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Author	Hee-Woon Lee[1,2#], Hae Rim Kim[3,#], Byunghoon Cha[1], Mirim Yu[4], Misun Ha[5], Sooho Choi[6], Yan Zhang[7], Xiangzi Li[8], Seong Ho Choi[9*] [#] HW Lee and HR Kim are co-first authors and contributed equally to this work.
Affiliation	[1] Mari Animal Medical Laboratory and Mari Animal Medical Center, Cheongju-si and Yongin-si, Gyeonggi 17178, Republic of Korea [2] School of Animal Science, Horticulture, and Food Engineering, Animal Science major, Chungbuk National University, Cheongju, Chungbuk, 28644, Repulic of Korea [3] Department of Statistical Data Science, University of Seoul, Seoul 02504, Republic of Korea [4] Department of Business Administration, University of Seoul, Seoul 02504, Republic of Korea [5] The Provincial Office of Gyeongsangbuk-do, Andong-si, Gyeongbuk 36759, Republic of Korea [6] Soo Animal Hospital, Yeongju-si, Gyeongbuk 36105, Republic of Korea [7] College of Animal Science and Technology, Jilin Agricultural Science and Technology College, Jilin 132109, China [8] Engineering Research Center of North-East Cold Region Beef Cattle Science & Technology Innovation Ministry of Education, Department of Animal Science, Yanbian University, Yanji 133002, China [9] Department of Animal Science, College of Agriculture, Life & Environment, Chungbuk National University, Cheongju, Chungbuk 28644, Republic of Korea
ORCID (for more information, please visit https://orcid.org)	Hee-Woon, Lee (ORCID: 0000-0003-1152-3993) Hae Rim, Kim (ORCID: 0000-0003-2186-8792) Byunghoon, Cha (ORCID: 0009-0009-8982-8720) Mirim, Yu (ORCID: 0009-0006-1316-4188) Misun, Ha (ORCID: 0009-0007-5061-1036) Sooho, Choi (ORCID: 0009-0005-4750-9984) Yan, Zhang (ORCID: 0000-0003-2752-0578) Xiangzi, Li (ORCID: 0000-0003-3061-3847) Seong Ho, Choi (ORCID: 0000-0001-8869-0218)
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CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Seong Ho Choi
Email address – this is where your proofs will be sent	seongho@cbnu.ac.kr
Secondary Email address	
Address	Department of Animal Science, College of Agriculture, Life & Environment, Chungbuk National University, Cheongju, Chungbuk 28644, Republic of Korea
Cell phone number	+82-10-5325-6192
Office phone number	+82-43-261-2545
Fax number	+82-43-273-2240

Abstract

Approaches including hormonal treatments and surgical interventions have been established to suppress estrus and thus improve the average daily gain and meat quality in female Hanwoo cattle. Ovarian ligation is one such approach which suppresses estrus without removal of the ovaries, thereby reducing complications such as hemorrhage and ovarian remnant syndrome. Ovariectomy and ovarian ligation have been increasingly applied in commercial farms to improve feed efficiency and meat quality; however, objective validation using large-scale data remains limited. To address this gap, sensitivity analysis was conducted by estimating odds ratios for superior carcass traits and grading through logistic regression models, including unadjusted models, models adjusted for covariates (season, age at slaughter, and parity), and models based on propensity score matching (PSM). Ovarian ligation significantly increased the carcass, weight, and marbling scores in both heifers and cows. Compared with their non-ligated counterparts, ligated female Hanwoo cattle had higher odds of receiving Quality Grade 1++ and ≥1+ in PSM-adjusted models. However, yield grade A was not improved in heifers and even decreased in cows. These findings suggest that ovarian ligation exerts a more pronounced effect on meat quality than on yield grade A, particularly given the lack of improvement or decline in yield grade A across parity groups. Restricted spline curve analysis showed that ligated female Hanwoo cattle consistently had higher probabilities of achieving superior quality grades (QG 1++ or ≥1+) than the non-ligated heifers and cows, with the largest differences at lower parity levels. In contrast, the probability of achieving a grade A yield was higher in non-ligated cows than in ligated cows. Ovarian ligation is an effective and minimally invasive strategy for enhancing meat quality in Hanwoo cattle, particularly heifers. However, its effect on the yield grade may be limited or negative in cows. These findings provide practical evidence for producers to consider ovarian ligation as a strategy to improve carcass traits and meat quality.

Keywords: Female Hanwoo cattle, Ovarian ligation, Carcass traits, Marbling score, Meat quality, Propensity score matching

Introduction

Female Hanwoo cattle (hereafter, female Hanwoo) were classified as nulliparous heifers (heifers) or parous cows (cows), according to their rearing purpose and calving history. The heifers had no calving experience, whereas the cows were slaughtered after a short fattening period. In recent years, the use of heifers for beef production has increased due to factors such as a decline in heifer prices, rising demand for female Hanwoo, and the need to regulate the Hanwoo population [1].

During the fattening period in females, estrus symptoms that occur periodically can reduce feed efficiency and negatively affect productivity [2]. During estrus, physiological changes such as increased activity, nervousness, and reduced feed intake lead to a decline in feed efficiency [3,4]. To improve feed efficiency and meat quality, estrus suppression is commonly applied to cows with a history of calving or to heifers during the pre-slaughter fattening period [5].

