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ORIGINAL ARTICLE

The effect of depression on bone fusion in a rabbit posterolateral bone fusion model

El efecto de la depresión en la fusión ósea en un modelo de fusión posterolateral en conejos

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Abstract

Objective: *The objective of this study was to evaluate the effect of depression on bone fusion in the rabbit spine using Katz's unpredictable chronic mild stress (UCMS) model.* **Methods:** *A total of 12 male New Zealand rabbits were divided randomly into 6 control and 6 experimental groups. Each rabbit underwent the same surgical procedure. An autograft was harvested from the iliac crest and put into the L5-L6 space, where the laminae and facets were decorticated. The animals of the control group were followed up for 8 weeks under standard laboratory conditions. The ones in the experimental group were put in Katz's UCMS. After 8 weeks, all rabbits were sacrificed, and the vertebra segments were evaluated with manual palpation and X-rays.* **Results:** *On manual palpation, fusion was detected in 83.34% in the control group compared to just 33.32% in the UCMS group. The model showed that depression had a significant negative effect on fusion on manual palpation (p = 0.045). Second, if no fusion was detected on X-rays, 0 points were given; callus formation received 1 point, and sclerosis received 2 points. In the control group, just 16.66% showed non-union, 49.98% had a callus formation, 16.66% showed sclerosis. In the experimental group, the non-fusion rate was 66.68%.* **Conclusions:** *Depression has a negative effect on the fusion of rabbit vertebrae. However, vertebrae that are evaluated as "fused" on palpation do not show meaningful relation in their radiologic scores. We believe that our model can work as a basis for further experimental trials.*

Keywords: *Unpredictable chronic mild stress. Depression. Fusion.*

Resumen

Objetivo: *Evaluar el efecto de la depresión en la fusión ósea de la columna vertebral en conejos utilizando el modelo de estrés leve crónico impredecible (UCMS) de Katz.* **Métodos:** *Se dividieron aleatoriamente 12 conejos machos de la raza New Zealand en dos grupos: seis en el grupo control y seis en el grupo experimental. Cada conejo fue sometido al mismo procedimiento quirúrgico. Se obtuvo un autoinjerto de la cresta iliaca y se colocó en el espacio L5-L6 tras la decorticación de las láminas y las facetas. Los animales del grupo control fueron monitoreados durante 8 semanas en condiciones estándar de laboratorio, mientras que los del grupo experimental fueron sometidos al modelo UCMS de Katz. Después de 8 semanas, todos los conejos fueron sacrificados y los segmentos vertebrales se evaluaron mediante palpación manual y radiografías.* **Resultados:** *En la palpación manual se detectó fusión en el 83.34% de los conejos del grupo control, en comparación con solo el 33.32% en el grupo UCMS. El modelo mostró que la depresión tuvo un efecto negativo significativo en la fusión según la palpación manual (p = 0.045). En segundo lugar, en la evaluación radiográfica se asignaron 0 puntos si no se detectaba*

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fusión, 1 punto para la formación de callo y 2 puntos para la esclerosis. En el grupo control, el 16.66% presentaron ausencia de fusión, el 49.98% mostraron formación de callo y el 16.66% presentaron esclerosis: en el grupo experimental, la tasa de no fusión fue del 66.68%. **Conclusiones:** *La depresión tiene un efecto negativo en la fusión de las vértebras en conejos. Sin embargo, las vértebras evaluadas como «fusionadas» mediante palpación no mostraron una relación significativa con sus puntuaciones radiológicas. Creemos que nuestro modelo puede servir como base para futuros estudios experimentales.*

Palabras clave: *Estrés leve crónico impredecible. Depresión. Fusión.*

Introduction

Spinal fusion is among the most frequently performed surgical procedures worldwide, particularly by spine surgeons. The Agency for Healthcare Research and Quality estimates that over 488,000 spinal fusion surgeries are performed annually in the United States, accounting for 3.1% of all surgical procedures.[1] Spinal fusion is indicated for various conditions, including degenerative disc disease, disc herniation, vertebral fractures, and spinal deformities. This procedure involves fusing two or more vertebral segments using screw-rod systems, plates, cages, or a combination of these techniques.[2]

