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Impact of intracystic ethanol instillation on ovarian cyst diameter and adjacent ovarian tissue

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ABSTRACT

Objective: To investigate the regression level of simple ovarian cyst size after local ethanol application and the damage level of adjacent ovarian reserve in rats.
Study design: This study was conducted at Firat University Animal Laboratory with 18 mature (12–14 weeks old) female Wistar albino rats weighing 200–220 g, with regular cycles. Ovarian cyst induction was performed with unilateral salpingectomy. Fourteen rats with ovarian cysts after a second laparotomy were divided into two groups as follows: Group 1 (n = 7): cyst aspiration group, and Group 2 (n = 7): intracystic 95% ethanol application group. One month after the cyst aspiration procedure a third laparotomy was performed. The cyst number and size were recorded for each rat. Right ovariectomy was performed and formalin-fixed/paraffin-embedded tissues were sectioned at 5 μm thickness. Under light microscopy, ovarian total follicle reserve and fibrosis were evaluated with Masson trichrome staining and apoptosis was evaluated with TUNEL staining. The groups were compared with the Mann–Whitney U test and Wilcoxon Rank test. $p < 0.05$ was considered significant.
Results: Ovarian cyst formation was observed in 85% (15/18) of rats. The mean diameter of ovarian cysts in Groups 1 and 2 were, respectively, 10.3 mm and 10.1 mm. After aspiration, there was no significant reduction in the cyst diameter (10.3 mm vs 8.1 mm), but after ethanol application the diameter significantly reduced (10.1 mm vs 3.4 mm, $p < 0.05$). Mean ovarian follicle count in Group 2 was significantly lower than in Group 1 (25 vs 42, $p < 0.05$), and mean fibrosis and apoptosis scores in Group 2 were significantly higher than in Group 1 (2.5 vs 0.9, $p < 0.05$).
Conclusion: Local ethanol application reduces cyst diameter but concomitantly decreases ovarian reserve due to increased fibrosis in rats. In humans, intracystic ethanol application should be performed cautiously.

1. Introduction

The incidence of adnexal masses among healthy women has been reported as 6%, of which 90% were cystic tumors, and the common form of these tumors was simple cysts [1]. Either laparoscopy or laparotomy can be performed for a cystectomy procedure, but on the other hand, ovarian cyst aspiration under ultrasound guidance could be the conservative treatment choice [2,3]. The aspiration procedure may bring the following complications: high recurrence, infection and lack of available histology [3–7]. As an option for treating benign cysts arising from various organs, e.g. the thyroid [8], parathyroid [9], liver [10], kidney [11], spleen [12] and heart [13], ethanol sclerotherapy has been shown to be efficacious and cost-effective. Where the cyst volume has exceeded 100 ml, surgical treatment by laparoscopy or laparotomy has been advised [14]. Ethanol application on the epithelial surface of the cyst capsule induces sclerosis by reactional fibrosis due to coagulation necrosis [15,16]. In this study we aimed to investigate the effects of intracystic ethanol application on ovarian cyst diameter, ovarian follicular reserve and apoptosis in an experimental model.

2. Materials and methods

This study was approved by Firat University Animal Use Committee and conducted at Firat University Animal Laboratory (FUTDAM). Twenty mature (12–14-week-old) female Wistar

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Albino rats, weighing 200–220 g, with regular cycles were used for all experiments. (The study population number was determined by the Animal Use Committee.) The rats were housed individually in groups of five in plastic cages with chip bedding, and ad libitum access to rat chow (pellet) and water. They were maintained on a 12:12 light:dark cycle (lights on at 07:00 AM) at room temperature.

Before surgical intervention, oral feeding except water intake was stopped. The rats on estrus cycle detected by vaginal smear were operated under intramuscular 60 mg/kg ketamine (Ketalar, Eczacibasi, Warner–Lambert, Istanbul, Turkey) and 7 mg/kg xylazine (Rompun, Bayer, Istanbul, Turkey) anesthesia and povidine–iodine surgical site antisepsis. After midline laparotomy, right total salpingectomy was performed for induction of ovarian cysts and then abdominal closure was done with 3/0 silk sutures. One month after first surgery, a second laparotomy was performed and a macroscopic ovarian cyst was observed in 15 rats (75%). The remaining five non-cystic rats were excluded from the study and one cystic rat was also excluded to equate the population number of groups. The 14 rats with ovarian cysts were randomly divided into two groups as follows; Group 1 (n = 7): the ovarian cyst was aspirated with an insulin injector and then the abdomen was closed. Group 2 (n = 7): the ovarian cyst was aspirated with an insulin injector and then the cystic cavity was irrigated with 95% ethanol equal to half of the aspirated cyst volume. After 10 min retention, the ethanol was aspirated and the abdomen was closed. One month after the second laparotomy, the 14 rats were decapitated after ketamine (75 mg/kg) + xylazine (10 mg/kg) intraperitoneal administration and exploratory laparotomy was performed. The presence and diameter of the ovarian cyst was recorded for each rat and then right oophorectomy was performed.

