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Ovarian Weight and Uterine Volume Index Are Useful for Age Estimation in Adult Women

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Abstract: Practically, when only parts of an unidentified human body are found, age estimation with limited materials is required. The purpose of this study was to investigate methods for estimating age using the uterus and ovaries. Among forensic autopsies performed between January 2011 and March 2022, 211 uteruses and 521 ovaries of 322 women were used for this study. Measured values for ovarian weight and uterine volume index were corrected by body surface area to consider the effect of body size. The corrected uterine volume index increased in teenage years and achieved its maximum in the 40–49 group, then gradually decreased with increasing age. The corrected ovarian weight increased until the twenties, after which it decreased with age. For women aged 20 years or more, receiver operating characteristic (ROC) curve analysis suggested that a uterine volume index of 41.2 cm^3/m^2 was the cutoff value for classifying the age as ≥60 years or <60 years, with an area under the ROC curve (AUC) value of 0.751. Ovarian weights of 2.27 g/m^2 and 1.92 g/m^2 were the cutoff values for classifying the age as ≥40 years or <40 years, or ≥50 years or <50 years, with AUC values of 0.935 and 0.930, respectively. These methods can help determine an unknown individual's age group simply and quickly, even for incomplete cadavers.

Keywords: age estimation; forensic autopsy; uterus; ovary; morphology

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1. Introduction

In forensic practice, the identification of an unknown person is a basic and important issue. Age and sex estimation is the first step when identifying a cadaver, so simple and concurrent scientific methods are required for this process. Classically, in infants and children, height and weight may be compared with standard tables. Then, we can use developmental methods, such as bone length, ossification centers of any bones, epiphysial union, and eruption of teeth [1,2]. In adults, if a tooth is obtained, then age estimation can be performed using any changes as follows: the attrition of the tooth by mastication; the deposition of secondary dentine in the pulp chamber; the level of the attached gingivae; the translucency of the root; the resorption of the root [1]. Adhesion of sutures between cranial bones can also be used when the skull is obtained [3–6]. A better guide to aging than suture closure is found in the symphysial surface of the pubic area. Pubic symphysis is assessed across six stages for the three components, and the age is determined by the total score [1]. Yet, these methods require examiners with sufficient experience and affected by individual differences in the cadavers. More objective methods include examining aspartic acid racemization in dentin or DNA methylation markers in the blood, saliva, and buccal

cells. With these, age can be estimated within a 3- to 4-year margin of error [7–11]. However, because these methods need complex techniques and facilities such as next-generation sequencing instruments, it is difficult to use them for estimating age in a quick and simple fashion.

When estimating age, using only one material, such as cranial bone, is not preferable because of the low accuracy of this approach. Therefore, in the field of forensic science, the combined use of various methods based on the available materials (i.e., bones, teeth, and abdominal organs) is required for age estimation [3,5]. Then, if artificial intelligence (AI) develops, it may be possible to estimate the age of the cadaver from various data at forensic autopsy. Therefore, more indicators are needed to improve the accuracy of age estimation. Previous studies have reported relationships between the various features of organs or tissues and the age. In the neck, the intima-media thickness of the common carotid artery is measured histologically, with a high correlation observed between age and this thickness value [12]. Some methods have been developed for estimating age from the organs or tissues in the thoraco-abdominal region. In the chest, the extent of lipofuscin accumulation in the myocardium was used for estimating age [13]. Furthermore, the biomechanical properties and circumference of the abdominal aorta correlated well with age [14,15]. For the lumbar region and retroperitoneal space, the proportion of glomeruli with hyaline deposits is known to increase with age [12]. Regarding male genital organs, the prostate was the focus for finding relations to the age. Tsuboi et al. measured the prostate weight at autopsy or calculated its weight from its size in 78 cases. They suggested that the prostate weight was significantly positively correlated with age (correlation coefficient of 0.283) [16]. Tanaka et al. examined the prostate volume by age and the effect of concomitant disease on prostate volume [17]. They suggested that although the mean-corrected prostate volume (divided by body surface area) was significantly higher in patients with atherosclerosis than in those without, multiple regression analysis revealed that only age influenced the corrected prostate volume. Therefore, they confirmed the adequacy of using prostate volume for age estimation.

