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TO THE ISSUE OF THE TECHNIQUE OF ENDOMETRIAL BIOPSY IN COWS

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The article presents the findings of research of morphometric parameters of the cow reproductive system. The length of the uterus sections is 28.53% (left) and 29.68% (right) in relation to the total length of the oviducts. The length of the uterine horns is 34% (left) and 32.52% (right), the uterine body is 6.53%, the uterine cervix is 2.31%, the vagina is 28.63% - 28.8%. The length of the left cow oviduct is less than the right oviduct by 9.69%, and the length of the left uterine horn exceeded that of the right horn by 9.53%. The diameter of the left oviduct is expanded in the caudal direction. The right oviduct is narrowed in the medial area, and has no significant differences in width in the cranial and caudal parts. In the caudal direction, the diameter of the uterine horns increases. At the same time, the width of the left uterine horn is greater than that of the right horn in the middle by 7.33%, and in the caudal by 4.43%. The uterine body and cervix are rectangular in shape. The vagina narrows in the caudal direction. The widest part of the vagina is cranial. The thickness of the wall of the uterine horns and body is uneven. Areas with a thinner wall predominate. The mucous, submucosal and muscular layers of the left uterine horn are inferior by 21.97%, 33.3% and 20.79% to the corresponding indicators of the right uterine horn.

Key words: *cows, biopsy, morphometry, endometrium, reproductive system.*

СИЫРЛАРДАҒЫ ЭНДОМЕТРИЯЛЫҚ БИОПСИЯ ӘДІСІ ТУРАЛЫ СҰРАҚ БОЙЫНША

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Мақалада сиырлардағы репродуктивті жүйенің морфометриялық параметрлерін зерттеу нәтижелері келтірілген. Жатыр бөліктерінің ұзындығы жалпы ұзындыққа қатысты: жұмыртқа жолдары 28,53 % (сол жақта) және 29,68% (оң жақта). Жатыр мүйіздерінің Ұзындығы – 34%(сол жақта) және 32,52% (оң жақта), жатыр денесі – 6,53%, жатыр мойны – 2,31%, қынап – 28,63% – 28,8%. Сиырлардағы сол жақ жұмыртқаның ұзындығы оң жақ жұмыртқадан 9,69%-ға аз, ал жатырдың сол жақ мүйізінің ұзындығы оң жақтағы көрсеткіштен 9,53%-ға артық. Сол жақ жұмыртқаның диаметрі каудальды бағытта кеңейеді. Оң жақ жұмыртқа сымы медиальды аймақта тарылған, ал краниальды және каудальды бөліктерінде ені бойынша сенімді айырмашылықтар жоқ. Каудальды бағытта жатыр мүйіздерінің диаметрі артады. Бұл ретте жатырдың сол жақ мүйізінің ені ортаңғы бөлігіндегі оң жақ енінен 7,33% - ға, ал каудальды бөлігінде 4,43% - ға артық. Дене және жатыр мойны тікбұрышты. Қынаптың ең кең аймағы – бас сүйек. Қынап каудальды бағытта тарылады. Мүйіз қабырғасы мен жатыр денесінің қалыңдығы біркелкі емес. Жұқа қабырғасы бар аймақтар басым. Жатырдың сол жақ мүйізінің шырышты, субмукозальды және бұлшықет қабаттары жатырдың оң мүйізіндегі сәйкес көрсеткіштерден 21,97%, 33,3% және 20,79% төмен.

