FULL PAPER ***Parasitology***

Comparative Study of the Reproductive Organs of *Fasciola* Groups by Optical Microscope

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ABSTRACT. Reproductive organs of stained and mounted whole specimens of different types of *Fasciola* (*F. hepatica*, *F. gigantica*, and parthenogenetic diploid and triploid flukes) were observed to clarify the structure of their reproductive organs. The results are as follows; 1. Basic structure differences could not be identified. 2. The flukes without sperm, or those with an extremely small quantity in the seminal vesicle, are parthenogenetic *Fasciola* sp. 3. It was newly discovered that the surface of the cirrus is surrounded by many shallow gutters, and that spines form a line in the gutters. 4. The structure of the reproductive organ on the genus *Fasciola* are shown in detail in the figures.

KEY WORDS: *Fasciola*, parthenogenesis, reproductive organ, spermatogenesis, triploid.

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The common liver flukes (the genus *Fasciola*) are one of the oldest known trematode, and descriptions of their reproductive organs has been reported since the 19th century. Reproductive organs are important for classification since the organs of these flukes have been observed for a long time, and the results agree on many points. However, the details differ slightly between some researchers. There are some contradictions and unclear parts in many textbooks, because they quoted from the reports of these researchers. One of the purposes of this research is to clarify the disagreements of the results of observations of the organ by some researchers.

Some species and some subspecies of the common liver flukes were proposed since the description of *F. hepatica*. Kendall [10] expressed, however, that the valid species of the genus *Fasciola* are *F. hepatica* Linnaeus, 1758 and *F. gigantica* Cobbold, 1856. But in Asia, there are cases of unclear identification of *Fasciola*, because both species are mixed [15, 30]. Furthermore *Fasciola* sp. which increase with parthenogenetic reproduction are known in Asia. In particular, the common liver flukes from Japan and Korea are all parthenogenetic *Fasciola* sp. [17, 22–24, 29]. There is almost no report which compared reproductive organs between groups of *Fasciola* [14]. Terasaki and Moriyama-Gonda [30] attempted the tentative classification of groups of the genus *Fasciola*. Another purpose of this research is the investigation of any possible differences in the structures of the reproductive organs between the groups of *Fasciola*.

MATERIALS AND METHODS

Living *Fasciola* sp. were collected from the livers of cattle from slaughterhouses in Fukuoka in Japan and Seoul in Korea. Using a razor blade, an incision was made at the rear part of the ventral sucker of each fluke, and germ cells from the testes were transferred to glass slides. A conventional air drying method was used to produce specimens for chromosome observation. Flukes were divided into two groups, i. e., diploids (2n=2x=20), and triploids (2n=3x=30).

After that the flukes were placed between two microscope slides and fixed in 70% ethanol, they were stained in alumcarmine, and whole mounted specimens were made using these flukes. Other *Fasciola* out of these flukes were collected from other countries with the cooperation of some research workers, and stained whole mounted specimens were made also from these flukes, which were *F. hepatica* from Russia (Vladivostok), Germany (Hamburg), Australia, USA (Washington), Mexico (Mexico City), and Peru (Cajamarca), *F. gigantica* from Kenya (Nairobi), and the flukes which are probably *F. gigantica* from the Philippines (Manila), Thailand (Bangkok), Nepal, India (Madras), and Pakistan (Rawalpindi). Ten flukes from each of these countries were used.

Male and female reproductive organs of each specimen were observed under the light microscope, and the fine specimens for observation were sketched with tracing each duct and tube.

RESULTS

In *Fasciola* sp. from Japan and Korea, the results of chromosome analysis were as follows; among the 19 specimens of *Fasciola* sp. from the Fukuoka slaughterhouse, only one (5.3%) was diploid, whereas the remaining 18 (94.7%) were triploids. Among the 84 specimens collected in Seoul, 65 (77.4%) were diploids the remaining 19 (22.6%) were triploids. Over all, 66 (64.1%) specimens were diploids and 37 (35.9%) were triploids. In the triploid flukes, spermatids in the spermiogenesis phase in the testes were identified, but their number was always extremely small. The condition of spermatids in these flukes could be clearly differentiated with the other flukes, *F. hepatica* and *F. gigantica*. There were no spermatids at all in any of the diploid flukes. Therefore, both flukes were decided to be parthenogenetic *Fasciola* sp. [31].

Results of the observation of each reproductive organ in the group of *Fasciola* (*F. hepatica*, *F. gigantica*, probably *F. gigantica*=undefinable geographic isolation from Asia, and parthenogenetic *Fasciola* sp. diploid and triploid) did not show the basic differences between the groups. The basic structures of the reproductive organs which were observed in this research are expressed as follows.

