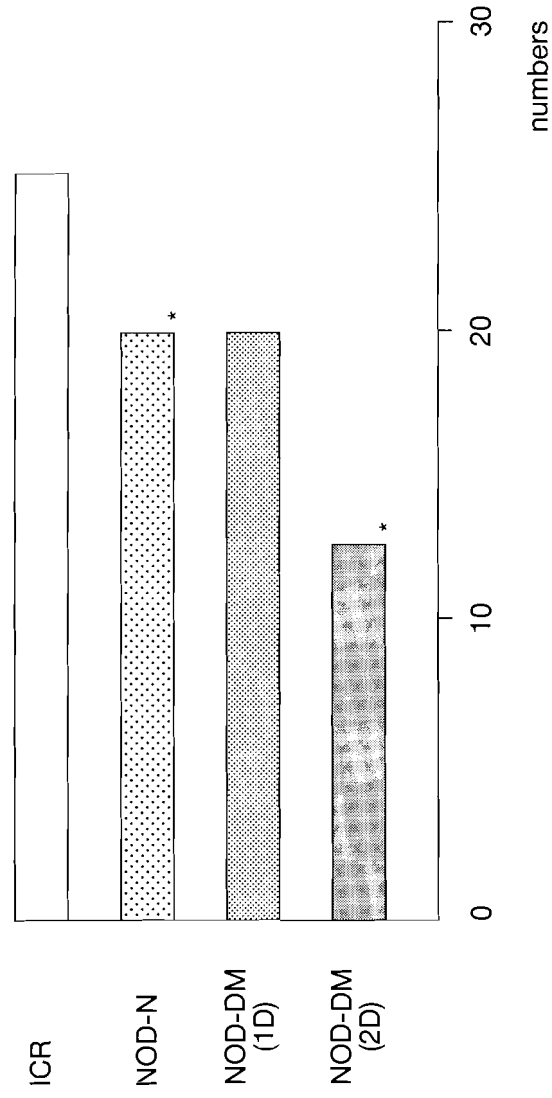
**Fig. 4.** TEM view of the diabetic uterine epithelium showing large amounts of lipid deposit in the basal pole. BM: basement membrane, L: lipid, A: apical side.  $\times 8,700$



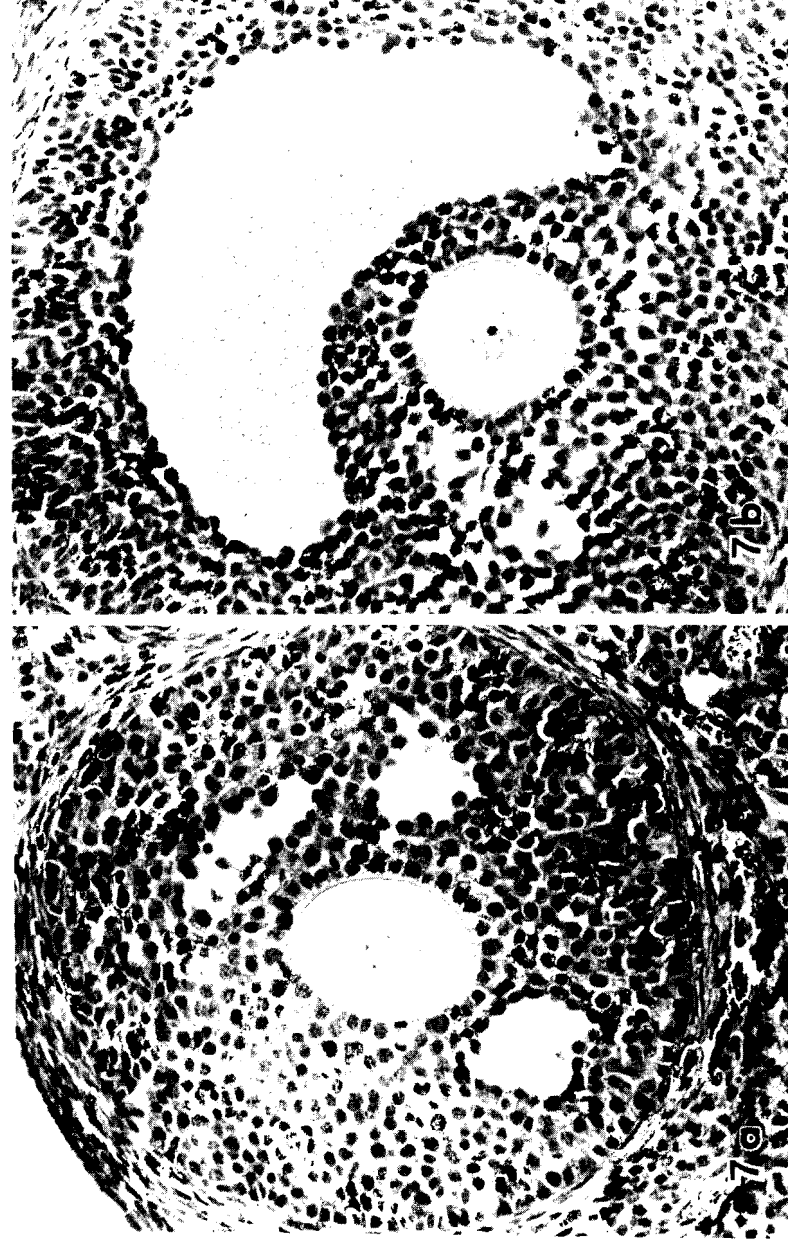
**Fig. 5.** The myometrium in ICR (a), NOD-N (b), NOD-DM(1D) (c) and NOD-DM (2D) mice (d).  $\times 530$ , H.E. staining. **a-b.** No change in ICR and NOD-N mice is observed. **c.** Change in NOD-DM (1D) mice is hardly ever found. **d.** Atrophy is observed in NOD-DM (2D) mice.