***Түйінді сөздер:** сиырлар, биопсия, морфометрия, эндометрия, репродуктивті жүйе.*

К ВОПРОСУ О МЕТОДИКЕ БИОПСИИ ЭНДОМЕТРИЯ У КОРОВ

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В статье приведены результаты исследований морфометрических параметров репродуктивной системы у коров. Длина отделов матки составляет по отношению к общей протяженности яйцепроводов: 28,53 % (левый) и 29,68% (правый). Длина рогов матки – 34% (левый) и 32,52% (правый), тело матки – 6,53%, шейка – 2,31%, влагалища 28,63% – 28,8%. Длина левого яйцепровода у коров меньше правого яйцепровода на 9,69%, а длина левого рога матки превосходила показатель в правом на 9,53%. Диаметр левого яйцепровода расширен в каудальном направлении. Правый яйцепровод сужен в медиальном участке, а в краниальной и каудальной частях достоверных отличий в ширине не имеет. В каудальном направлении диаметр рогов матки увеличивается. Ширина левого рога матки больше ширины правого в средней части на 7,33%, а в каудальной на 4,43%. Тело и шейка матки прямоугольной формы. Влагалище сужается в каудальном направлении. Самый широкий участок влагалища– краниальный. Толщина стенки рогов и тела матки неравномерная. Преобладают участки с более тонкой стенкой. Слизистый, подслизистый и мышечный слои левого рога матки уступают на 21,97%, 33,3% и 20,79% соответствующим показателям в правом роге матки.

***Ключевые слова:** коровы, биопсия, морфометрия, эндометрий, репродуктивная систем.*

Relevance: The livestock industry relies heavily on the consumption of milk and meat as essential products. Ensuring a consistent supply of these goods to the population necessitates an improvement in the reproductive qualities of cattle. Foremost, addressing this issue requires the development of diagnostic methods to assess the condition of the reproductive system in cows.

Diagnostic practitioners currently have access to a broad range of modern and progressive techniques, such as ultrasound and PCR. In recent scientific publications, there is a growing emphasis on

histological and electron-microscopic studies delving into the intricacies of the cow reproductive system [1, p.47]. Additionally, some studies provide valuable insights into the morphometric characteristics of the reproductive system in relation to different age groups [2, p.144].

Exploring the morphological and cytological features of the cattle endometrium allows for a comprehensive evaluation of the functional capacities of their reproductive systems. Notably, endometritis consistently correlates with salpingitis. In cases of moderate to severe inflammation, a significant increase in the thickness of tubal folds occurs ($p < 0.05$). Higher degrees of inflammation correspond to lower CBF levels ($p < 0.001$). Severe inflammation is associated with a decline in sperm motility, as spermatozoa get entrapped in mucus ($p < 0.001$) [3].

In recent years, there has been a growing importance placed on the intravital examination of the morphofunctional state of the endometrium in cows. This development aligns with the rapid advancement of in vitro fertilization (IVF) technology. Scientific literature now includes descriptions of experimental IVF studies aimed at evaluating reproductive technologies for Kazakh white-headed cattle, whose population has experienced a significant decline over the past thirty years [4, p.1632].

Researchers are diligently studying the synergy between biopsy and cytology to enhance diagnostic accuracy, not only for superficial material but also for deep tissues within the reproductive system. Authors discuss the biopsy of materials from both the uterine body and uterine horns, with subsequent histological and cytological examinations [5, p.195].

A comprehensive study of the endometrial biopsy process will contribute to the broader application of in vitro fertilization technology. Despite some authors referencing histological study results obtained through biopsy, regrettably, the existing scientific literature lacks a detailed methodology for biopsy sampling.

Research Objective: To determine key parameters of the cow reproductive system, with a focus on developing and refining the methodology for endometrial biopsy in cows.

Research Tasks: To identify morphometric indicators of the cow reproductive tract to provide a foundation for implementing the endometrial biopsy procedure.

Materials and Methods: The study was conducted at the prosectorium of Akhmet Baitursynuly Kostanay Regional University and the histology laboratory of the Emergency Care Institute in Chelyabinsk, Russia. The study material included reproductive organs from clinically healthy Kazakh white-headed cows (n=21) and Holstein cows (n=9) aged 5-7 years. Material was obtained from animals at the slaughterhouses of individual entrepreneurs "Parhomenko" and "Guseynov" in the Kostanay region. During anatomical examination, reproductive organs were examined carefully, focusing on tissue conditions such as elasticity, color, shape, cavity state, and mucous membrane. Measurements of uterine segment width and length (mm) were taken using a standard technique involving a measuring wire and caliper.

Histological studies of reproductive system tissues, including fixation in 10% formalin, embedding in paraffin, and staining with hematoxylin-eosin, followed standard procedures. The study employed equipment for tissue preparation, embedding, and staining of histological sections, such as the “Thermo scientific” tissue processing machine, TES 99 Medite medizintechnik tissue embedding system, Accu-cut SRM semi-automatic paraffin rotary microtome, and Tissue-Tek DRS automatic slide stainer. Histo-morphological examinations of uterine section walls were conducted using Leica DMRXA (Germany) and Biolam microscopes. Measurements included the thickness of uterine horns and body (μm), covering mucous, submucous, and muscular layers. Photographic documentation was obtained using a computer setup. Statistical analysis of digital material was carried out using the Excel 2023.