Female reproductive organ: Figure 1 shows a part of the female reproductive organ, highlighting the tracing of each duct in Mehlis' gland under the light microscope.

Ovary: The ovary (Fig. 1, OV) is constructed with branched tube-like antlers. It is located in the diagonal rear of the windering uterus, which is placed between the ventral sucker and Mehlis' gland (Fig. 1, MGC_1 and MGC_2), and its anterior end is piled up along with a part of the uterus in many cases. Its rear end is attached or piled up with the anterior end of the testis. Its isolated edge arrives at the vitelline gland, which is distributed laterally along the body of the fluke in many cases. The inside edge a tube approaches Mehlis' gland, and before reaching it, or after invading it, the ovary becomes a fine duct. This is the oviduct (Fig. 1, OD). Germ cells in various stages of development were recognized in it.

The results of the research reveal that the ovary is placed either on the left or on the right side of the body, as shown in Table 1. In many cases, ovaries in flukes from all countries are located on the right side of the body. In all, 75% of them are located on the right, 22.3% are on the left, and 2.7% are bilateral, which placed in the medial line of the body with half of the branches on the left and half on the right.

Mehlis' gland: Mehlis' gland is placed in the rear of the windering uterus. Its form is round, elliptical, or multilateral in shape, and it is composed of many secretory gland cells of two types (Fig. 1, MGC_1, and MGC_2). The gland is a unicellular gland. Each cell releases a fine duct from the tip of it, and the ducts run to the ootype (Fig. 1, OOT), which exists in the center of Mehlis' gland. These gland cells are rare in the area near the ootype. The ootype is buried radially by the fine ducts that are centralized from Mehris' gland. Mehlis' gland buries the ootype, a part of the ovary, oviduct, Lawrer's canal (Fig. 1, LC), ovo-vitelline duct (Fig. 1, O-VD), common vitelline duct (Fig. 1, CVD), a part of the vitelline reservoir (Fig. 1, VR), and the proximal part of the uterus (Fig. 1, UT).

Oviduct: The inner end of the ovary becomes the oviduct (Fig. 1, OD), but the border is smooth and has no recognizable special apparatus like an ovicapt. The thickness of the oviduct is continuous, and the organ runs straight to the rear toward the ootype. In some specimens, the oocytes were recognized in the oviduct. The oviduct branches off to yield Laurer's canal (Fig. 1, LC) near the ootype. The oviduct continues to run near the entrance of the ootype, and joins with the common vitelline duct (Fig. 1, CVD) which comes from the vitelline reservoir (Fig. 1, VR).

Laurer's canal: Laurer's canal (Fig. 1, LC) separated from the oviduct runs to the rear toward the ootype, and opens funnel-like at the lateral surface near the vitelline reservoir level. The thickness of Lawrer's canal is the same as the oviduct and the part joining the oviduct with the common vitelline duct. The seminal receptacle is not observed.

Vitelline glands: The vitelline glands exist in the body of the fluke near both dorsal and ventral surfaces, except for the anterior cone, the periphery of the ventral sucker, a part

