Gingival inflammation induction in pregnant Sprague-Dawley rats: A pilot study

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Abstract. Pregnancy gingivitis is an inflammation of the gingiva caused by dental plaque and exacerbated by hormonal changes. Gingival inflammation is often induced in laboratory animal models, such as Sprague-Dawley rats used for experimental models. This investigation was to establish inducing gingival inflammation in pregnant rats. The purpose of this study was to establish a reproducible method for inducing gingival inflammation in both incisive mandibles of pregnant Sprague-Dawley rats using ligation and dietary manipulation. Pregnant Sprague-Dawley rats were used as experimental subjects. As ligation, 4-0 non-resorbable silk thread was utilized and affixed using a "8"-shaped knot technique. The ligatures were inserted between the mandible's central incisors. The operation was carried out under anaesthesia. Once the rat's ligature was removed after 5th,7th,10th and 14th days. The clinical appearance and radiographic examination were evaluated. Gingival inflammation induction by ligation and dietary intake modification caused inflammation of gingival tissue, was seen at 5th day. Clinical examination showed getting worst at 14th day. In our study, gingival inflammation on pregnant rat was achieved five days after ligation and dietary intake modification.

Keywords: gingival inflammation, induction, ligation, dietary intake, animal model

1 Introduction

Animal models are of utmost importance in advancing information in the field of medical sciences, including periodontology. The experimental models provide notable advantages owing to their capacity to accurately reproduce the cellular features and reactions that take place in humans in vivo. The utilization of animal models in the study of periodontal disease is of utmost importance as it provides a necessary foundation for the establishment of a scientific framework aimed at understanding the underlying pathogenic mechanisms [1]. Commonly used animal models in periodontal research are rats and other rodents. The gingival anatomy of rats exhibits similarities to that of humans, characterized by a shallow gingival sulcus and the adherence of junctional epithelium to tooth surfaces. Furthermore, the junctional epithelium present in both gingivae functions as a pathway for the transportation of exogenous chemicals, bacterial toxins, and inflammatory cell exudates [2].

In the Classification of Periodontal and Peri-Implant Diseases and Conditions 2017, gingivitis is inflammation of the gingival tissue at one or more sites and is characterised by bleeding on probing. Bleeding on probing is the main parameter in diagnosing gingivitis [3]. Gingivitis is a mild periodontal disease that causes redness and swelling (inflammation) of the gingiva. Gingivitis is a disorder that primarily affects the gingival area and does not entail damage to the periodontal tissues. However, it is important to note that gingivitis has the potential to progress into periodontitis, a more serious condition compared to gingivitis [4]. Pregnancy gingivitis is an inflammation of the gingiva caused by dental plaque and exacerbated by hormonal changes, particularly during the second and third trimesters [5]. Estrogen and progesterone are hormones that undergo significant fluctuations during pregnancy. These hormonal changes will influence the clinical appearance of periodontal tissue [6].

Periodontal diseases can be created in rats through the inoculation of bacteria, administration of a diet rich in carbohydrates, and the placement of ligatures around the teeth [7]. Male rodents had their second maxillary molars ligated and were fed moist food and 10% sucrose water. Two weeks later, the gingival inflammation model was effectively established [8]. Not much has been research regarding the induction of periodontal diseases in pregnant rats. An experimental study [9] was conducted periodontitis on pregnant rats by ligation around second upper molars until the last day of breast feeding. Another experiment using female rats were received an oral inoculation containing $1 \times 10^9$ CFU of Porphyromonas gingivalis for 4 consecutive days/week.

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After 24 inoculations over a 12-week, female rats mated with male, and pregnancy was determined by vaginal swab [10].

Due to the lack of information about conducting experiments on pregnant rats, it is proposed to modify the existing model by ligating the central incisor and manipulating the food intake.

The present study ligated the cervical region of the lower incisors using silk thread. The incisor is utilized more frequently due to its simple accessibility and straightforward operatory technique [1, 7, 11, 12].

The objective of this study was to establish a reliable experimental method for producing inflammation in the gingival tissue surrounding the incisive mandible teeth of pregnant Sprague-Dawley rats. This was achieved by employing a ligation approach in combination with a modified nutritional regimen.

2 Material and methods

2.1 Animals modeling

All procedures were conducted after approval from the ethics committee of the Faculty of Dentistry-Prof Soedomo Dental Hospital, Universitas Gadjah Mada with the number 2/UN1/KEP/FKG-RSGM/EC/2023. The experimental protocol was carried out in accordance with current animal welfare and experimentation ethics laws. Four female adults of Sprague-Dawley rats obtained from Laboratory animal facility at LPPT unit IV Universitas Gadjah Mada, aged three months with a weight of (200 to 250) g, were included in this study. A controlled environment was established to house a group of five rats in individual wire cages. The room was maintained at specific temperature and humidity levels, and a 12-hour light/dark cycle was implemented. The rats were provided with normal rat pellets and had unrestricted access to water. After a week of acclimation, female rats in estrus stage were placed overnight with a male for mating. Vaginal examinations determined the cycle of estrus or pregnancy. The following morning, if spermatozoa were detected in the vaginal smear as seen in Figure 1, pregnancy was determined [10, 13].

Spermatozoa were observed using a microscope.