To optimize surgical outcomes, extensive research has focused on surgical techniques, fusion materials, and adjunctive therapies such as grafting, electromagnetic stimulation, and pharmacological agents. Insufficient bone fusion can result in complications such as pseudoarthrosis, non-union, instrumentation failure, and iatrogenic deformities, significantly diminishing patients' quality of life.[3] Bone grafting remains a cornerstone of fusion surgery, with options including autografts, allografts, xenografts, and synthetic materials.[4] In addition, biological agents such as RANKL inhibitors (e.g., denosumab), parathyroid hormone analogs, growth factors (e.g., Insulin-like growth factor, fibroblast growth factor, and B-epidermal growth factor), and sonic hedgehog genes have demonstrated efficacy in promoting bone healing.[5,6]

Equally critical is understanding factors that hinder bone fusion. Metabolic disorders (e.g., diabetes mellitus and osteoporosis), vitamin deficiencies, vascular issues (e.g., anemia and hypoxia), mechanical factors (e.g., inadequate immobilization and instrumentation failure), and lifestyle factors (e.g., tobacco and alcohol use) have been shown to impair bone healing.[7] Furthermore, psychological factors such as depression and distress are known to affect wound healing adversely.[8] However, the impact of depression on spinal fusion remains unexplored.

Katz's unpredictable chronic mild stress (UCMS) model, developed in the 1980s, serves as a widely used model for studying depression.[9] Given that bone fusion is a form of wound healing, this study aims to investigate the effect of psychological distress and depression on spinal fusion using a rabbit posterolateral fusion model.

Methods

Setting and ethics committee

This study was conducted in the Department of Experimental Studies at a university hospital with ethics approval was obtained from the same institution (approval number: 776). The experiment was designed to last for a total duration of 8 weeks.

Selection criteria for the study and grouping of the animals

A total of 12 male New Zealand rabbits, aged 8 months with a mean weight of 3250 g, were selected for the study. Each rabbit was housed individually in separate cages. The animals were randomly divided into two equal groups: an experimental group and a control group, each consisting of six rabbits.

The control group was maintained under standard laboratory conditions, with *ad libitum* feeding, a 12-h light-dark cycle, a controlled room temperature of 20-24°C, and 60% relative humidity. In contrast, the experimental group was subjected to the conditions of UCMS, which will be described in detail later. All rabbits underwent identical surgical procedures as part of the study protocol.

Exclusion criteria

To ensure adherence to ethical standards and animal welfare while minimizing potential bias in the results, the following exclusion criteria were established:

- Rabbits that died before the completion of the 8-week study period, either preoperatively, intraoperatively, or postoperatively
- Rabbits that failed to eat or drink adequately during the study
- Rabbits that were unresponsive to external stimuli
- Rabbits deemed unsuitable for the study by the attending veterinarian.

Fortunately, no adverse events occurred during the study, and all rabbits met the inclusion criteria, enabling the collection of optimal data.

Anesthesia

In an operating room environment with air exchange every 5 min, a controlled temperature of 22° C, and 60% humidity, pre-anesthesia was administered intramuscularly using 8 mg/kg xylazine (Rompun 2%©, Bayer AG, Germany). Once the animals were sedated, anesthesia was induced with 40 mg/kg ketamine (Ketasol 10%©, Richter Pharma AG, Austria) administered intraperitoneally. Proper anesthetic depth was confirmed by the veterinary team through the absence of a pain response to foot pinching. To prevent corneal dryness during the procedure, eye drops (Viscotears©, Alcon Pharma, Switzerland) were applied.

First surgery

Once anesthesia was confirmed, the rabbits were positioned prone. The surgical field was shaved and thoroughly cleansed with an antiseptic solution. To minimize intraoperative bleeding and post-operative pain, 2 cc of prilocaine hydrochloride (Pricain 2%©, Polifarma İlaç, Turkey) was administered subcutaneously and intramuscularly into the surgical area. The left and right iliac crests were palpated, and a 2 cm skin incision was made parallel to the left iliac crest. The area was exposed through blunt dissection, and 2 g of bone was harvested from the left iliac crest using a rongeur (Fig. 1A).

A midline skin incision was then made from L4 to S2 to access the rabbit spine. The paravertebral muscles were dissected, exposing the posterior spinal elements (Fig. 1B). As no instrumentation was planned, the left L5 and L6 facets and transverse processes were decorticated using a rongeur to create a stable environment for fusion. The harvested iliac crest bone graft was placed in the prepared area, and the wound was closed with 3/0 silk sutures (Fig. 1C).

Postoperatively, the rabbits were monitored until full recovery from anesthesia. All animals retained normal motor function following the procedure. Wounds were inspected and cleaned daily. For pain management, the rabbits received 2 mg/kg carprofen (Rimadyl XL©, Zoetis, USA) for 5 days.

