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Short Communication

Stereolithographic biomodeling of equine ovary based on 3D serial digitizing device

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The 3D internal structure microscopy (3D-ISM) was applied to the equine ovary, which possesses peculiar structural characteristics. Stereolithography was applied to make a life-sized model by means of data obtained from 3D-ISM. Images from serially sliced surfaces contributed to a successful 3D reconstruction of the equine ovary. Photopolymerized resin models of equine ovaries produced by stereolithography can clearly show the internal structure and spatial localizations in the ovary. The understanding of the spatial relationship between the ovulation fossa and follicles and/or corpora lutea in the equine ovary was a great benefit. The peculiar structure of the equine ovary could be thoroughly observed and understood through this model.

Keywords: biomodeling, horse, ovary, stereolithography

The kidney shaped equine ovary has a very unique structure among mammals, as there is a very prominent depression, the ovulation fossa, on the surface or ventral border [3]. This is the only area from which normal ovulation occurs. [3] The corpus luteum does not project from the greater surface of the ovary as in other species. The inverted location of the cortex and medulla is also a unique characteristic which only equidae and the nine-banded armadillo (*Dasypus novemcinctus*) possess [7].

The ovary of an adult mare is structured so that the medullary or vascular zone is superficial, and the cortical zone, which contains the oocyte and follicles (parenchyma), is partly in the interior of the gland. The cortex reaches the surface only at the depression (ovulation fossa) on the free border [1]. The mean dimensions of the equine ovary from a non pregnant riding-type horse during the ovulatory season are 51.6 mm in length, 28.5 mm in width, and 32.7 mm in height, which are the average of the data collected from 131 mares [1]. Because of this extremely large size, it has been difficult to observe their internal structure by conventional histological techniques, such as paraffin sections of the whole equine ovary [1].

There has been a need for a device to analysis the spatial arrangement, number and the size of the follicles and corpus luteum within the ovary. To do so, we have developed three-dimensional internal structure microscopy (3D-ISM) and applied this device to the equine ovary [5]. In a previous report, we have shown serially sliced images and some simple three dimensional reconstructed images of the equine ovary from more than 1,000 sliced images [5]. Follicles can be extracted from the ovary and their spatial arrangement can be roughly observed. A more sophisticated image processing method for reconstruction to show the internal structure of the equine ovary more clearly was also attempted. The spatial arrangement of the internal structure of the equine ovary was clarified by the reconstruction of serially sliced images with the aid of 3D-ISM and the sophisticated image processing technique [4].

In addition to these previous studies, it has been necessary to develop methods or materials by which people can observe the structure more distinctly. Stereolithography has been most widely used as the technology for rapid prototyping. Stereolithography is applied to both industrial and medical purposes as a rapid prototype model [6,9] and builds plastic parts or objects a layer at a time by tracing a laser beam on the surface of liquid photopolymer. This class of materials quickly solidifies wherever the laser beam strikes the surface of the photopolymer. Once one layer is completely traced, it is lowered a small distance into the vat and a second layer is traced right on top of the first. The self-adhesive property of the material causes the layers to bond to one another and eventually form a complete, three-dimensional object after many such layers are formed [6].

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The production of a substantial geometric model of the equine ovary was attempted in this study to understand the spatial localization of the internal structure of the ovary.

The preparation of samples was performed by the method reported by Kimura *et al.* [4,5]. Briefly, bilateral ovaries were excised from two mares after the euthanasia of thoroughbreds from equine farms. These ovaries were fixed in 10% formalin until use. The ovaries which contained large follicles or corpora lutea was chosen by the palpation and the naked-eye observation of the ovaries to be used at this attempt in a preliminary study.

