ORIGINAL ARTICLE

A new ovarian denervation technique and it's effect on sexual cycle, conception rates and offspring numbers in rats

Ali Risvanli [1], Huseyin Timurkan [1], M. Faik Ozveren[2] & Cahit Kalkan [1]
[1] Deparment of Obstetric and Gynecology, Faculty of Veterinary, University of Firat, Elazig, TURKEY.
[2] Deparment of Neurosurgery, Faculty of Medicine University of Firat, Elazig, TURKEY.

Correspondence to: Ali Risvanli
Deparment of Obstetric and Gynecology,
Faculty of Veterinary, University of Firat,
Elazig 23159, TURKEY
TEL: + 90 424 237 00 00/ 6169
FAX: + 90 424 238 81 73
EMAIL: arisvanli@yahoo.com

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Abstract

OBJECTIVES: A novel denervation technique of ovarian was used in rats to investigate its effects on sexual cycle, pregnancy rates and offspring numbers.
SETTING AND DESIGN: A total of 34 female albino rats were included. Animals were divided into 3 groups.
METHODS: In group 1, ovaries were bilaterally denervated. Animals in group 2 underwent sham operation and group 3 comprised of controls. Sexual cycles in animals were followed by vaginal irrigations. Gestations were determined with parturition of rats.
RESULTS: Results indicated that in denervated rats, frequency of estrus decreased and period of estrus increased resulting in a decrease in conception rates and offspring numbers when compared to control rats.
MAIN FINDINGS: Although the results were consistent with previous denervation techniques, the operational procedure described herein is simpler and requires no sophisticated equipment, suggesting this to be a method of choice in reproductive physiology studies.
CONCLUSION: In this study, we denervated the ovary with a technique other than classical in which the tissues except vascular structures over ovarian suspensory ligament were excised. We found that, estrus count decreased, duration of sexual cycle increased, conception rates and the offspring numbers reduced. This is thought to be a result of blockage of ovarian neural control due to denervation.

Abbreviations
SON: Superior Ovarian Nerve
HE: Hemotoxylene Eosine

1. Introduction

Ovarian maturation and functions in female animals are regulated directly by nerve, especially by extrinsic innervation. Ovarian follicles are innervated with sympathetic and sensory nerve fibers. The neurotransmitters like norepinephrine, which is found in ovarian nerves, play a major role in steroidogenesis. Elimination of sympathetic innervation was reported to reduce follicular development, to block the effects of gonadotrophins on steroids, to delay puberty and to lead the ovaries into the atrophy [1–6].

The rat ovary receives innervation from three main sources: the ovarian plexus nerve that travels along the ovarian artery, the SON that is associated with suspensory ligament and, nerves vagus [7, 8]. The neural control of ovary is mediated in greater percentages by SON. The SON fibers innervating thecal and interstitial cells. Other cells such as luteal and granulosa ovarian cells, in spite of not being directly innervated. The effects of the SON on the ovarian cell steroidogenesis has been demonstrated using electrical stimulation or transection and guanethidine applications which is an adrenergic blocker that capable of degenerating sympathetic nerves [9–12]. The ovarian plexus associated with the vascular ovarian system [13, 14].

Laboratory rats are polyestrous all year. The estrus cycle averages 4 to 5 days. Proestrus, estrus, metaestrus and diestrus periods in these animals take 12, 12, 21 and 57 hours respectively. Ovulation occurs 8–11 hours after the beginning of estrus and the length of gestation is 21 to 23 days [15–19].

As for other animals, determination of cytological alterations in vagina can be used as a marker of sexual cycle and the prediction of pregnancy in rats. For this purpose, smear and vaginal irrigation techniques were used. Giemsa, Methylen blue, Papanicolau and Toluidine blue staining were used. Different types of cells are encountered depending on the stage of sexual cycle in vaginal smear. In estrus period, a decrease in superficial cells with nucleus and increase keratinized superficial cell count and crystallization and no leukocytes have been reported [18, 19].

Objective of the present study was to develop a new denervation procedure of ovarian nerves in rats that is different from previously reported techniques and to investigate its effects on neural control of ovarian functions.

2. Materials and Methods

2.1. Animals

A total of 34 female albino rats that were 3 months old, weighing 200–250 g were used. During the study, the rats were kept in consisting of 4–5 rats. Animals had free access to food and water. They were housed in cages under controlled circumstances (lights on from $07.^{00}$ to $19.^{00}$ h) and temperature (24 ± 2 °C). The rats divided into 3 groups: group 1 (n=14) were exposed ovarian denervation bilaterally, group 2 (n=10) as sham operation group and group 3 (n=10) served as controls.

