Anatomy. — *"The foetal membranes and the placenta of Phoca vitulina."* By A. J. P. VAN DEN BROEK. (Communicated by Prof. L. BOLK).

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Some time ago a seal in an advanced state of gestation was sent to the anatomical Laboratory of the Amsterdam University. As neither the foetal envelopes nor the placenta of the pinnepedal carnivores have been described in detail, I was very gratified to be entrusted by the Director of the Laboratory with the task of carefully examining these organs. The preparation turned out to be in very good condition and well enough preserved for microscopic examination.

Whereas a more detailed description will be published in another place, the following may be given here as the principal results of my investigations.

The gravid uterus was U-shaped, the convexity being on the cranial side. The organ, which was somewhat flattened dorso-ventrally was lying in transverse direction, so that the fundus uteri was situated in the righthand part of the body. This U-shaped curve was accompanied by a twisting of the organ, so that the left ovarium, entirely enveloped by an ovarial bag, was medially situated.

The greater part of the ligamentum latum had become absorbed; the ligamentum rotundum, a very powerful cord, ran from the front-, respectively hind-wall of the uterus, that is: from the spot where the ovarium was lying against the uterine wall, running down in a slanting direction across the latter, towards the abdominal wall. The ostium uteri, filled with a mucous clot, showed an aperture of ± 3 cm.

Close to the top of this ostium, which formed an oval foramen in the middle of the portio vaginalis, the foetal sac, rather strained, could be felt. All this seemed to indicate that the animal, being in labour, had gone ashore, when it was caught.

On opening the uterus by means of a longitudinal incision in the organ, along the convexity, the following was noticed: The wall of the uterus is extremely thin in proportion to the voluminous organ, a little more than 1 mm., but increases in thickness towards the vagina. The muscularis of the wall of the latter, however, is rather thick (1 cm.).

The chorion, as well as the placenta, is only loosely attached to the walls of the uterus all over. It appears that the foetal sac reaches down to the ostium uteri. On detaching the placenta I found septa of cellular tissue adhering to the inner surface of the wall of the uterus, which, just there, is somewhat thicker.

The placenta forms a perfectly closed girdle, situated about the middle of the foetal sac, or, with regard to the uterus, in the curve of that organ.

The uterine surface presents a lobated structure, the various irregular lobes being separated by pretty wide fissures, and as moreover, the placenta is comparatively thin, it is not very compact.

The circumference, measured at the surface of the uterus, is 66 cm., the width, which is the same all over, 32 cm. The edges are not stumpy and thick; rather thin. Several thick bloodvessels emerge from the sides of the placenta, branching off into the chorion so abundantly as to leave not a single part of it without a supply of vessels. On splitting the chorion and the placenta along the convex curve of the foetal sac, the amnion becomes visible. This is quite disconnected. There was no direct contact between the two tissues anywhere, nor with the placenta, the amnion not covering the foetal surface of the placenta, but branching off at the placentary end of the umbilical cord. The amnion, like a bag with thin walls, invests the foetus pretty closely, especially along the back. Only behind the tail-end of the foetus, which is turned towards the fundus uteri, we find another part of the amnion that is not taken up by the foetus, but filled with a substance that is soft to the touch. The amnion is partly vascularized, partly devoid of any vessels; especially in the immediate vicinity of the umbilical cord a vascular part is noticeable. Here the amnion membrane seems also to be somewhat thicker and to consist of two layers, which are moveable one over the other. Here we find the umbilical sac, in the shape of an elongated organ, connected with the amnion right along, lying against this membrane.

As the amnion has not coalesced with the foetal surface of the placenta, the latter can be at once examined after opening the chorion. Of an insertion proper of the umbilical cord into the placenta there can hardly be any question, the vessels dividing and branching off long before they reach the placenta.

These ramifications enter the placenta at a rather considerable distance from each other. Slips of the allantois, which lines the placenta, cover these vessels, which therefore are found in duplicatures in this membrane as soon as they leave the cord. The main canals of each of the two umbilical arteries branch off into a girdle-shaped half of the placenta, and with fairly large ramifications spread further into the chorion. When the convex surface of the amnion is slit open very little foetal fluid runs out. The foetus is found to be lying with its muzzle towards the ostium

uteri, its back turned upwards. It also appears that the available space behind the tail-end of the foetus is filled up with about 3 liter of lanugo, some of which was also adhering in places to the body of the foetus. In the skin itself there were no remains of the lanugo-covering, which consisted of short, straight hairs, all of the same pearl grey colour. Speckled or black hairs were not found.

