OVARIAN FAILURE IN DIABETIC RAT MODEL; NF-KAPPAB, OXIDATIVE STRESS AND PENTRAXIN-3

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- Tissue damage mechanism of hyperglycaemia is overproduction of mitochondrial reactive oxygen species (ROS)
- Oxidative stress → pathogenic mechanism for DM and its complications

Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005;54:1615–1625

- AGEs → nonenzymatic reaction of glucose and other glycating components derived from glucose and fatty acid oxidation in endothelial cells

- AGEs → ↑ in extracellular matrix in DM

- RAGE → production of ROS → pleiotropic transcription factor nuclear factor (NF)-kappaB

Wautier JL, Schmidt AM. Protein glycation: a firm link to endothelial cell dysfunction. *Circ Res* 2004;95:233–238

Stitt AW, Moore JE, Sharkey JA, et al. Advanced glycation end products in vitreous: structural and functional implications for diabetic vitreopathy. *Invest Ophthalmol Vis Sci* 1998;39:2517–2523

- Vascular endothelial cell damage → ↑ levels of AGE and ROS. Endothelial dysfunction → vascular complications of DM

- Resveratrol → antioxidant, anti-platelet, anti-cancer, anti-inflammatory in various experimental models

- Resveratrol → DM-induced vasculopathy through attenuation of RAGE for AGE NF-kappaB signalling pathway

Olas B, Wachowicz B, Tomczak A, Erler J, Stochmal A, Oleszek W. Comparative anti-platelet and antioxidant properties of polyphenol-rich extracts from: berries of aronia melanocarpa, seeds of grape and bark of yucca schidigera in vitro. *Platelets* 2008; 19: 70–77

Jing YH, Chen KH, Yang SH, Kuo PC, Chen JK. Resveratrol ameliorates vasculopathy in STZ-induced diabetic rats: role of AGE–RAGE signalling. *Diabetes Metab Res Rev* 2010; 26: 212–222

- PTX3 → a member of long pentraxin family and has C-terminal sequence showing homology with CRP

- PTX3 → produced in different cell components of atherosclerotic lesions, including endothelial cells, macrophages, fibroblasts, vascular smooth muscle cells, adypocytes

- As PTX3 synthesis occurs at sites of inflammation it is believed to be a independent indicator of disease activity

Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol* 2005;23:337–366.

Fazzini F, Peri G, Doni A, et al. PTX3 in small-vessel vasculitides: An independent indicator of disease activity produced at sites of inflammation. *Arthritis Rheum* 2001;44: 2841–2850

- Ovarian reserve and function in DM play important role in fertility, cardiovascular disease risk, osteoporosis

- We aimed to investigate the effects of DM on ovarian reserve and injury in terms of laboratory and histopathologic findings in a rat model

Schweiger BM, Snell-Bergeon JK, Roman R, McFann K, Klingensmith GJ. Menarche delay and menstrual irregularities persist in adolescents with type 1 diabetes. *Reprod Biol Endocrinol* 2011;9:61

Materials & Methods

- 24 female Sprague Dawley albino mature rats at 8 weeks, weighing 200–220 g
- fed ad libitum
- 22 ± 2 °C
- 12-h light/dark cycles
- Committee for Animal Research of Gaziosmanpasa University
- Animal experiment guidelines of the Committee for Human Care

Experimental protocol

- Diabetes → i.p. injection of STZ (Sigma-Aldrich, Inc.; Saint Louis, MO, USA) (60 mg/kg in 0.9% NaCl, adjusted to a pH 4.0 with 0.2M sodium citrate) in 16 rats
- Diabetes was verified by evaluating blood glucose levels with glucose oxidase reagent strips (Boehringer- Mannheim, Indianapolis) after 24 hours
- The rats with 250 mg/dl and higher blood glucose levels were accepted as diabetic and included in this study
- Eight rats with normal blood glucose levels (< 120 mg/dl) were taken as control group

Experimental protocol

- STZ → 7 weeks → to develop diabetic complications
- 16 diabetic rats were randomly divided into 2 groups;
- Group 1 → 1 ml/kg/day 0.9% NaCl i.p. (n=8) [non-treated diabetic group]
- Group 2 → 20 mg/kg/day resveratrol i.p. (Sigma Aldrich) (n=8) for 4 weeks [resveratrol treated diabetic group]