Applying the UCMS model

The rabbits in the control group were monitored under standard laboratory conditions for 8 weeks. Those in the experimental group were subjected to the UCMS model, implemented as follows:

Day 1

A tub was filled with tap water at 24° C to a depth of 1 cm. The rabbits in the experimental group were placed in the tub for 4 h (Fig. 2). Afterward, they were removed, dried, warmed under a 37° C heating lamp, and returned to their cages.

Day 2

The standard 12-h light and dark cycle was altered. The animals were kept in complete darkness for 24 h.

Day 3

The light-dark cycle was disrupted again, with the lights left on continuously for 24 h.

Day 4

A 5 cm box was placed under one side of the shelf holding the cages, tilting the cages to create an inclined position. The rabbits remain in this setting for 4 h (Fig. 3).

Day 5

The rabbits were confined to a cage typically designed for rats, restricting their movement and inducing a claustrophobic environment for 4 h (Fig. 4).



Figure 1. A: *harvesting of the iliac bone graft.* **B:** *anatomic orientation and visualization of the L5-L6 intervertebral space.* **C:** *inserting the bone grafts and closing the wound.*



Figure 2. *A water tub in which the rabbits were kept for 4 h.*



Figure 3. *Elevation construct with a 5 cm box providing an inclination of the cages.*

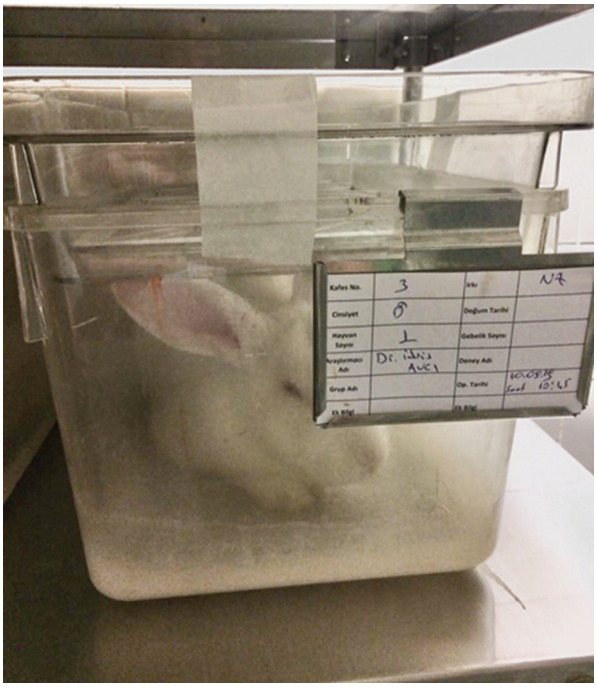


Figure 4. *Putting the rabbits in a rabbit cage to achieve claustrophobia.*

Day 6

Each rabbit was placed in another rabbit's cage for 4 h before being returned to its own.

Day 7

No stress-inducing intervention was performed.

To prevent habituation, the cycle was altered each week. During the experimental period, the rabbits exhibited behavioral changes, including reduced food and water intake and decreased activity, which are consistent with the core clinical symptom of depression: anhedonia.

Second surgery

After 8 weeks, all animals from the experimental and control groups were sedated with 8 mg/kg intramuscular xylazine (Rompun 2%©, Bayer AG, Germany) and euthanized via intracardiac injection of 100 mg/kg potassium chloride. The midline incision was reopened, and macroscopic observations in some rabbits revealed fusion between the harvested bone graft and the facet and transverse processes.

The vertebral segments were carefully dissected free of surrounding soft tissue and removed *en bloc* using a wire saw. The extracted vertebrae were palpated and assessed for evidence of fusion by manually applying flexion and extension maneuvers. The results were categorized as "fusion" or "no fusion" (Fig. 5).

Subsequently, the vertebrae were preserved in formaldehyde and transported to the radiology department. Anterior-posterior images were obtained using a fluoroscopic C-arm (Ziehm Solo©, Ziehm Imaging, Germany). Radiographic evaluation focused on signs of fusion. A scoring system was applied: vertebrae without callus formation or sclerosis, indicating no evidence of fusion, received 0 points. Vertebrae displaying callus formation, an early marker of fusion, were assigned 1 point. Complete fusion, characterized by sclerosis, received 2 points (Fig. 6).

Results

Statistical analysis

The results were analyzed using the Z-test to compare categorical measurements between the two independent groups. Numeric and percentage values were applied as complementary statistical tools for categorical variables. Statistical analysis was performed using the Statistical Package for the Social Sciences v.24 (IBM, USA), and $p < 0.05$ was considered statistically significant.