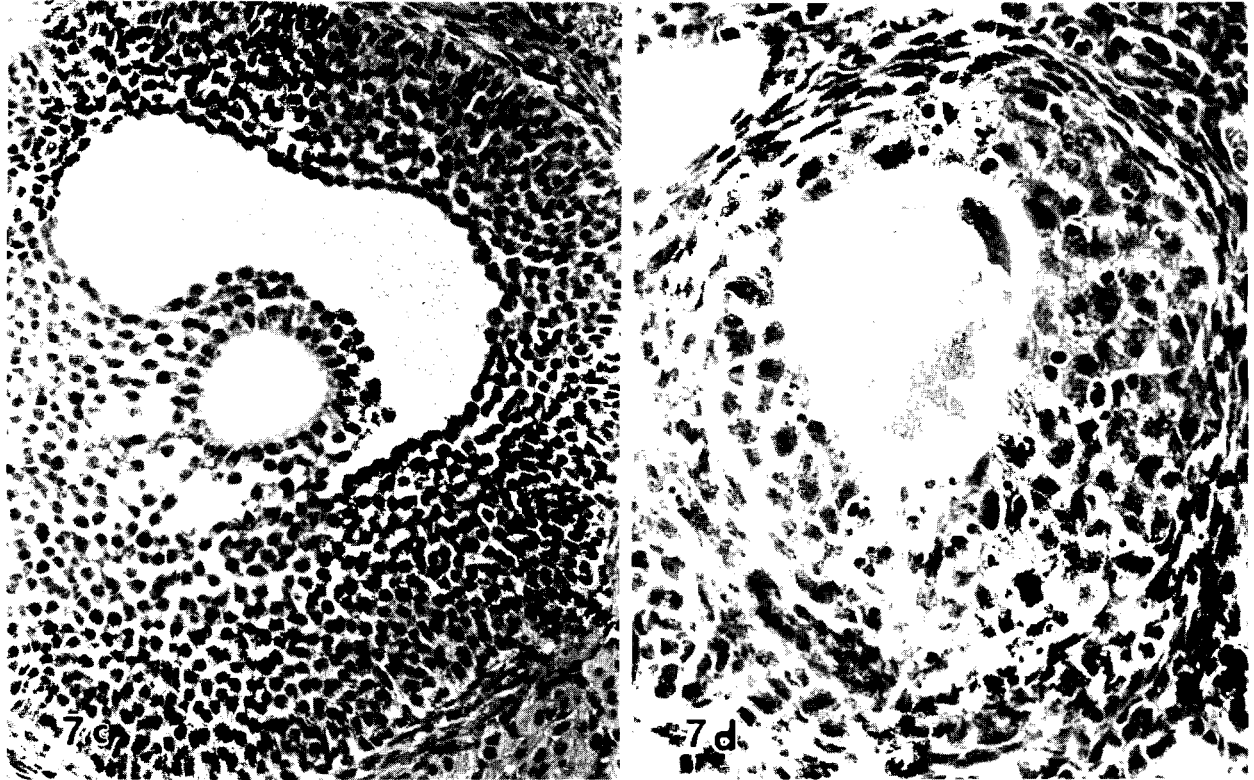


*Change of diabetic reproductive organ*

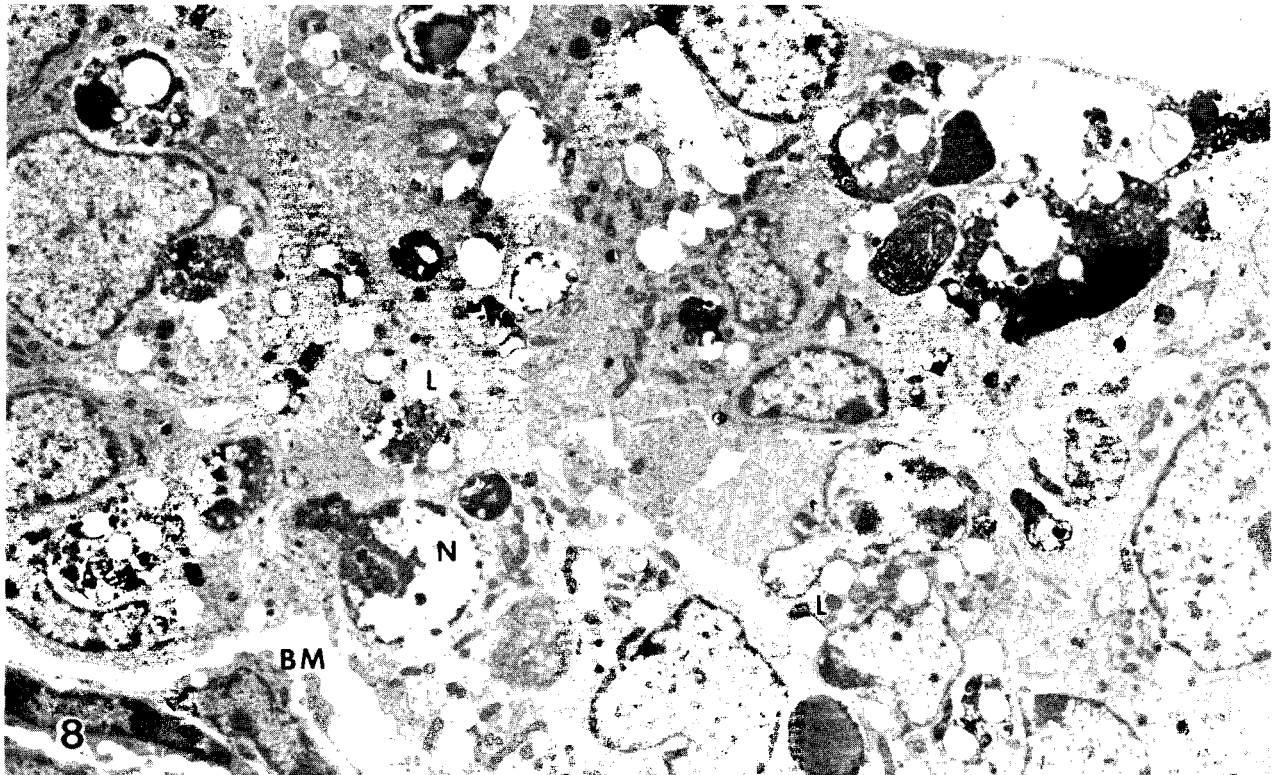


**Fig. 6.** Numbers of uterine circular muscle fibers in ICR and NOD mice. The number of NOD-N shows a significant decrease in comparison with that of ICR mice. In comparison with NOD-N mice, the number in NOD-DM (2D) mice decreases more markedly than that of NOD-N mice, although that of NOD-DM (1D) shows no significant difference from NOD-N mice. \* $p < 0.01$





**Fig. 7.** Ovarian follicles in ICR (a), NOD-N (b), NOD-DM (1D) (c) and NOD-DM (2D) mice (d).  $\times 530$ , H.E. staining. **a-b.** Healthy follicles are observed in ICR and NOD-N mice. **c.** Follicles of  $\sim 450 \mu\text{m}$  diameter are still observed in NOD-DM (1D) mice, although many atrophic follicles are found. **d.** Many follicles in NOD-DM (2D) mice exhibit granulosa cell atresia and ovum degeneration.