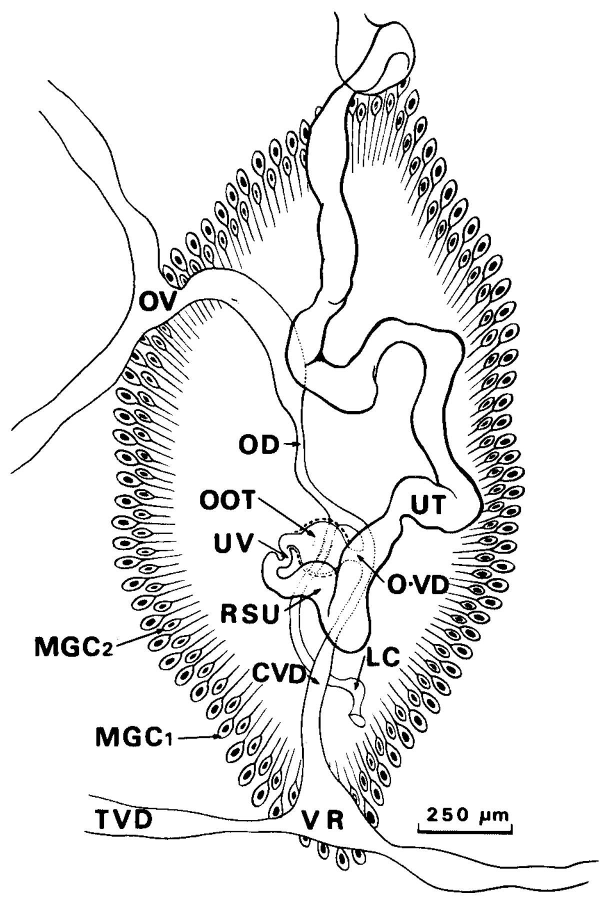

Fig. 1. Female reproductive organs, highlighting the tracing of each duct in Mehlis' gland under the light microscope. Abbreviations-C: Cirrus, CC: Cirrus canal, CG: Cirrus glans, CP: Cirrus pouch, CS: Cirrus spine, CVD: Common vitelline duct, ED(PG): Ejaculatory duct (Pars glans), ED(PP): Ejaculatory duct (Pars prostatica), EP: Ejaculatory pore, GP: Genital pore, LC: Laurer's canal, MGC_1: Mehlis' gland cell 1, MGC_2: Mehlis' gland cell 2, OD: Oviduct, OOT: Ootype, OV: Ovary, O-VD: Ovo-vitelline duct, PGD: Prostate gland, RSU: Receptaculum seminis uterinum, S: Scale, SD: Sperm duct, SV: Seminal vesicle, TVD: Transverse vitelline duct, UP: Uterine pore, UT: Uterus, UV: Uterine valve, VR: Vitelline reservoir, VS: Ventral sucker.

Table 1. Location of ovary in the genus *Fasciola* (%)

Taxa	Locality	Right	Left	Bilateral	Total
F. hepatica	Russia (Vladivostok)	9 (90)		1 (10)	10
F. hepatica	Germany (Hamberg)	9 (90)	1 (10)		10
F. hepatica	Australia	8 (80)	2 (20)		10
F. hepatica	U.S.A. (Washington)	9 (90)	1 (10)		10
F. hepatica	Mexico (Mexico city)	8 (80)	1 (10)	1 (10)	10
F. hepatica	Peru (Cajamarca)	8 (80)	1 (10)	1 (10)	10
F. gigantica	Kenya (Nairobi)	8 (80)	2 (20)		10
F. gigantica (UGI[a)])	Philippines (Manila)	8 (80)	2 (20)		10
F. gigantica (UGI[a)])	Thailand (Bangkok)	8 (80)	1 (10)	1 (10)	10
F. gigantica (UGI[a)])	Nepal	6 (60)	4 (40)		10
F. gigantica (UGI[a)])	India (Madras)	8 (80)	2 (20)		10
F. gigantica (UGI[a)])	Pakistan (Rawalpindi)	5 (50)	4 (40)	1 (10)	10
Fasciola sp. (parth. Diploid[b)])	Japan and Korea	47 (70.1)	19 (28.4)	1 (1.5)	67
Fasciola sp. (parth. Triploid[c)])	Japan and Korea	27 (73.0)	10 (27.0)		37

a) The flukes considered *F. gigantica,* and UGI means undefinable geoglaphic isolation.
b) Parthenogenetic diploid flukes.
c) Parthenogenetic triploid flukes.
Refer to report of Terasaki and Moriyama-Gonda [30].

of the uterus, and the center of the testes, and cover dorsiventrally the branches of the intestines and testes. They are like bunches of grapes, and a single bunch of them includes from a few to many vitelline cells. Each bunch puts out a fine duct, and the ducts combine to form larger ducts, until finally the ducts are collected on the mid-line of the fluke, and become two longitudinal vitelline ducts.

Transverse vitelline duct: Both sides of the longitudinal vitelline ducts branch at the posterior end of Mehlis' gland and become transverse vitelline ducts (Fig. 1, TVD). The transverse vitelline ducts cross the fluke and become the vitelline reservoir to join at the mid-line of the fluke between Mehlis' gland and the anterior testis.

Vitelline reservoir: The vitelline reservoir (Fig. 1, VR) lies rear end of Mehlis' gland. It appears in many kinds of shapes, but most of it is an inaccurate triangle. Two corners of it are connected with the transverse vitelline ducts and the rest of it becomes the common vitelline duct (Fig. 1, CVD) which runs to the center of Mehlis' gland to become thin either suddenly or gradually. The vitelline reservoir always includes a large amount of vitelline cells.

Common vitelline duct: The common vitelline duct (Fig. 1, CVD) that comes from the vitelline reservoir runs anteriorly to the ootype in the center of Mehlis' gland, and joins the oviduct which appears posteriorly. The common vitelline duct crosses often with Laurer's canal, but is always located on the ventral side of it.

Ovo-vitelline duct: The oviduct joins the common vitelline duct near the ootype, and the joining duct is called the ovo-vitelline duct (Fig. 1, O-VD). It connects immediately with the ootype. This duct is very short and sometimes barrel-like.

