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# The Histomorphometric Effects of Maternal Diabetes on Rat Offspring's Ovaries at the Puberty

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**Abstract**

In pregnant mothers, maternal diabetes occurs when the pancreas cannot produce enough insulin, which leads to increased blood glucose concentration in the mother and consequently in the foetus, causing various neonatal problems. This study was conducted to evaluate the effects of maternal diabetes on foetal ovarian structure. Sixteen adult female rats were allocated into two equal groups. Diabetes was induced in one group by alloxan agent. Both groups became pregnant by natural mating. 60 days after birth, the female offspring were terminated, the body weight and blood glucose of the animals measured and their ovaries removed. Various histological parameters were determined using histological techniques. Results revealed a significant increase in body weight and blood glucose in the offspring of the diabetic mothers (ODM) compared to that of controls. The weight, volume and diameter of the ovary and ovarian capsule thickness were decreased in the ODM group. The number and diameter of primary, preantral, antral and preovulatory follicles and corpora lutea were decreased in ovaries in the ODM. Maternal hyperglycaemia exhibited deleterious effects on the reproductive system of their offspring.

**Keywords:** Gestational Diabetes, Rat, neonates, Ovary, Follicle

## Introduction

Diabetes mellitus is the most common metabolic and endocrine disorder (1). Diabetes mellitus is characterized by hyperglycemia and associated with disturbances in carbohydrate, protein and fat metabolism (2). In diabetic subjects, the pancreas produces insufficient amounts of insulin, causing blood sugar levels to rise (3). Diabetes mellitus is usually associated with glycosuria, polyuria and polydipsia (4). It have been reported that diabetes has deleterious effects on female reproductive system (5) and on the development of the blastocysts (6), and results in abnormalities on reproductive function (7). Diabetes is also associated with increased risk of reproductive problems (8). Data also have indicated that streptozotocin-induced diabetes mellitus inhibits the feedback action of gonadal steroids and this could account for both the loss of oestrous cyclicity and the reduced pituitary sensitivity to LHRH (9). Moley and co-workers have reported that hyperglycaemic conditions, either *in vivo* or *in vitro*, modulate the expression of an apoptosis regulatory gene as early as the pre-implantation blastocyst stage in the mouse (10).

The most convincing evidence comes from the commonly observed association of premature ovarian failure with some autoimmune disorders in diabetes mellitus (11). Data have suggested that diabetes mutation-induced ovarian structural and functional involution is a direct reflection of the cellular metabolic shift towards lipogenesis (12). The diabetes mutation induced a hyperglycaemic-hyperinsulinaemic endometabolic environment that promotes hypercytolipidaemic, utero-ovarian involution in mice, resulting in reproductive sterility and eventual organoatrophy (13). Maternal diabetes

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increased apoptosis in mice oocytes (14). Ovarian dysfunction in a diabetic mutant mouse is associated with follicular atrophy, adiposity, impaired steroidogenesis, and imbalanced glucose utilization (15). The purpose of this investigation is to evaluate the possibility of congenital ovarian malformations in the offspring of diabetic rats at day 60 after birth.

## Materials and Methods

Sixteen adult female Sprague Dawley rats (200–230 g and 4–5 months old) were housed in an air-conditioned room (22 ± 2°C) and supplied with standard pellet food, with tap water *ad libitum* (16). Animals were separated allocated into two equal groups: diabetics and normal (control). This study was approved by the Animal Care and Use local ethical Committee Guidelines.

Diabetes was induced in eight rats by using a single intra-peritoneal injection (150 mg/kg) of alloxan tetra hydrate agent (Sigma, St. Louis, MO) according to our previous study (17). The animals were fasted 12 h before and after alloxan injection. Rats with blood glucose above 200 mg/dL, as well as with polydipsia, polyuria and polyphagia for at least 1 week, were considered to be diabetic and were selected for the experiment (18).

Females in both groups at the oestrus stage of the reproductive cycle were caged with male rats for mating. Mating was confirmed by observation of vaginal plugs (19). Female offspring of both groups were reared in similar conditions in an animal house for 60 days. At the end of the experiment, the animals were anaesthetized using diethyl ether and terminated by whole blood collection via cardiac puncture. Body weight and blood glucose of the offspring were measured in both the control and the test groups. The volume, diameter and weight of the freshly isolated ovaries were measured (20), and the ovaries were fixed in 10% buffered formalin solution.

Formaldehyde-fixed samples were embedded in paraffin and then sectioned at 4–5 micrometer. They were further deparaffinised with xylol, and histologic observations were performed after staining by the Haematoxylin–Eosin or Green Masson's trichrome method (21). For histomorphological and histomorphometric study, the sections were observed under a light microscope, and the average of the following parameters were evaluated in ovaries of both control and test groups: (1) Thickness of the ovarian capsule (micrometer), (2) Ratio of medulla to cortex, (3) Diameter of primary, preantral, antral and preovulatory follicles and the diameter of the corpora lutea (micrometer), (4) Number of primary, preantral, antral and preovulatory follicles and the number of corpora lutea(/$mm^2$).

