

## Recovery of Estrus and Ovulatory Response in Cows after Intrauterine Injection of Chitin Suspension

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**Abstract:** The purpose of the present study was to evaluate the therapeutic effect of intrauterine chitin suspension on endometritis in cows. In experiment 1, 50 ml chitin suspension (60 and 80 mg/ml in saline), definitely induced estrus. Therefore, 50 ml of the 60 mg/ml suspension was injected into the uteri of Japanese Black cows (3-5 years old, average weight 530 kg) with a normal estrus cycle or endometritis in further experiments. In experiment 2, 21 parous Japanese Black cows with a normal estrus cycle received chitin on day 8-12 of estrus (the day on which estrus occurred was designated day 0). Nineteen of the 21 (90.5%) came into estrus 5-8 days after administration, and 3 control cows receiving saline solution came into estrus 13 days after administration (21 days after the preceding estrus). Progesterone levels in 3 randomly selected cows were reduced from 6.3, 6.3 and 2.6 ng/ml on the day of chitin administration to 0.3, 0.2 and 0.2 ng/ml, respectively, 6 days after administration. In addition, many leucocytes were detected in uterine mucosal biopsy specimens 2 to 3 days after chitin administration. In experiment 3, estrus was induced in 5 cows with endometritis 6-8 days after chitin administration and the endometritis was improved with the disappearance of pus. Normal embryos were obtained in 2 of 4 cows (8/12 ova; 75%) that received superovulatory treatment during the estrus cycle induced by chitin. These findings suggest that the intrauterine injection of chitin can induce estrus and subsequent ovulatory response by its therapeutic effect on endometritis in cattle.

**Key words:** Cow, Chitin, Uterus, Estrus, Endometritis, Superovulation

Chitin and chitosan are polysaccharides that occur naturally in the skeletons of crustacea and fungi. Chitin, a straight chain polymer of  $\beta$ -1,4-coupled *N*-acetyl-*D*-glucosamine, is used as a wound healing accelerator in veterinary practice and its efficacy has been confirmed [1-3]. It increases tissue regenerating activity and induces local biological defense mechanisms. The mechanism underlying these actions is thought to involve a fluid factor-mediated increase in the migration activity and chemiluminescence ability of neutrophils and macrophages [2, 4]. Most reports state that this effect of chitin is produced after subcutaneous administration. Recently, however, a similar general body reaction has been reported after administration to the bovine gland system [5]. No information is available in or outside Japan concerning the administration of chitin into the uterine cavity. Therefore, in the present study, chitin was injected into the uteri of healthy cattle with a normal sexual cycle and those of cows with endometritis to determine the reactivity of the endometrial epithelial cells to this substance.

### Materials and Methods

#### *Experiment 1: Determination of the optimal concentration of chitin*

A preliminary study was conducted to determine the most appropriate concentration of chitin. Chitin was suspended in saline to obtain suspensions of 20, 40, 60 and 80 mg/ml and each dose was injected into two cows with a normal estrous cycle. Fifty ml of chitin suspension was injected into the cornua uteri on the luteinizing ovary side via a metal injector 4 mm in diameter and 55 cm in length (FHK, Tokyo, Japan). As estrus was definitely induced in cows injected with the

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**Table 1.** Induction of estrus by the intrauterine administration of chitin in Japanese Black Cattle

Injection Medium	No. of cows	Estrus cycle before treatment (day)	Day of induction of estrus (%)												
			5	6	7	8	9	10	11	12	13	14	15	16	
Saline	3	8	—	—	—	—	—	—	3 (100)	—	—	—	—	—	
Chitin	21	8-12	6 (28.6)	11 (52.4)	1 (4.8)	1 (4.8)	—	—	—	—	1 (4.8)	—	1 (4.8)	—	

60 and 80 mg/ml suspensions, we used the 60 mg/ml chitin suspension in further studies.