However, there have been no reports of using the tissues or organs in the female pelvic cavity for age estimation. Therefore, we focused on use of the pelvic organs, particularly the uterus and ovaries. Because the uterus and ovaries are female reproductive organs, they can reportedly change according to hormone levels [12–18]. Therefore, the morphological features of the uterus and ovaries can depend on a person's age. Accordingly, the purpose of this study was to investigate the feasibility of estimating age using the uterus and ovaries.

2. Materials and Methods

2.1. Subjects

This study involved 75 females from forensic autopsies performed at Dokkyo Medical University in Tochigi Prefecture, Japan, between January 2011 and March 2014 and 340 females from forensic autopsies performed at Shiga University of Medical Science in Shiga Prefecture, Japan, between April 2014 and March 2022. For the Dokkyo Medical University cases, forensic autopsy was performed by one of the authors (MH). In the Shiga University of Medical Science, autopsies were performed by some of the authors (MH, MN, or MT). Among the total 415 females, we excluded cases that were not successfully identified and those with significant damage, such as severe burns, skeletonization, decomposition, and pregnancy cases. The median (interquartile range) post-mortem interval (PMI) was 1.5 (1.0, 2.5) days. After the initial investigation, each cadaver was stored in a dark room at a temperature of 4 °C until autopsy. Cases in which the uterus and both ovaries had been extracted or in which diseases were found macroscopically were also excluded. Even if one myoma or cyst was found in the uterus or ovary, the case was excluded. Overall, 211 uteruses and a total of 521 ovaries (285 women) from 322 women were included in this analysis.

2.2. Measurement of Uterus and Ovaries

At forensic autopsy, the age, height, and weight of the person were examined. The body surface area (BSA) was calculated using the following formula: (BSA (m^2) = height (cm) $^{0.725}$ × weight (kg) $^{0.425}$ × 0.007184) [19]. The uterus was placed on a flat plate, and its height, width, and length were directly measured with a ruler (Figure 1). The weight of the ovaries was measured using an automatic balance (CJ-3200, SHINKO DENSHI, Itabashi-ku, Tokyo). In this study, we propose calculating the index of uterine volume as height × width × length. Although this index is not an actual volume, the height, width, and length of the uterus are measured practically during forensic autopsy. We can, therefore, establish a simple index as described above. If both the left and right ovary could be evaluated, then the average weight of the two was used. If only one ovary could be evaluated, then this weight value was used.

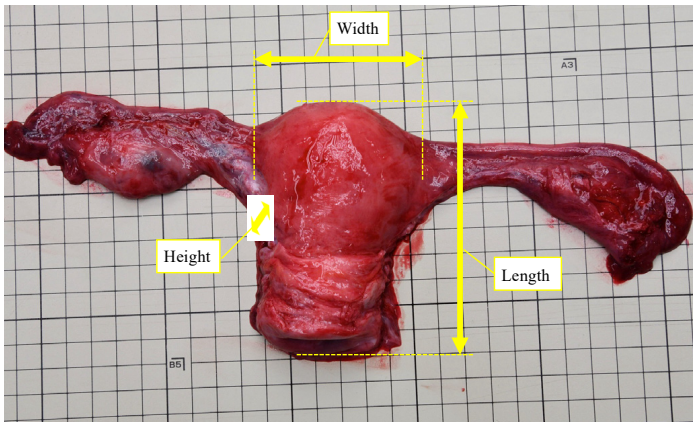

Figure 1. How to measure the uterus.