Ovariectomy is performed to suppress ovarian function, and various surgical instruments have been developed for this purpose [6]. These instruments include the Kimberling-Rupp Spaying Device [7], an ovarian removal instrument for mammals [8], the Willis-drop Spay Instrument [9], and the Meagher Ovary Flute [10]. All these procedures involve intra-abdominal ovarian removal. However, this approach poses the risk of severe hemorrhage due to transection of the ovarian artery and vein, and if ovarian tissue remains in the abdominal cavity, it may lead to complications such as ovarian remnant syndrome [11].

Ovarian ligation has garnered attention as a safe technique with minimal adverse effects [12]. As this method does not involve transection of the ovarian artery and vein, the risk of severe hemorrhage is significantly reduced, and postoperative clinical observations are minimized [12]. The large animal ovarian ligation device used in this procedure is a novel surgical instrument patented in the Republic of Korea [13]. Unlike traditional ovariectomy, which involves complete removal of the ovary, ovarian ligation induces regression without excision. Consequently, secretion of major hormones such as estrogen and progesterone from the ovary is not entirely suppressed [12].

In the participating commercial farms, ovarian ligation has been applied under field conditions with the expectation of improving feed efficiency, enhancing meat quality, and increasing carcass traits. However, statistical analyses based on field-level ligation procedure and slaughter records for evaluating the effectiveness of ovarian ligation are limited. Although numerous studies have reported the effect of ovariectomy on carcass traits and grade, quantitative evidence regarding the effects of ovarian ligation remains limited [15,15]. Previous studies have suggested that parity in female Hanwoo cattle may influence meat quality [16]. In the present study, analyses were conducted separately for heifers and cows.

This study aimed to evaluate the effects of ovarian ligation in female Hanwoo cattle quantitatively. After adjusting for baseline differences between the ligated and non-ligated groups using propensity score matching (PSM), statistical comparisons were conducted to assess differences in carcass traits and meat quality. The findings of this study provide practical evidence to support nutritional management strategies on commercial farms.

Materials and Methods

Ovarian ligation

In this study, ovarian ligation procedures were performed at two Large Animal Hospitals: Mari Animal Medical Center and Soo Animal Hospital. Eligibility for the procedure was first assessed through rectal examination, and an appropriately sized large animal ovarian ligation device was selected based on the size of the left and right ovaries. A specially designed silicone ligation ring corresponding to the ovarian size was attached to the device. A disinfected vaginal wall perforator was used to create an insertion site in the heifer's or cow's vaginal wall. The ligation device equipped with a ring was inserted through the perforated site, and the ring was gently deployed onto the left and right ovaries using an appropriately sized ligation device. The ligation ring constricted the ovarian artery and vein, inducing ovarian regression. Upon completion of the ovarian ligation procedure, antibiotics and anti-inflammatory agents were administered to prevent infection and inflammation.

Data source

The data were obtained from the Cow Chronicle (www.cowchronicle.com), an electronic medical record system specialized for bovine health management, which was developed and operated by the Mari Animal Medical Center. The dataset included records related to ovarian ligation procedures and slaughter outcomes.

This study uses data collected from commercial farms in Korea between 2013 and 2024 (n = 29,751). Excluding non-Hanwoo (n = 17,577) and non-female (n = 3,007) cattle, 9,167 female Hanwoo were included in this study. Cases in which only one ovary was ligated were excluded (n=5) because the function of the contralateral ovary remained intact. Cases with duplicate identification numbers (ID) caused by the procedure being performed twice due to failure (n = 39) were excluded. A total of 9,123 female Hanwoo cattle raised and slaughtered on farms where ovarian ligation was conducted were classified into two groups: ligated (n = 5,746) and non-ligated (intact; n = 3,377) (Figure 1). The required sample size calculated using G*Power was 343 animals, and the achieved statistical power was 0.9504, indicating that the sample size was sufficient to meet the pre-specified power requirements.

Korean carcass grading system

In Korea, carcass traits are evaluated according to the Korean Carcass Grading Procedure [17] and classified into yield grade (YG) and quality grade (QG).

YG was determined based on backfat thickness, rib-eye area, and carcass weight, using a yield index calculated according to the specified formula for breed and sex. YG was categorized into three grades: A, B, and C. For female Hanwoo cattle, the yield index was calculated using the formula shown in Equation 1 [18], and the evaluation criteria included backfat thickness, rib-eye area, and carcass weight.

The QG was determined using the marbling score (1–9, where 1 = devoid and 9 = abundant), meat color (1–7, where 1=very bright red and 7 = very dark red), fat color (1–7, where 1 = creamy white and 7 = yellowish), texture score (1–5, where 1 = firm and 5 = soft), and maturity score (1–9, where 1 = very youthful and 9 = very mature) [17]. Among these, the marbling score plays a primary role in determining QG, which is classified into grades 1 + +, 1 +, 1, 2, and 3. A marbling score of 9, 8, or 7 corresponds to grade 1++, a score of 6 to grade 1+, a score of 5 or 4 to grade 1, a score of 3 or 2 to grade 2, and a score of 1 to grade 3 [17]. Grade 1++ represents the most desirable quality, whereas Grade 3 indicates the lowest quality (Supplementary Table 1).

