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Original Article

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Comparison of Transverse and Vertical Incision Techniques in Ventral Approach for Bilateral Ovariectomy in Rats

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Abstract

Objective: Ventral and dorsal surgical approaches are widely used for bilateral ovariectomy in animal experiments. The outcome of the surgery depends largely on the approach and incision used for conducting surgery. The present study compared the transverse and vertical incisions in the ventral approach with regard to the duration of the procedure, accessibility for the surgeon, number of sutures required, and time taken for wound healing and post-surgical mortality in rats. ***Design:*** A prospective study, conducted on 30 female wistar rats, at SGRRIM & HS, Dehradun for 6 months after approval from Institutional Animal Ethics Committee. Rats were randomly divided into 2 groups of 15 rats each. In Group A, rats underwent bilateral ovariectomy using transverse incision in ventral approach and in Group B, using vertical incision in ventral approach. Ketamine (80 mg/kg) and xylazine (10 mg/kg) were used intraperitoneally for anaesthesia. Comparison of surgical and post-surgical outcomes was done using Student T-test and Mann-Whitney U Test. The p-value < 0.05 was statistically significant. ***Results:*** The duration of surgical procedure in Group A was 15 ± 2.48 minutes and Group B was 29.47 ± 3.54 (p-value- 0.001), size of the incision in Group A was 1.35 ± 0.22 cm and Group B was 2.57 ± 0.40 (p-value- 0.001), number of sutures in Group A were 3.73 ± 0.80 and in Group B were 4.87 ± 0.92 (p-value- 0.003) and wound healing time in Group A was 2.87 ± 0.83 days and in Group B was 5.40 ± 0.83 days (p-value- 0.001). ***Conclusion:*** Study indicated that surgical and post-surgical outcomes by transverse incision for bilateral ovariectomy were significantly better than those by vertical incision. Further studies using a larger number of animals are needed to determine better incision in ventral approach.

Keywords: ***Bilateral ovariectomy, transverse incision, ventral approach, vertical incision.***

Introduction

Rats (Rattus norvegicus) are commonly used laboratory animals in experimental studies including pharmacology, pathology, biological assay and nutritional as well as behavioural research due to the structural and functional similarity of most of the organs and tissues to those of humans. They are inexpensive to maintain, can grow rapidly, have a relatively short life span and are widely available [1-3]. Experimental animal models play a vital role in improving the knowledge of the aetiology, pathophysiology, diagnosis, and preventive and therapeutic techniques regarding the disease it is being used for. Choice of approach to conducting surgery is also important to save on time, minimum handling of tissues and quick location of ovaries and largely influence the outcome by determining the post-operative healing time, contamination of the wound, loss of blood and factors about the surgeon including feasibility and ease of conducting surgery [4,5]. Ovariectomy in female rats can be performed in different ways and the selection of the operative method for ovariectomy is very important, especially when the number of animals is very high and the duration of the experiment is short. The various approaches for ovariectomy in female rats are single midline dorsal skin incisions, double dorsolateral incisions, transverse ventral incisions and vertical ventral incisions. In the present study, we have compared transverse and vertical incisions using the ventral approach for ovariectomy in rats for inducing osteoporosis in female rats [5,6].

Methods

The prospective study was conducted at SGRRIM & HS, Dehradun for 6 months from 1st July 2023 to 1st January 2024 following the approval from the Institutional Animal Ethics Committee. The procedures followed were by the ethical standards of the responsible committee on human experimentation (institutional and national) or regulations for laboratory animals. The current study was conducted as a part of the project titled, 'Evaluation of probiotic bacillus isolated from fermented soybean for anti-osteoporotic attributes

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against postmenopausal osteoporosis, conducted in the institution. The surgical procedure of bilateral ovary removal in rats was done to procure animal models for conducting a study on postmenopausal osteoporosis.

30 Wistar albino rats, housed and cared for in the Institutional Animal House were procured and kept in separate cages in pathogen-free rooms of the institutional animal house under standardized living conditions at a temperature of 25 °C ± 2 ºC. Female rats aged 12-14 weeks and weighing an average of 250-300 g were selected for the study. All the necessary animal handling and housing guidelines were followed. Rats were randomly divided into 2 groups; Group A and Group B comprising 15 rats each. In Group A, rats were subjected to bilateral ovariectomy using the transverse incision by ventral approach and in Group B, bilateral ovariectomy was performed using vertical incision by ventral approach. Animals were acclimatized for 1 week before the surgical procedure. Animals were anesthetised using the general anaesthetic agents; Ketamine (80 mg/kg) and Xylazine (10 mg/kg) by intraperitoneal route (i.p). General anaesthesia was considered achieved once the animal stopped responding to the pedal withdrawal reflex caused by firm pressure on the hind paws or pinch on the ear or the skin between digits [5,7].