**Fig. 8.** The granulosa cell layers of a follicle from diabetic mice as seen by TEM. Lipid deposits (L) and degenerative nuclei (N) in the granulosa cells are observed.  $\times 4,200$ , BL: basal lamina.

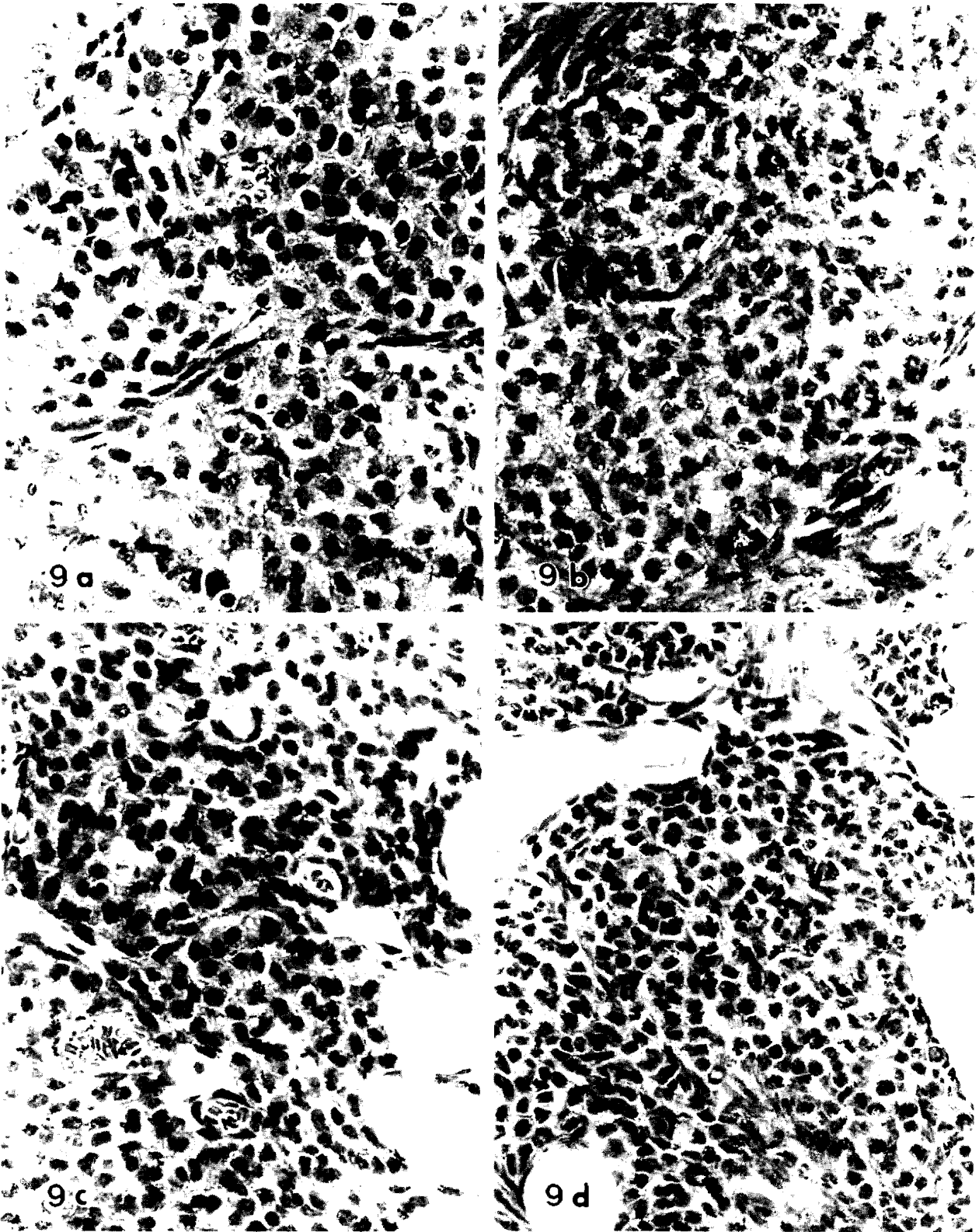
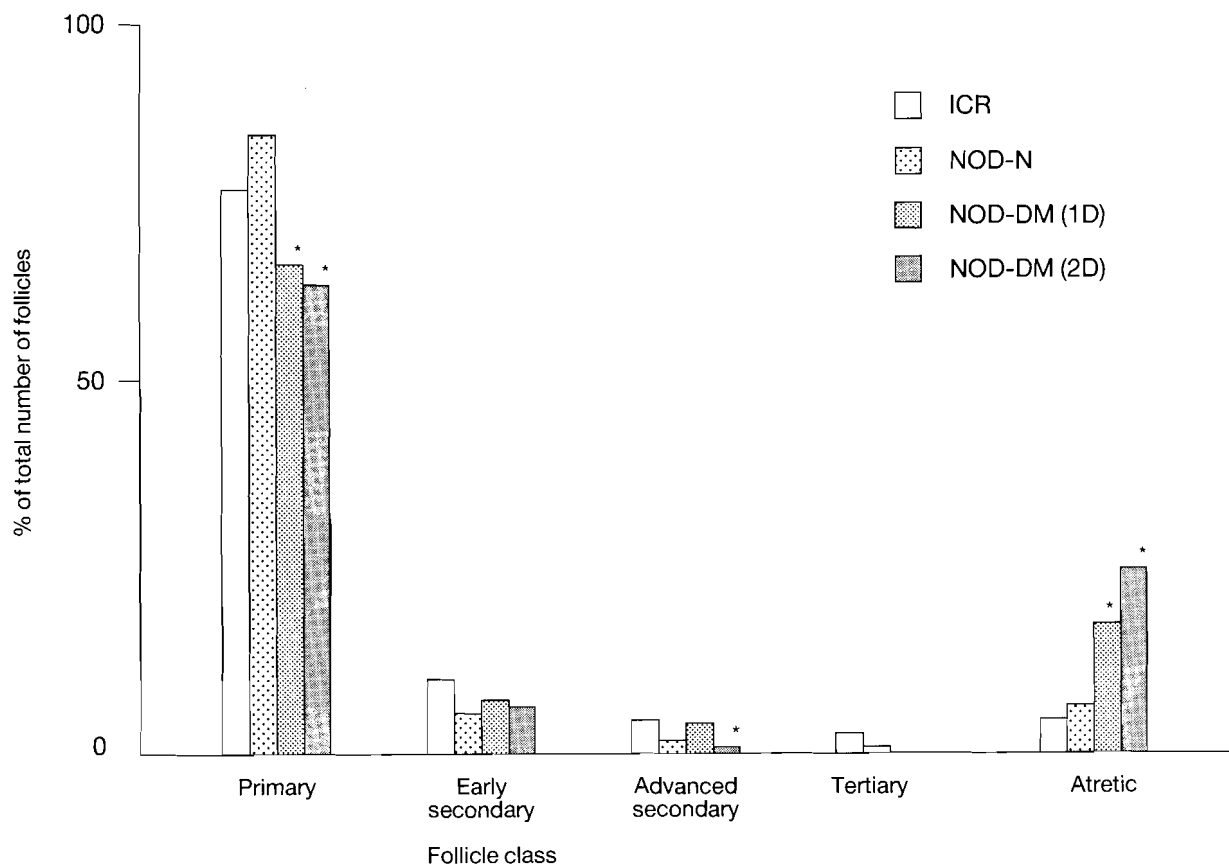