Following the confirmation of pregnancy, general anesthesia was induced through intramuscular injection using a solution consisting of Ketamine 10% and Xylazine 2% in a ratio of 2:1, at a dosage of 0.12 ml per 100 g of body weight. The animals were positioned on an operating table to enable unobstructed access to the rats' mouths, hence facilitating maintenance of the teeth. Both mandibular incisive were ligated with 4-0 silk thread non resorbable. The silk thread was knotted around three circles with "8"-shaped and pressed into the subgingival as much as possible (Figure 2). After placing ligatures, the animals were kept in cage. The animals were feed with moist feed and drank 10% sucrose water. Control of ligatures and animal models was performed daily.

2.2 Clinical and radiographic examination

The rats were divided into five groups. Before the extraction of the silk thread, a standard procedure of general anesthesia was administered through intramuscular injection using a solution consisting of 10% Ketamine and 2% Xylazine in a ratio of 2:1. The dosage administered was 0.12 ml per 100 grams of weight. On days 5th, 7th, 10th, and 14th, ligatures were removed in each rat. Clinically, the examination was made on day 5th until day 14th. The parameters gingival index, bleeding on probing, and probing depth were evaluated. Gingival Index (GI) as described by Löe and Silness was used, with scores ranging from 0 to 3, score 0: healthy gingiva; score 1: mildly inflamed, slight change in color, slight edema, no bleeding on probing; score 2: moderately inflamed, redness, edema, glazing, bleeding on probing; score 3: severely inflamed, marked redness and edema, ulceration, spontaneous bleeding [14]. The probing depth (PD) and bleeding on probing were evaluated using a periodontal probe (UNC-15 probe, Osung, Korea). Lower incisors were examined in the gingival sulcus and their depth was measured. The bleeding on probing (BOP) was determined to be positive if bleeding occurred within thirty seconds of probe placement [15]. All parameters were documented in the chart for each subject.

![Fig. 1. Spermatozoa on vaginal smear as shown by the arrow](image1)

![Fig. 2. The macroscopic appearance after the placement of the ligature.](image2)
After clinical examination, radiographic examination was taken using a digital periapical radiograph machine for animals. Conus was positioned upright to the mandible of rat.

## 3 Results

### 3.1 Clinical

The pregnant gingivitis model in Sprague-Dawley rats was established experimentally with the use of silk thread ligatures and dietary modifications. Observable clinical changes become apparent during a span of five days subsequent to the initiation of induction. The gingival tissue exhibited erythema and edema, with a probing depth of 1 millimeter. The gingival tissue exhibits bleeding upon probing. Indicating that the gingivitis model was effectively established. At two weeks, the gingival tissues were swollen, bleeding was observed during BOP examination, PD was 3 mm and gingival recession occurred on the mandibular left incisor (Figure 3 and Table 1).

![Fig. 3. Clinical appearances](image)

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Days</th>
<th>5th</th>
<th>7th</th>
<th>10th</th>
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<tr>
<td>BOP</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>1</td>
<td>2</td>
<td>3</td>
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</table>

*GI by Löe & Silness; + bleeding

### 3.2 Radiographic

Rats' mandibles were evaluated using digital periapical radiographs for radiographic examination. The decrease in radiopacity and widening of periodontal ligament was seen at fourteenth day, as illustrated in Figure 4.

![Fig. 4. Radiographic appearances](image)

## 4 Discussion

The experimental model of gingivitis in pregnant rats was generated with the implementation of ligation, a diet consisting of moist food, and the provision of sugary beverages. The ligation line serves as a structural framework facilitating the aggregation and adherence of bacteria [1, 16]. In addition, the process of ligation leads to gingival irritation. Moist feed exhibits a notable level of viscosity, which facilitates its adherence to the tooth surface and thus enhances the adhesion of dental plaque [16].

The gingivitis model was established by daily administration of moist feed and sugary drinks. On the fifth day following the establishment of the gingivitis...
animal model, the rat mandibular incisor exhibited red and swollen gums, as well as gingival bleeding during exploration. Bleeding on probing is primary indicator to set the threshold for gingivitis [3]. The research demonstrates that the gingival inflammation models were success. Besides that, rat pregnancy gingivitis may be influenced indirectly by progesterone and its receptor [11].

In this present study, clinical changes of gingival inflammation can be seen on fifth day after induction started. Consistent with the findings of previous research, the obtained data were in accordance with those of earlier studies [12], although was performed ligation on male rats with additional injection of bacteria. Studies conducted by Shi et al. [11] showed that gingival inflammation on pregnant rats were established after ligation for two weeks.

Visual manifestations of gingival inflammation are highly responsive indicators of early-stage gingivitis. Consequently, gingival index that focus on bleeding have been given significant emphasis [17]. The current investigation demonstrated a positive correlation between the gingival index and the presence of blood on probing. The clinical assessment revealed that there was an increase in probing depth values when the duration of ligation was extended.

The present study has limitations by a low sample size and reliance solely on clinical and radiographic assessments. Consequently, further investigation is warranted to assess the histopathological examination, weight, and hormone levels in pregnant rats. In the context of this study, gingival inflammation was achieved five days after ligature placement and dietary intake modification.

5 Conclusion

The present study elucidates the clinical and radiographic assessment of gingival inflammation provoked in pregnant rats with the application of ligation in conjunction with food modification. The short-term induction demonstrated gingival inflammation that occurred on the fifth day.

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References