Backfat thickness, rib-eye area, marbling score, meat color, fat color, and texture score were measured on the cut surface of the longissimus dorsi muscle between the last thoracic and first lumbar vertebrae of the left half of the carcass. The maturity score was assessed by evaluating the degree of cartilage ossification in the spinous processes of the vertebrae of the left half-carcass.

$$Equation\ 1 = \frac{[6.90137 - 0.9446 \times BFT(mm) + 0.31805 \times RA(cm^2) - 0.54952 \times CW(kg)]}{CW(kg)} \times 100$$

$BFT;\ BAKFAT\ THICKNESS, RA;\ RIBEYE\ AREA, CW;\ CARCASS\ WEIGHT$

Statistics

Statistical power was calculated a priori to ensure sufficient sample size estimation for the Wilcoxon signed-rank test (matched pairs) using G*Power version 3.1.9.7. The parameters were set as a small effect size (dz = 0.2), a significance level (α) of 0.05, and a statistical power (1–β) of 0.95, assuming a two-tailed test. The effect size of 0.2 was selected as a conservative assumption to avoid overestimation of the true effect, while the power level of 0.95, which is more stringent than the commonly used 0.80, was chosen to enhance the robustness and reliability of statistical inference in this large-scale retrospective study.

Continuous variables are presented as mean and standard deviation or median (interquartile range: Q1–Q3) for each group based on Shapiro–Wilk test. Slaughter age in months satisfied the normality assumption and was analyzed using the t-test, and other continuous variables did not satisfy the normality assumption and were analyzed using non-parametric tests. The Wilcoxon signed-rank test was performed based on the unique identification code (Cow ID) matched using PSM. Additionally, the median difference and 95% confidence intervals (CI) were calculated. Categorical variables are presented as N and percentages, and the McNemar test was used as a paired test for categorical comparisons.

Logistic regression analysis was performed to calculate odds ratios (ORs) and 95% CIs. Three modeling approaches were applied. First, the unadjusted model (n=9,123) was a univariate logistic regression using the original dataset, including only ligation status as the independent variable. Second, in Adjusted Model 1 (n=9,123), multivariable logistic regression analysis was performed by including the slaughter season, birth season, slaughter age in months, and parity as covariates using the original dataset. In this analysis, slaughter season and birth season were treated as categorical variables, whereas slaughter age in months and parity were included as continuous variables. Third, Adjusted Model 2 (n=2,890) used a PSM approach to adjust for potential confounding factors between the ligated and non-ligated groups, thereby ensuring comparability. Propensity scores were calculated using a logistic regression model that included the same covariates as in Adjusted Model 1. A 1:1 nearest-neighbor matching without replacement was performed (caliper = 0.001), and the standard mean difference (SMD) was calculated to evaluate covariate balance between groups. Multicollinearity among covariates was evaluated using the variance inflation factor (VIF), and all VIF values were below 5.

Using ligated heifers as the reference group, the predicted ORs for the association between ligation status, carcass traits, and meat quality were estimated across the different parity levels. Restricted spline curves were plotted using the rcsplot function from the plotRCS package with four nodes (5th, 33rd, 66th, and 95th) specified for the spline.

All statistical analyses and visualizations were performed using R statistical software (version 4.5.0; R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set at a two-sided p-value <0.05.

Results

Table 1 presents the distributions of slaughter season, birth season, slaughter age in months, and parity before and after PSM between the ligated and non-ligated groups stratified by heifers and cows. Before PSM, significant differences were observed between ligated (n = 4,302) and non-ligated (n = 839) heifers in terms of slaughter season, birth season, and age at slaughter in months ($p<0.001$). Specifically, the mean slaughtered age in months was 31.08 months (SD=3.45) in the ligated group and 43.55 months (SD=22.65) in the non-ligated group. In cows, significant differences were also found between the ligated (n=1,444) and non-ligated (n=2,538) groups for slaughter season, birth season, slaughter age in months, and parity ($p<0.001$). The mean age in months was 60.85 months (SD=20.76) in the ligated group and 65.99 months (SD=26.08) in the non-ligated group. After PSM, 509 heifers and 936 cows were matched in each group (ligated vs. non-ligated). Following matching, no significant differences were observed between the two groups across any of the covariates, indicating good balance (SMD<0.05).

Table 2 summarizes the comparison of carcass traits and meat quality between the matched ligated and non-ligated groups (509 heifers and 936 cows in each group) after PSM. Among heifers, the ligated group showed a significantly higher carcass weight than the non-ligated group (median 369.0 kg vs. 355.0 kg, median difference 14.00 kg [95% CI: 7.00, 20.50], $p<0.001$). The rib-eye area was also significantly greater in the ligated group (86.0 cm^2 vs. 84.0 cm^2, median difference 2.00 cm^2 [95% CI: 0.00, 3.50], $p=0.024$). Marbling score was higher in the ligated group (median 6.0 vs. 5.0, median difference 1.50 [95% CI: 1.50, 2.00], $p<0.001$). The yield index was slightly lower in the ligated group (median difference –1.92 [95% CI: –2.28, –1.55], $p<0.001$). However, no statistically significant differences in backfat thickness, fat color, or texture score were found between the groups. Although meat color was significantly different ($p=0.003$), the median value was the same in both groups (5.0 vs. 5.0). The maturity score was lower in the ligated group (median difference: –1.00 [95% CI: –1.00, –0.50], $p<0.001$).

