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3 **Transrectal guidance of the ovaries reduces operative time during bovine**
4 **laparoscopic ovariectomy**

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21 Running head: LAPAROSCOPIC OVARIECTOMY IN COWS

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25 **ABSTRACT**

26 The main objective of this study was to evaluate the effects of transrectal guidance of the
27 ovaries by an assistant on operative time during bovine laparoscopic ovariectomy. Twenty four
28 clinically healthy Holstein dairy cows were divided randomly into two groups. In the transrectal
29 guidance group, an assistant grasped the ovaries via the transrectal route and pulled them to a
30 position where they could be visualized with a camera. On the other hand, the control group
31 was operated without guidance. The time required to remove both ovaries in the guidance group
32 was shorter than that in the control group ($P < 0.01$). We concluded that laparoscopic ovariec-
33 tomy with transrectal guidance of the ovaries can substantially shorten operative time, thereby
34 greatly contributing to animal welfare and to reducing the burden on the operator.

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36 KEY WORD: cow, laparoscopy, ovariectomy, transrectal

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49 Bovine ovariectomy is considered a necessary farm animal management technique, not only
50 for research on reproductive endocrinology [10, 13], but also for improving feeding efficiency
51 in feeder cattle [15] and for treating ovarian diseases, such as ovarian tumors and granulosa cell
52 tumors [3, 7, 15]. Ovariectomy used to be performed by colpotomy or laparotomy [2, 5, 12]. In
53 these methods, the operator pulls the ovary into the vagina, where it is ligated and ablated under
54 direct vision or with the aid of a Willis spay instrument or Kimberling-Rupp (K-R) spay instru-
55 ment. During ovariectomy by colpotomy, postoperative bleeding can easily go unnoticed and
56 can thus be fatal. Ovariectomy by laparotomy is performed under direct vision, making it pos-
57 sible to reduce the risk of complications; however, the size of the cow or a small uterus (such
58 as during non-pregnant stages) may make it difficult to confirm the ovary position under direct
59 vision, sometimes making it impossible to complete the desired treatment. Furthermore, ovar-
60 iectomy by colpotomy or laparotomy increases the risk of adhesions or bleeding from the ovar-
61 ian pedicle; in colpotomy, peritonitis may result in life-threatening complications [5, 12].

62 In contrast, laparoscopic ovariectomy offers various advantages, which are being increas-
63 ingly recognized. Minimal invasion of the abdominal cavity is not only esthetically advanta-
64 geous, but also therapeutically beneficial. Because this method causes little invasiveness and
65 pain, patients are admitted to a hospital for shorter periods and return to normal production
66 levels quicker [8, 9, 11]. In addition, the use of a laparoscopic camera and specialized forceps
67 makes it possible to perform ovariectomy in a reliable manner.

68 However, in laparoscopic ovariectomy, the uterus and ovaries are difficult to visualize when
69 over fattening results in pronounced fat deposits in organs or when insufficient withholding of
70 food makes it impossible to secure sufficient intra-abdominal space; as a result, the surgery is
71 prolonged, thereby placing significant burden on the cow.

72 Rectal palpation is a physical examination commonly used for the diagnosis of reproductive

73 disturbance and pregnancy [6]. Using this technique, the ovary or uterus can be palpated within
74 a few sec. Thus, we investigated the effect of transrectal guidance of the ovaries by an assistant
75 on operative time during laparoscopic ovariectomy.

76 All animal experiments were performed in compliance with the Guide for the Animal Care
77 and Use Committee at Azabu University, School of Veterinary Medicine (No. 160829-3). In
78 total, 24 Holstein dairy cows (28.2–98.9 months, 1-5 parities, weight 406–698 kg, BCS 2.75–
79 4.00) from a commercial dairy farm were included in the present study (Table 1). These cows
80 were randomly divided into two groups: with (guidance group) and without (control group)
81 transrectal guidance of the ovaries by an assistant (12 cows each). All cows were submitted to
82 general status examinations and blood tests prior to surgery and on days 1 and day 14 after
83 surgery. All surgeries were performed by a veterinary surgeon who performs laparoscopic ex-
84 aminations and surgeries on a daily basis, a camera assistant, and a surgical technician.
85 Transrectal manipulation of the ovaries in the guidance group were also performed by a veter-
86 inary technician who performs transrectal examinations on a daily basis.