Manual palpation results

Bone fusion was evaluated using two methods. The first involved manual flexion-extension maneuvers performed on the *en bloc* extracted vertebral segments. In the experimental group, subjected to the UCMS model, fusion was observed in only 2 out of 6 rabbits (33.3%). In contrast, in the control group, which was not exposed to stress, 5 out of 6 rabbits (83.3%) exhibited fusion.

The results from the manual palpation method indicated that UCMS had a significant negative effect on bone fusion compared to the control group ($p = 0.045$) (Table 1a-c).

Table 1a. Evaluation of fusion after manual palpation. Evaluation of fusion in the experimental group

Rabbit number	Fusion
1	No
2	No
3	Yes
4	No
5	No
6	Yes

Table 1b. Evaluation of fusion in the control group

Rabbit number	Fusion
1	Yes
2	Yes
3	No
4	Yes
5	Yes
6	Yes

Table 1c. Comparison of fusion between the experimental and control groups

	Experimental group	Control group	Total	z	p
Fusion	2/6 = 0.33	5/6 = 0.83	7/12 = 0.58	2.020	0.045
No fusion	4/6 = 0.67	1/6 = 0.17	5/12 = 0.42		

Radiological evaluation results

The second method involved evaluating the harvested vertebrae under a fluoroscopic C-arm. Vertebrae showing no signs of fusion, such as callus formation or sclerosis, were assigned 0 points. Vertebrae with callus formation were given 1 point, whereas those with sclerosis, indicative of complete fusion, received 2 points.

In the experimental group, only 2 out of 6 rabbits demonstrated fusion. One rabbit exhibited callus



Figure 5. *Manual palpation and applying flexion-extension movements on the extracted spine segment.*

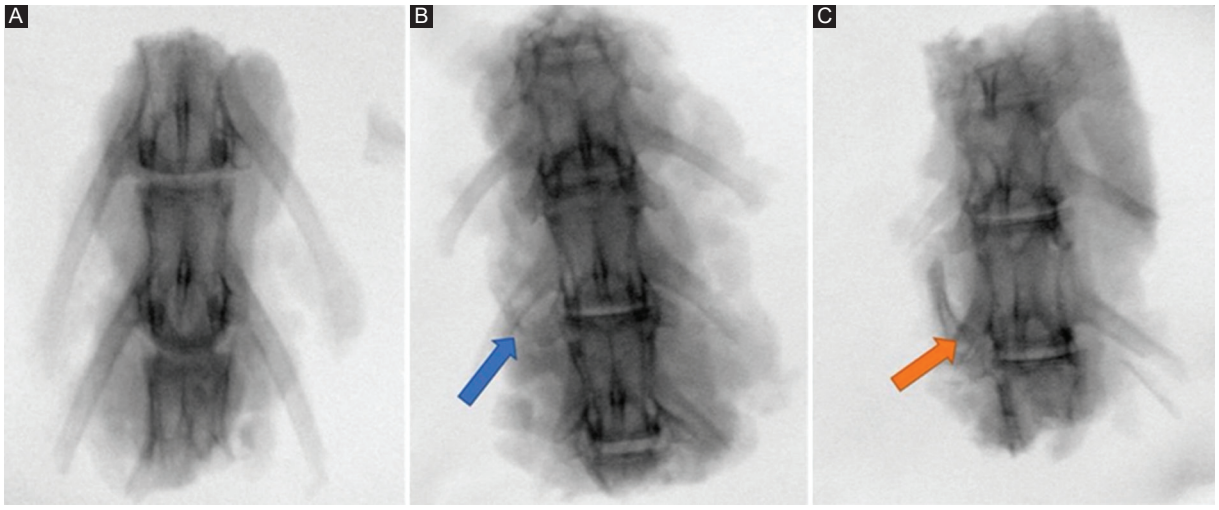


Figure 6. *Radiological X-ray images of the extracted spine segment.* **A***: no sign of fusion = 0 points.* **B***: callus formation = 1 point.* **C***: sclerosis formation = 2 points.*

formation (1 point), and another showed sclerosis formation (2 points). In the control group, only one rabbit displayed no evidence of fusion (0 points), whereas 3 rabbits exhibited callus formation (1 point) and 2 demonstrated sclerosis formation (2 points).

These results suggest that UCMS negatively impacts the overall rate of bone fusion. However, it did not have a statistically significant effect on the degree of fusion, whether callus or sclerosis was present ($p = 0.180$) (Table 2a-c).

