Effect of Renal Extracorporeal Shock Wave Lithotripsy on Ovarian Insulin-Like Growth Factor-1 Expression in a Rat Model

Short title: Renal ESWL and Ovarian IGF-1 Expression

ABSTRACT

Background:

Extracorporeal shock wave lithotripsy (ESWL) is a well-established non-invasive treatment modality for urinary stone disease. Although its direct effects on renal tissues have been extensively investigated, the possible indirect effects of ESWL on distant reproductive organs remain unclear. Insulin-like growth factor-1 (IGF-1) is a key regulator of cellular proliferation, tissue regeneration, and ovarian follicular development. This experimental study aimed to investigate whether renal ESWL exposure induces alterations in ovarian IGF-1 expression.

Methods:

Fifteen female rats were randomly allocated into three groups: a control group (n=6), an ESWL day 14 group (n=5), and an ESWL day 28 group (n=4). A total of 2000 shock waves were applied to the renal region of the experimental groups. Ovarian tissues were collected at predetermined time points and evaluated immunohistochemically for IGF-1 expression. The staining patterns in epithelial, stromal, and adipose tissues were classified as positive or negative due to the weak staining intensity. Statistical analyses were performed using Chi-square and likelihood ratio tests, and $P \leq 0.05$ was considered statistically significant.

Results:

A significant difference in stromal IGF-1 expression was observed among the groups ($P=0.021$). The control group demonstrated a higher rate of positive stromal staining compared with the ESWL-treated groups. No statistically significant difference was detected in epithelial IGF-1 expression ($P=0.973$). Overall, IGF-1 immunoreactivity was weak in ovarian tissues.

Conclusions:

Renal ESWL may induce limited indirect alterations in ovarian stromal IGF-1 expression. However, the biological significance of these findings remains uncertain because of the small sample size and low staining intensity. Further studies including larger experimental populations, different ESWL protocols, and simultaneous serum IGF-1 measurements are required to clarify the underlying mechanisms.

Keywords:

Extracorporeal shock wave lithotripsy; IGF-1; ovary; immunohistochemistry; rat model

Introduction

Extracorporeal shock wave lithotripsy (ESWL) is a widely used non-invasive treatment method for urinary stone disease and remains one of the first-line therapeutic options, particularly in selected pediatric and adult patients (Alić et al., 2022). The mechanism of ESWL is based on the generation of high-energy acoustic waves that fragment urinary calculi (Akin & Yucel, 2014). Although ESWL primarily targets renal stones, the propagation of shock waves may also influence adjacent and distant tissues through mechanical stress, oxidative injury, inflammatory responses, and alterations in cellular signaling pathways (Alić et al., 2022; Akin & Yucel, 2014).

Previous experimental studies have demonstrated that ESWL may increase oxidative stress and apoptosis in renal and surrounding tissues (Baba et al., 2020; Gecit et al., 2014). Although clinically significant damage to neighboring organs is uncommon, experimental evidence has shown that structures such as the adrenal gland and diaphragm may exhibit histopathological changes following ESWL exposure. Conversely, extracorporeal shock wave therapy has also been utilized in regenerative medicine because of its ability to modulate angiogenesis, cellular proliferation, and tissue repair processes (Lv et al., 2023; Piau et al., 2023; Salcedo-Jiménez et al., 2020).

Insulin-like growth factor (IGF) signaling is an important regulator of cell growth, differentiation, and survival. The IGF system consists of two ligands (IGF-1 and IGF-2), their receptors, and several IGF-binding proteins that regulate their biological activity. In the ovary, IGF-1 contributes to follicular growth by enhancing the actions of gonadotropins and regulating communication among granulosa, theca, and stromal cells (Piau et al., 2023; Salcedo-Jiménez et al., 2020). Furthermore, IGF-mediated signaling pathways play essential roles in oocyte maturation, cellular metabolism, and the maintenance of normal ovarian physiology (Lv et al., 2023; Piau et al., 2023; Salcedo-Jiménez et al., 2020).