Research Results and Discussion: Upon reviewing literature on intravital diagnostics, it is observed that available scientific sources briefly mention the examination of biopsy specimens from the reproductive system of cows. Notably, there is a lack of information on the methodology for obtaining these biopsies.

Particular interest was placed on data regarding the length and width of uterine segments to establish parameters for the safe introduction of a biopsy tool into the uterine cavity. Consequently, morphometric indicators of reproductive system segments in cows, encompassing the length and width of segments in the cranial, middle, and caudal parts, were examined.

Figure 1 presents data on the length of uterine segments in clinically healthy cows. Findings reveal that the length of the oviducts constitutes 28.53% (left) and 29.68% (right) of the total length of tubular reproductive organs in cows. The length of the uterine horns is 34% (left) and 32.52% (right), the uterine body is 6.53%, and the cervix is 2.31% of the total length. The length of the vagina is 28.63%–28.8%. Digital data processing revealed that the length of the left oviduct is 9.69% less than the corresponding measurement of the right oviduct, while the length of the left uterine horn exceeds that of the right by 9.53%.

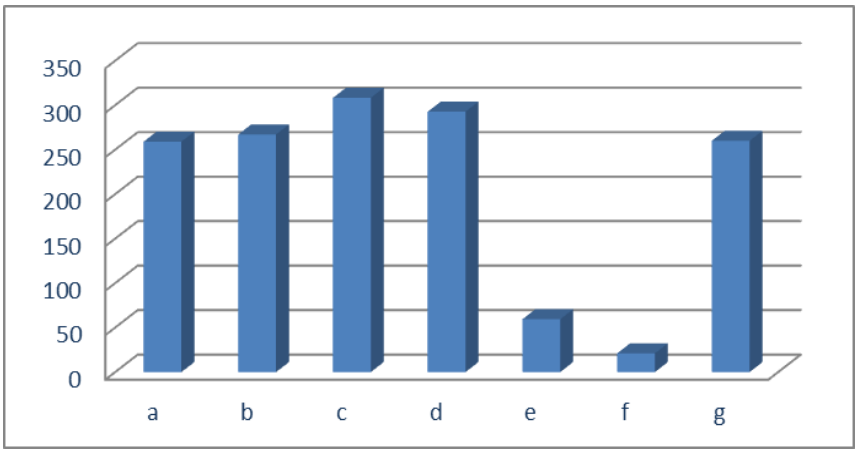


Figure 1 – Length measurements of cow uterine segments.
a- Left oviduct, b- Right oviduct, c- Left uterine horn,
d- Right uterine horn, e- Body, f- Cervix, g- Vagina

The width of the left oviduct in the cranial section was recorded as 5.2±2.30 mm, showing expansion in both the middle (12.4±0.32 mm) and caudal sections (12.0±0.57 mm) (Figure 2).

The right oviduct exhibits narrowing in the medial section, with no significant differences in width observed in the cranial and caudal regions (3.3±0.30 mm and 3.2±0.57 mm, respectively).

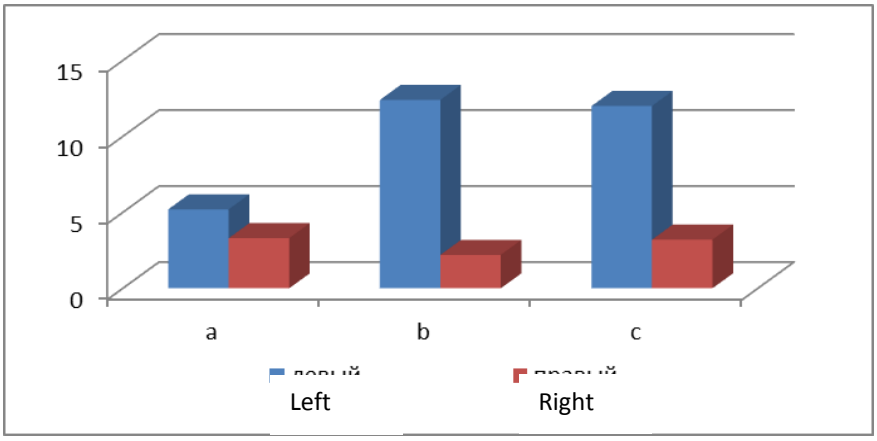


Figure 2 – Diameter of cow oviducts (mm)
a- Cranial, b- Medial, c- Caudal segments