2.2. Ovarian Denervation Procedures

Animals underwent an operation by the technique other than described by Lawrence and Burden [7] under xysilasine and ketamine anaesthesia. Bilateral dorsal incisions were done over the kidneys in anaesthetized rats. Suspensory ligament distal to the kidney was found and then all the tissues around ovarian artery were dissected by the aid of a stereoscopic microscope and all of tissues except ovarian vasculature were transected. The same procedure was repeated on contralateral side (Figure 1).

2.3. Sexual Cycle and Conception

Postoperatively at one week, all of animals underwent daily vaginal irrigations. Smears were stained with HE. Sexual cycle of animals were followed up for 1 month. The density of cell types were regarded +, ++, +++. When superficial cells at +++ density and crystallization was encountered, animals were considered as in estrus period [18]. At the end of 1 month, total estrus numbers were recorded and then male rats were placed for 12 hours into the cages of the female animals in which estrus was recorded.

Gestations were determined with parturition of rats. The offspring numbers were recorded.

2.4. Histopathological Examination and The Demonstration of Ovarian Artery

For this purpose, four female rats other than the test group were used. Animals underwent xysilasine and ketamine anaesthesia and cannulated via cardiac left ventricule and right atrium and evacuated their bloods entirely. First, wash-out with physiological saline 3–4 times was performed and then given latex by colored with red. Later, ovarian artery was photographed. At the end of the procedure, ovaries and suspensory ligament were excised and placed into formaldehyde of 10% solution and prepared histopathological slides. Nerves structures over the ligament was demonstrated (Figure 2).

The statistical analysis of the results were made by SPSS for Windows [20].

3. Results

Procedures were resulted to lead decrease in estrus count, increase duration of sexual cycle and reduce in conception rates and the offspring number (Table 1).

4. Discussion

There are several factors that effect the sexual cycle and conception rates. However, these has been no clear understanding about mechanisms. Studies on ovarian dysfunction mainly relate endocrinological aspects;