2.3. Statistical Analysis

To examine the relationship between uterine volume index or ovarian weight and age, the cases were divided into the following age groups: 0–9, 10–19, 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, 80–89, and 90–99 years. Because Bartlett's tests showed that the population of each group was not normally distributed, Steel–Dwass multiple comparison tests were performed to compare the mean uterine volume index and ovarian weight values between the age groups. A *p*-value of less than 0.05 was considered statistically significant.

Receiver operating characteristic (ROC) curve analysis was performed to determine the best uterine volume index cutoff value for predicting an age of 60 or more. Additionally, ROC curve analysis was also performed to determine the best ovarian weight cutoff value for predicting an age of 40 or more, or 50 or more. For statistical analysis, Bartlett's test and Steel–Dwass multiple comparison tests were performed using Statcel-4th edition-the Useful Add-in Forms on Excel 2011 (Microsoft Corporation, Redmond, WA, USA). IBM SPSS Statistics v25 (IBM, Armonk, NY, USA) was used for ROC curve analysis. The ethics committee of Shiga University of Medical Science approved this study (R2021-055).

3. Results

3.1. General Aspects

The ages of the 322 women analyzed were distributed from 0 to 99 years, with a mean and standard deviation (SD) of 56.7 ± 26.6 years. The median and interquartile range of the height, weight, and BSA were as follows: height of 153.0 (147.0, 158.0) cm; weight of 45.0 (37.3, 53.2) kg; BSA of 1.4 (1.3, 1.5) m^2. The median with the interquartile range of the post-mortem interval was 1.5 (1.0, 2.5) days. The ages of the 211 women from which a uterus could be evaluated had mean and SD values of 53.3 ± 29.1 years (range, 0 to 96 years). The mean and SD of the uterine volume index were 66.7 ± 48.7 cm^3 (range, 0.4 to 247.5 cm^3). This was 47.2 ± 32.8 cm^3/m^2 (range, 1.09 to 210.8 cm^3/m^2)

when corrected by BSA. Ovaries were obtained from 285 women that had a mean and SD age of 57.9 ± 25.6 years (range, 0 to 99 years). The mean and SD of ovarian weight were 61.8 ± 25.8 g (range, 6 to 160.0 g). This was 2.01 ± 1.64 g/m^2 (range, 0.31 to 11.5 g/m^2) when corrected by BSA. When conducting statistical analyses, we used the values corrected by BSA to consider the effect of body size.

The corrected uterine volume index by age group is shown in Figure 2. The corrected volume index rapidly increased in teenage women, 53.4 ± 21.9 cm^3/m^2 in the 10–19 age group, and achieved a maximum volume index of 73.5 ± 46.1 cm^3/m^2 in the 40–49 age group. Then, the corrected volume index gradually decreased with increasing age, 59.4 ± 29.8 cm^3/m^2 in the 50–59 age group; 44.4 ± 26.2 cm^3/m^2 in the 60–69 age group; 43.7 ± 24.3 cm^3/m^2 in the 70–79 age group; 41.0 ± 24.3 cm^3/m^2 in the 80–89 age group; 36.7 ± 15.2 cm^3/m^2 in the 90–99 age group. The corrected ovarian weight based on age group is shown in Figure 3. The corrected weight increased until the 20–29 age group, 4.8 ± 2.1 g/m^2, then decreased with aging: 4.4 ± 2.1 g/m^2 in the 30–39 age group and 2.8 ± 1.1 g/m^2 in the 40–49 age group. After 50 years old, it remained approximately constant as 1.4 ± 0.68 g/m^2 in the 50–59 age group; 1.6 ± 0.82 g/m^2 in the 60–69 age group; 1.4 ± 0.82 g/m^2 in the 70–79 age group; 1.3 ± 0.92 g/m^2 in the 80–89 age group; 1.1 ± 0.38 g/m^2 in the 90–99 age group.