The thickness of the ovarian capsule was measured at 100 magnification using Olysia software (Olysia soft imaging system provided by Olympus 2000) and an Olympus BX51 light microscope. At least six points of the capsule sections were chosen randomly and measured for each test. The diameters of the ovarian follicles were measured at 100 magnification using Olysia software (Olysia soft imaging system provided by Olympus 2000) and an Olympus BX51 light microscope. At least six points of the cortex sections were chosen randomly and measured for each test. Ovarian follicles were counted at 400 magnification using a 441-intersection grid placed in the ocular of the light microscope (Olympus BX51). Ten sections were chosen at random from each ovary, and the number of round or nearly round ovarian follicles in square millimeters ($mm^2$) was obtained.

Morphometric data are presented as the mean ± standard deviation (SD), and were analyzed by Student's *t* test using SPSS software (22). Significant differences were considered when P value was $\leq 0.05$.

## Results

Body weight and blood glucose of the offspring of diabetic mothers (ODM) were greater ($p<0.05$) than that of controls 60 days after birth (144.2±6g and 126.8±3.4g and, 120.3±6.8mg/dL and 92.5±4.3mg/dL, respectively).

Values for weight, volume, ovarian diameter, medulla to cortex ratio and ovarian capsular thickness for both groups are presented in Table 1. The weight, volume and diameter of ovary, and ovarian capsule thickness were decreased ($p<0.05$) in ODM compared to that of controls. Ovarian weight was 42mg in ODM and 43.5mg in the controls. Volume of ovary was decreased in ODM in comparison to control, which was 39.5 $mm^2$ in ODM and 40.9 $mm^2$ in the control group.

Figure 1 has compared the diameter of different follicles of the ovary in ODM and controls 60 days after birth. The diameter of the primary (51.2 vs. 53.07 micrometer), preantral (142.8 vs. 149.83 micrometer), antral (297.94 vs. 306.96 micrometer) and preovulatory (514.5 vs. 535.1 micrometer) follicles showed a non-significant decrease ($p<0.05$) in ODM ovaries compared to that of controls. The diameter of the corpora lutea showed a non-significant decrease in ODM ovaries compared to that of controls (801.3 and 884.7 micrometer, respectively).

Figure 2 has demonstrated the comparison between the number of different follicles in the ovaries of the ODM and control groups 60 days after birth. The number of primary and preovulatory follicles showed a non-significant decrease ($p<0.05$) in ovaries of ODM compared to that in controls. The number of preantral and antral follicles showed a significant decrease ($p<0.05$) in ODM ovaries. The numbers of primary, preantral, antral and preovulatory follicles were 0.14, 0.26, 0.15 and 3.1 in ODM ovaries and 0.16, 0.32, 0.19 and 3.3 in control ovaries, respectively. The number of corpora lutea was decreased in ODM ovaries (3.4) compared to that of controls (4.1).

Comparison of the antral follicle in ovaries of control and ODM groups has been demonstrated that, in control group, the ovum cytoplasm is clear and the nucleus is distinct, whereas in the ovum of ODM group, the cytoplasm is cloudy and vacuolated, and the nucleus is indistinct (Figure 3).

Comparison of the preovulatory follicle in control and ODM groups has been shown that the diameter of the follicle of ODM group was less than that in control group (Figure 4).

Table1: Ovarian histomorphometric parameters in offspring of the diabetic mothers (ODM) and controls 60 days after birth. Values are presented as mean± SD.

| Group | ODM | Control |
|---|---|---|
| **Volume of ovary ($mm^2$)** | 39.5±3.5 | 40.9±4.1 |
| **Diameter of ovary (mm)** | 4.2±0.4 | 4.3±0.4 |
| **Weight of ovary (mg)** | 42±3.8 | 43.5±4.4 |
| **Ovarian capsular thickness (µm)** | 10.7±1.1 | 11.1±1.2 |
| **Medulla to cortex ratio** | 0.27±0.02 | 0.26±0.02 |