In the cranial segment, the uterine horns exhibit a diameter of 9.7±1.02 mm (left) and 8.8±0.93 mm (right). Progressing caudally, the diameter shows an increase. The width of the left uterine horn surpasses that of the right in the middle segment by 7.33% and in the caudal segment by 4.43% (Figure 3).

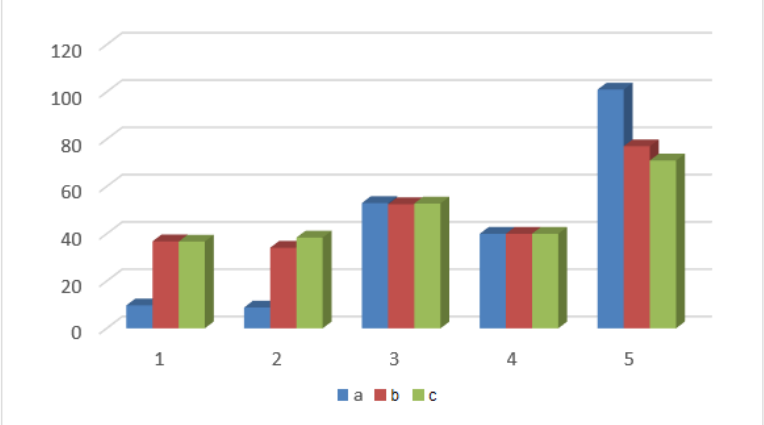


Figure 3 – Width of cow uterine segments (mm)
1- Left horn, 2- Right horn, 3- Body, 4- Cervix, 5- Vagina (a- Cranial, b- Medial, c- Caudal segments)

The uterine body has a rectangular shape, measuring 59.3±1.9 mm in length and 52.5±1.7 mm in width. The cervix is notably shorter, at 64.59% of the uterine body's length, and narrower by 23.8%.

The vaginal length of cows is recorded at 260±13.1 mm. Our research findings align with reports from scientists studying the refinement of vaginal examination techniques in cows [6, p.52]. The widest segment of the vagina is observed cranially (101±0.15 mm), followed by a gradual reduction in diameter in the caudal direction: 77±0.74 mm and 71±0.25 mm, respectively (Figure 3).

Throughout the study, we examined the morphological characteristics of the mucous membrane within the reproductive tract. Sections of the vagina near the cervix, the body of the uterus near the bifurcation of uterine horns, and the cavities within uterine horns were carefully studied. Analysis of the uterine horns diameter at the bifurcation highlighted a noticeable predominance of the left horn over the right horn (Figure 4). Well-defined longitudinal folds were evident in the mucous membrane of the vagina, uterus body, and uterine horns.

These findings are crucial for guiding the biopsy catheter's direction during the collection of mucous membrane biopsies from the cow uterus.