In cows, the ligated group also showed significantly higher carcass weight than the non-ligated group (median 373.0 kg vs. 354.0 kg, median difference 20.00 kg [95% CI: 15.00, 24.50], $p<0.001$), as well as a larger rib-eye area (86.0 cm^2 vs. 84.0 cm^2, median difference 2.50 cm^2 [95% CI: 1.50, 3.50], $p<0.001$). The marbling score was also significantly higher in the ligated group (median 5.0 vs. 4.0, median difference, 1.00 [95% CI: 1.00, 1.50]; $p<0.001$). Unlike in heifers, the ligated group of cows showed significantly greater backfat thickness (median 13.0 vs. 12.0, median difference 1.50 [95% CI: 1.00, 2.00], $p<0.001$). No significant differences were observed among the groups in fat color, maturity score, or texture score. The meat color was statistically different despite having the same median value (5.0 vs. 5.0, median difference –0.50 [95% CI: –1.00, –0.00], $p<0.001$). The yield index was also significantly lower in the ligated group (median difference –2.76 [95% CI: –3.06, –2.45], $p<0.001$).

As shown in Table 3, the distribution of QG significantly increased in the ligated group in both heifers and cows ($p<0.001$, McNemar's test). In contrast, among heifers, no statistically significant difference in YG was found between the ligated and non-ligated groups ($p=0.767$, McNemar's test). However, in cows, the proportion of grade A meat was significantly higher in the non-ligated group than in the ligated group ($p<0.001$, McNemar test).

Table 4 presents the OR in QG (1++, ≥1+) and YG (A). ORs were calculated using the non-ligated group as the reference group, using the original and PSM datasets (Table 4). Restricted spline curves using the original dataset and ORs were calculated using the ligated group with heifers as the reference group (Figure 2, Supplementary Table 3).

In the analysis of heifers (Table 4), the ligated group showed a significantly higher QG of 1++ than the non-ligated group. Specifically, the unadjusted model showed an OR of 5.90 [95% CI: 4.70, 7.52], whereas the model adjusted by slaughter season, birth season, and slaughtered age in months (Model 1) showed an OR of 5.53 [95% CI: 4.32, 7.19]. The PSM-adjusted OR was 3.61 [95% CI: 2.66, 4.96]. A similar pattern was observed for QG ≥ 1+, where the PSM-adjusted OR was 3.35 [95% CI: 2.60, 4.35], indicating a statistically significant improvement in meat quality. However, no significant difference was observed between the ligated and non-ligated groups for YG = A (Model 2 OR: 0.88 [95% CI: 0.66, 1.16]).

A similar trend was observed in cows (Table 4). The ligated group had a significantly higher likelihood of achieving a QG of 1++ (PSM-adjusted OR: 3.00 [95% CI: 2.11, 4.34]) and QG≥1+ (PSM-adjusted OR: 2.46 [95% CI: 2.00, 3.03]). In contrast, for YG = A, the PSM-adjusted OR was 0.71 (95% CI: 0.57, 0.88), indicating that the likelihood of achieving grade A was significantly lower in the ligated group of cows.

Restricted spline curve analysis (Figure 2, Supplementary Table 3) of the ligated group, with heifers as the reference group, showed similar patterns. As parity increased, the likelihood of achieving a QG of 1++ or ≥1+ decreased sharply. Across the entire parity range, the ligated group consistently demonstrated higher probabilities than the non-ligated group, with the most pronounced differences observed at lower parity levels (1–3). In contrast, for YG = A, the probability was higher in the non-ligated group than in the ligated group.

Discussion

Various techniques have been introduced to enhance meat quality in female Hanwoo cattle, ranging from hormonal treatments to surgical interventions. The underlying principle involves suppressing estrus to enhance average daily gain and meat quality. Studies have reported that dietary supplementation with melengestrol acetate, an orally active progestin, suppresses estrus in female cattle and improves both average daily gain and meat quality [19]. While ovariectomy is the most commonly used surgical method, ovarian ligation has been developed as a safer alternative with a minimal risk of hemorrhagic complications [12,13]. Among the 5,746 procedures performed in this study, 39 (0.68%) required re-intervention due to estrus recurrence or the presence of non-regressed ovaries. These cases included instances in which a portion of the ovary was not ligated, leaving residual ovarian tissue; the broad ligament covered the ovary at the time of ligation, resulting in the silicone ring constricting only the broad ligament and allowing the ovary to slip out; neovascularization occurred over the ring, thereby re-establishing normal blood flow to the ovary; or the ring itself fractured. Although these issues were resolved through repeat procedures, the corresponding cases were excluded from the final dataset.

As this study was based on retrospective data, it was necessary to adjust for covariates that could potentially cause bias. Previous studies have demonstrated that slaughter season, birth season, and slaughtered age in months are statistically significant factors influencing carcass traits in Hanwoo cattle [20-24]. We compared the two groups using the original data and PSM dataset for sensitivity analysis, an unadjusted model, and a model adjusted for slaughter season, birth season, slaughtered age in months, and parity as covariates.