Appropriate aseptic measures were taken to maintain the sterility of the surgical procedure. Each rat was weighed using a digital weighing machine and anaesthesia was then induced on a separate workstation. The rats were placed in a dorsally recumbent position and were then shaved using an easy glide blade and sterile drapes were laid down on the rat. The surgical field was disinfected using Betadine solution and spirit following all the aseptic precautions. The scalpel and back of 23 number blade were used to mark the area in the surgical field where the incision was to be made. The same blade was used to make the incision thereafter. Following the incision, the muscles were then retracted and the adipose tissue was then pulled away making it easy to identify the uterine tube with ovary. The ovary and associated ovarian fat pad were gently retracted using sterile blunt forceps, following which through the same incision, the other ovary was identified and withdrawn similarly. Ligature and severing of the ovary, ovarian fat pad, oviduct and part of the uterus were performed as discussed above. The remaining part of the uterus is inserted back into the abdominal cavity and the incisions in the muscle and the skin were sutured in two layers using chromic catgut, using a swaged needle.

Transverse incision ventral approach: An incision of approximately 0.7-1 cm was made, in the middle part of the abdomen and slightly to the right, adjacent to the cranial inguinal nipple [5,8].

Vertical incision ventral approach: An incision of approximately 2-3 cm was made on the ventral midline at the caudal edge of the ribcage, behind the lumbar vertebrae [8,9].

Povidone Iodine was applied to the area to disinfect the area after suturing. The high degree of aseptic procedure was maintained throughout the operation. After surgery, the rats were housed individually in cages to allow recovery.

Results

An analysis of the following surgical and postsurgical outcomes was conducted in the study groups:

Surgical outcomes:

1. Duration of surgery- Duration of surgery refers to the time from weighing the rat and inducing anaesthesia to completion of the surgical procedure and suturing the rats.
2. Number of sutures required
3. Incision size
4. Accessibility for the surgeon
5. Blood loss during surgery

Post-surgical outcomes:

1. Wound healing time
2. Mortality post-surgery

In Group A, accessibility to the surgical site was better as compared to Group B. Blood loss during surgery was more in Group B as compared to Group A. No post-surgical mortality was seen in both the groups till 10 days post-surgery.

Data is described in terms of mean ± standard deviation (± SD) and p-value. Comparison of quantitative variables between the study groups was done using the student t-test and Mann-Whitney U test for independent samples for parametric and non-parametric data respectively. For the normally distributed data, the student T-test was applied and other than that Mann Whitney U test was applied. A probability value (p-value) less than 0.05 was considered statistically significant. All statistical calculations were done using (Statistical Package for the Social Science) SPSS 21.0 version (SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows.

Comparative evaluation of surgical outcomes was performed and it was found that the duration of surgery was recorded for each case in both the groups and the outcome in terms of mean ± standard deviation in Group A was 15 ± 2.48 minutes and in Group B was 29.47 ± 3.54 minutes. The average size of incision in Group A was 1.35 ± 0.22 and in Group B was 2.57 ± 0.40. The number of sutures required was significantly less in Group A than in Group B. Blood loss during surgery was more in Group B. The average time required for wound healing was 2.87 ± 0.83 in Group A and 5.40 ± 0.83 in Group B. On statistically analyzing the outcomes of duration of surgery, size of incision, number of sutures and wound healing time it was seen that there is a statistically significant difference observed between the two groups.

Table 1: Results of surgical and non-surgical outcomes in Group A (n=15)

Group A	Duration of Surgery (in minutes)	Size of incision (in cm)	No. Of sutures required	Wound Healing Time (in days)
Rat 1	15	1.3	3	2
Rat 2	18	0.8	3	2
Rat 3	16	1.5	5	3
Rat 4	15	1.4	4	2
Rat 5	19	1.4	4	3
Rat 6	14	1.6	5	3
Rat 7	12	1.2	3	3
Rat 8	16	1.5	4	4

Rat 9	18	1.6	4	4
Rat 10	13	1.1	3	2
Rat 11	12	1.5	4	4
Rat 12	15	1.3	3	2
Rat 13	18	1.6	5	4
Rat 14	12	1.3	3	3
Rat 15	12	1.2	3	2

Table 2: Results of surgical and non-surgical outcomes in Group B (n=15)