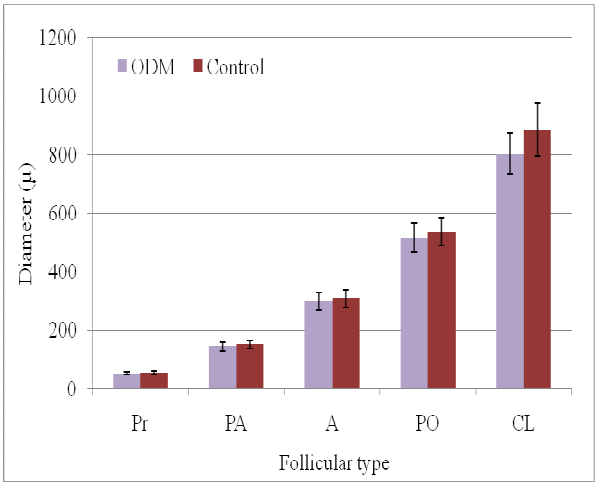


Fig 1: Diameters of different follicles of the ovaries of offspring of the diabetic mothers (ODM) and controls 60 days after birth. Pr (Primary follicle), PA (Preantral follicle), A (Antral follicle), PO (Preovulatory Follicle), CL (Corpus Luteum).

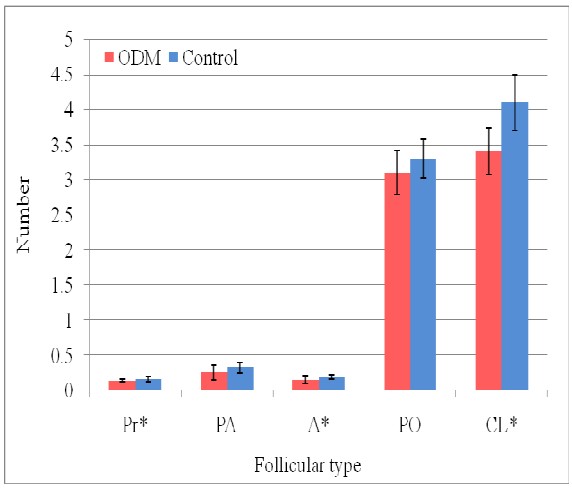


Fig 2: Number of different follicles in offspring of the diabetic mothers (ODM) and control ovaries 60 days after birth. Pr (Primary follicle), PA (Preantral follicle), A (Antral follicle), PO (Preovulatory Follicle), CL (Corpus Luteum).* Represents a significant difference at $p<0.05$.

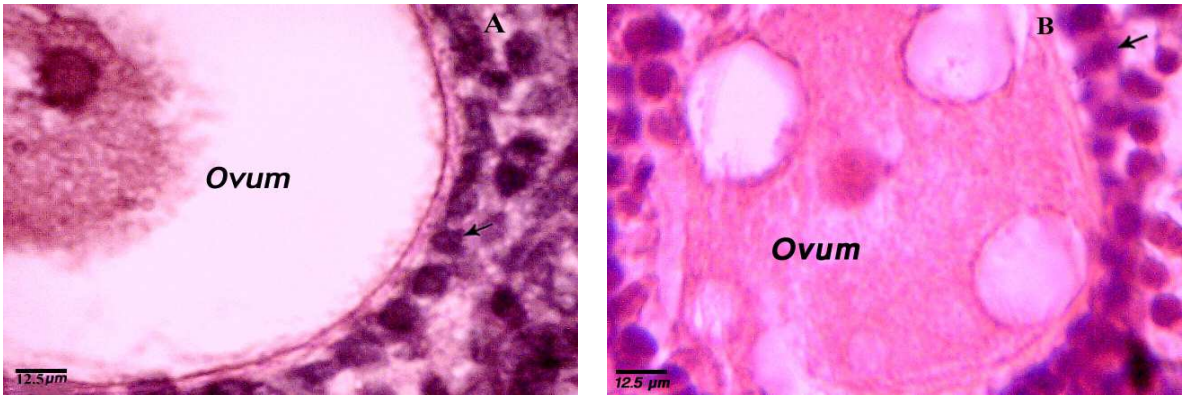


**Figure 3**: Comparison of the antral follicle in control (A) and offspring of the diabetic mothers (ODM) (B) groups. Arrows demonstrate granulosa cells (Staining: Haematoxylin-Eosin).