The regenerative effects of shock wave therapy in various tissues suggest that ESWL may influence the expression of growth factors involved in tissue remodeling (Lv et al., 2023; Piau et al., 2023; Salcedo-Jiménez et al., 2020). However, data regarding the indirect effects of renal ESWL on ovarian tissue and IGF-1 expression remain limited.

Therefore, the present experimental study aimed to evaluate the indirect effects of renal ESWL on ovarian tissue by assessing immunohistochemical IGF-1 expression in epithelial, stromal, and adipose compartments of rat ovaries.

Materials and Methods

Animals and experimental design

This experimental study was conducted following approval from the institutional experimental animal ethics committee. Fifteen female rats were randomly assigned into three groups: a control group (n=6), an ESWL day 14 group (n=5), and an ESWL day 28 group (n=4).

The experimental groups underwent renal extracorporeal shock wave lithotripsy. A total of 2000 shock waves were delivered to the renal region. The animals in the ESWL day 14 and day 28 groups were sacrificed 14 and 28 days after treatment, respectively. Ovarian tissues were excised and processed for histopathological and immunohistochemical examinations.

Immunohistochemical evaluation of IGF-1 expression

Ovarian tissue sections were stained immunohistochemically using an anti-IGF-1 antibody and examined under a light microscope. IGF-1 immunoreactivity was assessed separately in ovarian epithelial, stromal, and adipose tissue compartments.

Because the staining intensity was generally weak, the immunohistochemical findings were classified qualitatively as positive or negative. The percentages of positive and negative staining among the experimental groups were compared statistically.

Statistical analysis

Categorical variables were expressed as frequency and percentage. Group comparisons were performed using the Pearson Chi-square test. When the assumptions required for Pearson Chi-square analysis were not fulfilled, the likelihood ratio test was applied. Statistical analyses were conducted using SPSS version 28.0 (IBM Corp., Armonk, NY, USA), and a P value ≤ 0.05 was considered statistically significant.

Results

The immunohistochemical evaluation demonstrated differences in IGF-1 expression among the experimental groups. Stromal IGF-1 positivity showed a statistically significant variation between groups (P=0.021). The control group exhibited a higher rate of positive stromal staining compared with the ESWL day 14 and ESWL day 28 groups. No positive stromal staining was observed in the ESWL day 28 group.

In contrast, epithelial IGF-1 expression did not differ significantly among groups (P=0.973). Although mild positive staining was observed in some epithelial samples, the overall level of IGF-1 immunoreactivity was low.

The findings indicate that renal ESWL was associated with a reduction in stromal IGF-1 expression, particularly during the early post-treatment period; however, the biological significance of this observation requires further investigation.

Discussion

The present study investigated whether renal ESWL induces indirect changes in ovarian tissue by evaluating IGF-1 expression. The principal finding was a significant reduction in stromal IGF-1 immunoreactivity after renal ESWL, whereas epithelial IGF-1 expression remained unaffected.

The biological effects of ESWL are complex and may involve mechanical stress, oxidative injury, inflammatory responses, and alterations in cellular signaling pathways (Lv et al., 2023; Piau et al., 2023; Salcedo-Jiménez et al., 2020; Suh et al.,2013). Previous studies have reported that ESWL may induce oxidative damage and apoptotic changes not only in renal tissue but also in adjacent organs. These findings support the hypothesis that shock waves may influence distant tissues through indirect biological mechanisms (Lv et al., 2023; Piau et al., 2023; Salcedo-Jiménez et al., 2020; Suh et al.,2013).

The IGF system has an essential role in ovarian physiology. IGF-1 enhances the effects of gonadotropins and contributes to follicular growth, granulosa cell function, and oocyte maturation. Therefore, alterations in ovarian IGF-1 expression may potentially affect local tissue remodeling and follicular homeostasis (Gecit et al.,2014; Hogg et al.,2004; Barnes et al.,2014; Zulu et al.,2002).