Ootype: The ootype (Fig. 1, OOT) lies in the center of Mehlis' gland. The shape of this structure is tubular, spindle, heart, pear-shaped, etc., but most commonly appears pouch-like with a characteristic thick wall. Fine ducts from Mehlis' gland cells (Fig. 1, MGC_1 and MGC_2) collect radially to the ootype. The vitelline cells and oocytes were found in the ootype, but no formed egg was recognized in any specimen.

Uterine valve: The distal part of the ootype becomes a very narrow duct, and this fine duct, called the uterine valve (Fig. 1, UV), was recognized in all observed specimens. However, the appearance of lips in the valve was not recognized; only very fine duct was observed.

Uterus: The next part is the uterus, a wide chamber in which vitelline cells, shell globules, masses of cells, formed eggs, and sperm mass in except parthenogenetic *Fasciola* sp. were found. However, the formed eggs were rare in this part. It is placed more ventrally than the ootype. In many *Fasciola* excluding parthenogenetic *Fasciola*, many stained sperm were found, forming the receptaculm seminis uterinum. The more distal part is a large tube having two or three convolutions under Mehlis' gland, and the uterus runs forward, then goes out the anterior part of the ventral side of Mehlis' gland. Furthermore, the uterus twists right and left, meandering between Mehlis' gland and the ventral sucker, and it leads to a female genital pore (Figs. 2 and 3, GP) behind the ventral sucker. When the cirrus (Fig. 3, C) is everted from the cirrus pouch (Fig. 2, CP), the uterus opens on the ventral surface near the posterior part of the cirrus base with a funnel-like shape, known as the uterine pore (Fig. 3, UP). Many brown eggs are found in the uterus, whereas they were white near Mehlis' gland. In most *Fasciola* excluding parthenogenetic *Fasciola*, many masses of sperm are found in the uterus.

Male reproductive organ: Figure 2 shows a sketch of the male reproductive organ near the cirrus pouch (Figs. 2 and 3, CP) when the cirrus is under invagination. Figure 3 shows a sketch of the same area when the cirrus is everted from the cirrus pouch.

Testes: Testes are constricted within tubes composed of

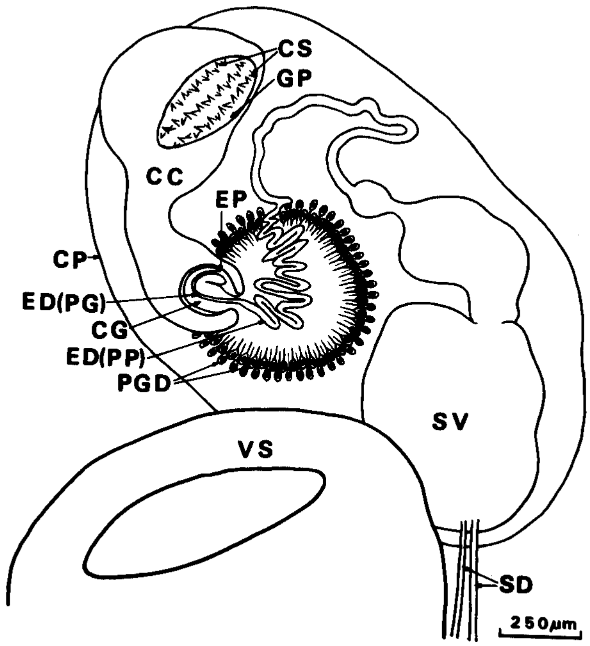

Fig. 2. Male reproductive organs near the cirrus porch when the cirrus is under invagination. See the abbreviations in Fig. 1.

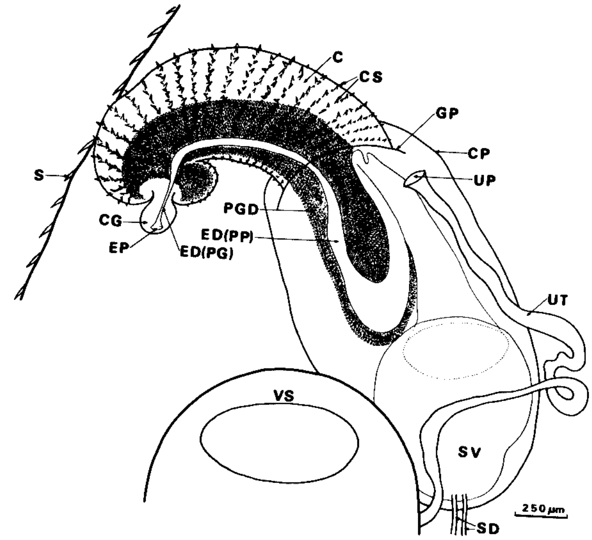

Fig. 3. Male reproductive organs near the cirrus porch when the cirrus is everted from the cirrus porch. See the abbreviations in Fig. 1.