In the dataset (Table 1), the number of heifers that underwent ovarian ligation (n = 4,302) was significantly higher than that of cows (n = 1,444). This discrepancy may reflect the differences in the levels of interest in fattening technologies between beef and breeding farms. Beef farms actively pursue strategies to improve both meat quality and carcass traits, and tend to adopt various technologies for this purpose. In such settings, heifers are often purchased for fattening purposes rather than breeding, and ovarian ligation is commonly performed to prevent calving. In contrast, breeding farms primarily focus on improving fertility efficiency and generally show less interest in fattening technologies. Consequently, cows raised for breeding on these farms are typically fattened only after they have calved at least once, and there is less motivation to apply technologies aimed at enhancing meat quality or carcass traits.

Before PSM, slaughter age in months was significantly lower in the ligated heifer group than in the non-ligated group (Table 1). In the commercial farms that provided data for this study, non-ligated (intact) heifers were primarily raised for breeding purposes and were often fattened at a later stage following reproductive failure or abortion. Therefore, the observed difference in slaughter age likely reflects a delayed transition from breeding to fattening, resulting in increased slaughter age in the non-ligated group.

PSM was applied to adjust for baseline differences between the ligated and non-ligated groups. The matching procedure confirmed that there were no significant differences between the two groups for all analytical variables. In both heifers and cows, carcass weight, rib-eye area, and marbling score were higher in the ligated group than in the non-ligated group. These results indicated the positive effects of ovarian ligation, as the ligated group exhibited improvements in carcass traits related to yield (carcass weight and rib-eye area) and meat quality (marbling score). Furthermore, the proportions of QG 1++ and 1+ were higher in the ligated group than in the non-ligated group, suggesting that ovarian ligation had a positive influence on meat quality (Table 3). However, a statistically significant decrease in the yield index was observed in cows, most likely because of the computational method of the yield index (Supplementary Table 2). According to this equation, an increase in the rib-eye area contributes to a higher yield index, whereas increases in the carcass weight and backfat thickness contribute to a lower yield index. This is because, in cows, although rib-eye area, which increases yield index, was elevated, concurrent increases in carcass weight and backfat thickness, which reduce yield index, were also observed. Despite the statistically significant difference in meat color between the two groups, the median values were virtually identical (Table 2). In cows, a median difference in meat color of –0.50 [95% CI: –1.00, 0.00] was observed. The ligated group showed a tendency toward a slightly brighter meat color than the non-ligated group. In the ligated group, the 95% CI for the meat color was 4–5 for heifers and 5–5 for cows. In contrast, the non-ligated group showed a consistent 95% CI of 5–5 for both heifers and cows. According to the Korean carcass grading system, a meat color score of 3–5 was classified as QG 1++ [17]. Therefore, the actual influence of meat color on QG between the two groups appears to be limited. However, because a meat color score of 6 or higher results in a decrease in quality to QG 1+, even a slight reduction in meat color may have a positive impact on QG. No significant differences in fat color or texture score were observed between the groups in either heifers or cows (Table 2).

Backfat thickness and maturity score showed different patterns in heifers and cows (Table 2). In cows, the backfat thickness score was higher in the ligated group than in the non-ligated group. This outcome can be considered a negative change in terms of its effect on lowering the yield index. The effect of ovarian ligation on increasing backfat thickness was more pronounced at a higher slaughter age (Table 1). In previous studies on Hanwoo cattle, an increase in slaughter age in months was significantly associated with greater backfat thickness scores [25]; however, as the effect of slaughter age in months was adjusted for in the present analysis, the increase in backfat thickness observed only in cows after ovarian ligation could not be fully accounted for by previously reported associations. Previous studies have shown that extended fattening periods are associated with increased backfat thickness and marbling score [26]. In the commercial farms that provided data for this study, cows were frequently slaughtered at approximately 6 months postpartum. By contrast, ovarian ligation was generally conducted at around 2 months postpartum in ligated cows, and these animals were subsequently subjected to fattening management for at least 6 months following the procedure. Accordingly, farms performing ovarian ligation may have placed greater emphasis on marbling score, which could indicate a longer fattening period in ligated cows. In heifers, the ligated group exhibited a lower maturity score. The maturity score is determined by the color, shape, and degree of bone and cartilage ossification [27]. Higher maturity scores are associated with increased yellow fat deposition and tougher meat texture [28]. Therefore, an increase in the maturity score has a negative impact on meat quality. According to the Korean meat grading system, a maturity score of 8 or 9 indicates a one-grade reduction in QG [17]. Thus, a median difference of –1.00 [95% CI: –1.00, –0.50] in maturity score observed in the ligated group of heifers can be considered a positive contributor to QG assessment. However, because the 95% CI for non-ligated heifers was 3–4, it is difficult to conclude whether this difference had a substantial impact on QG assessment. In cows, no difference in maturity score was observed between the ligated and non-ligated groups.

In heifers, the likelihoods of achieving QG 1++ and 1+ were 3.61 [95% CI: 2.66, 4.96] and 3.35 [95% CI: 2.60, 4.35] times higher, respectively, in the ligated group compared with the non-ligated group (Table 4). In cows, the corresponding odds were 3.00 [95% CI: 2.11, 4.34] and 2.46 [95% CI: 2.00, 3.03], respectively. These results indicated a statistically significant improvement in meat quality associated with ovarian ligation in both heifers and cows, with a more pronounced effect observed in heifers. In contrast, the odds of achieving YG = A were not significantly different among heifers, whereas a negative association was observed among cows. This suggests that the effects of ovarian ligation on yield outcomes differ according to parity.