The umbilical cord has a length of only 12 cm. so that the animal, at the time of birth, is bound to pull out the placenta along with the membranes. The skin is continued for about 1 cm. along the umbilical cord, which is compressed sideways along its whole length. Taking into consideration the thickness of the umbilical cord there is only a small quantity of connective tissue. The three umbilical vessels are not twisted around each other. The outer surface of the umbilical cord is yellowish, shining, and feels hard.

A microscopical examination gave the following results:

The umbilical cord is covered with an epithelium forming a pavement of several layers, which forcibly reminds one of the epithelium of the epidermis. The lower layer of cells consists of high cells of a somewhat cylindrical shape, lying close together, with large round nuclei. Towards the surface the cells grow flatter, their limits being well defined in the layers immediately following the basal one. As the cells flatten down, the nuclei grow less prominent. Finally, forming the outside layer, there is a horny surface with lamelliform structure, in which, (by means of staining with haematoxyline) the remains of nuclei can be traced in places. This horny layer is sharply marked off from the epithelium-layer.

Where the amnion leaves the umbilical cord, this epithelium of many layers changes into a simple epithelium.

In the cord itself there are in section five channels of unequal width and with a wall of uneven thickness.

The two umbilical arteries and the vena umbilicalis have a very thick muscular coating of circular muscle-fibres. The muscle-cells show a lamelliform arrangement, the lamelli being separated by connective tissue.

Elastic filaments cannot be traced. Towards their lumen the blood-vessels are covered with an intima, which is not clearly defined towards the periphery.

In the walls of the blood-vessels we find, almost right up to the intima, the lumina of vasa vasorum, belonging to the system of the vessels proper of the umbilical cord, to be mentioned presently.

A fourth lumen is that of the second vena umbilicalis. The wall of it clearly shows an intima, round this there is a circular muscular

coating, and then, right round this, a coating of bundles of muscles running lengthways, but not adhering close together. In the walls of this vena I have not noticed any bloodvessels. In the lumen I found here and there some blood-corpuscles. In following up this partly obliterated vena towards the foetus, it is found to divide itself into two branches shortly before the foetal insertion of the umbilical cord; the two branches run together a little way down, then one of them splits up again and three branches can be traced right up to the front abdominal wall, where they lose themselves. Towards the placenta this vena also splits up into ramifications, which grow finer and finer and finally branch off into the tissue of the umbilical cord.

The fifth lumen in the umbilical cord is situated in the foetal half between the two umbilical arteries; towards the placenta these approach each other and the vessel runs alongside of them. This lumen is of an irregular shape, somewhat compressed and provided with an epithelium composed of cells that are flattened and arranged in several layers, the respective limits not being distinctly defined.

This lumen runs right through the cord till close to the placenta where it suddenly stops. There is no communication between this lumen and the yolksac. That we have here a continuation of the allantois-channel is proved by its original position between the two umbilical arteries, which position I also noticed inside the abdominal wall. I did not notice any remains of a yolk duct with certainty.

The stroma of the umbilical cord consists of cellular tissue with exceedingly fine fibrils pursuing a circular course. Underneath the epithelial coating this circular direction is deviated from and the curve becomes irregular. Round the vessels there is no distinct system of circular fibres. Between the two arteries the character of the stroma changes somewhat, it is of a looser construction and contains a few longitudinal bundles of smooth muscular tissue. These can best be seen by staining with polychrome methylen-blue (Unna) and can be traced right through the whole cord. By the side of this the profusion of the bloodvessels belonging to stroma funiculi proper seems remarkable.

These *vasa propria funiculi umbilicalis* are met with right along the funiculus, most of all in the foetal part. They appear to be connected with the vasa of the subcutaneous cellular tissue of the abdominal wall. Arteries as well as large veins filled with blood are noticeable. The distribution is somewhat irregular. Especially round the umbilical vessels they are heaped up together, entering the walls close up to the intima. I have not been able to ascertain the existence of a connection between the vasa propria funiculi and the vasa

umbilicalia. As far as I know, a case of the presence of vasa propria funiculi in any animal was not on record.

This isolated case suggests the general peculiarity of the vascular system of seals, to which attention has already been drawn by HYRTL [1]). I may add that the immediately surrounding parts of the allantois were also very rich in vasa propria, which, however, were generally of smaller size.

A microscopic examination of the chorion, or rather of the wall of the outermost and widest embryo-sac, shows us that there are two layers, an outer one and an inner one, connected by extremely loosely woven cellular tissues; between the two layers the blood-vessels are situated. The outer, or uterine layer, the chorion proper, is lined with a simple cylindrical epithelium about 20 μ high. The nuclei are oval-shaped and in the basal half of the cell; the protoplasm is finely granulated. Cell limits are distinctly visible. The inner lining consists of one layer of flat cells with much-flattened nuclei. The inner coat is nothing but the outer surface of the allantois. (This connection will be referred to bye and bye). We cannot, therefore, call this membrane the chorion, strictly speaking, the name of outer foetal envelope is more to the point. The bloodvessels in the wall of this sac are of unequal caliber, in the centre, between the two layers, larger vessels are met with, the arteries have a thick muscular coating, smaller vessels are found partly in the stroma of the allantois, in greater quantity however right under the chorion-ectoderm.