complicated branches. They are placed in the center of the body and lie directly behind each other. Only the posterior portion of the forward testis and the anterior end of the rear testis overlap one another. Only the anterior portion of the forward testis overlaps with the posterior end of the ovary. In the opposite side of the ovary, the anterior end of the forward testis is in front of the median level of the ovary, but does not come out at the level of the anterior end of the ovary. The tubes of the testes sometimes have swellings, and these swellings are recognized in all groups of *Fasciola*, so there is no difference between the groups. The germ cells in many stages of the developing process filled the testes, but among the parthenogenetic *Fasciola* diploid, no spermatids in the spermiogenesis process were found, while among triploid, spermatocytes and spermatids were found. However their number are few compared with other *Fasciola*, and cells with differing sizes of nucleus and abnormal flagella of spermatids were found.

Sperm duct: Two sperm ducts (Figs. 1 and 2, SD) come out from near the anterior end of each testis and go forward through the uterus and behind the ventral sucker, and both ducts lead to the seminal vesicle (Figs. 2 and 3, SV), passing through the cirrus pouch (Figs. 2 and 3, CP) without joining. It was difficult to trace sperm ducts due to their thinness, but in the liver flukes excluding parthenogenetic *Fasciola*, sometimes the sperm filled the ducts, and in rare cases the ducts could be traced perfectly. In parthenogenetic *Fasciola*, rosettes of spermatocytes and spermatids were often found in the sperm ducts.

Cirrus pouch: In observing the mounted specimens, the cirrus pouch (Figs. 2 and 3, CP) was found obliquely in front of the ventral sucker on the ventral side of the fluke. However, in an observation under natural conditions, almost all of the cirrus pouch was hidden behind of the ventral sucker, and only the anterior end of the cirrus pouch was found in front of the ventral sucker. The cirrus pouch is a big saccular body surrounded by a thin membrane when the cirrus is under invagination (Fig. 2). The seminal vesicle (Fig. 2, SV), prostate gland (Fig. 2, PGD), ejaculate duct (Fig. 2, ED), cirrus (Fig. 3, C) or cirrus canal (Fig. 2, CC), and cirrus glans (Fig. 2, CG) were found in the cirrus pouch.

Seminal vesicle: The seminal vesicle (Figs. 2 and 3, SV) occupies the posterior half of the cirrus pouch, and the shape is undefined, but often the posterior half of the seminal vesicle is a saccular body, having some winding narrow parts in the anterior half, gradually becoming slender, and changing into the ejaculate duct (Figs. 2 and 3, ED) with no border between the two organs. In the *Fasciola* excluding parthenogenetic *Fasciola*, many sperm were found here. However, in the parthenogenetic *Fasciola* diploid, no sperm were found, and in the triploid the sperm were either absent or present in only a few of them. Therefore, parthenogenetic *Fasciola* clearly differs from other *Fasciola*. In parthenogenetic *Fasciola*, spermatogonia, along with rosettes of spermatocytes and spermatids, were found.

Prostate gland: The prostate gland (Figs. 2 and 3, PGD) is a unicellular gland similar to Mehlis' gland. A fine duct from the end of each cell leads toward the ejaculate duct, which runs through center of the gland. When the cirrus is under invagination (Fig. 2), the gland lies in front of the seminal vesicle within the center of the cirrus pouch. Its shape is roughly circular. The anterior end of it is attached to the bottom of the cirrus canal (Fig. 2, CC). However, when the cirrus is everted from the cirrus pouch, the gland invades in cirrus, so it occupies the ventral half of the cirrus (Fig. 3, PGD).

Ejaculate duct: The ejaculate duct (Figs. 2 and 3, ED), which continues from the seminal vesicle, invades the prostate gland when the cirrus is under invagination, convoluting greatly in the gland, and arrives at the cirrus glans (Fig. 2, CG) at the bottom of the cirrus canal. It is very difficult to completely trace the winding of the ejaculate duct due to the full development of the prostate gland of the *Fasciola*. This part of the ejaculate duct is called the pars prostatica (Fig. 2, ED(PP)). After the pars prostatica arrives at the cirrus glans, the ejaculate duct becomes the pars glans (Figs. 2 and 3, ED(PG)), an extremely fine duct. When the cirrus is everted from the cirrus pouch (Fig. 3), the ejaculate duct is included in the cirrus (Fig. 3, C) together with the prostate gland (Fig. 3, PGD), and the winding duct becomes a longer, straight duct(Fig. 3, ED(PP)). The pars prostatica of the ejaculate duct is surrounded by the prostate gland running along the ventral side of the cirrus, and arrives at the cirrus glans (Fig. 3, CG). In the specimens containing a full mass of sperm, the duct is clearly observed. On the cirrus glans, the ejaculate duct becomes an extremely fine duct (Fig. 3, ED(PG)) the same as when the cirrus is under invagination, and the fine duct opens at the top of the cirrus glans, called the ejaculatory pore(Figs. 2 and 3, EP).