Experimental protocol

- Animals → euthanized
- Blood samples → cardiac puncture for biochemical analysis
- Bilateral oophorectomy

Histpathological examination

- Follicular degeneration
- Stromal degeneration
- Stromal fibrosis

  scored from 0 to 3 according to the injury severity

- 0 → no pathologic findings
- 1 → 33% ↓
- 2 → 33% - 66%
- 3 → 66% ↑

Histopathological examination

- Follicles were counted according to the follicle morphology
- ***Primordial Follicle:*** surrounded by thin, single layer of follicular epithelial cells
- ***Primary Follicle:*** Follicular epithelium surrounding the oocyte → prismatic
- ***Secondary Follicle:*** Follicular epithelium encompassing multiple rows → stratum granulosum. Zona pellucida and thecal cells
- ***Tertiary Follicle:*** → fluid-filled cavity and antral follicle.

NF-kappaB immunoexpression

- Brown cytoplasmic staining in ovarian stromal cells and oocyte was scored as positive for NF-kappaB.
- The number of NF-kappaB (+) cell was assessed by systematically scoring at least 100 ovarian stromal cells per field in 10 fields of tissue sections at 10x objective.

Measurements

- Lipid peroxidation → plasma samples by measuring malondialdehyde (MDA) levels as it is thiobarbituric acid reactive substance (TBARS).
- Glutathione (GSH) → in plasma samples was measured spectrophotometrically according to Ellman's method
- Plasma AMH levels were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kit (Biosciences)
- Plasma PTX3 levels were measured in each 100 µl sample by standard ELISA apparatus at 450 nm using a PTX3 kit (Uscn Life Science Inc., Wuhan, China)

Statistical analysis

- SPSS version 20.0 for Windows
- Parametric variables → Student's t test and ANOVA
- Nonparametric → Mann Whitney U test
- Cathegorical variables → x^2 test
- Results → mean ± SEM
- $p<0.05$ → statistically significant
- $p<0.001$ → statistically highly significant