Group B	Duration of procedure (in minutes)	Size of incision (in cm)	No. of sutures required	Wound Healing Time (in days)
Rat 1	28	2	4	5
Rat 2	25	2.2	4	5
Rat 3	32	2.9	5	6
Rat 4	30	2.8	5	6
Rat 5	26	2.1	4	4
Rat 6	25	1.9	3	4
Rat 7	34	2.8	5	6
Rat 8	35	2.9	5	6
Rat 9	32	3	6	6
Rat 10	30	2.5	5	5
Rat 11	29	2.6	5	6
Rat 12	29	2.8	6	6
Rat 13	33	3	6	6
Rat 14	23	2.1	4	4
Rat 15	31	2.9	6	6

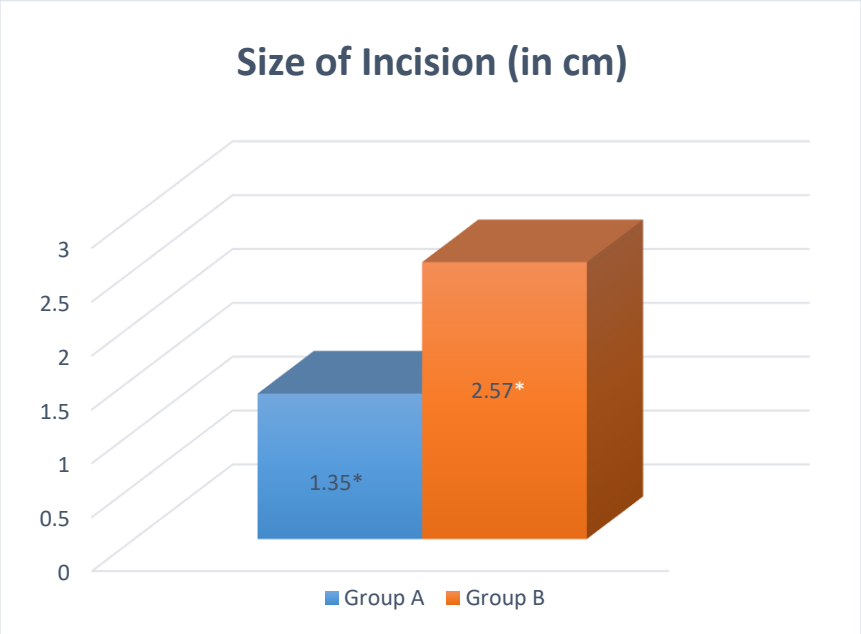
Table 3: Comparative analysis of parameters in Group A and Group B.

Parameters	Group A n=15 (Mean ± S.D)	Group B n=15 (Mean ± S.D)	p- Value
Duration of surgery (in Minutes) *	15 ± 2.48	29.47 ± 3.54	0.001
Size of incision (in cm) **	1.35 ± 0.22	2.57 ± 0.40	0.001
No. of sutures required**	3.73 ± 0.80	4.87 ± 0.92	0.003
Wound Healing Time (In Days) **	2.87 ± 0.83	5.40 ± 0.83	0.001

*- Student- T Test was used for analysis.

**- Mann Whitney U test was used for analysis.

P-value <0.05 was considered statistically significant.



*Data is represented as Mean.

Figure 1: Number of sutures required in each group.