Cirrus and cirrus canal: The protrusion of the cirrus (Fig. 3, C) everts like a glove. Therefore, when the cirrus depresses completely, a canal where the top of the cirrus becomes the bottom is created. This is the cirrus canal (Fig. 2, CC), a large irregular cave. The cirrus canal occupies the anterior half of the cirrus pouch. It contains the cirrus glans underneath. The ejaculate duct (Fig. 2, ED) opens out here. The inner wall of the cirrus canal consists of complicated wrinkles, and many spines grow on its surface (Fig.2, CS). An opening of the cirrus canal is called the genital pore (Figs. 2 and 3, GP).

The protrusion of the cirrus occurs conversely with the depression, and the cirrus canal everts itself, so that the inner wall of the cirrus canal becomes the surface of the cirrus. The protrusion process can be understood well through the observation of the movement of the cirrus glans. The everted cirrus is shaped like a banana, with the terminal portion recurved posteriorly. The surface of the cirrus, except for the cirrus glans and part of its adhesion, is surrounded by many shallow gutters and spines form a line along the gutters. The spines differ in scale (Fig. 3, S) that grow along the surface of the fluke's body, being smaller in size, and appear as isosceles triangles with the tops pointed. The dorsal half of the cirrus is constructed like a muscle, and the ventral half is filled by the prostate gland.

Cirrus glans: When the cirrus is depressed (Fig. 2), the cirrus glans (Fig. 2, CG) is located at the bottom of cirrus canal, and is mushroom-like. A very fine duct (the pars glans of the ejaculate duct, Figs. 2 and 3, ED(PG)) runs in the middle of it. When the cirrus protrudes from the cirrus pouch, the cirrus glans (Fig. 3, CG) is located at the top of the cirrus, does not evert, and does not change its shape at the protrusion. The wall of the cirrus glans does not have cirrus spines. The ejaculate pore opens up at the top of the cirrus glans.

DISCUSSION

Female reproductive organs: The position and shape of Mehlis' gland are well known. Mehlis' gland is formed by the gland cells [5]. The gland cells are separated into two types [8, 26, 33, 35]. We recognized two types of cells that are differentiated by their size and color.

It is agreed by many researchers that the ovary is located diagonally in front of Mehlis' gland. However, their opinions differ regarding its position, either right or left of the mid-line of the fluke. There are many who report that the ovary is located on the right side in the fluke, i. e. Belding [1], Dawes [2], Engbert [3], Faust [4], Miyagawa [16], Olsen [18], Yokogawa and Morishita [38] etc., while others state that it is on the left, Pantelouris [19], Yoshimura [39]. Yoshimura [39] mentioned that it is on the left side in many trematodes. Hoffman [6] reported a condition of a bilateral ovary as an abnormal case of *F. hepatica*. Henneguy [5] quotes a report by Sommer as follows; in *F. hepatica*, most of the flukes have ovaries on the right side, 20% of flukes have them on the left, and 6% bilaterally. Tandon [27] stated that in *F. indica*, the ovary is placed either on the left or on the right side of the body, whereas in some flukes, there are bilateral ovaries. Watanabe [37] reported that from a ventral view, in 93.2% of *F. hepatica*, the ovary is situated on the left, while in Japanese species and *F. gigantica*, 80.4% on the left; furthermore in the latter two, ovaries in both sides are not sparse. The results of this research from a dorsal view in *F. hepatica*; right side 85%, left side 10%, and bilateral 5%; in the rest of the flukes including parthenogenetic *Fasciola*; right side 71.3%, left side 26.8%, and bilateral 1.8%. These results show that in *F. hepatica* the ovary occurs on the right side more often than in other flukes. In all flukes, the ovary is usually placed on the right side, but in some flukes it is located on the left side, and there is a small percentage of flukes that have the condition of a bilateral ovary as Watanabe's report [37].