Although extracorporeal shock wave therapy has demonstrated regenerative and angiogenic effects in several experimental models, the response appears to be highly dependent on the target tissue, energy level, treatment duration, and biological environment. In contrast to the regenerative effects observed in musculoskeletal tissues, our findings suggest that renal ESWL may be associated with a decrease in stromal IGF-1 expression in ovarian tissue (Hogg et al., 2004; Barnes et al., 2014; Zulu et al., 2002).

The current study has several limitations. The small number of experimental animals reduces statistical power. In addition, serum IGF-1 concentrations were not measured, follicular stages were not analyzed separately, and complementary molecular markers related to apoptosis, oxidative stress, or fibrosis were not evaluated.

Despite these limitations, the present study provides preliminary evidence that renal ESWL may induce indirect changes in ovarian stromal IGF-1 expression. Further experimental studies with larger sample sizes, different ESWL energy protocols, and comprehensive molecular analyses are required to determine the clinical relevance of these findings.

Conclusions

The present experimental study demonstrated that renal extracorporeal shock wave lithotripsy may induce limited indirect alterations in ovarian IGF-1 expression, particularly within stromal tissues. However, the biological importance of this alteration remains uncertain because of the low intensity of immunohistochemical staining and the limited sample size.

The absence of significant changes in epithelial IGF-1 expression suggests that the effects of renal ESWL on ovarian tissue may be compartment-specific. Future studies involving larger experimental populations, different ESWL energy protocols, serum IGF-1 measurements, and additional molecular markers associated with oxidative stress, apoptosis, and tissue remodeling are required to clarify the underlying mechanisms and possible reproductive implications.

Funding

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Ethical approval

All experimental procedures were approved by the General Directorate of Nature Conservation and National Parks (Approval No. E-26137614-280.01.01-16654555/2024) and were performed in accordance with institutional guidelines for animal care and use.

Consent for publication

Not applicable.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

Figure legends

Figure 1. Control group

(A) Absence of IGF-1 immunostaining in the ovarian epithelial component (×400).
(B) Mild IGF-1 immunoreactivity in the stromal component (×100).
(C) Mild stromal IGF-1 staining (×200).
(D) Mild epithelial IGF-1 staining (×100).

Figure 2. ESWL Day 14 group

(A) Mild stromal IGF-1 staining (×400).
(B) Mild epithelial IGF-1 immunoreactivity (×100).

(C) Absence of stromal IGF-1 staining (×40).
(D) Mild epithelial IGF-1 staining (×200).

Figure 3. ESWL Day 28 group

(A) Mild epithelial IGF-1 staining (×400).
(B) Mild stromal IGF-1 immunoreactivity (×10).
(C) Mild stromal IGF-1 staining (×200).
(D) Mild IGF-1 staining in stromal and adipose tissues (×200).

References

Alić, J., Heljić, J., Hadžiosmanović, O., Kulovac, B., Lepara, Z., Spahović, H., et al. (2022). The efficiency of extracorporeal shock wave lithotripsy in the treatment of distal ureteral stones: An unjustly forgotten option? *Cureus, 14*, e28671. https://doi.org/10.7759/cureus.28671

Akin, Y., & Yucel, S. (2014). Long-term effects of pediatric extracorporeal shock wave lithotripsy on renal function. *Research and Reports in Urology, 6*, 21–25. https://doi.org/10.2147/RRU.S40965

Baba D, Cam, K., Senoglu, Y., Yuksel, A., Erdem, H., & Basaran, E. (2020). The efficacy of N-acetylcysteine against renal oxidative stress after extracorporeal shock wave treatment: An experimental rat model. *Journal of Urological Surgery, 7*(1), 8–15. https://doi.org/10.4274/jus.galenos.2019.2941