#### Experiment 2: Induction of estrus and progesterone analysis

Fifty ml of 60 mg/ml chitin suspension was injected into the cornua uteri on the luteinizing ovary side in 21 Japanese Black cows (3 to 5 years of age). In 3 control cows 50 ml saline solution was injected into the cornua uteri on the luteinizing ovary side. Investigations to diagnose estrus were started 5 days after the chitin injection.

From these 21 cows, 3 were randomly selected for blood progesterone level measurements: blood was collected from the jugular vein via an indwelling catheter at 09:00 every other day from the initiation of chitin treatment until the day on which estrus occurred. The blood samples were transferred into centrifuge tubes and cooled on ice immediately after collection, then stored overnight at 4°C. Progesterone levels were assayed by radioimmunoassay (RIA) according to the procedures described and validated by Troxel *et al.* [6] and Pope *et al.* [7]. Three more cows were selected at random from the remaining animals, and uterine mucosal epithelial specimens were taken for biopsy (FHK, Tokyo, Japan) every 24 hours for 6 days after the administration of chitin. The specimens were placed on to slides and Giemsa-stained for microscopic examination.

#### Experiment 3: Therapy of endometritis

Fifty ml of 60 mg/ml chitin suspension was injected into the cornua uteri on the luteinizing ovary side of 5 cows with endometritis. Three of these were 2–5 year-old cows in which no estrus had occurred within 50 days of delivery and in which a diagnosis of endometritis had been made on the basis of the presence of pus and mucosal cell degeneration in uterine washings and corpora lutea in the ovary. The remaining two were 3-year-old cows in which pus exudation was noted during collection of ova 7 days after treatment to induce superovulation. A reduced dose (24 mg) of a follicle-stimulating hormone (FSH) preparation was administered to induce superovulation in accordance with an earlier report [8] on day 9 of the estrus induced by chitin, and 5 mg

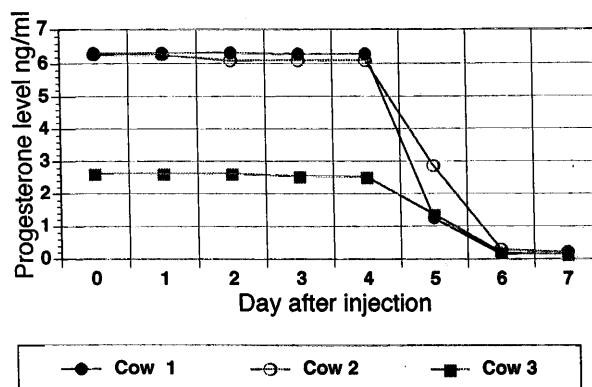


Fig. 1. Blood progesterone levels in cows after chitin treatment.

prostaglandin (PG)  $F_{2\alpha}$  was injected into the uterus 3 days after FSH administration to induce estrus. Embryos were collected 7 days after artificial insemination.

## Results

In experiment 1, estrus occurred 8 to 10, 6 to 8, 5 to 6 and 5 to 6 days after the administration of chitin, 20, 40, 60 and 80 mg/ml, respectively.

In experiment 2, estrus occurred 5, 6, 7 and 8 days after the administration of chitin in 6 cows (28.6%), 11 cows (52.4%), 1 cow (4.8%) and 1 cow (4.8%), respectively (Table 1). In two of these cows (9.5%), uterine contractions were noted during estrus, but no ovulation occurred and the ovarian follicles remained closed. In the controls, which received saline solution on day 8 of the estrous cycle, estrus occurred 13 days after administration. Fig. 1 shows that the blood progesterone levels in these animals, which showed definite estrus, were reduced from 6.3, 6.3 and 2.6 ng/ml on the day of chitin administration to 0.3, 0.2 and 0.2 ng/ml, respectively, 6 days after administration. Biopsy specimens taken from 3 cows on 2 to 3 days after the administration of chitin revealed the infiltration of a large number of leukocytes into the uterine mucosal tissues.