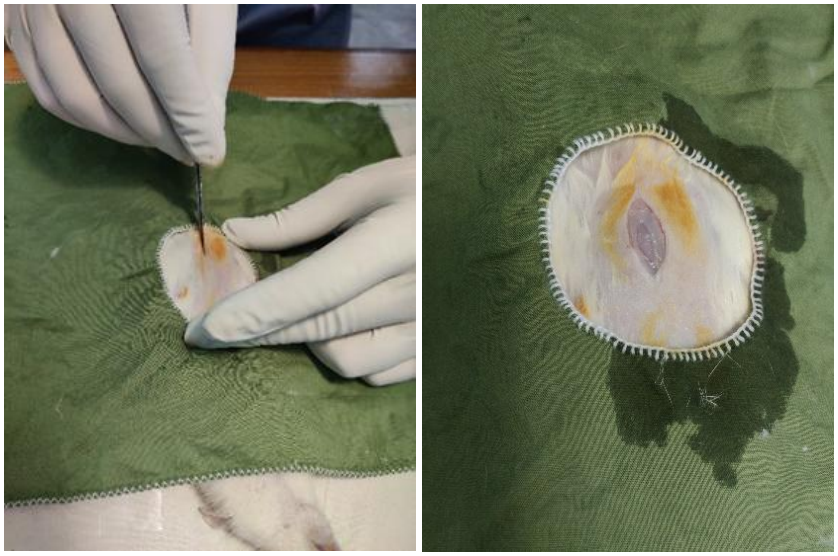


Figure 2: Vertical Incision: Ventral Approach

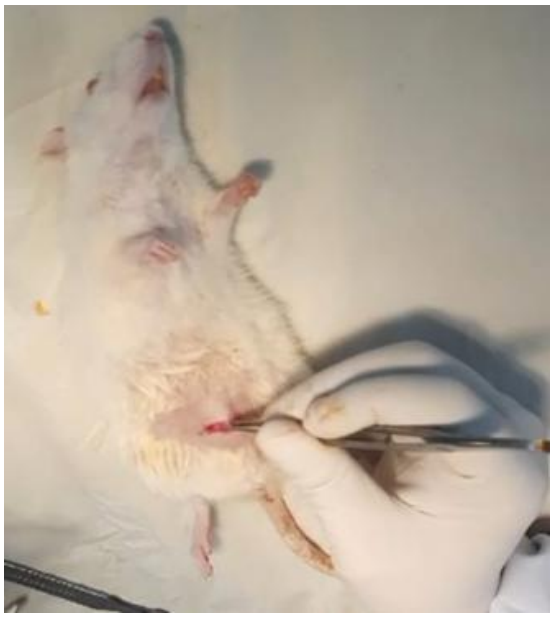


Figure 3: Transverse Incision: Ventral Approach

Discussion

The present study was conducted to compare transverse and vertical incisions using the ventral approach for performing bilateral ovariectomy in female rats. Through the current study, we wanted to establish the differences amongst the ventral approach of bilateral ovariectomy using the transverse and vertical incisions depending on the parameters of importance. For conducting the surgery in animals one of the challenges faced by the surgeons is the exposure to the operative field due to the small size of the animal. To ensure minimal pain to the animal, damage to tissues and ease of the animal, minimisation of surgical duration and limited handling of internal structures should be targeted. The size of the incision in our study in group A was 0.8 to 1.6 cm whereas it was 0.4 to 0.6 cm long in the study conducted by Khajuria *et al.* This could be due to the size of the animal in their study [5]. The size of the incision in our study in Group B was 1.9 to 3 cm which is significantly more than the incision size in Group A. Various studies have highlighted the many advantages of transverse incisions for abdominal surgery like, better cosmetic results, less pain and low incidence of hernia formation [5].

The wound healing time in Group A of our study was significantly less than Group B which is comparable to the study conducted by Khajuria et al, Grantcharov and Sarkar *et al.* [3,5,-10]. This could be due to the smaller size of the incision in Group A. Other studies conducted time and again also insisted that transverse incisions are both less time-consuming and suggest a shorter duration of wound healing [5,11]. As mentioned in a study conducted by Sophocleous *et al.* access to the abdomen is excellent with ventral midline incisions [8,12]. Similarly, in our study also accessibility was more in a ventral vertical incision in comparison with the ventral transverse incision. Duration of surgery was less than 10 minutes in our study as comparable to studies conducted by Saadat *et al.*, Khajuria *et al.*, Sankar *et al.*, Popović *et al.* and Rigalli *et al.* [3,11,13-15]. In this study while comparing the two approaches we extracted that the duration of surgery, size of incision, number of sutures and wound healing time was significantly more in Group B. It was also seen that the accessibility for the surgeon was more in Group A and there was more loss of blood in Group B. Due to the smaller size of the incision, a lesser number of sutures were required in Group A. Whereas, there was more loss of blood during the surgery in Group B, no mortality was seen up to 10 days post-surgery in both groups. The results obtained in this study suggest the importance of the size of the incision, which in turn determines the number of sutures required and also seems to affect the duration required for wound healing.

The present study concluded on the above-mentioned key points, but there were many limitations. The study was conducted at a single centre among a limited number of rats. Hence, it won't be appropriate to establish the results achieved, on bilateral ovariectomy being conducted worldwide. Hence, it is suggested that further detailed elucidation on the usage of both incisions in the ventral approach should be performed for a clearer assessment of outcomes.

Conclusion

The comparison of transverse and vertical incisions used in the ventral approach of bilateral ovariectomy in female Wistar rats indicates the importance of the choice of the incision on the various surgical and non-surgical parameters. They essentially determine the outcomes in terms of wound healing, blood loss, accessibility and feasibility of the surgeon and time taken to perform the surgery. The study indicated that transverse incision in ventral approach was significantly better than vertical incision in ventral approach in terms of time taken for a surgical procedure, size of incision, wound healing, feasibility and accessibility of the surgeon during the procedure and there was less blood loss also.

Declarations

Ethical Clearance

Ethical clearance was obtained from Institutional Animal Ethics Committee prior to beginning of study.

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Conflict of Interest

The authors declare that there was no conflict of interest.

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