Even with the naked eye an accumulation of small villi, conspicuous by their velvety aspect, could be seen in some places on the uterine surface of the outer foetal sac. A microscopic examination confirmed that these were rudimentary villi. The epithelium did not differ from the other parts, but the stroma was a much more compact tissue and the interior was filled with very numerous capillaries the central ones of which had a fairly large lumen.

The amnion, too, or rather the inner foetal sac, is found to consist of two layers, lying against each other along the whole surface; only where the umbilical vesicle is situated they separate to envelop this. The inner layer is covered with a cubic epithelium of 10 μ., the outer one is identical with the inner layer of the outer foetal sac and must be considered to be a layer of the allantois. On comparing foetal sacs of Phoca vitulina with those of other carnivores, Phoca

[1]) HYRTL. Ueber einige Eigentümlichkeiten der arteriellen Gefässverästelungen bei den Seehunden.

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is found to exhibit somewhat different conditions. In dogs, for instance, according to the investigations of BISCHOFF [1]), STRAHL [2]) and others, the allantois-sac forces its way between the chorion and the amnion. While the allantois is thus making its way further and further between the two membranes, gradually separating them almost entirely, the edges of the allantois are coming closer together. But these edges, according to the drawings of BISCHOFF (l.c. Plate XV, fig. 8) remain separated; according to STRAHL [3]), they establish contact by means of cellular tissue, chorion and amnion being thus connected at this point of contact. In *Phoca* I found neither the edges of the allantois-sac, nor a connection like STRAHL noticed in dogs; I must therefore assume that in Phoca the development has gone a little further and that through the elimination of the partition between the two edges of the allantois-sac, mentioned by STRAHL, the outer and inner sac have become entirely isolated. The whole of the original extra-embryonal coelom-cavity has in this way disappeared; the space between the inner and outer foetal sac is the allantois-cavity.

I have already pointed out that the umbilical cord was connected with the placenta by means of a so-called insertio velamentosa. The vessels running towards the placenta or in the inverse direction, are enclosed by the membrane covering the foetal side of the placenta, viz. the outer layer of the allantois-sac, which continues on the chorion along the edge of the placenta.

As far as the inner foetal membrane is vascularised, the vessels show the same proportions as in the outer foetal sac.

The umbilical sac is situated in the inner wall of the foetal sac, between the amnion and the allantois, connected by thin cords of cellular tissue with both. It is a much-elongated, narrow bag, with folded walls.

The walls consist of strongly vascularized connective tissue, its limits are not sharply separated from the stroma of the amnion- and allantois membranes. Only here and there we find the remains of an original coating of epithelium. The bloodvessels, some of which have a fairly large lumen, run almost entirely lengthways through the organ. The majority of these vessels have a thick muscular wall and were filled with blood. These vessels and the umbilical ones are connected with one another.

With regard to the placenta the following may here be stated.

[1]) BISCHOFF, Die Entwickelungsgeschichte des Hundeëies.

[2]) STRAHL, Untersuchungen über den Bau des Placenta. III. Archiv für Anatomie und Entwickelungsgeschichte. 1890.

[3]) l.c. p. 199.

As already mentioned, the seal has a placenta zonaria, which, compared with that of the dog or the cat, is of much looser construction. The uterine side shows many more or less deep grooves, which divide the organ into a number of lobes. The deeper grooves, as a rule, run through the placenta in a longitudinal direction.

In the seal a green border-zone, such as we find described in the placenta of several carnivores, does not exist. Yet there is something like it. On detaching the placenta from the wall of the uterus, one could see, even with the naked eye, that both edges of the placenta were freely dotted with small, light-orange coloured particles, some as large as a pin's head; found, on examination, to consist of bilirubine. This pigment was found in enormous quantities and a microscopical investigation showed that the whole edge was saturated with these orange coloured bits. Owing to them, the narrow border-seam, instead of being a dark red, like the other parts of the placenta, was of a dirty brown colour.

As far as the blood-pigment penetrates in the placentary tissue — for these corpuscles may safely be taken to be transformed blood — the whole tissue, when seen under the microscope, is tinged a light yellow.

On following up this tinged zone under the microscope, we shall see that along the outer edge the amorphous pigment is found in great quantities, but no newly ejected blood is found. Only at some distance from the edge the clots of pigment become smaller, but at the same time they are found to be lying in ejected blood that can still be easily recognised as such, until finally, at the placental end of the brown zone, the blood predominates and here and there a glittering orange-coloured pigment clot is met with.