After fixation, ovaries were dipped into an embedding solution (OCT compound; Sakura Finetek Japan, Japan), encapsulated in a metallic chamber, frozen at -80°C in the deep freezer. After the solution frozen, the embedded ovaries were removed from the metallic chamber and subjected to 3D-ISM. 3D-ISM consists of a system linking a computed drive cryotome, a high-sensitive CCD camera (DXC-H10; Sony, Japan), a HDD digital image recorder (Totsu Sangyo, Japan). The embedded ovaries were sliced serially by the precise horizontal rotary slicer continuously without missing sections. The thickness of the slices can be mechanically adjusted and was set at 30 μm in this study. Each cross- section was viewed through a CCD camera and was recorded by a laser video disk processing more than 1,000 stored images of serially sliced surfaces of each frozen and embedded ovary. After all the sections were collected, the internal structure of the ovary (e.g. follicles and corpora lutea) was extracted by the automatic threshold selection method [8] which had been modified for full-colored serially sliced images.

Two separate ovaries were reconstructed three-dimensionally by the full-colored, ray-casting volume-rendering method using Voxel Viewer (Toshiba Machine, Japan), and its anatomical structure thoroughly observed. By using the digitalized three dimensional data obtained by the abovementioned 3D-ISM technique, two dimensional data slices of a distance of 0.1 mm each other were produced. Laser stereolithography was performed using liquid photopolymer resin (HS-661S; ADEKA, Japan) with the stereolithography device (SOUP250GH; CMET, Japan). Ultraviolet (325 nm) laser beams were irradiated onto the surface of the liquid photopolymer resin through a scanning mirror. The photoinitiator containing resin was solidified layer by layer at a thickness of 0.1 mm. The total time of irradiation for each specimen was 7 h for the ovary with large corpora lutea and 13 h for the ovary with large follicles.

The area of follicles and/or corpora lutea was left as an empty space in the solidified structure. The follicles, the total volume of greater than 100 mm^3, were left as empty spaces for visualization purposes as it was difficult to include small follicles into this model. In the same manner, the medium to large sized corpora lutea, the volume of which is more than 50 mm^3, was also left as an empty space.

Four dye-silicone rubber mixtures (blue: K-COLOR-BI-70, green: K-COLOR-GN-60, red: K-COLOR-R-20 and white: K-COLOR-W-10; Shin-Etsu Chemical, Japan) were diluted with liquid silicone rubber (KE-108; Shin- Etsu Chemical, Japan) and mixed by a defoaming mixer (AR-250; Thinky, Japan). These dyes were injected into the empty spaces through the passage. Color was selected according to the size of the follicles or corpus luteum. Follicles or corpora lutea larger than 10,000 mm^3 were marked in red, $3,000 \sim 9,999$ mm^3 marked in green, $1,000 \sim 2,999$ mm^3 marked in blue and $100 \sim 999$ mm^3 marked in white. The empty spaces for the follicles and/or corpora lutea are connected to the surface of the whole structure through a narrow passage with an inner diameter of 0.5 mm. The outer surface of the solidified models was polished with the aid of different grits (G80-180) of sand papers and an electric grinder to facilitate the visibility of the model.

Two equine ovaries, one with follicles in varying sizes and the other with a large corpora luteum, were processed to make serially sliced images by three dimensional internal microscopy, and more than a thousand sliced images were obtained and stored as digital data. The size and the spatial localization of the follicle and corpus luteum were expressed by computational simulation with the aid of an RV editor (Riken, Japan) after analysis by 3D internal microscopy. Fig. 1 shows the location and size of the follicles in variable sizes in the ovary in the follicular phase. Six surfaces can be seen from different angles. Colors indicate the size of the follicles as mentioned previously in the paper. Based on the digital data obtained by image processing, manufacture of the stereolithographs of equine ovaries with the aid of the laser stereolithography device was successful in these two examples (Fig. 2). The model is to a 1 : 1 scale and the space for the follicles or corpus luteum was created. The injection of dye-silicone mixtures into these spaces was successful through the narrow passages specifically made for this purpose. The injected dyes into the empty spaces were very effective for visualization of the follicles (Fig. 2B) and corpus luteum (Fig. 2C).

Stereolithographic production of a substantial geometric model of an equine ovary was successful in this study. The ovary was at a 1 : 1 scale with a real one and internal structures were easily observed from the outside due of high transparency of the resin. The shape, structure and localization of both follicles and corpora lutea can be distinctly determined. In particular, the understanding of the spatial relationship between the ovulation fossa and follicles and/or corpus luteum in the equine ovary was a great benefit. The peculiar structure of the equine ovary could be thoroughly observed and understood through this model.