Fig. 9. Ovarian stroma cells of ICR and NOD mice. There is no change in ICR (a), NOD-N (b) and NOD-DM (1D) mice (c). But atrophy is observed in NOD-DM (2D) mice (d).  $\times 530$ . H.E. staining.

## Change of diabetic reproductive organ



**Fig. 10.** Classification of ovarian follicles in ICR and NOD mice. The percentages of classified groups are compared. There is a significant decrease in primary follicles of NOD-DM (1D) and NOD-DM (2D) mice, and in advanced secondary follicles of NOD-DM (2D) mice. There are significant increases in atretic follicles of NOD-DM (1D) and NOD-DM (2D) mice in comparison with ICR and NOD-N mice. \* $p < 0.01$

many degenerated ova (i.e., fragmentation of ovum) that differed from the physiological follicle atresia. The granulosa cells of NOD-DM mice had a large accumulation of intracellular lipid and included a disruption of many cells (Fig. 7d). Follicles of non-diabetic (NOD-N and ICR) mice showed healthy ova surrounded by variable layers of round granulosa cells that were characterized by distinct cytoplasmic membranes (Figs. 7a-c). From the TEM observation, the lipid deposits in the granulosa cells of diabetic mice obliterated the cytoplasmic space (Fig. 8), and the nuclei of granulosa cells of diabetic animals appeared degenerative.