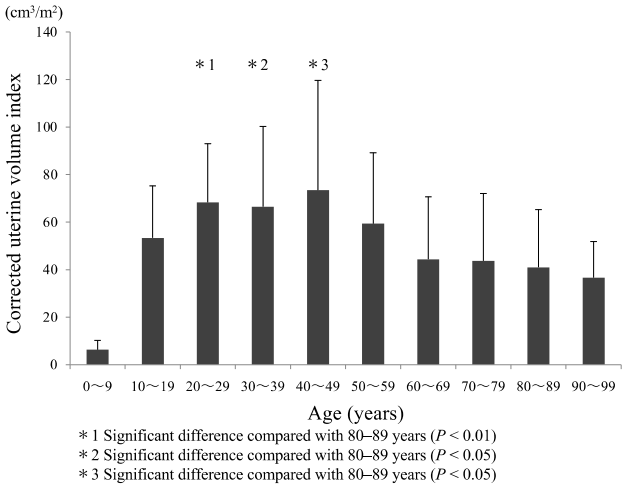

Figure 2. Age distribution of corrected uterine volume.

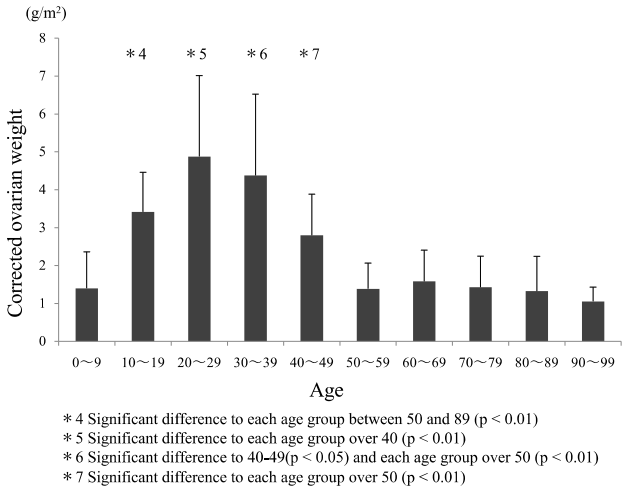

Figure 3. Age distribution of corrected ovarian weight.

3.2. Comparisons between Age Groups

The mean values of the corrected uterine volume index and ovarian weight were compared between the age groups. For the mean uterine volume index values, significant differences were found between the 20–29 and 80–89 groups ($p < 0.01$), 30–39 and 80–89 groups ($p < 0.01$), and 40–49 and 80–89 groups ($p < 0.01$) (Figure 2).

For the mean ovarian weight values, significant differences were found between the 10–19 group and 40–49 group, 50–59 group, 60–69 group, 70–79 group, and 80–89 group ($p < 0.01$). Also, significant differences were found between the 20–29 group and 40–49 group, 50–59 group, 60–69 group, 70–79 group, 80–89 group, and 90–99 group ($p < 0.01$); the 30–39 group and 40–49 group ($p < 0.05$), 50–59 group, 60–69 group, 70–79 group, 80–89 group, and 90–99 group ($p < 0.01$); the 40–49 group and 50–59 group, 60–69 group, 70–79 group, 80–89 group, and 90–99 group ($p < 0.01$) (Figure 3).

3.3. ROC Curve Analysis

Using the uterus or ovaries of women aged 20 years or more, we examined the borderlines for predicting age. These are the values used to determine whether an individual was older or younger than a certain age. To explore the cutoff points for the corrected uterine volume index or ovarian weight values, we determined an optimal point having higher sensitivity and lower 1- specificity using the ROC analysis results. With the uterus, for determining the age as more or less than 60 years, the cutoff value for the corrected uterine volume index was determined to be 41.2 cm^3/m^2, with an area under the ROC curve (AUC) of 0.751, sensitivity of 78.7%, and specificity of 64.1% (Figure 4). With the ovary, for determining the age as more or less than 40 years, the cutoff value for the corrected ovarian weight was determined to be 2.27 g/m^2, with an AUC of 0.935, sensitivity of 92.9%, and specificity of 80.1% (Figure 5). Next, for determining the age as more or less than 50 years, the cutoff value of the corrected ovarian weight was 1.92 g/m^2, with an AUC of 0.930, sensitivity of 89.7%, and specificity of 82.2% (Figure 6).