The results of this study confirmed that ovarian ligation improved marbling score, rib-eye area, and carcass weight. The effect of ovarian ligation was greatest in heifers, whereas in cows, the effect diminished with increasing parity compared with that observed in heifers (Figure 2). As parity, defined at the time of ovarian ligation, increased, the probabilities of achieving QG 1++ and QG 1+ showed a markedly decreasing trend. Across all parity levels, the ligated group consistently demonstrated higher odds than the non-ligated group (Figure 2A, B, Supplementary Table 3). The difference between the ligated and non-ligated groups was particularly pronounced at lower parity levels, suggesting that ovarian ligation may have a beneficial effect in improving QG at lower parity levels. However, compared to ligated heifers, female Hanwoo cattle in the ligated group were more likely to achieve YG=A up to the third parity, whereas those in the non-ligated group maintained a higher probability up to the fourth parity. This suggests that ovarian ligation may have a negative effect on the yield index. These results highlight the need to establish breeding and fattening strategies that consider the relationship between QG and YG. From an economic perspective, this trade-off may still be acceptable in pricing systems where carcass weight and QG premiums contribute more substantially to carcass value than YG discounts. Previous pricing analyses have demonstrated that carcass weight and QG account for a considerably greater share of revenue variation than yield grade [29], suggesting that the observed increases in carcass weight and marbling in ligated cows offset the economic loss associated with a lower yield index. Further studies are warranted to determine the optimal timing of ovarian ligation to maximize improvements in carcass traits, including QG and YG.

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Tables and Figures

Table 1
Baseline characteristics of heifers and cows

		Before PSM				After PSM			
		Ligated	Non-ligated	SMD	*p*	Ligated	Non-ligated	SMD	*p*
Heifers		**(n=4,302)**	**(n=839)**			**(n=509)**	**(n=509)**		
Slaughtered season	Spring (3–5)	726 (16.90%)	204 (24.30%)	0.227	<0.001	121 (23.80%)	121 (23.80%)	0.006	1
	Summer (6–8)	1,041 (24.20%)	228 (27.20%)			131 (25.70%)	131 (25.70%)		
	Fall (9–11)	1,217 (28.30%)	189 (22.50%)			125 (24.60%)	124 (24.40%)		
	Winter (12–2)	1,318 (30.60%)	218 (26.00%)			132 (25.90%)	133 (26.10%)		
Birth season	Spring (3–5)	2,059 (47.90%)	327 (39.00%)	0.234	<0.001	213 (41.80%)	213 (41.80%)	0.006	1
	Summer (6–8)	927 (21.50%)	183 (21.80%)			109 (21.40%)	110 (21.60%)		
	Fall (9–11)	412 (9.60%)	136 (16.20%)			76 (14.90%)	76 (14.90%)		
	Winter (12–2)	904 (21.00%)	193 (23.00%)			111 (21.80%)	110 (21.60%)		
Slaughtered age in month		31.08 (±3.45)	43.55 (±22.65)	0.77	<0.001	31.96 (±5.99)	31.96 (±5.99)	<0.001	1
Cows		**(n=1,444)**	**(n=2,538)**			**(n=936)**	**(n=936)**		
Slaughtered season	Spring (3–5)	397 (27.50%)	615 (24.20%)	0.151	<0.001	260 (27.80%)	260 (27.80%)	<0.001	1
	Summer (6–8)	422 (29.20%)	650 (25.60%)			264 (28.20%)	264 (28.20%)		
	Fall (9–11)	318 (22.00%)	590 (23.20%)			190 (20.30%)	190 (20.30%)		
	Winter (12–2)	307 (21.30%)	683 (26.90%)			222 (23.70%)	222 (23.70%)		
Birth season	Spring (3–5)	700 (48.50%)	1,038 (40.90%)	0.165	<0.001	466 (49.80%)	466 (49.80%)	<0.001	1
	Summer (6–8)	301 (20.80%)	563 (22.20%)			191 (20.40%)	191 (20.40%)		

	Fall (9–11)	181 (12.50%)	349 (13.80%)			100 (10.70%)	100 (10.70%)		
	Winter (12–2)	262 (18.10%)	588 (23.20%)			179 (19.10%)	179 (19.10%)		
Slaughtered age in month		60.85 (±20.76)	65.99 (±26.08)	0.218	<0.001	57.59 (±17.46)	57.59 (±17.46)	<0.001	1
Parity (%)	1	413 (28.60%)	560 (22.10%)	0.242	<0.001	286 (30.60%)	286 (30.60%)	<0.001	1
	2	389 (26.90%)	627 (24.70%)			261 (27.90%)	261 (27.90%)		
	3	319 (22.10%)	575 (22.70%)			215 (23.00%)	215 (23.00%)		
	4	135 (9.30%)	323 (12.70%)			87 (9.30%)	87 (9.30%)		
	5	92 (6.40%)	188 (7.40%)			53 (5.70%)	53 (5.70%)		
	6	43 (3.00%)	102 (4.00%)			20 (2.10%)	20 (2.10%)		
	7	24 (1.70%)	68 (2.70%)			9 (1.00%)	9 (1.00%)		
	8	24 (1.70%)	55 (2.20%)			5 (0.50%)	5 (0.50%)		
	9	4 (0.30%)	19 (0.70%)			0 (0.00%)	0 (0.00%)		
	10	1 (0.10%)	11 (0.40%)			0 (0.00%)	0 (0.00%)		
	11	0 (0.00%)	6 (0.20%)			0 (0.00%)	0 (0.00%)		
	12	0 (0.00%)	2 (0.10%)			0 (0.00%)	0 (0.00%)		
	13	0 (0.00%)	2 (0.10%)			0 (0.00%)	0 (0.00%)		

Abbreviations: SMD = standard mean difference.