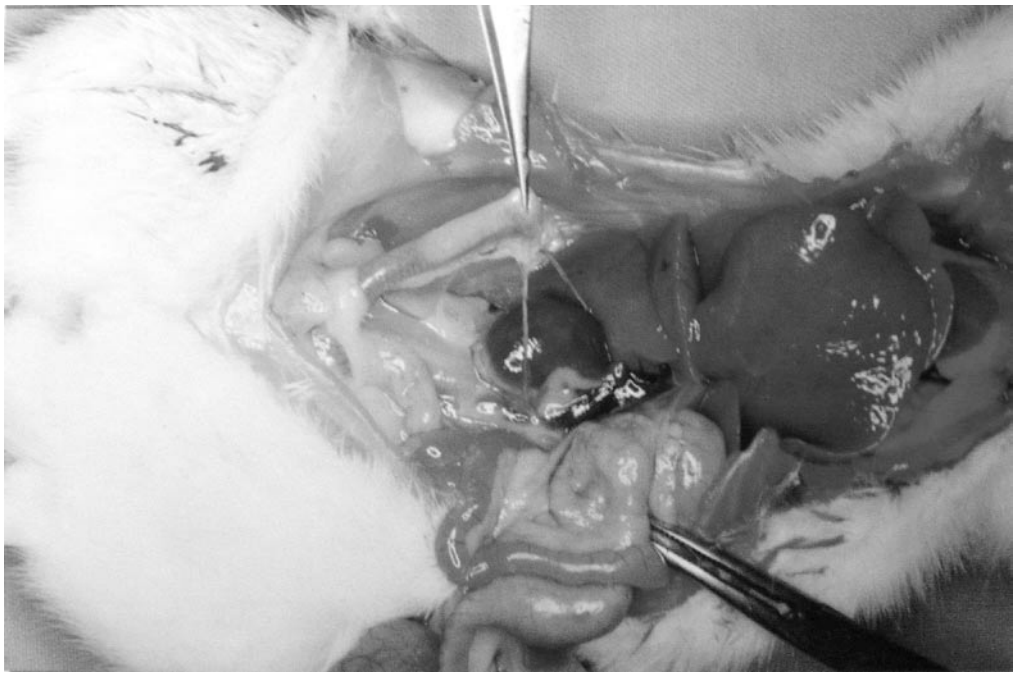

Figure 1. All of tissues except ovarian vasculature were transected

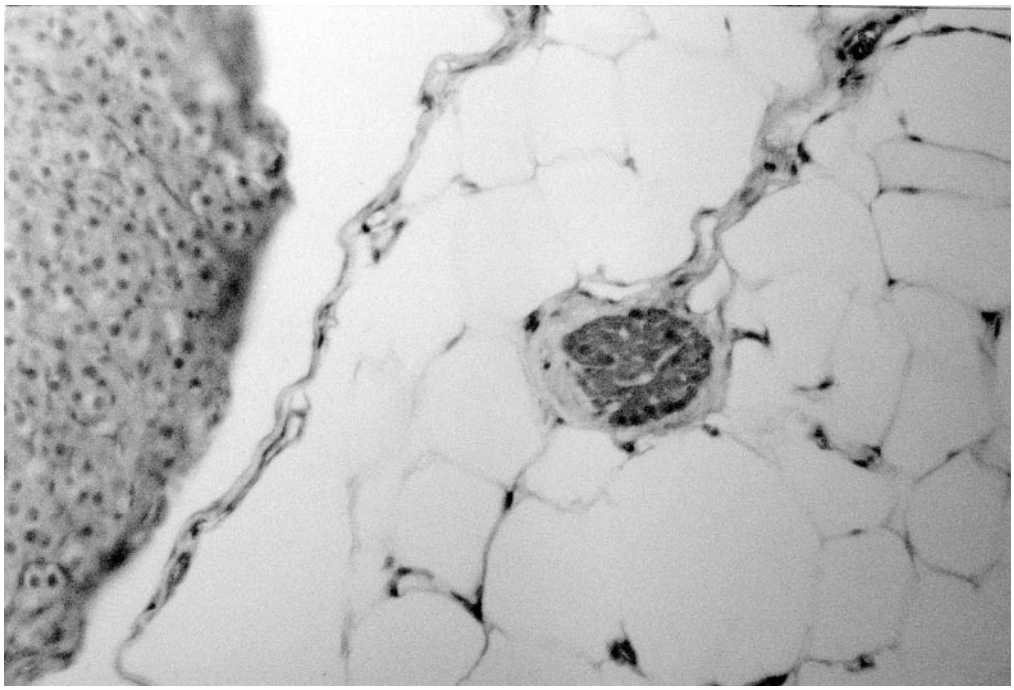

Figure 2. Nerves structures over the ligament and ovarian tissue HE x 80.

Table 1: Effects of ovarian denervation on sexual cycle, conception rates and offspring numbers in rats.

Data	Group 1 (n=14)	Group 2 (n=10)	Group 3 (n=10)	P
Estrus count	4.7±1.10[a]	5.6±0.75[b]	6.6±1.05[c]	*
Duration of sexual cycle (Day)	6.2±1.44[a]	5.7±0.45[b]	4.5±0.92[c]	*
Percentage of animals having parturation (%)	42.86[a]	60.00[b]	70.00[c]	*
Mean offspring numbers	5.4±2.27[a]	8.2±1.45[b]	7.6±0.87[b]	**

*The difference between the group percentages is significant (P<0.01).
** The difference between the group percentages is significant (P<0.05).
a, b, c: The difference between values shown with different letters in the same column is important.

but, there have been no sufficient studies regarding efficiency of neural control. However, it has been well known that, nerves, especially ovarian ones have dramatic effects on sexual cycle. This effect is particularly evident in synthesis, secretion of steroidal hormones and the maturation of follicles [21–25]. Because, nerve fibers like SON innervate terminate in hormone producing cells like theca intestina [26]. Besides, ovarian denervation was reported to reduce significantly 3 B-hydroxysteroiddehydrogenase activity in corpus luteum [5, 8]. However Riboni [21] reported that ovarian innervation played major role in follicular maturation and atresia but had no effects on steroid production.

Forneris and Aguado [27] a study about the relationship effects of the gonadotropins on ovarian activity and SON reported that, bilaterally transection of this nerve had no role on the gonadotrophin stimulation of ovulation.

Myriam et al [28] reported transection of SON in earlier ages in rats played major role in ovarian maturation in cyclic and prepubertal rats.

In this study, we denervated the ovary with a technique other than classical ways in which the tissues except vascular structures over ovarian suspensory ligament were excised. We found that, estrus count decreased, duration of sexual cycle increased, conception rates and the offspring numbers reduced. This is thought to be a result of blockage of ovarian neural control due to denervation.

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