87 The cows were allowed free access to water at all times but were prevented from feeding for
88 24 hr prior to surgery. Although one study reported that a sufficient visual field could not be
89 obtained if food was withheld in the rumen for less than 36 hr [1], we were able to procure
90 sufficient intra-abdominal space by withholding food for only 24 hr prior to surgery. First, 5,000
91 IU/kg procaine penicillin G (Kyoritsu Seiyaku Inc., Tokyo, Japan) was injected into the muscles
92 1 hr before surgery and for 3 days after surgery. The cows were restrained in a standing position.
93 Local anesthesia consisted of a lumbar epidural injection of 2% lidocaine hydrochloride (Pfizer
94 Japan Inc., Tokyo, Japan) 0.2 mg/kg and 2% Xylazine hydrochloride (Selactar 0.2%; Bayer,
95 Ltd., Tokyo, Japan) 0.05 mg/kg were administered between the first and second lumbar ver-
96 tebrae using the epidural needle (16G, 12 cm in length; Hakko syoji., Tokyo, Japan).

97 With a left abdominal incision, the risk of tissue damage is lower than if a right abdominal
98 incision was utilized, and the ovaries and uterus can be viewed more easily [14]. An approach
99 from the right abdominal wall is obstructed by the omentum majus; however, an approach from
100 the left flank enabled sufficient visualization of the uterus and ovaries. We clipped the hair,
101 cleaned and disinfected the region extending from the lumbar spine in the left abdomen to the
102 lower abdomen across a width of 25 cm, from the last rib to the tuber coxae; at the site of the
103 ports, an infiltration anesthesia with lidocaine 2% was performed. The laparoscopic camera port
104 (port 1) site was approximately 15 cm rostral from the left tuber coxae and approximately 10
105 cm towards the lower abdomen from the transverse processes of the lumbar vertebrae. After
106 inserting the trocar (11 mm in diameter, 20 cm in length; KARL STORZ GmbH & Co. KG,
107 Tuttlingen, Germany), CO₂ gas was injected into the abdominal cavity with a pressure of 10
108 mmHg. There were two surgical access ports: one (port 2) situated 10–15 cm towards the lower
109 abdomen from port 1 and the other (port 3) situated approximately 10 cm towards the lower
110 abdomen from port 2 and approximately 5 cm cranial to the vertical line from the tuber coxae
111 (Figures 1A and 1B).

112 First, a 30° laparoscope (10 mm in diameter, 57 cm in length; KARL STORZ GmbH & Co.
113 KG, Tuttlingen, Germany) was inserted through port 1. In the control group, two pairs of grasp-
114 ing forceps (HOPKINS Forceps, 43 cm in length; KARL STORZ GmbH & Co. KG, Tuttlingen,
115 Germany) were introduced through ports 2 and 3 to identify the uterus, which was then grasped
116 with forceps; we then followed along the right and left uterus horn until we confirmed the ova-
117 ries. In the guidance group, the designated assistant grasped the ovaries via a transrectal ap-
118 proach and pulled them to a position where they could be visualized with the laparoscopic
119 camera. In both groups, once the ovaries were identified with the laparoscopic camera, the left
120 ovarian parenchyma was grasped with the grasping forceps inserted through port 2; an injection

121 cannula (43 cm in length; KARL STORZ GmbH & Co. KG, Tuttlingen, Germany) was then
122 inserted through port 3, and 10 ml of lidocaine was injected into the mesosalpinx and the
123 mesovarium. The ovary was pulled with the grasping forceps, and the extended mesovarium
124 was cauterized close to the ovary with a vessel-sealing device (Ligasure Maryland 44; Med-
125 tronic plc, Dublin, Ireland) inserted through port 3. In the present study, we used a vessel-seal-
126 ing device that can seal blood vessels of up to 7 mm in diameter. The ovarian artery is expected
127 to have a diameter of 2.5 mm [4]. Once the cauterization site turned white, the mesovarium was
128 ablated with an organ cutter (Figure 1C); while confirming that there was no bleeding from the
129 ablation surface, this process was repeated until the ovary was completely detached. While
130 continuing to grasp the detached ovary with the forceps, the ovary was removed from the ab-
131 dominal cavity through port 2. The right ovary was also resected with the same procedure.