Barnes, P. J. (2014). Cellular and molecular mechanisms of chronic obstructive pulmonary disease. *Clinics in Chest Medicine, 35*(1), 71–86. https://doi.org/10.1016/j.ccm.2013.10.004

Gecit, I., Kavak, S., Oguz, E. K., Pirincci, N., Gunes, M., Kara, M., et al. (2014). Tissue damage in kidney, adrenal glands and diaphragm following extracorporeal shock wave lithotripsy. *Toxicology and Industrial Health, 30*(9), 845–850. https://doi.org/10.1177/0748233712462481

Hogg, J. C., Chu, F., Utokaparch, S., Woods, R., Elliott, W. M., Buzatu, L., et al. (2004). The nature of small-airway obstruction in chronic obstructive pulmonary disease. *The New England Journal of Medicine, 350*(26), 2645–2653. https://doi.org/10.1056/NEJMoa032158

Lv, F., Li, Z., Jing, Y., Sun, L., Li, Z., & Duan, H. (2023). The effects and underlying mechanisms of extracorporeal shock wave therapy on fracture healing. *Frontiers in Endocrinology, 14*, 1188297. https://doi.org/10.3389/fendo.2023.1188297

Piau, T. B., de Queiroz Rodrigues, A., & Paulini, F. (2023). Insulin-like growth factor performance in ovarian function and applications in reproductive biotechnologies. *Growth Hormone & IGF Research, 72–73*, 101561. https://doi.org/10.1016/j.ghir.2023.101561

Salcedo-Jiménez, R., Koenig, J. B., Lee, O. J., Gibson, T. W. G., Madan, P., & Koch, T. G. (2020). Extracorporeal shock wave therapy enhances the in vitro metabolic activity and differentiation of equine umbilical cord blood mesenchymal stromal cells. *Frontiers in Veterinary Science, 7*, 554306. https://doi.org/10.3389/fvets.2020.554306

Suhr, F., Delhasse, Y., Bungartz, G., Schmidt, A., Pfannkuche, K., & Bloch, W. (2013). Cell biological effects of mechanical stimulation generated by focused extracorporeal shock wave application on cultured human bone marrow stromal cells. *Stem Cell Research, 11*(3), 951–964. https://doi.org/10.1016/j.scr.2013.05.010

Zulu, V. C., Sawamukai, Y., Nakada, K., Kida, K., & Moriyoshi, M. (2002). Relationship among insulin-like growth factor-I, blood metabolites and postpartum ovarian function in dairy cows. *Journal of Veterinary Medical Science, 64*(10), 879–885. https://doi.org/10.1292/jvms.64.879

Table 1. Positivity rates of study groups according to epithelial, stromal and adipose tissue

		Epithelial		Stromal		Adipose tissue		*Total*		p
		n	%	n	%	n	%	*n*	*%*	
SHAM	Negative	4	80.0	2	40.0	2	40.0	*8*	*53.3*	0.322[b1]
	Positive	1	20.0	3	60.0	3	60.0	*7*	*46.7*	
TOES 14	Negative	6	75.0	7	87.5	7	87.5	*20*	*83.3*	0.751[b1]
	Positive	2	25.0	1	12.5	1	12.5	*4*	*16.7*	
TOES 28	Negative	6	75.0	8	100	5	62.5	*19*	*79.2*	0.083[b1]
	Positive	2	25.0	0	0.0	3	37.5	*5*	*20.8*	
p		0.973[b2]		**0.021**[b2]		0.184[b2]		*0.090*[a3]		

[a]: Pearson Chi-square

[b]: Likelihood ratio Chi-square

Table 2. Positivity rates of study groups according to epithelial, stromal and adipose tissue