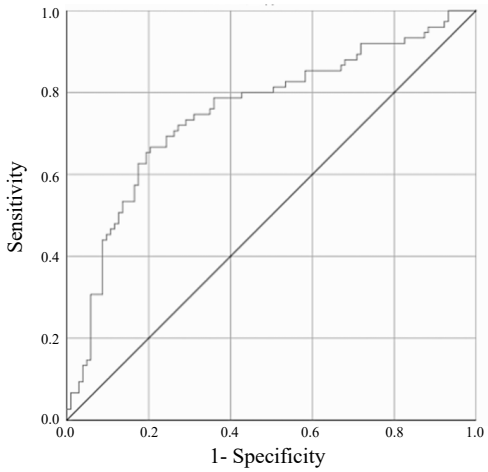

Figure 4. Receiver operating characteristic (ROC) curve between corrected uterine volume and age for predicting the age as ≥60 in individuals 20 years of age or more.

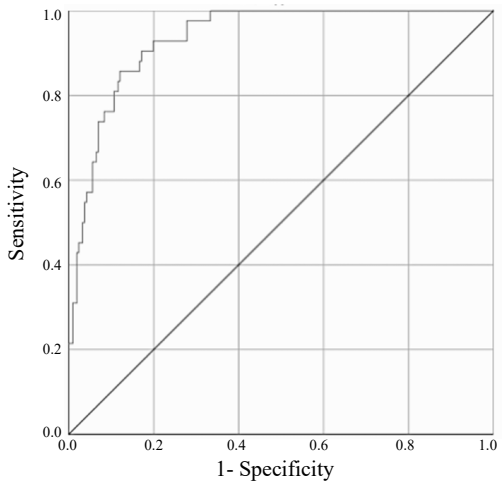

Figure 5. Receiver operating characteristic (ROC) curve between corrected ovarian weight and age for predicting the age as ≥40 in individuals 20 years of age or more.

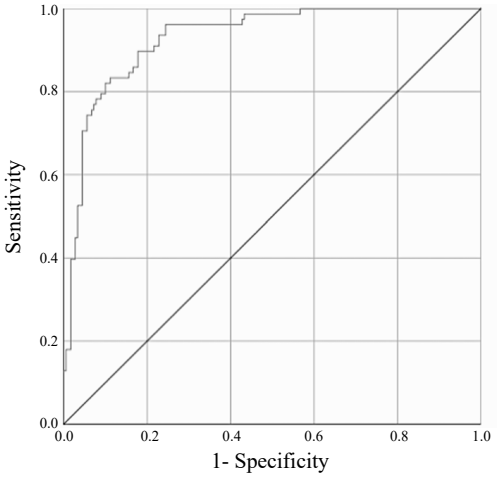

Figure 6. ROC curve between corrected ovarian weight and age for predicting the age as ≥50 years in individuals 20 years of age or more.

4. Discussion

Previous studies have shown that the uterus and ovaries begin to grow in size following increased hormonal activity with the onset of puberty. After middle age, the uterine volume index and ovarian weight start to decrease with increasing age. Some investigators have examined the uterine and ovarian sizes by ultrasound. Sokalska et al. studied longitudinal changes in the uterine and ovarian sizes over two years before menopause and two years after menopause. Significant reductions in both uterine and ovarian sizes were found between before and after menopause [17]. Additionally, Flaws et al. observed a reduced ovarian volume associated with age in postmenopausal women when compared with premenopausal women [20]. However, Oppermann et al. described a decrease in ovarian volume after age 40, even in pre- and perimenopausal women [21]. Pavlik et al. examined the relationship between ovarian volume and age, observing a significant decrease in ovarian volume with each ten-year period from 30 to 70 years of age [22]. As the impending exhaustion of the primordial follicle endowment triggers the menopausal transition, Merz et al. showed a significant reduction in the uterine size related to years since menopause [23].