132 In the control group, if more than 90 min passed after the skin incision, the designated assis-
133 tant performed transrectal guidance of the ovaries. Following surgery, the abdominal cavity was
134 deflated through port 1, and the skin incision sites were closed with a stapler (WiSM Skin Sta-
135 pler; Keisei Ika KK, Tokyo, Japan). For cows with large ovaries which therefore required a
136 longer incision in the muscle in access port 3 for removal from the abdominal cavity, the muscle
137 layer was continuously sutured with synthetic absorbable suture material (USP 4) before sta-
138 pling the skin incision.

139 Blood samples were obtained from the jugular vein prior to surgery and 1 day and 14 days
140 after surgery. Collecting samples were used for counting of red blood cells (RBC) and white
141 blood cells (WBC), Platelet (PLT) and hematocrit (Ht) analysis by automated cell counter (PCE-
142 170, ERMA Inc, Tokyo, Japan) within 30 min after collection.

143 All statistical analyses were performed using the statistical software (Statcel, 4th edition,

144 OMS Publishing, Saitama, Japan). Data were tested normality distribution and as this was con-
145 firmed, Student's *t*-test was used to compare the mean values resulting from both treatments
146 groups (control and guidance groups). Data in the present study were expressed as mean \pm
147 standard deviation of the mean. Statistical significance was defined as $P < 0.05$.

148 The time required from skin incision to the completion of bilateral ovariectomy in the control
149 group (63 ± 25.2 min) was significantly longer than that in the guidance group (24 ± 6.6 min)
150 ($P < 0.01$; Figure 2). In a previous study, the duration of ovariectomy in cows was reported to
151 be 120–150 min [1]. In the present study, however, transrectal examination enabled us to com-
152 plete ovariectomy in a much shorter period of time.

153 None of the 24 cows demonstrated any abnormalities at general status examinations or blood
154 tests prior to surgery or at day 1 or day 14 after surgery; furthermore, no differences were ob-
155 served in blood tests between the groups (Table 2).

156 In general, it was necessary to use large traumatic forceps with relatively large teeth to avoid
157 losing hold of the uterine horn; this procedure can cause bleeding and lacerations in the uterine
158 serosa [1]. However, in the present study, an assistant grasped the ovaries via a transrectal ap-
159 proach, which enabled us to avoid injuring the uterine serosa. We can therefore confirm that the
160 transrectal assistance for ovariectomy by laparoscopy in cows helps to protect the cow from
161 physical damage.

162 The local anesthesia is necessary when the ovariectomy is done even the method excluding
163 the laparoscopy. The ovariectomy by colpotomy or laparotomy increases the risk of adhesions
164 or bleeding from the ovarian pedicle [5, 12]. Even if the laparoscopic ovariectomy need special
165 instruments, it is reduce the risk of complications because performed under direct vision. Ad-
166 ditionally the small incisions and little pain are return to normal activity level quickly [8, 9, 11].

167 In conclusion, our results indicate that the combined technique of using laparoscopy and a

168 transrescal assistant is effective for ovariectomy in cows because it reduces operative time,
169 physical damage, and the burden on the operator.

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216 **FIGURE LEGENDS**

217 **Fig. 1** Images showing portals for laparoscopic ovariectomy via the left flank.

218 Image (A) and illustration (B) showing the surgical site after insertion of the laparoscope and
219 forceps. The laparoscope (①) and two forceps portals (②, ③) were established. The port (①)
220 was approximately 15 cm rostral from the left tuber coxae (a) and approximately 10 cm towards
221 the lower abdomen from the transverse processes of the lumbar vertebrae (b). The port (②)
222 situated 10–15 cm towards the lower abdomen from port 1 (c) and the port (③) situated ap-
223 proximately 10 cm towards the lower abdomen from port 2 (d) and approximately 5 cm cranial
224 to the vertical line from the tuber coxae (e). The ovary was grasped with forceps and cauterized
225 by a vessel-sealing device (C). Cran = cranial, Caud = caudal, LUH = left uterus horn, RUH =
226 right uterus horn, OP = ovarian pedicle, OV = ovary, TC = tuber coxae.

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228 **Fig. 2** Bar graph (mean + SD) showing the surgery time of laparoscopic ovariectomy (control
229 vs guidance group). Statistically significant differences ($P < 0.01$), as determined by Student 's
230 *t*-test.