		Epithelial		Stromal		Adipose tissue		*Total*		p
		n	%	n	%	n	%	*n*	*%*	
SHAM	Negative	4	80.0	2	40.0	2	40.0	*8*	*53.3*	0.322^{b1}
	Positive	1	20.0	3	60.0	3	60.0	*7*	*46.7*	
TOES 14	Negative	6	75.0	7	87.5	7	87.5	*20*	*83.3*	0.751^{b1}
	Positive	2	25.0	1	12.5	1	12.5	*4*	*16.7*	
TOES 28	Negative	6	75.0	8	100	5	62.5	*19*	*79.2*	0.083^{b1}
	Positive	2	25.0	0	0.0	3	37.5	*5*	*20.8*	
p		0.973^{b2}		**0.021^{b2}**		0.184^{b2}		*0.090^{a3}*		

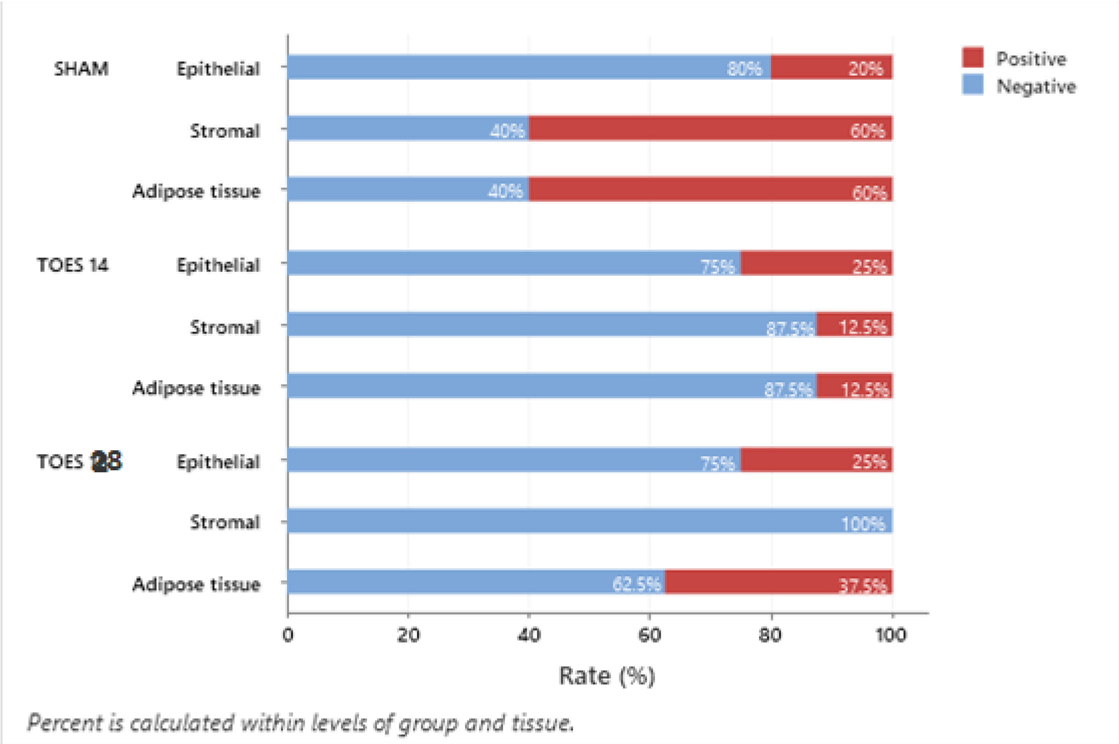
a: Pearson Chi-square; b: Likelihood ratio Chi-square