## 2) Stroma cells

Fat staining, using  $O_3O_4$ , showed the following consequence: although non-diabetic (NOD-N and ICR) mice had no lipid in healthy follicles and some lipid deposits in stroma cells, diabetic mice, especially NOD-DM (2D) mice, exhibited a high accumulation of lipid in degenerated follicles and no lipid in stroma cells. The stroma cells of diabetic mice also appeared to undergo atrophic degeneration, and the nuclei of the stromal cells were reduced and condensed (Fig. 9d) in comparison with the large, round nuclei in non-diabetic (NOD-N and

ICR) mice (Figs. 9a,b). The degenerative change in NOD-DM (1D) mice (Fig. 9c) was milder than that in NOD-DM (2D).

## 3) Morphometric analysis of the follicles

The results of morphometric analysis agreed with the progress of lesion in diabetes mellitus.

The morphometric analysis of the ovaries revealed a large difference in the follicular pattern between diabetic and non-diabetic mice. The primary, secondary, tertiary and atretic follicles (Table 1) classified by size and granulosa layer number are expressed as a percentage, as shown in Fig. 10. The percentages of the primary follicles showed that those of diabetic groups, NOD-DM (1D) and NOD-DM (2D), decreased in comparison with non-diabetic (NOD-N and ICR) mice. The early secondary follicles were almost the same in diabetic and non-diabetic groups. The healthy advanced secondary and tertiary follicles in NOD-DM mice, especially NOD-DM (2D), were fewer than those of the other groups, and there were no healthy tertiary follicles in NOD-DM (2D) mice. Significant differences were found statistically. In contrast, the percentage of atretic follicles was significantly increased in NOD-DM (1D) and NOD-DM (2D) compared with NOD-N and ICR mice. Especially

in NOD-DM (2D) mice, the percentage of viable follicles declined and that of atretic follicles increased as the stage of diabetes mellitus progressed. The change in NOD-DM (LD) mice was milder than in NOD-DM (2D).

#### 4. Changes in the organs in NOD-DM (LD) mice.

NOD-DM (LD) mice exhibited the highest percentage of destroyed islet  $\beta$ -cells (Table 2), and the change in the liver was milder than that in NOD-DM (2D) mice. The vacuolar degeneration in the liver in NOD-DM (LD) was milder than that in NOD-DM (2D) mice, but an increase in Bowman's capsules was observed only in NOD-DM (LD) mice. The changes in the ovary were slight atrophies of follicles and stroma cells. The change in the uterus was a high accumulation in the luminal and glandular epithelium; there was no change in the endometrium and myometrium.

As NOD-DM (LD) mice were 30 weeks old or more, the influence of age was studied with age-matched NOD-N mice. Similar, but milder, changes than in NOD-DM (LD) mice were detected in the organs of age-matched NOD-N mice.

Considering alterations caused by aging, the influence of diabetes mellitus on reproductive organs in NOD-DM (LD) mice appeared milder than that in NOD-DM (2D) mice.

## Discussion

### *I. The pancreas and the liver*

Histopathological changes in the pancreas (Makino et al., 1980; Fujita et al., 1982; Miyazaki et al., 1985; Asamoto et al., 1986; Fujita and Yui, 1986) and kidney (Kohama et al., 1981; Funakawa et al., 1984) are recognized to occur in association with the diabetes syndrome in NOD mice. Changes in hepatic structure, however, have not been described in this literature. Alteration in the liver is considered to be affected by a systemic metabolic disorder associated with diabetes. The vacuolar degeneration of the hepatic cell differed from that of fatty liver associated with the diabetic condition, but might be a characteristic of lesion in NOD mice. In our observations, the change in the pancreas in NOD mice increased in proportion to the duration of diabetes mellitus. The change in the liver was equivalent to that of the pancreas. Therefore we are able to consider the above changes, in place of the glucose levels, as indicators of the severity of diabetes mellitus.