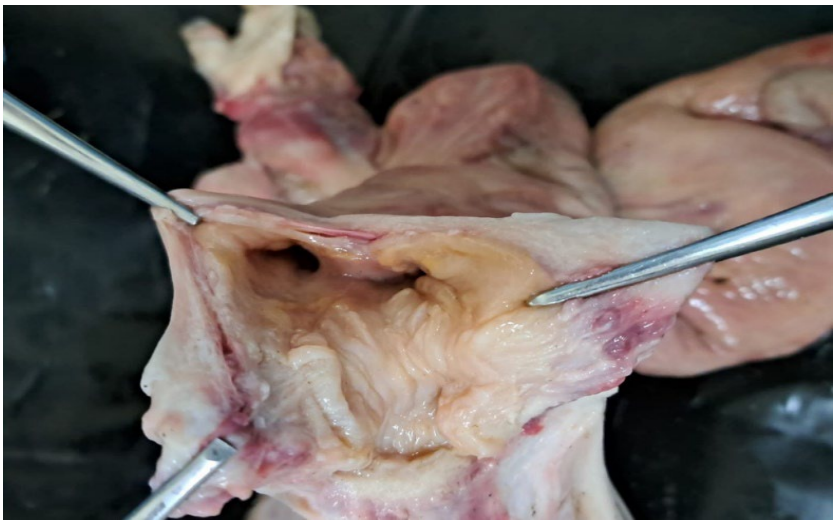


Figure 4 – Cow uterine horn bifurcation a – Left Uterine horn, b – Right horn

Researchers find particular significance in the histological details of the uterine horns and body structure when obtaining a biopsy. It is essential, primarily, to consider the uneven thickness of the wall layers in these segments for determining the depth of the biopsy catheter insertion.

Cytometric examinations of these segment walls have revealed non-uniform average thickness, especially in the mucous membrane. For instance, the mucous layer in the left uterine horn is 27.95% thinner than in the right, the submucosal layer is 22.07% thinner, and the muscular layer is 9.73% thinner (Figure 5).

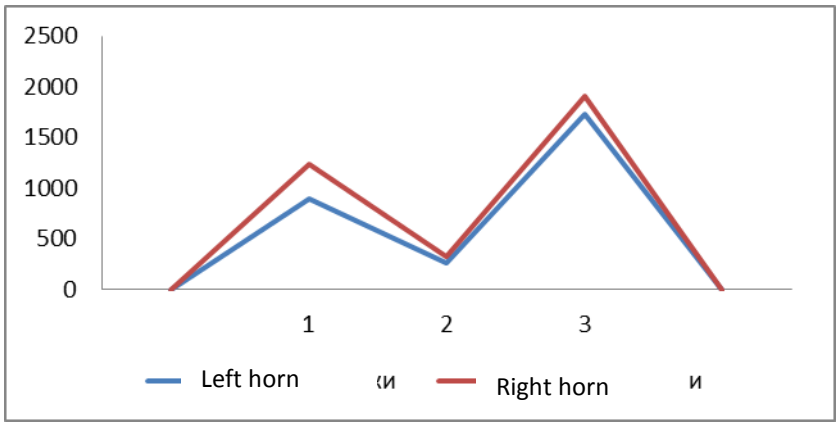


Figure 5 – Wall thickness of the cow uterine horns (mm)
1 - mucous layer, 2 - submucosa, 3 - muscular layer

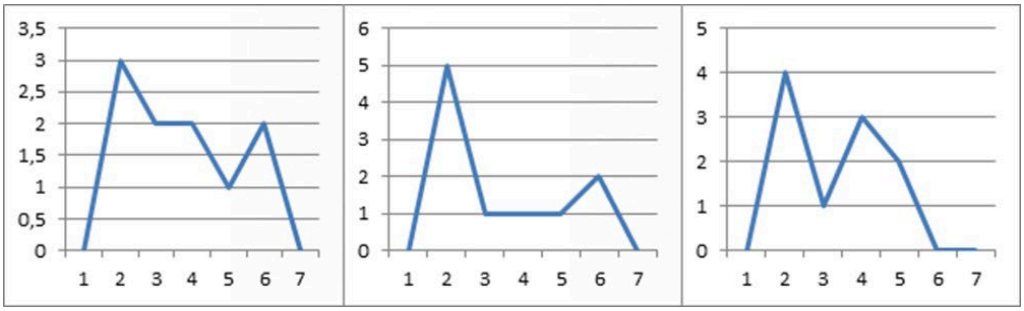
The thickness of the mucous layer exhibits uneven distribution along the uterine horns, featuring alternating segments of thinning (down to 874 μm) and thickening (up to 1490 μm). Predominantly, the regions with a thinner mucous layer take precedence.

Notably, despite an average thickness of 995.7±128.4 μm in the left uterine horn of cows, there are areas of thinning reaching 874 μm, interspersed with regions of a thicker mucous layer up to 1160 μm. Figure 5 illustrates two distinct and clearly defined generations, displaying leftward and rightward modal shifts, with a prevalence of areas featuring a thinner mucous layer. In the *right uterine horn*, the average mucous layer thickness is 1276.0±111.5 μm, ranging from a minimum of 1110 μm to a maximum of 1490 μm. Linear analysis reveals two generations, exhibiting leftward and centrally located modalities, where areas of thinning mucous layer predominate.