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239 **Table 1.** Description of the experimental cows (n=24; 12 control cows and 12 guidance cows) used for laparoscopic ovariectomy.

| 240 | No. | Group | Breed | Age (Months) | Parity | Body weight (kg) | BCS* | |
|-----|-----|-----------|-----------|-----------------|-------------|---------------------|--------------|------------|
| 241 | 1 | | Holstein | 40.0 | 2 | 406.0 | 2.75 | |
| | 2 | | Holstein | 28.2 | 1 | 454.0 | 2.75 | |
| | 3 | | Holstein | 33.8 | 2 | 478.0 | 3.00 | |
| 242 | 4 | | Holstein | 36.1 | 1 | 488.0 | 2.75 | |
| | 5 | | Holstein | 29.6 | 1 | 555.0 | 3.50 | |
| 243 | 6 | control | Holstein | 57.8 | 2 | 559.0 | 3.00 | |
| | 7 | | Holstein | 37.6 | 1 | 582.0 | 3.50 | |
| 244 | 8 | | Holstein | 52.0 | 3 | 587.0 | 3.25 | |
| | 9 | | Holstein | 38.2 | 1 | 593.5 | 3.50 | |
| 245 | 10 | | Holstein | 92.9 | 3 | 600.0 | 3.75 | |
| | 11 | | Holstein | 78.4 | 4 | 643.0 | 3.75 | |
| 246 | 12 | | Holstein | 40.8 | 1 | 698.0 | 4.00 | |
| | | | mean ± SD | | 47.1 ± 20.1 | 1.8 ± 1.0 | 553.6 ± 83.3 | 3.29 ± 0.4 |
| 247 | 13 | | | Holstein | 29.3 | 1 | 462.0 | 2.75 |
| | 14 | | | Holstein | 36.6 | 2 | 413.0 | 2.75 |
| 248 | 15 | | | Holstein | 39.7 | 1 | 456.0 | 2.75 |
| | 16 | | | Holstein | 31.3 | 1 | 481.0 | 2.75 |
| 249 | 17 | guidance | Holstein | 58.6 | 2 | 527.0 | 3.00 | |
| | 18 | | Holstein | 55.2 | 2 | 530.0 | 2.75 | |
| 250 | 19 | | Holstein | 66.1 | 3 | 545.0 | 3.50 | |
| | 20 | | Holstein | 45.1 | 1 | 560.0 | 3.50 | |
| 251 | 21 | | Holstein | 71.5 | 3 | 575.0 | 3.50 | |
| | 22 | | Holstein | 98.9 | 5 | 595.5 | 3.75 | |
| 252 | 23 | | Holstein | 46.8 | 1 | 617.0 | 4.00 | |
| | 24 | | Holstein | 66.8 | 3 | 645.0 | 3.75 | |
| 253 | | mean ± SD | | 53.8 ± 20.0 | 2.1 ± 1.2 | 533.9 ± 70.2 | 3.23 ± 0.5 | |

*Body condition Score

254 **Table 2.** Profile of blood examinations prior to surgery and on day 1 and day 14 after surgery.

| | | pre | | 1d | | 14d | |
|------------|---------------------------|-------------------|--------------------|-------------------|--------------------|-------------------|--------------------|
| | | control (n=12) | guidacne (n=12) | control (n=12) | guidance (n=12) | control (n=12) | guidance (n=12) |
| RBC | $\times 10^4/\mu\text{L}$ | 697 \pm 115 | 609 \pm 246 | 679 \pm 141 | 726 \pm 122 | 653 \pm 93 | 631 \pm 109 |
| WBC | / μL | 8,308 \pm 2,627 | 8,392 \pm 6,829 | 9,042 \pm 3,909 | 8,325 \pm 4,336 | 8,517 \pm 3,852 | 8,217 \pm 2,750 |
| PLT | $\times 10^4/\mu\text{L}$ | 49 \pm 13 | 43 \pm 38 | 59 \pm 27 | 40 \pm 22 | 30 \pm 20 | 42 \pm 22 |
| Ht | % | 35 \pm 9 | 33 \pm 10 | 31 \pm 6 | 34 \pm 5 | 33 \pm 4 | 33 \pm 4 |
| Fibrinogen | mg/dL | 500 \pm 148 | 500 \pm 341 | 583 \pm 199 | 492 \pm 198 | 267 \pm 98 | 327 \pm 257 |

RBC = red blood cell, WBC = white blood cell, PLT = platlet, Ht = hematocrit

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