In experiment 3, one of the 5 cows with endometritis

**Table 2.** The effects of chitin by intrauterine administration on endometritis

Case no.	Age of cows	Disease	Day of estrus after injection of chitin	Diagnosis	Treatment on day 9 after estrus	No. of normal/Recovered embryos	A.I. Treatment
1	3	endometritis	8	normal	Superovulation	6/7	—
2	3	endometritis	6	normal	Superovulation	2/5	—
3	5	endometritis	6	normal	Superovulation	0/12	—
4	2	endometritis	6	normal	Superovulation	0/1	—
5	5	endometritis	8	pus	Chitin injection	—	Yes (pregnant)

treated with chitin came into estrus 8 days after chitin administration and ovulated on day 9 of estrus, and the other 3 came into estrus 6 days after and ovulated on day 8 (Table 2). The last cow came into estrus 8 days after the chitin injection, but a further injection was necessary since the exudation of pus persisted. She was given a further injection of chitin suspension on day 9 of the estrus cycle, and conceived by artificial insemination during the estrus thus induced. After superovulatory treatment in 4 cows, normal late morulae were recovered from six of seven ova (85.5%) in animal No. 1 and from two of five ova (40%) in animal No. 2. In the remaining 2 cows, one unfertilized ovum and 12 unfertilized or degenerated ova were recovered, respectively.

### Discussion

In the present study, chitin was administered into the uterus to evaluate the endometrial reaction to this substance. Estrus was induced seven days after administration or thereabouts and was confirmed as normal in view of the decreased blood progesterone levels and maturation of oocytes observed. The mechanism whereby estrus induction occurs is probably as follows: when chitin is given, complement activation occurs at the site of administration, thereby leading to vasodilatation and acceleration of blood flow. These changes activate the arachidonic acid cascade system to accelerate  $\text{PGF}_{2\alpha}$  secretion [9]. Indeed, it has been shown that when the uterine epithelial cell membrane (which is composed of a bilayer of a fatty substance called "lecithins") is stimulated, phospholipase catalyzes the cleavage of arachidonic acid from the membrane and releases it into the cell, where it is converted to PG by cyclooxygenase [9, 10].

In the present study, estrus took about 6 days to occur after the chitin injection, and the infiltration of a large number of leucocytes into the uterine mucosal tissues was detected 2–3 days after administration. This was probably because 3–4 days were required for the concentration of  $\text{PGF}_{2\alpha}$ , which triggers the regression of

the corpus luteum, to rise. Their induction of estrus by this mechanism can therefore be considered effective not only for artificial insemination, but also for the synchronization of the estrous cycle.

The cows with endometritis used in the present study were restored to normal health after further treatment and yielded normal embryos after superovulatory treatment given during the synchronized estrous cycle. The efficacy of the chitin suspension is supported by the development of embryos, especially in 2 cows (Nos. 1 and 2 in Table 2) in which endometritis had been caused by previous superovulatory treatment.

The efficacy of chitin as a therapeutic agent for wounds has been confirmed, and the local level of  $\text{PGE}_2$  induced by chitin has been reported to reach 6 times the normal level [5]. Moreover, experimentally induced abscesses in dogs have been reported to be almost completely healed 6–8 days after the administration of chitin [11]. In the present study, pus associated with endometritis in cattle disappeared after the use of chitin. This effect was thought to have been produced in the following manner.  $\text{PGF}_{2\alpha}$  was induced 6–8 days after the administration of chitin and caused the elimination of foreign bodies. Neutrophils and macrophages migrated into the uterine mucosal blood supply and their activation induced an acute inflammatory reaction in the endometrium; rapid resolution then occurred. It has been suggested that complement activity is involved in the migration of phagocytes [12] and protection of the host against bacterial infection has been shown to be stimulated by chitin [13, 14]. It is therefore interesting to observe that the immune system is activated in the uterine cavity under such circumstances.

No previous work has been done concerning the induction of estrus or treatment of endometritis in cattle by the intrauterine administration of chitin. If the administration of chitin can reliably induce estrus and ovulatory response in cattle, this substance could be recommended from the viewpoint of safety because there is no risk of drug retention.

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