In addition to 3D-ISM, sterolithography can also be a valuable educational tool to facilitate a better anatomical understanding of animals. Stereolithographic biomodeling technique was
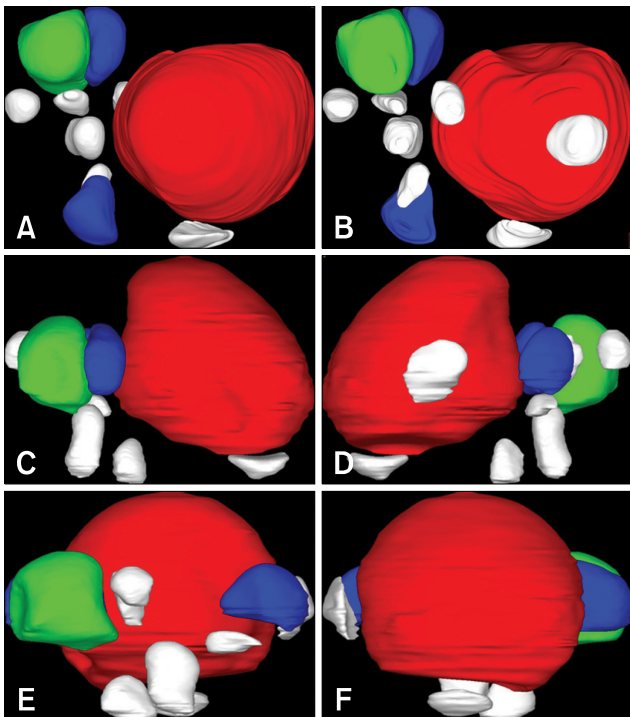
Fig. 1. The computer simulation of the spatial arrangement of follicles in the equine ovary. These images were created by 3-dimensional reconstruction from serially sliced images. Assuming that the ovary is in the hexahedron box, six views are illustrated from the bottom surface (A), the top surface (B), the front surface (C), the back surface (D), the left side surface (E) and the right side surface (F). Follicles whose size is bigger than 10,000 mm^3 marked in red, $3,000 \sim 9,999$ mm^3 marked in green, $1,000 \sim 2,999$ mm^3 marked in blue and $100 \sim 999$ mm^3 marked in white.
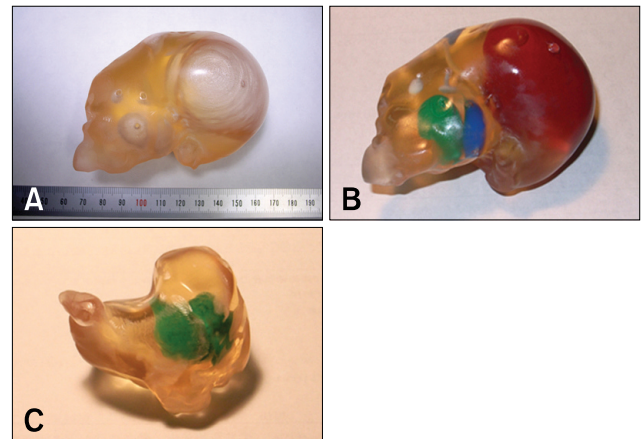

Fig. 2. The stereolithography product with empty spaces for the follicles (A), the follicles where the dye materials were injected (B) and the corpus luteum where the dye material was injected (C).

initially developed in the engineering sciences to manufacture prototype models, improve design and reduce product development time. Now this technique has been applied to many medical specialties [6,9,11]. The application for soft tissues was limited to several studies including the structure of blood vessels and heart [2,10] because of difficulties in obtaining high contrast images by MRI and CT scans. The manufacture of colored biomodels was also attempted in this study and successful. The spatial localization of the follicles in different sizes was easily visualized. This can be used to display local regions of interest. There have been recent demands to diminish the number of sacrificed animals for research and education, and biomodeling has proven to be a good alternative. Stereolithography is suitable for the education of students and trainees as an alternative educational tool.

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