Discussion

In the United States of America, vertebra fusion surgery had increased by 62.3% in total between 2004 and 2015. Especially in the elderly population over 65 years of age, fusion surgery has risen by 138.7%.[10] As this type of surgeries become a part of every spinal surgeon's practice, achieving maximal satisfactory outcomes is extremely important. In 1995, Boden and Sumner, and in 2012, Hudec and Camacho developed a classification of factors that

Table 2a. Evaluation of fusion after radiodiagnostics. Evaluation of fusion in the experimental group

Rabbit number	Rate of fusion
1	0
2	0
3	1
4	0
5	0
6	2

Table 2b. Evaluation of fusion in the control group

Rabbit number	Fusion
1	1
2	1
3	0
4	1
5	2
6	2

Classification of fusion rates. 0 = no fusion, 1 = partial fusion (callus), 2 = complete fusion (sclerosis)

Table 2c. Comparison of fusion between the experimental and control groups

Group	n	Mean	Standard deviation	z	p
Experimental	6	1.1667	0.75277	–1.450	0.180
Control	6	0.5	0.83666		

negatively affected bone fusion. They stated that the 4 main elements which interfere with bone fusion: vascular factors, mechanical factors, metabolic factors and other factors. Anemia, hypoxia and infections, part of vascular factors, insufficient immobilization and fixation or abundant soft tissue between the fusion parts were defined as mechanical factors. The metabolic category consisted of osteoporosis, Vitamin C and D deficiency and steroid use. Tobacco and alcohol consumption were accepted as "other'' elements slowing down or completely interrupting bone fusion.[7,11] Besides these, lots of many more factors that obstruct bone healing have been published throughout the years. In contrast, using allografts or xenografts, growth hormone derivatives or electrophysiological stimulation were shown to enhance bone fusion[7]. Although there are numerous publications on how psychological stress has a negative outcome on wound healing, there are no significant reports of how it influences bone healing. In 2013, GBD 2017[12] stated that post-traumatic stress showed negative results in patients after arthrodesis, but a specific paper about how depression affects bone fusion could not be found in any database. The World Health Organization predicts that over 264 million people in the world suffer from depression.[12] We believe that investigation the connection between such a common public health issue and one of the most practiced surgeries of a spinal surgeon would be an interesting topic for further understanding bone healing. To project depression on humans and study their surgical outcome is nearly impossible to accomplish, either due to ethical reasons or to have a homogenous group without any bias or external affecting stimuli. That is why we developed a model to have the best possible results with the minimum number of animals to take part in it. The UCMS model was developed by Katz in the 1980's to induce depression in laboratory animals and was further modified by other authors. For the surgery, we used the standardized posterolateral fusion model, which is commonly chosen by many researchers throughout the years. The duration of the study was limited to 8 weeks because it is the minimum amount of time needed for bone fusion. We wanted to evaluate bone healing in two steps. The first is manual palpation by forcing extension and flexion maneuvers, which is thought to be a simple but subjective way. The other was a more objective way by radiodiagnostics under the C-arm, where we graded the extracted vertebra on its callus and sclerosis formation. The results were rather surprising, in that depression negatively interferes with proper bone fusion but does not affect the degree or quality of it *per se*. These findings were followed by some unanswered further questions. Will the bones without any signs of fusion or with callus formation, so the ones that began the fusion process but did not fully heal, later develop sclerosis despite being under chronic stress? To achieve this, the study time can be prolonged to 12 or maybe 24 weeks. Furthermore, additional diagnostic tests, such as computed tomography and histopathological evaluation, may be used. An interesting topic for another study would be whether this negative effect of depression on bone healing can be reduced by antidepressant agents.

Key results

Depression mimicked in the UCMS model negatively affects bone fusion.

Limitations

Prolonged study time and more advanced radiologic and histopathologic evaluations more detailed results might be achieved despite being extortionate.

Conclusion

Our findings demonstrated that depression significantly impairs bone fusion in rabbits but does not alter the quality or degree of fusion. We believe this model provides a promising foundation for future research. Extending the study duration and incorporating advanced radiologic and histopathologic evaluations could yield more comprehensive results, although these approaches may involve higher costs.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of human subjects and animals. The authors declare that the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the World Medical Association and the Declaration of Helsinki. The procedures were authorized by the Institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. The authors have followed their institution's confidentiality protocols, obtained informed consent from all patients, and secured approval from the Ethics Committee. SAGER guidelines have been followed as applicable to the nature of the study.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing or creation of the content of this manuscript.

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