On the shape of the ovary, the opinions of researchers agree with each other, i. e. unpaired, highly branched, or tubular organs like antlers. However, it is called that the branches of the ovary in *F. gigantica* were more complicated than those in *F. hepatica*. Watanabe [37] also reported that the ovary of Japanese *Fasciola* sp. is the same as in *F. gigantica*, being thinner and more complex than in *F. hepatica*. From this observation, the branches of the ovaries vary greatly among individuals and developing situations, so it might be difficult to identify the species of each fluke by the shape of its ovary.

There are differences of opinion among researchers concerning the ducts (oviduct, Laurer's canal, and common vitelline duct) in Mehlis' gland. In this observation, the inside end of the ovary becomes thin, and the oviduct begins. A special organ like the ovicapt [2, 27] was not found. Iijima [7] suggested that Laurer's canal joined with the common vitelline duct. Henneguy [5] thought that Laurer's canal combines with the oviduct, uterus, and the (common) vitelline duct. Yosufzai [40] reported that there is a wide elliptical chamber in the center of Mehlis' (shell) gland, and the common vitelline duct and the oviduct joins the chamber. However, he failed to observe the connection between Laurer's canal and the duct in Mehlis' gland. The figure of the report of Lal and Johri [13], who observed *F. gigantica*, might show the connection of Laurer's canal and the ootype. Our observation agreed with Engbert [3], Madhavi and Rao [14], Rao [20], Stephenson [26], in the observation of *F. hepatica*, Rao and Madhavi [21] in that of *F. gigantica*, and Tandon [27] in that of *F. indica*.

In our observation, after the junction of the oviduct and common vitelline duct, the duct is very short, and reaches the ootype. The duct is called the ovo-vitelline duct by Rao and Madhavi [21]. Stephenson [26] thought that the uterus begins at the junction of the oviduct and the common vitelline duct, but he called the central chamber the beginning section, and found fine ducts from Mehlis' gland cells gathering in the chamber. It might be the ootype that is named by other workers [8, 14, 21, 27]. However, Stephenson [26] stated that the chamber was very narrow, thus indicating that the chamber differs from the ootype observed by us. Yosufzai [40] stated it was wide elliptical chamber, and joined the vitelline duct and oviduct. He thought that the chamber was narrower towards its anterior end, where it almost reaches the center of the shell gland. Engbert [3] stated that the part of the duct, whose wall is perforated like a sieve by the openings of Mehlis' gland, is the space for fertilization, and he called it fertilization space. Regarding the shape of the ootype, the opinions of researchers differ slightly. Rao and Madhavi [21] recognized a short blind caecum at the end of ootype in *F. gigantica*. They also recognized it in *F. hepatica* [14]. However, our observations did not discern a caecum in any *Fasciola*.

It is said that there is a uterine valve (non-return valve) in a funnel-like development between the ootype and the uterus [14, 21, 26,], but Henneguy [5] and Tandon [27] stated that the "valve" is merely narrow beginning portion of the uterus. In our observations of all specimens, we recognized the constricted duct, but could not recognize the funnel-shaped valve.

It is well known that the uterus opens to touch the genital pore through the dorsal phase of the ventral sucker. However, when the cirrus is everted from the cirrus pouch, the pore opens the surface at the base of the cirrus body. In the genus *Fasciola*, it is known that the seminal receptacle does not exist [3, 14]. The proximal coils of the uterus act as a seminal receptacle in *Fasciola* [25]. Panthelouris [19] showed this part in a figure to be a seminal receptacle. Kobayashi [12] called this part the receptaculm seminis uterinum.

Concerning both the position of the vitelline glands and the direction of the vitelline duct, almost all researchers agree with the results of our observations. As Tandon [27] stated, the vitelline gland spread over both the dorsal and ventral sides of the intestinal caeca. However, the reports that the vitelline glands present on the surface of both the ventral and dorsal area of the body to envelop other organs (testes, ovary, uterus, intestinal caeca etc.) are few. The vitelline reservoir is formed at the rear of Mehlis' gland, and the common vitelline duct [13, 27] protruding from the end of it goes to the ootype. This duct is also called the anterior vitelline duct [26], median vitelline duct [20], and median yolk duct [3].

Male reproductive organ: It is well known that the testes are present at the rear of the transverse vitelline duct and occupy the central body except the tail and the margin of the body, that the two testes lie one behind the other with their median axes nearly in the same line, and that they are very branched [2, 3, 16, 19, 27, 38]. The sperm ducts begin at the top of the two testes. Tandon [27] stated that the sperm ducts beginning from the two testes unite together near the ventral sucker and lead to the seminal vesicle. However, Iijima [7] showed in his figure that the two sperm ducts enter the seminal vesicle separately. Engbert [3] and Pantelouris [19] also stated that the two sperm ducts enter the seminal vesicle without joining. They are consistent with our observations.