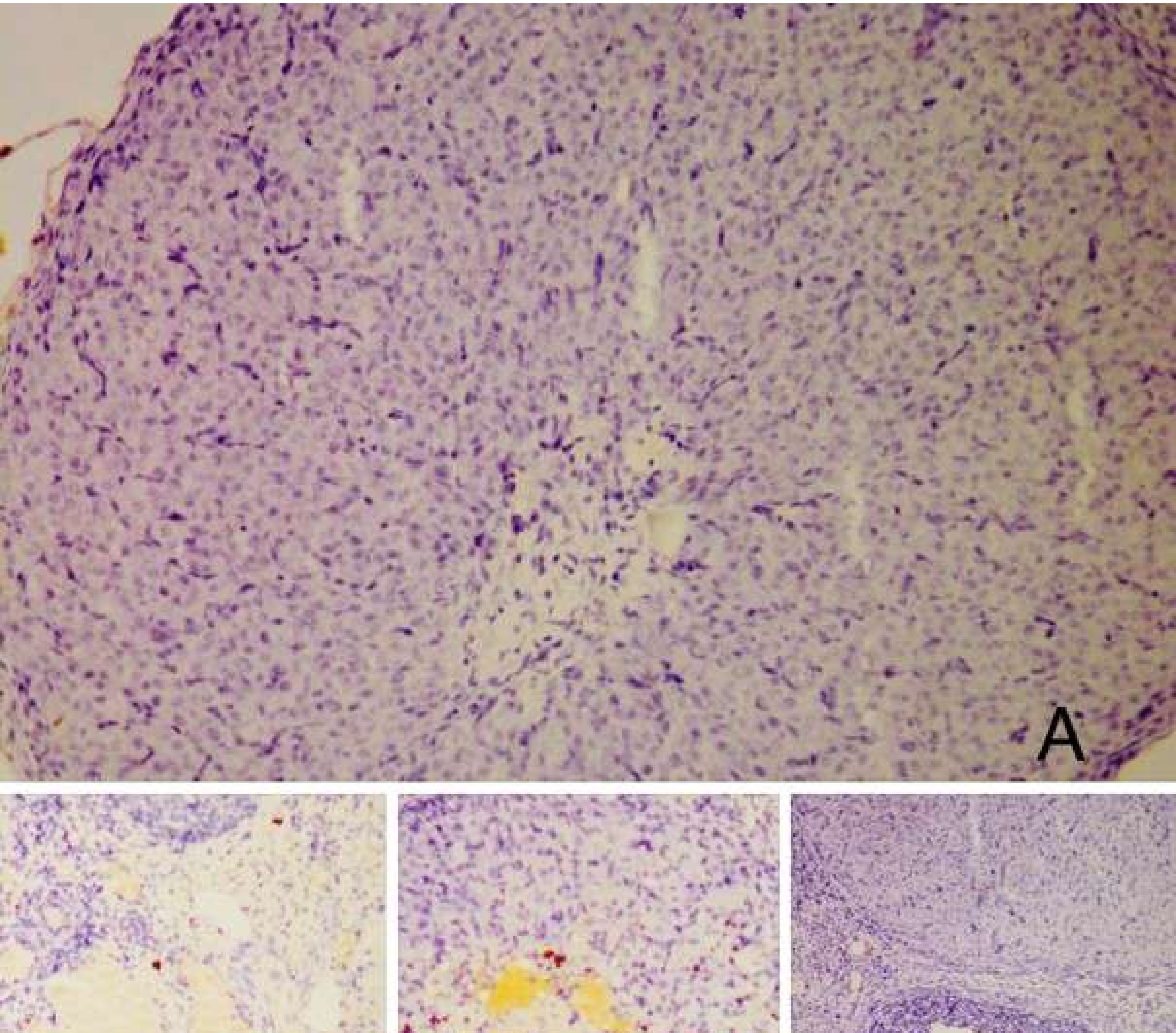
1: Regions (Epithelial, Stroma and Adipose Tissue) were compared in each group

2: Groups (SHAM, TOES 14 and TOES 28) compared in each region

3: Groups were compared without considering the regions (Epithelial, Stroma, Adipose Tissue)