### *II. The reproductive organs*

The more the pancreas and the liver in NOD-DM mice changed histopathologically, the more damaged the reproductive organs became.

#### 1. The ovary

Changes in follicles in the present study agreed with

those in the papers reported by Garris (1984) and Garris et al. (1985, 1986), and appeared to be caused by a hyperglycemic condition as described.

It was suggested that a decline in the function of ovarian granulosa cells arose from an accumulation of lipid and a disruption of some granulosa cells which were stained to a lighter color, in comparison with healthy cells, by hematoxylin-eosin staining. The alteration in ovarian granulosa cells appeared to be related to an increase in atretic follicles. It might also have decreased the numbers of ova ovulated normally in NOD-DM (1D) and NOD-DM (2D) mice, because the number of eggs in the fallopian tubes (oviduct) at the third day of pregnancy is significantly decreased in 95% of pancreatectomized rats (Chieri, 1969). The diabetic condition also seemed to induce atrophy and a lack of lipid in ovarian stroma cells, while normal stroma cells had some lipids. This change in NOD-DM, especially NOD-DM (2D) mice, might exhibit a characteristic of NOD mice, and/or might suggest that the lipid metabolism in these animals had fallen into disorder. These degenerations in granulosa cells and stroma cells are not only directly related to an impaired follicular ability (Chieri et al., 1969), but seem to influence the production of some steroid hormones and other substances (Garris et al., 1986). This is because granulosa cells physiologically take part in the production of steroid hormones and of non-steroidal regulatory factors. These substances operate on the feedback system to the diencephalon and the hypophysis, on the proliferation in uterine endometrium, on the regulatory process of growth of the ovum and so on (Suzuki, 1981; Garris et al., 1986). Therefore the decline in the steroidogenesis might influence the structure and function of the uterus in NOD-DM mice.

#### 2. The uterus

The uterus in NOD-DM mice, especially NOD-DM (2D) ones, exhibited involution as described by Lawrence and Contopoulos (1960), who used the alloxan-induced diabetic rat, and as reported by Garris (1985), who used C57BL/KsJ mouse. According to the report of Garris (1985), the decrease in uterine weight appears to be caused by the impairment of estrogen-stimulated water inhibition. Uterine weight loss in NOD-DM mice may be related to a decline in steroid hormone. The decline of steroid hormone from the ovary was strongly suggested by the structural alteration in NOD-DM mice described above.

#### Epithelium

Our results on lipid deposits in the uterine epithelium agreed with those in the paper of Garris (1985) and Garris et al. (1986). The change in uterine epithelium is caused by the hyperglycemic condition associated with diabetes mellitus (Garris, 1982, 1984, 1985; Garris and Smith, 1983), and it occurs only in the estrogen-sensitive epithelial cells (Finn and Martin, 1976; Finn and Publicover, 1981). Their conclusions might apply to the alterations in NOD-

DM mice; the degeneration of ovarian granulosa cells induced the decline of steroidogenesis, thereby the deficiency of estrogen is considered to have direct effects upon the luminal and glandular epithelium under a hyperglycemic condition, as reported by Marcus (1974) and Kirkland et al. (1981). The hyperglycemic condition, furthermore, exacerbates the deleterious effects of lowered ovarian steroid levels in diabetic animals (Garris et al., 1983, 1984; Garris and Smith, 1983). Therefore, the rate of implantation might decrease due to this change in uterine epithelium by a hyperglycemic condition together with a probable decline of steroidogenesis in NOD-DM mice.

#### Endometrium

Uterine stromal cells in NOD-DM, especially NOD-DM (2D) mice, appeared atrophic, although the atrophy was not so severe as that reported by Garris et al. (1984). In addition, the uterine glands appeared to decrease as described by Garris et al. (1984). This degeneration would probably also prevent a normal implantation reaction, because a proper endometrial structure is necessary for proper uterine-blastocyst contact (Swigart et al., 1961; Garris and Whitehead, 1981; Garris et al., 1983, 1984; Garris, 1984).

#### Myometrium

The progress of disease, furthermore, exhibited an atrophic myometrium and a decrease in the number of smooth muscle fibers in NOD-DM mice. The value of smooth layers in NOD-DM (2D) was significantly lower than that in NOD-N mice, probably due to an effect associated with diabetes mellitus, although the difference observed between ICR and NOD-N mice might indicate a characteristic of NOD mice which have an immunodeficiency (Fujita, 1982; Asamoto et al., 1986; Hanafusa et al., 1986).