Results

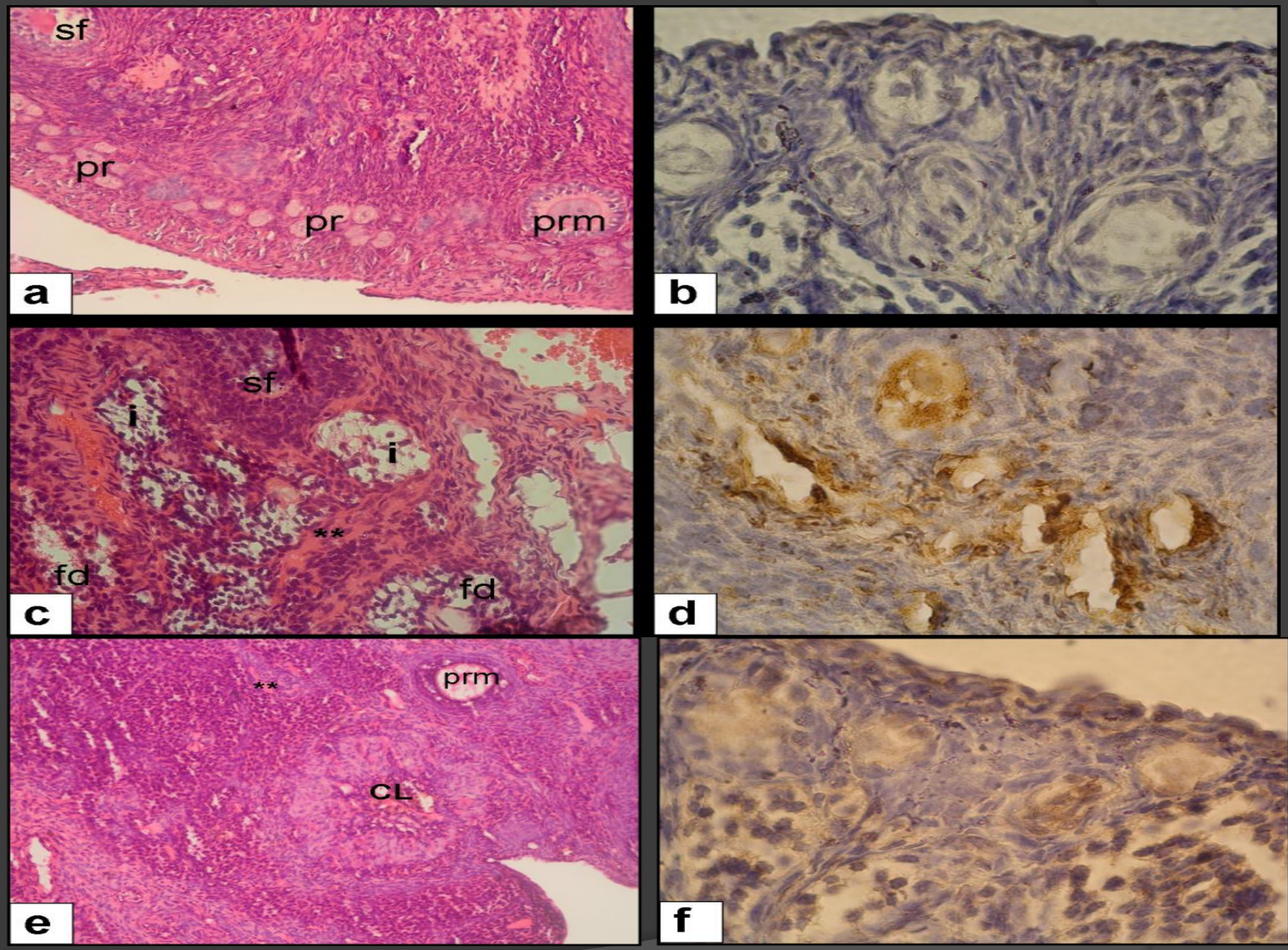
	GSH (µM)	MDA (µM)	AMH (ng/ml)	PTX-3 (ng/ml)
Control Rats	12.26 ± 2.18	0.14 ± 0.09	2.45 ± 0.18	1.17 ± 0.16
Non-treated Diabetic Rats	1.55 ± 0.53**	0.37 ± 0.04**	1.59 ± 0.23**	2.58 ± 0.09**
Resveratrol-treated Diabetic Rats	10.19±2.15*	0.26 ± 0.05*	2.14 ± 0.16*	1.98 ± 0.33*

Results

	Control Rats	Non-treated Diabetic Rats	Resveratrol-treated Diabetic Rats
Stromal Degeneration	0.10 ± 0.08	2.48 ± 0.25**	1.12 ± 0.16*
Follicle Degeneration	0.18 ± 0.10	2.62 ± 0.18 **	0.93 ± 0.11*
Stromal Fibrosis	0.35 ± 0.09	2.37 ± 0.53 **	0.56 ± 0.32*
NF-kappaB Immunoexpression (%)	5.86 ± 2.21	43.54 ± 5.75 **	28.55 ± 6.24**

Results

Follicle	Control Rats	Non-treated Diabetic Rats	Resveratrol-treated Diabetic Rats
Primordial	17.32 ± 4.65	7.84 ± 0.86*	12.25 ± 2.14*
Primary	12.86 ± 3.24	6.24 ± 2.85*	10.48 ± 1.16*
Secondary	10.26 ± 2.50	8.32 ± 1.15	8.45 ± 2.53
Tertiary	5.32 ± 1.04	4.45 ± 0.52	5.18 ± 1.75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- In this study we found that DM effects ovarian follicle reserve especially primordial and primary
- Follicle maturation is effected in early stages of the period in DM rats
- This phenomenon most likely occurs at the oocyte–cumulus cell complex and leads to injury through several pathways

- We speculate that a non-immune mechanism; NF-kappaB pathway plays a role in the pathophysiology of this ovarian complication
- Inhibiting NF-kappaB pathway by antagonists ameliorates the negative effects of oxidative stress on ovaries
- Further studies should evaluate this precise mechanism that leads a decline in AMH levels. As well as the relationship between this abnormality and reproductive function in DM patient should be further analysed