2.1. Histologic evaluation

After oophorectomy, the adherent tissues were removed in culture medium, ovarian tissues were fixed with 10% formaldehyde and then paraffin-embedded tissue samples were cut into 4 μm sections for estimation of mean ovarian follicle count. The sections were stained with Masson trichrome to determine ovarian follicle reserve, fibrosis and angiogenesis in the corpus luteum under light microscopy. The 4 μm step sections were mounted at 50 μm intervals onto microscope slides to prevent counting the same structure twice, according to the method described previously [17]. Follicles were classified as primordial, primary, secondary, and tertiary follicles. The definitions of follicle types were as follows: primordial follicle = oocyte partially or completely surrounded by flattened pregranulosa cells; primary follicle = enlarged oocyte with one layer of cuboidal cells, or at least one cuboidal cell among flattened granulosa cells; secondary follicle = two layers of granulosa cells or at least one layer and one cell of the second layer; tertiary follicle = oocyte enclosed by more than two layers of granulosa cells with antrum formation. An atretic follicle was defined as a follicle that presented more than ten pyknotic nuclei per follicle; in the smallest follicles, the criterion for atresia was a degenerate oocyte, precocious antrum formation, or both [18–20]. The scoring for fibrosis was applied as follows: 0 = none, +1 = mild, +2 = moderate, +3 = intense [21,22]. Angiogenesis in the corpus luteum was defined according to previous studies and $H\text{-SCORE} = \sum P_i(i+1)$, where i = intensity of staining with a value of (±, minimal), (+, mild), (++, moderate) and (+++, strong) was applied [23,24].

To detect cells undergoing apoptosis, we applied the TUNEL method. We used the technique of Terminal-Transferase dUTP Nick End labeling (TUNEL Apoptag plus peroxidase in situ Apoptosis detection kit, S7101, Chemicon, USA). Sections were

Table 1
Histopathological findings of right ovary in all rats.

Parameters	Group 1 (n = 7)	Group 2 (n = 7)	p value
Primordial follicle count	17.1 ± 2.9	7.0 ± 3.3	*
Primary follicle count	13.2 ± 1.8	9.7 ± 4.3	*
Secondary follicle count	9.0 ± 1.6	6.0 ± 2.7	Ns
Tertiary follicle count	3.1 ± 0.7	2.5 ± 0.8	Ns
Ovarian follicle reserve	42.5 ± 6.5	25.2 ± 10.9	*
Corpus luteum count	1 ± 0.6	0.8 ± 0.4	Ns
Corpus albicans count	0 ± 0	0 ± 0	Ns
Angiogenesis score in corpus luteum	1.8 ± 0.4	0.6 ± 0.2	*
Fibrosis score	0.9 ± 0.2	2.5 ± 0.5	*
Apoptosis score	0.4 ± 0.5	2.3 ± 0.7	*

Note: Values are presented as mean ± SD.
*$p < 0.05$, Mann–Whitney U test; Ns = non-significant.

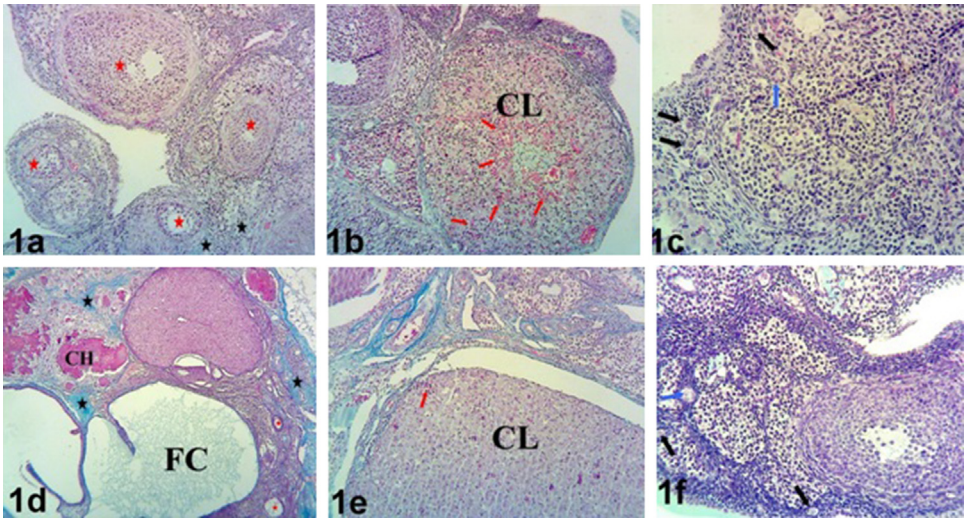

Fig. 1. Masson trichrome staining under light microscope. Normal ovarian tissue of cyst aspiration group (1a, 1b, 1c). Decrement in ovarian follicle reserve, increment in fibrosis and decreased angiogenesis in corpus luteum of ethanol instillation group (1d, 1e, 1f). Red star, different types of follicles; black star, fibrosis areas; red arrow, angiogenesis; black arrow, primordial follicle; blue arrow, primary follicle; CL, corpus luteum; CH, corpus hemorrhagicum; FC, follicle cyst. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)