The submucosal layer of both the *left and right uterine horns* also demonstrates uneven thickness. In the left horn, the average thickness is 276.9±57.2 μm, varying from 200 μm to 367 μm. Meanwhile, the *submucosal layer* thickness in the right uterine horn surpasses that in the left by 33.3%, ranging from 299 to 567 μm.

Similarly, the thickness of the muscular layer in both the left and right uterine horns shows non-uniformity. In the left horn, it measures 1680.0±278.3 μm (ranging from 1280 μm to 2140 μm), while in the right horn, it is 2121.0±200.5 μm (from 1730 μm to 2410 μm).

Upon examining the mucous layer characteristics in the uterine body wall of cows, it becomes evident that its thickness varies across the entire area, ranging from 589 μm to 962 μm. On average, this measure is 759.5±133.6 μm. Linear analysis identifies two distinct generations, with the larger generation exhibiting an extremely leftward modal shift, indicative of a prevalence of thin mucous membrane areas (Figure 6).



1. Mucous layer 2. Submucosa 3. Muscular layer

Figure 6 – Wall thickness of the cow uterine body (μm)

The submucosal layer demonstrates noticeable variability in thickness, ranging from 128 to 201 μm, with an average of 157.9±27.5 μm. Areas with thinner thickness prevail, similar to the pattern observed in the uterine horn walls.

The thickness of the muscular layer in the uterine body varies from 1020 μm to 1700 μm, with an average thickness of 1295.0±207.3 μm. Linear analysis identifies two distinct generations in this layer.

Conclusion. Our investigation into the fundamental morphometric parameters of the reproductive system of cows, with the aim of refining the in vitro endometrial biopsy technique, revealed well-defined longitudinal folding of the mucous membrane in the vagina, uterine body, and uterine horns. Notably, the left uterine horn diameter at the bifurcation site significantly exceeds that of the right uterine horn.

The length distribution of uterine segments, relative to the total length, is as follows: oviducts 28.53% (left) and 29.68% (right), uterine horns 34% (left) and 32.52% (right), uterine body 6.53%, cervix 2.31%, and vagina 28.63% – 28.8%. The left oviduct length of cows is 9.69% shorter than that of the right oviduct, while the left uterine horn length exceeds that of the right by 9.53%. The width of the left oviduct in the cranial part is 5.2±2.30 mm, showing expansion in the middle (12.4±0.32 mm) and in the caudal part (12.0±0.57 mm). The right oviduct narrows in the medial part, with no significant width differences in the cranial and caudal parts (3.3±0.30 mm and 3.2±0.57 mm).

In the caudal direction, the diameter of the uterine horns increases. The width of the left uterine horn is larger than that of the right in the middle by 7.33%, and in the caudal part by 4.43%. The uterine body is of rectangular shape, with a length of 59.3±1.9 mm and a width of 52.5±1.7 mm. The length of the cervix is 64.59% less than the length of the uterine body, and the width is 23.8% less.

The widest part of the vagina is in the cranial region (101±0.15 mm), narrowing in the caudal direction (77±0.74 mm and 71±0.25 mm, respectively). The mucous layer of the *uterine horns* displays uneven thickness throughout, with regions of thinning (874 μm) prevailing and interspersed with thickened areas (up to 1490 μm).

The submucosal layer of both the *left and right uterine horns* also exhibits non-uniform thickness. In the left horn, the average thickness is 276.9±57.2 μm, ranging from 200 μm to 367 μm. The thickness of the *submucosal layer in the right uterine* horn exceeds that in the left by 33.3%, with values ranging from 299 μm to 567 μm.

The thickness of the muscular layer in both the left and right uterine horns is also non-uniform throughout, with areas of thinner walls prevailing. The mucous, submucosa, and muscular layers of the left uterine horn are thinner by 21.97%, 33.3%, and 20.79%, respectively, compared to the corresponding measurements in the right horn.

Examining the thickness characteristics of the mucous layer in the uterine body of cows, we observed variation across the entire area, ranging from 589 μm to 962 μm. On average, this measure is 759.5±133.6 μm. Linear analysis reveals predominant areas of thin mucous membrane in the uterine wall.

The data obtained in our study are crucial for determining the direction and depth of tissue receiver insertion during the collection of endometrial mucosa biopsy samples in cows.

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