Graph-1: Graphical evaluation of all results

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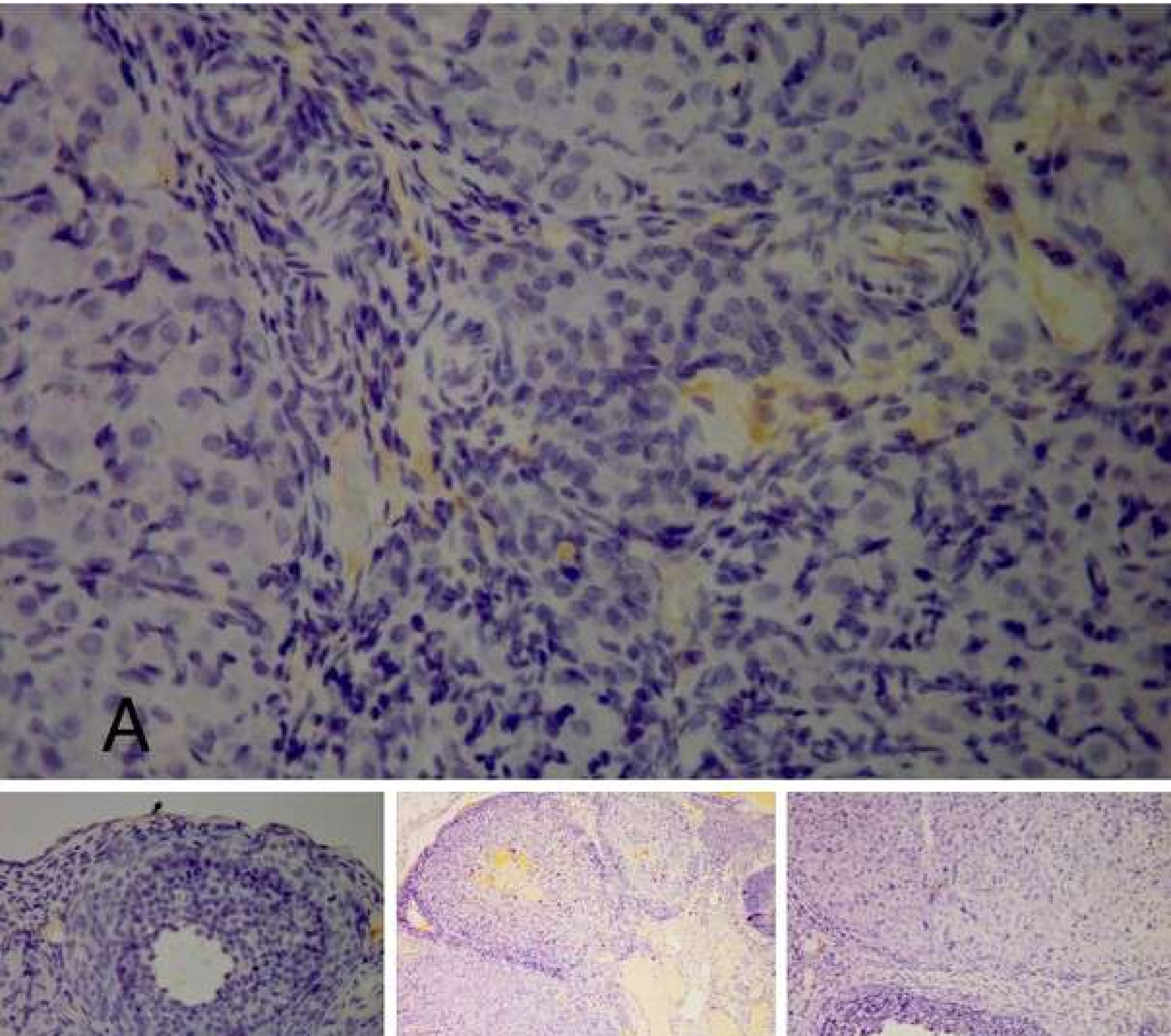