Most researchers agree about the position and contents of the cirrus pouch (cirrus sac) [3, 19, 27]. Kobayashi [11] stated that the cirrus pouch is a long elliptical bursa, and is located obliquely in the dorsal part in front of the ventral sucker, and holds the prostate gland, seminal vesicle, ejaculate duct, cirrus, and cirrus apex. His description is consistent with our observations. However, Dawes [2] noted that the cirrus pouch is absent.

The interesting thing about the seminal vesicle is that Tandon [27], who observed *F. indica*, and Engbert [3], who observed *F. hepatica*, noticed many sperm in it, but Japanese researchers [11, 12, 38] do not note any sperm in it. The reason might be that Japanese *Fasciola* sp. are parthenogenetic flukes [9, 29]. In our observations, the common liver flukes, both *F. hepatica* and *F. gigantica*, were filled with sperm in the seminal vesicle, but in the parthenogenetic *Fasciola* sp., the sperm were either absent or sparse. This fact shows that the spermatogenesis is abnormal [30, 31], and the parthenogenetic *Fasciola* sp. can be differentiated clearly from other *Fasciola* including *F. hepatica* and *F. gigantica* by observation of the seminal vesicle. The seminal vesicle is one of the largest organs, so the organ of even the living flukes can be easily observed by a loupe; the failure of observing of sperm in it is rare. Of particular note, in the parthenogenetic *Fasciola* diploid, sperm were not found. However, a part of the triploid had some sperm in it. Terasaki *et al.* [32] state their hypothesis that these sperm fertilized the egg of the parthenogenetic diploid, resulting and the mixoploid is created. The seminal vesicle becomes an increasingly thinner duct toward the end of it, and proceeds to the ejaculatory duct with a lack of a clear border [11].

The ejaculatory duct differentiates two parts, pars prostatica and pars glandis (pars apex) [11]. When the cirrus is under invagination, the pars prostatica appears U-shaped and winding [12], and is surrounded by the prostate gland [3, 27]. These shapes agree with our observations. When the cirrus is everted from the cirrus pouch, the prostate gland enters the cirrus, occupying the ventral side of the cirrus. The pars prostatica of the ejaculate duct along with the prostate gland, also enters the cirrus, and appears without winding. Kobayashi [11] stated the pars glans becomes a very fine duct, agreeing with our observation, but the reports that showed the pars glans or pars apex are few.

Tandon [27] recognized spines on the surface of the cirrus in observations of *F. indica*, and stated that the spines have not been noted so far on the cirrus in any of the species of *Fasciola*. However, Kobayashi [11] already found the cirrus spine in *Fasciola*, and after that, other researchers also reported its presence in *F. hepatica* [3, 34]. Therefore, these spines are not distinctive features of only *F. indica*. Tandon [27] also stated that the spines are spread over the entire organ in an irregular way, and other researchers have not expressed the state of the spines. In our observation, we found that the surface of the cirrus is covered by many ring-like shallow gutters, and the spines grow regularly side by side along the gutters.

In the tip of the cirrus in *F. indica*, Tandon [27] recognized a finger-like process which he called the "cirrus glans", which was not noted in any other species of the genus *Fasciola*. However, Kobayashi [11] already reported its existence, and called it the "Cirrus-verus" or "Cirrus-appex". We also recognized its existence in observed flukes of all *Fasciola* groups.

Reports of the comparison of the reproductive organs between groups of the genus *Fasciola* are few. Almost all of the reports only stated that there are no differences in the organs between *F. indica*, *F. hepatica* and *F. gigantica* [13, 14, 21, 36]. Taylor [28] stated that the remarkably distinguishable shapes between *F. hepatic* and *F. gigantica* are not recognized. The results of our observation and consideration show that there is no difference on the structure of the reproductive organs among the *Fasciola* group (*F. hepatica*, *F. gigantica*, flukes which are probably *F. gigantica* from Asia, and parthenogenetic *Fasciola* diploid and triploid).

The new discovery in this research is only the finding that the surface of the cirrus is covered by many ring-like shallow gutters, and that spines grow orderly side by side along the gutters. Though there is no new discovery regarding structure of the reproductive organs, the condition of the testes, the sperm duct, the seminal vesicle, the ejaculatory duct, and the uterus are different between parthenogenetic *Fasciola* and other *Fasciola*, especially in the seminal vesicle, both groups can be easily and clearly distinguished by the existence of sperm. However, it is thought that the sperm in the uterus are from other individual flukes, so the existence of sperm in the uterus of a fluke does not reveal that the fluke is not parthenogenetic *Fasciola*.

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