According to our data, the corrected uterine volume index peaked in the 40–49 age group, then gradually decreased with age (Figure 2). Considering that menopause typically occurs around age 50, our data showed a similar trend to the previously published results.

The observed decrease in uterine size after menopause is likely explained by lower estrogen levels [24]. Because estrogen levels drop dramatically after menopause, substantial changes in uterine volume occur only after menopause [25]. Histologically, the decrease in uterine size is reflected by the thinner myometrium, the thickness of the vascular wall, and collagen deposition. Furthermore, the corrected ovarian weight decreased with age from the 30–39 to 50–59 age groups, then remained constant with increasing age (Figure 3). Thus, our data showed a similar trend to the previous findings that ovary size decreases after approximately 30 years old. This shrinkage of the ovaries is likely caused by the depletion of follicles [26]. In addition, the number of ovarian follicles is well correlated with the level of the anti-Müllerian hormone (AMH) [27]. In a study of Japanese women, AMH serum values decreased from 28 to 48 years of age with polynomial expression [28]. Similarly, AMH values in American women decreased steadily in a manner highly correlated with advancing age from 24 to 50 [29]. Observations consistent with these trends were obtained in European studies [30,31]. Thus, a decrease in ovarian size is likely related to the number of follicles and AMH levels. Histologically, the decrease in ovarian weight is reflected by a decrease in the number of follicles, fibrosis associated with higher permanent collagen deposition, and vascular wall thickness.

However, these previous data of uterine and ovarian sizes are difficult to apply to age estimation in forensic practice because they were obtained from indirect measurements using ultrasounds. In our study, we directly measured the uterine size and ovarian weight from age 0 to 99 years at forensic autopsy. Therefore, it is suggested that the age-related changes in the uterus and ovaries are more accurately reflected. Furthermore, the previously measured sizes of the uterus and ovaries were not corrected for the individual's body size. One study suggested that taller women have larger mean ovarian volumes [19]. Other studies have suggested that uterine size is positively correlated with body mass index (BMI) [32,33]. Additionally, the uterine length, width, and anteroposterior diameter became larger with increased height in nulligravid infertile women [34–36]. Therefore, the uterine volume and ovarian weight were corrected by BSA in our study, providing more precise data for age estimation.

From the above findings, we propose some cutoff points to determine age, such as more or less than 40, 50, or 60 years. Among them, the highest accuracy was obtained when estimating an age of 40 or more by the ovarian weight. If both ovarian weight and uterine volume index are obtained, we can effectively estimate the age of a cadaver.

As previously mentioned, if only some body parts of an individual are found, we can estimate the person's age using several materials, such as the myocardium, abdominal aorta, and common carotid artery, with higher accuracy [12–15]. Horny et al. measured the length of the abdominal aorta during autopsies before and after segment resection. Then, the ratio between in situ length and the length after the excision was calculated. When using biomechanical properties and the circumference of the abdominal aorta, a high correlation was observed with age ($r = -0916/-0.921$, male/female) [15]. Kakimoto et al. examined the lipofuscin accumulation in the myocardium for autopsy cases and investigated the correlation between the lipofuscin accumulation and age. As a result, the lipofuscin accumulation in the myocardium correlates well with age ($r = 0.82$) [13]. Additionally, Lehmann-Leo et al. measured the intima-media thickness of the common carotid artery and examined the correlation between the age and this thickness. The result suggested that the intima-media thickness of the common carotid artery was highly correlated with age ($r = 0.887$) [12].

Although we cannot estimate a more specific age using our method, we can narrow down the age of the cadaver to a ten-year range using both uterine size and ovarian weight. The strengths of our age estimation method include its simplicity of application and its ability to quickly obtain results, even for incomplete bodies.