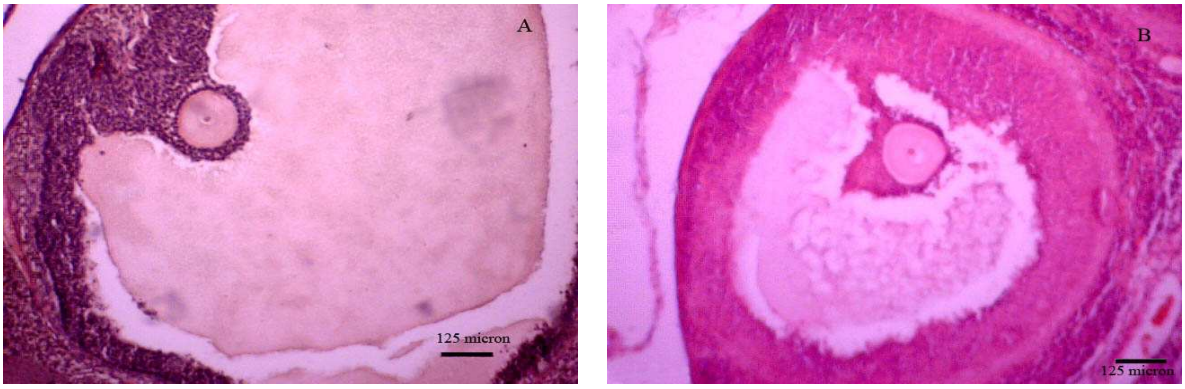


**Figure 4**: Comparison the preovulatory follicle in control (A) and offspring of the diabetic mothers (ODM) (B) groups (Staining: Haematoxylin-Eosin).

**Discussion**

The body weight of ODM was greater than that of controls (macrosomia), which is due to an increase in placental transport of glucose and other nutrients to the foetus (3). The blood glucose of ODM was higher than that of the controls. This condition, accompanied by a moderate increase in fasting blood glucose in ODM, may be due to maternal hyperglycaemia leading to foetal hyperglycaemia and hypoinsulinaemia (17).

There was a decrease in the weight, volume and diameter of ovary and thickness of ovarian capsule in ODM. Ovarian atrophy and reproductive tract incompetence are recognized consequences of the progressive expression of the overt, diabetes-obesity syndrome (DOS) in diabetic mutant mice. In both humans and experimental models, utero-ovarian structural, functional, and metabolic parameters are altered in response to the progressive hyperglycemic-hyperinsulinemic systemic conditions that characterize noninsulin dependent (Type II) (12). Diabetes can play a role in ovarian atrophy, suggesting that ovarian involution in these mutants is directly related to an impaired follicular ability to properly metabolize the elevated intracellular glucose concentrations that develop in the diabetic mice (15). The increase in medulla to cortex ratio seen in the ODM group may be due to a decline in cortical elements such as oocytes and follicles. Previous studies demonstrated that maternal diabetes increases oocytes apoptosis (14) and follicular atrophy (15) in mice.

A reduction in the diameters (Figure 1) and number (Figure 2) of primary, preantral, antral and preovulatory follicles as well as corpora lutea was observed in ODM. It has been indicated that diabetes causes alterations in the timing of the estrous cycle, associated with modifications in ovary function, which

induces a decrease or even absence of ovulated oocytes and oocyte maturation in female rats (8). Garris et al. indicate that follicular atrophy appears in diabetes (15). One study demonstrated that both models of maternal hyperglycemia and hypoinsulinemia may have a detrimental effect on oocyte maturation and development as detailed by the smaller sizes of oocytes and developing ovarian follicles and the greater amount of apoptosis (23). Lin et al. reported that maternal diabetes increase oocyte apoptosis (14). Wang et al. demonstrated that the mitochondrial impairments induced by maternal diabetes lead to cumulus cell apoptosis, at least in part, through the release of cytochrome *c*. Together, the deleterious effects on cumulus cells may disrupt trophic and signalling interactions with the oocyte, contributing to oocyte incompetence and thus poor pregnancy outcomes in diabetic females (24). Cumulus cells and the oocyte are metabolically coupled throughout follicular development by membrane specializations known as gap junctions. An important point, particularly in relation to diabetes, is that oocytes are deficient in their ability to use glucose as an energy substrate and require cumulus cell-provided products of glycolysis like pyruvate for their own development (25). Egg, zygote or blastocyst derived from diabetic parents may develop into offspring with high risk of any type of diabetes, even if placed in a normal uterus, producing developmental delay, embryopathy, geno- and cyto-toxicity, teratogenic changes, free radicals and apoptosis. These early insults may then lead to an increased rate of miscarriage and congenital anomalies depending on free radicals signaling and cell-death pathways involved with the diabetogenic agents. Furthermore, egg, zygote or blastocyst from normal parents will have an increased risk of diabetes if placed in a diabetic uterus (6). Garris and Garris demonstrated that enhanced lipid deposition and cellular metabolic indices promote a very no homeostatic, hyperlipogenic metabolic environment within all ovarian compartments of a diabetic mutant rat. The progressive deposition of enhanced interstitial and follicular lipid pools compromises the functional and structural characteristics of all ovarian cellular and tissue compartments, ultimately inducing a hypercytolipidemia, which contributes to premature tissue involution and ovarian failure (12).

**Conclusions**

We have concluded that foetal gonads of females may be affected by maternal hyperglycaemia which remains after birth such as reduction in their ovarian weight, volume and diameter, and the follicular numbers and diameters.

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