The uterus at estrus stage exhibited a dramatic increase in uterine blood flow, a rise in estrogen and a different ratio of estrogen levels to progesterone compared with other cyclic stage (Nequin, 1979; Garris and Foreman, 1984). Estrogen stimulates cell divisions in the surface and a superficial glandular epithelium and progesterone induces stroma cell division (Marcus, 1974). The uterus in NOD-DM mice histologically manifested a gradual fall toward a reproductive acycle, probably inducing reproductive failure in association with the progress of disease. The cause of the reproductive acycle in NOD-DM mice is probably epithelial and stromal degeneration associated with diabetes mellitus, and also the degradation of steroidogenesis in the ovary, because the results from NOD mice resembled those from Chinese hamsters (Garris and Smith, 1983) and from rats (Garris et al., 1983).

The uterine atrophy and the related inability to respond to estradiol treatment probably account for the low incidence of post-ovulatory development of the conceptus (Coleman, 1978; Garris, 1985). Therefore the

structural changes in the uterus that occur in association with the hyperglycemic condition, and the probable decline of steroid hormone from the ovary may decrease not only the ratios of implantation but also viable embryos (Otani et al., 1987; Tatewaki et al., 1987).

#### *III. The influence of body weight loss*

Weight loss causes loss of menstrual function and weight gain restores menstrual cycles in humans (Frish and McArthur, 1975), and weight loss causes a consistently lower LH (luteinizing hormone) and FSH (follicle stimulating hormone) responsiveness to LH-RH (luteinizing hormone-releasing hormone) and the responsiveness returns with weight gain (Warren et al., 1975). Furthermore, increasing body weight is accompanied by a sharp decrease in 2-hydroxylation of estradiol that caused amenorrhea, and an increase in 16  $\alpha$ -hydroxylation that produced estriol (Fishman et al., 1975). NOD-DM mice became very lean and showed a progressive lack of lipid in ovarian stroma cells with the progress of disease. Therefore, the disturbance of hormone secretion and impaired metabolism of steroid hormone may be caused by a deficiency of body fat.

#### *IV. Comparison with other diabetic animals*

As described above, NOD-DM mice exhibited uterine weight loss, accumulation of lipids in the luminal and glandular epithelium of the uterus, decrease in numbers of smooth muscle fibers in the myometrium, a higher concentration of lipid deposits in ovarian granulosa cells, atretic follicles and atrophy and lack of lipids in ovarian stroma cells. In comparison with C57BL/KsJ mouse (Garris, 1985; Garris et al., 1985, 1986), the changes in NOD-DM mice agreed with them, except for those in myometrium and stroma cells. Particularly, stroma cells of C57BL/KsJ mouse were occupied by a hyaline type of ground substance of unknown composition or origin. There is no description of the myometrium in C57BL/KsJ mouse. Alterations in Chinese hamsters (Garris, 1984; Garris et al., 1984) differed from those in NOD-DM mice, except for the decline of the uterine glands and the increase of atrophic follicles. In particular, changes in the endometrium in Chinese hamster are severer than those in NOD-DM mice.

The structural changes in the reproductive organs in NOD-DM mice corresponded to the changes in the pancreas and the liver which appeared to reflect the hyperglycemic condition and secondary metabolic disorders, respectively. The histopathological alteration in the ovary strongly suggested a degradation of steroidogenesis and ovulation (Chieri, 1969). In the uterus, the morphological changes which suggest impaired hormone levels may be related to the decrease in ratios of implantation and of viable embryos in post implantation stage (Otani et al., 1987; Tatewaki et al., 1987, 1988).

In conclusion, the present studies suggest that the environment in the reproductive organs associated with diabetes mellitus causes a disturbance in oocyte maturation and implantation as well as an abnormal development at post-implantation stage.

*Acknowledgements.* We are deeply indebted to Professor Morikawa S. of the Department of Pathology, Shimane Medical University, for the discrimination of degenerative tissue and to Dr. Yoshioka T. of the Department of Anatomy, Shimane Medical University, for generous help in the transmission electron studies.

We are also very grateful to Dr. Suzuki S. of the Institute of Experimental Animals, and Dr. Tanigawa K. of the Department of Internal Medicine, Shimane Medical University, for provision of NOD mice.

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