However, this study has several limitations. First, the results of this study only apply to cadavers with a short PMI (around 1.5 days) because we excluded heavily degraded cadavers. As the PMI increases, the size of the uterus and the weight of the ovaries decrease

owing to autolysis. In the future, our method should be used to evaluate the changes, size, and weight of these organs over a longer PMI in moderately or severely degraded cadavers. Second, as this was a cross-sectional study, we cannot obtain detailed information about the menstrual cycle status of the individuals. However, because we collected many of the actual sizes, this effect can be minimized. In the future, we would collect such information by measuring the thickness of the myometrium and endometrium, assessing the content of the uterus, and performing a histological evaluation of the uterus. Third, both ovarian weight and uterine volume index were corrected for BSA. However, when only body parts are found, the height and weight of the cadaver are not known. Therefore, this method cannot be applied to only body parts. In the future, a revised method that would be useful for cases in which only parts of the body are found is required. Fourth, these data were obtained from Japanese subjects, so our determined cutoff values may only be useful for Japanese victims. Despite this, however, our results were consistent with the observed trends for both uterine size and ovarian weight by age described in previous reports. Therefore, our method may still be able to be applied to victims in other countries. Fifth, our analysis excluded victims with macroscopic abnormalities of the uterus or ovary. Therefore, age estimation for victims with diseases such as myoma or ovarian cysts is difficult. Because this study is the first to use uterine size and ovarian weight, further studies are required that include individuals with such diseases. Finally, according to our results, we could not discriminate the victims as child or aged person because the values of the corrected ovarian weight were similar in such cases. Therefore, when applying the present methods, children need to be excluded with other findings, such as size or other organ/tissue features.

5. Conclusions

We applied a method of age estimation using the uterus and ovary by analyzing the uterine size and ovarian weight of forensic autopsy cases. The corrected uterine volume index (divided by BSA) rapidly increased in teenage women and achieved a maximum in the 40–49 age group. Then, the corrected uterine volume index (divided by BSA) decreased from the age group of 50–59 years. The corrected ovarian weight increased until the 20–29 age group, then decreased from the age group of 30–39 years with aging. For the mean uterine volume index values, significant differences were found between the 20–29 and 80–89 groups, 30–39 and 80–89 groups, and the 40–49 and 80–89 groups. For the mean ovarian weight values, significant differences were found between the 10–19 group and each age group between 40 and 89 years, the 20–29 group and each age group over 40 years, the 30–39 group and each age group over 40 years, and the 40–49 group and each age group over 50 years. ROC analysis using cases aged 20 years or more showed that the cutoff value of 41.2 cm^3/m^2 for the corrected uterine volume index determines, with high accuracy, the age of cadavers as more or less than 60 years. Also, ROC analysis showed that the cutoff values of 2.27 g/m^2 and 1.92 g/m^2 for the corrected ovarian weight determine, with high accuracy, the age of cadavers as more or less than 40 and 50 years. Although estimating detailed age requires the combination of a variety of other methods, our methods can help determine an unknown individual's age group simply and quickly, even for incomplete cadavers. Currently, there is no established standardized method of age estimation in forensic practice by using the uterus or ovary. We would recommend combining some of the possible age estimation methods from the obtained samples for more accurate estimation.

Author Contributions: Conceptualization, T.M. and M.H.; data curation, S.K.; formal analysis, T.M. and T.T.; investigation, M.T. and M.N.; methodology, Y.T. and M.H.; supervision, M.H.; validation, M.T., A.T. and M.N.; visualization, T.M., M.T. and M.N.; writing—original draft, T.M. and M.H.; writing—review and editing, T.T. and S.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was approved by the Ethics Committee of Shiga University of Medical Science (R2021-055).

Informed Consent Statement: We performed this research with the written informed consent of the next of kin.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

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