[1] Categorical variables are presented by N (%), and continuous variables are presented as means ± std;

Table 2
Comparison of carcass traits between ligated and non-ligated female Hanwoo

	Heifers				Cows			
	Ligated **(n=509)**	Non-ligated **(n=509)**	Difference Median (95% CI)	*p*	Ligated **(n=936)**	Non-ligated **(n=936)**	Difference Median (95% CI)	*p*
Carcass weight	369.00 (335.00,401.00)	355.00 (322.00,385.00)	14.00 (7.00, 20.50)	<0.001	373.00 (343.00,403.00)	354.00 (321.00,386.50)	20.00 (15.00, 24.50)	<0.001
Backfat thickness	13.00 (10.00,16.00)	12.00 (10.00,16.00)	0.00 (-0.50, 1.00)	0.686	13.00 (10.00,17.00)	12.00 (10.00,16.00)	1.50 (1.00, 2.00)	<0.001
Rib-eye area	86.00 (78.00,94.00)	84.00 (77.00,92.00)	2.00 (0.00, 3.50)	0.024	86.00 (79.00,93.00)	84.00 (76.00,91.00)	2.50 (1.50, 3.50)	<0.001
Marbling score	6.00 (5.00,8.00)	5.00 (3.00,6.00)	1.50 (1.50, 2.00)	<0.001	5.00 (4.00,6.00)	4.00 (3.00,6.00)	1.00 (1.00, 1.50)	<0.001
Meat color	5.00 (4.00,5.00)	5.00 (5.00,5.00)	-0.00 (-0.00, -0.00)	0.003	5.00 (5.00,5.00)	5.00 (5.00,5.00)	-0.50 (-1.00, -0.00)	<0.001
Fat color	3.00 (3.00,3.00)	3.00 (3.00,3.00)	-0.00 (-0.00, 0.00)	0.284	3.00 (3.00,3.00)	3.00 (3.00,3.00)	0.00 (-0.00, 0.00)	0.349
Maturity score	3.00 (3.00,3.00)	3.00 (3.00,4.00)	-1.00 (-1.00, -0.50)	<0.001	7.00 (5.00,8.00)	7.00 (5.00,7.00)	0.00 (-0.00, 0.00)	0.284
Texture score	1.00 (1.00,2.00)	2.00 (1.00,2.00)	0.00 (-0.00, 0.00)	0.204	2.00 (1.00,3.00)	2.00 (1.00,2.00)	0.00 (-0.00, 0.00)	0.084
Yield index	61.32 (60.12,62.46)	62.70 (60.87,66.53)	-1.92 (-2.28, -1.55)	<0.001	60.97 (59.76,62.19)	63.30 (61.04,66.97)	-2.76 (-3.06, -2.45)	<0.001

[1] The dataset after propensity score matching (PSM) was used. Continuous variables were analyzed using the paired Wilcoxon signed-rank test

Table 3

Comparison of carcass grade between ligated and non-ligated female Hanwoo

	Heifers			**Cows**		
	Ligated **(n=509)**	Non-ligated **(n=509)**	*p*	Ligated **(n=936)**	Non-ligated **(n=936)**	*p*
Yield grade (%)			0.767			0.001
A	125 (24.56%)	138 (27.11%)		196 (20.94%)	254 (27.14%)	
B	260 (51.08%)	247 (48.53%)		483 (51.60%)	488 (52.14%)	
C	124 (24.36%)	124 (24.36%)		257 (27.46%)	194 (20.73%)	
Quality grade (%)			<0.001			<.001
1++	184 (36.15%)	69 (13.56%)		119 (12.71%)	44 (4.70%)	
1+	155 (30.45%)	121 (23.77%)		256 (27.35%)	161 (17.20%)	
1	103 (20.24%)	163 (32.02%)		303 (32.37%)	285 (30.45%)	
2	62 (12.18%)	133 (26.13%)		195 (20.83%)	319 (34.08%)	
3	5 (0.98%)	23 (4.52%)		63 (6.73%)	127 (13.57%)	

[1]The dataset after propensity score matching (PSM) was used. Categorical variables (grades) were analyzed using the McNemar's test.

Table 4
Odds ratio for carcass grade in heifers and cows

	Heifers			**Cows**		
	Unadjusted OR (95% CI) **(n=9,123)**	Model1 OR (95% CI) **(n=9,123)**	Model2 OR (95% CI) **(n=2,890)**	Unadjusted OR (95% CI) **(n=9,123)**	Model1 OR (95% CI) **(n=9,123)**	Model2 OR (95% CI) **(n=2,890)**
QUALITY GRADE=1++						
Non-ligated	ref	ref	ref	ref	ref	ref
Ligated	5.90 (4.70, 7.52)	5.53 (4.32, 7.19)	3.61 (2.66, 4.96)	4.09 (3.15, 5.35)	3.73 (2.86, 4.91)	3.00 (2.11, 4.34)
QUALITY GRADE≥1+						
Non-ligated	ref	ref	ref	ref	ref	ref
Ligated	5.32 (4.53, 6.27)	4.42 (3.71, 5.29)	3.35 (2.60, 4.35)	3.22 (2.77, 3.75)	3.06 (2.61, 3.58)	2.46 (2.00, 3.03)
YIELD GRADE="A"						
Non-ligated	ref	ref	ref	ref	ref	ref
Ligated	0.78 (0.66, 0.93)	0.74 (0.61, 0.90)	0.88 (0.66, 1.16)	0.80 (0.69, 0.93)	0.76 (0.65, 0.89)	0.71 (0.57, 0.88)

