Effects of Splenectomy on Luteolysis in Pseudopregnant Rabbits

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ABSTRACT. The effects of splenectomy on luteolysis in pseudopregnant rabbits were observed. Pseudopregnancy was induced in the rabbits by mating with vasoligated mature males and injection of human chorionic gonadotrophin (D0). In these rabbits, the concentration of serum progesterone increased for 7 days after the induction of pseudopregnancy. In the control group (sham-operation), the concentration of serum progesterone returned to the level of the pre-ovulatory levels by 14 days of pseudopregnancy. On the other hand, in the splenectomized group, in which splenectomy was performed at D7, the serum progesterone concentration was maintained at the level of the functional luteal phase (D7) at least until 21 days of pseudopregnancy. These findings indicate that the spleen is concerned with luteolysis of the rabbit.—**KEY WORDS:** luteolysis, rabbit, spleen.

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Immunocytes such as macrophages (M ϕ) and T-lymphocytes exist in the luteal tissue in several mammals [3, 6, 10]. A significant increase in the number of M ϕ has been observed in the corpus luteum at the end of the pseudopregnant and pregnant luteal phases in rabbits [1, 2], and the cytokines released from these immunocytes might regulate progesterone production of the luteal cells [4, 5, 9]. These reports also suggest that immunocytes, M ϕ in particular, take part in the regulation of luteinization and luteolysis.

The spleen has been assumed to be a source of M ϕ for regulating luteal functions [8, 11]. In rodents with an incomplete estrous cycle, the spleen has been believed to play an important role in the regulation of their estrous cycles [8, 11]. But, no information on the relationship between the spleen and the luteal functions in the other species has been obtained.

Matsuyama *et al.* [8] reported that splenectomy caused delayed luteolysis in the luteal tissue of rats. To determine the role of the spleen in the regression of the corpus luteum in the ovaries of rabbits, we examined whether or not the corpus luteum would also regress in rabbits after splenectomy in pseudopregnant rabbits.

Sexually mature and nulliparous female Japanese white rabbits, each weighing 2.8 to 3.2 kg, were purchased from a commercial breeder (Saitama Experimental Animal Supply Co., Ltd., Saitama, Japan). The rabbits were housed individually in 68 × 82 × 70 cm wire netting cages (R3 type; Okazaki Sangyo Co., Ltd., Saitama, Japan). The animal room was air-conditioned: 23 ± 2°C, 55 ± 5% relative humidity, 15 air changes per hour, and illuminated for 14 hr (05:00–19:00) with 300 lux day light fluorescent lamps. They were given commercial pellets (Labo R Stock; Nihon Nosan Kogyo Co., Ltd., Yokohama, Japan) and tap water *ad libitum*. Each animal had been isolated in a separate cage for at least 31 days prior to the experiment.

Pseudopregnancy was induced in the rabbits by mating with vasoligated mature males and by injecting 75 iu of human chorionic gonadotrophin (hCG; Gonatropin, Teikoku Hormone Mfg. Co., Ltd., Tokyo, Japan) into the ear vein. The day of this treatment was designated as day 0 of pseudopregnancy (D0). On D7, D14 and D21, all of the

animals examined were laparotomized with a lower midline incision under anesthesia with sodium pentobarbital; NEMBUTAL (Abbot Laboratories, North Chicago, IL, U.S.A.) for macroscopy of the corpora lutea. When the corpora lutea were macroscopically observed on D7, the splenectomy or sham-operation was also performed. Under anesthesia with NEMBUTAL, seven rabbits were splenectomized with an upper midline abdominal incision in the splenectomized group. On the other hand, in the control group, sham-operation without splenectomy was performed in the same way. As the control group, five rabbits were used.

Blood samples were collected from the ear vein for assay of serum levels of progesterone at D0 (just before hCG injection), D3, D7 (before splenectomy or sham-operation), D14 and D21 in both groups. Sera obtained from these animals were stored in a deep freezer (–20°C) until assayed. Progesterone was assayed with a radioimmunoassay kit (Progesterone Kit “Daiichi”, Daiichi Radioisotope Laboratory Co., Ltd., Tokyo, Japan). With this kit, the range of assayed progesterone was found to be between 15 pg and 2 ng per tube. The coefficients of variation for intra- and inter-assay were less than 9.8% and less than 9.3%, respectively.

Fig. 1. Changes in concentration of progesterone in the serum during pseudopregnancy in the rabbits. Each point represents the mean ± SEM. N=5 (Control Group) and N=7 (Splenectomized Group). *Significantly ($p<0.05$) different from control group (Cochran-Cox test).

The data on the serum progesterone concentration were statistically analyzed by analysis of variance followed by Student's *t*-test or Cochran-Cox test. Differences of $p < 0.05$ were considered to be statistically significant.

As shown in Fig. 1, the concentrations of the serum progesterone markedly increased until D7 not only in the splenectomized group but also in the control group. In the

control group, the peak of the progesterone concentration was observed on D7. The concentration of serum progesterone in the control group decreased from D7. By D14, the progesterone concentration returned to the level of the pre-ovulatory state in the control group, but, in the splenectomized group, in which the spleen was removed on D7, the progesterone concentration was maintained at

Fig. 2. Macroscopic observation of the ovaries. The functional corpora lutea (arrows) on D7 in the splenectomized group (a) and in the control group (d). The remaining functional corpora lutea (arrows) in the splenectomized group on D14 (b) and on D21 (c). The regressive corpora lutea (arrows) on D14 in the control group (e). The corpora albicantia (arrows) on D21 in the control group (f).

the D7 (functional luteal phase) level until at least D21 (Fig. 1).

As the ovaries were observed macroscopically, there were functional corpora lutea with very clear blood vessels in the ovaries of both groups on D7. In the control group, the corpora lutea had regressed by D14, and the corpora albicantia existed in the ovaries on D21. On the other hand, functional corpora lutea were observed in the ovaries of the splenectomized group on D21 (Fig. 2). The serum progesterone concentration on D21 in the splenectomized group was not significantly different from that on D7 (Fig. 1), indicating that these corpora lutea are functional not only morphologically but also endocrinologically.

The corpora lutea regresses by 15 days on average after ovulation in pseudopregnant rabbits [7]. M ϕ has been shown to participate in the regression process of the corpus luteum [1–3, 8, 10]. M ϕ play a role as phagocytes for degenerative luteal cells [3, 10]. Cytokines such as tumor necrosis factor- α (TNF- α) produced by M ϕ may be one of the factors for regression of the corpus luteum [1, 2]. TNF- α makes luteal cells synthesize prostaglandin F $_{2\alpha}$ to regress luteal cells [5]. Matsuyama *et al.* [8] and Saito *et al.* [11] have shown that the spleen is one of the sources of the M ϕ which play a role in luteolysis. In the present data, when splenectomy was performed in the functional luteal phase (D7), prolongation of the pseudopregnant luteal phase was observed.

A relationship between ovarian functions and immune systems has been reported [4, 5, 9, 12]. M ϕ and cytokines, such as TNF- α and interferon- γ , have been shown to play

a role in the control of the ovarian cycle and steroidogenesis [1, 2, 4, 5, 8, 9, 11]. The absence of splenic M ϕ caused by splenectomy delays luteolysis in rats with an incomplete estrous cycle [8]. The present findings clearly indicate that the spleen plays an important role in the regulation of the luteal functions, especially luteolysis in rabbits.

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