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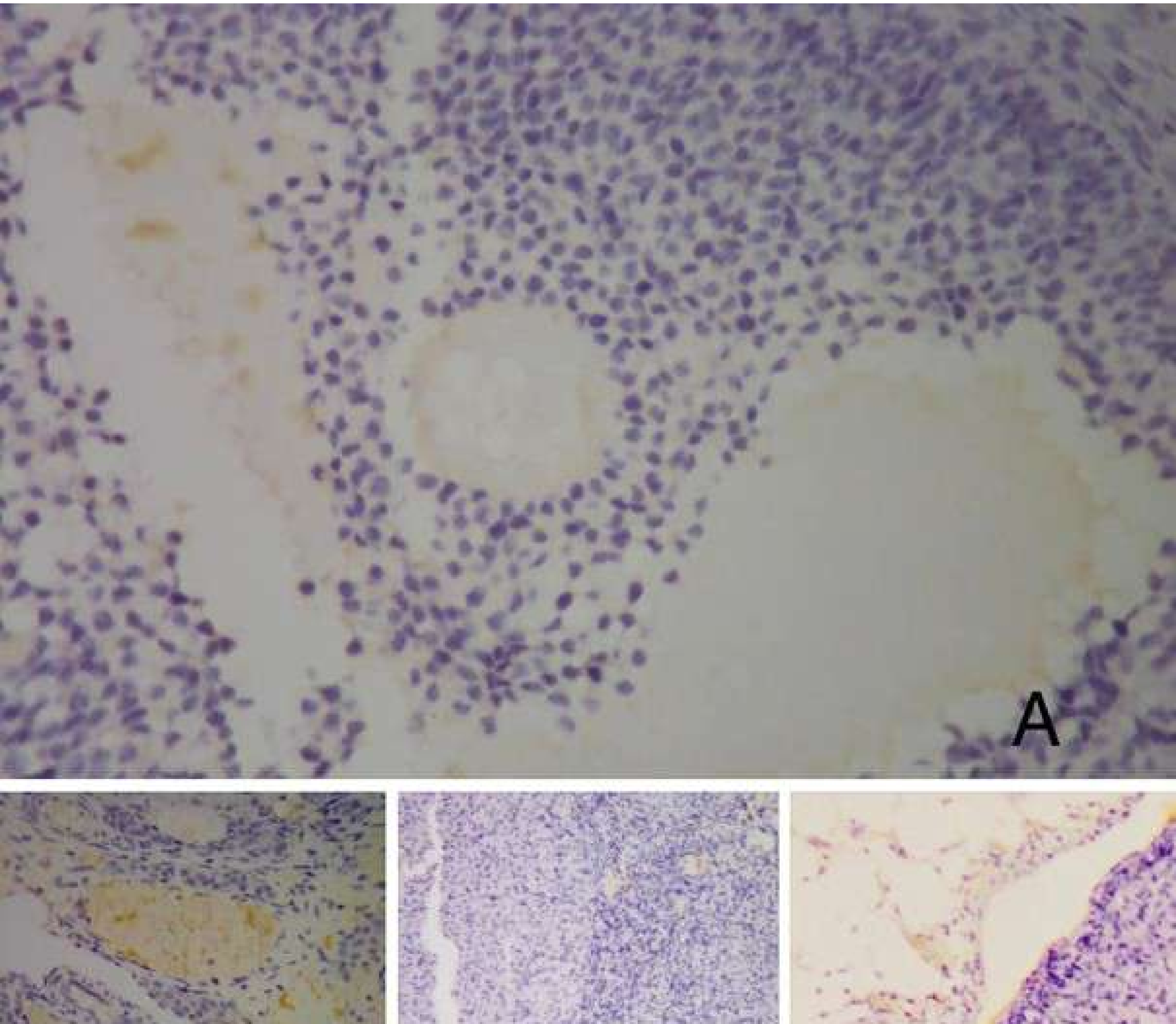
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