Abbreviation: OR = odds ratio
Unadjusted Model: original dataset was used

Figure captions

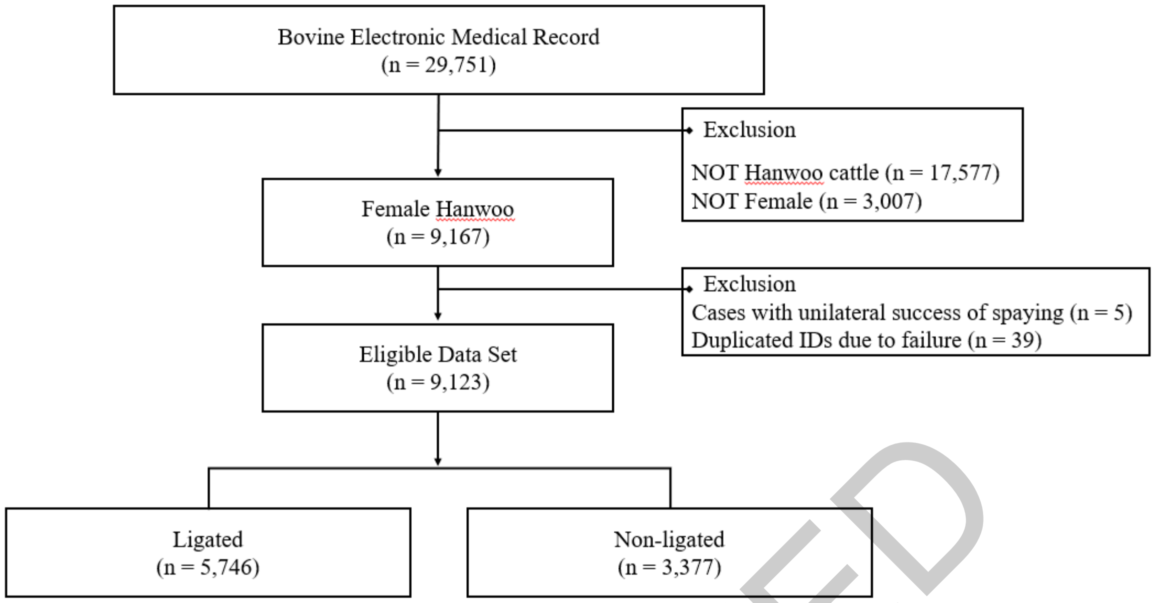

Fig. 1. Flow chart of the study design

* Missing Value and Duplicated cows

1) Entire rows duplicated (n = 13)

2) Duplicate IDs (n = 605, from 598 cows)

3) Missing values in carcass traits (n = 26)

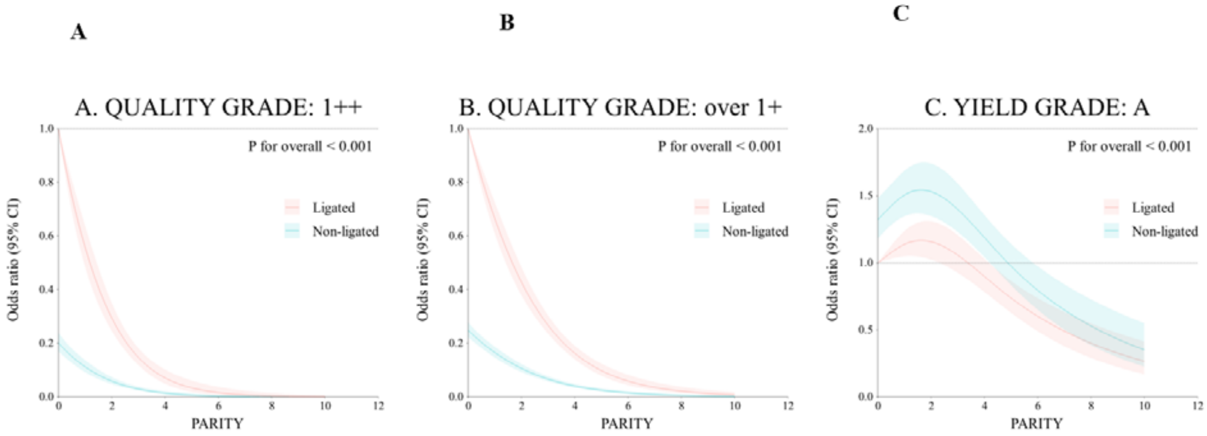

Fig. 2. Restricted spline curve of odds ratios. Quality grade 1++, over 1+, and yield grade A by parity.

* Missing values and Duplicated cow IDs

1) Entire rows duplicated (n = 13)
2) Duplicate the unique identification code (Cow ID) (n = 605, from 598 cows)
3) Missing values in carcass traits (n = 26)