From this we may infer that the hemorrhage, during the development of the foetus, first occurs on the outer limits of the placenta and gradually more towards the centre.

The bleedings at the edge are not the only ones I noticed in the placenta.

In dogs, for instance, STRAHL describes some so-called green islets in the placenta, spots corresponding to the green edge-zone. These islets do not occur in seals either, at least they are not visible macroscopically. Under the microscope, however, we can see in many places, under the foetal coating of the placenta, some uniformly coloured light yellow spots. On close inspection it is seen that in such places, situated immediately below the foetal surface, hemorrhage has taken place, and that round this seat of bleeding the placental tissue is of a light yellow hue all over. Blood-pigment in

amorphous condition I have not been able to trace in such spots. The discoloration of the tissue may be explained in this way that the blood-corpuscles give off their haemoglobine to the surrounding parts and that this is absorbed gradually by the surrounding placenta-tissue. This latter point is the most significant part of the process, for, why should this surrounding tissue, which, unlike the corpuscles, can hardly be regarded as having died off, absorb the altered blood-pigment so uniformly? That we have here no post-mortem phenomenon appears clearly on examination of the blood-vessels at the edge of the placenta. As already stated, this is of a more intense yellow hue than that of the spots under the foetal surface, partly through the more considerable amount of pigment. Now, we find in the tissue of the border of the placenta, several vessels, the lumen of which, in addition to blood-corpuscles, is filled with pigment. These are foetal vessels. In the vasa umbilicalia I only found some blood, but no pigment.

The presence of this pigment in the vessels proves, that this yellow hue of the placenta-tissues is a vital reabsorption-process, and not one of post-mortem diffusion. There is another fact in favour of this view: it is my discovering (in the border-zone, where the villi are not very much elongated and the villous epithelium, or chorion ectoderm, is still intact), some distinct pigment-particles in these cells, which had been absorbed from the pigment situated close against this epithelium.

And on further comparing the structure of the placenta of Phoca with that of fissipede carnivores (e. g. the dog), it is found that the spongy layer is entirely missing; all along the thickness of the placenta the same uniform structure is maintained.

The foetal surface of the placenta is covered with a layer of the allantois, coated with a single layer of flat endothelium, as already described. Underneath this, there is a thin layer of fairly firm connective tissue, in which the larger ramifications of the umbilical vessels are found.

From this tissue coarse septa of connective tissue find their way into the placenta, which gradually diminish as they run towards the foetal surface, although they usually succeed in reaching it. In these septa the ramifications of the foetal vessels are found. Coarse septa send out finer compartments in every direction. The coarser ones divide the very compact mass of placenta into smaller sections.

From the maternal side too, some septa of connective tissue filling up the aforesaid grooves on the foetal side of the placenta, enter the latter; they are however less voluminous than those coming

from the foetal side. The compact cellular tissue of the placenta reaches right up to the wall; between the latter's coating of muscles and the compact placenta tissue a very narrow, loosely woven layer of connective tissue runs. In this layer, lying against the muscular coat, we find the diameters of the very wide uterine vessels and also in several places some uterus-glands. These glands, in the shape of elongated tubes with short ramifications and parallel with the surface of the uterus, are squeezed in between the muscular coat and the placenta. They have a distinct lumen, the epithelium is highly cylindrical. They are glands that have obviously fully developed, but have not been invaded by villi. On the foetal surface of the placenta moreover, distinct extremities of glands are met with, which, like those just described, have bent round and which run parallel with the muscular coat, in which a foetal villus is plainly visible. These villi are not lying close to the inner surface of the follicle, but are somewhat retracted from it. I did not succeed in observing a distinct epithelium on these villi.

Some of these uterus glands appear in another form: the cells have very much increased in volume, have swollen and are bulging out in several places in the lumen. The protoplasm of these cells is of a well defined reticular structure, the cell nuclei are in the basal part of the cells. In the lumen of such glands we find some independent cells, or combinations of only a few, some with nuclei, the larger ones without any; moreover some conglomerations of deeply stained fine particles may be observed here and there.

The compact mass of the placenta, when magnified, turns out to contain a great number of bloodvessel-lumina, adhering close together, each one of them surrounded by a distinct endothelium-wall. The ramifications of the foetal vessels, together with some connective tissue, run between these branches of the placental vessels. Forming a separating layer between these two systems of blood-vessels, we find in the greater part of the placenta one single layer of nuclei, situated in a mass of protoplasm, in which, however, no cell limits could be traced. This layer of nuclei, is to be regarded as belonging to a syncytium. In some places I counted two, sometimes even more, rows of nuclei between the branches of the placental- and those of the foetal vessels, without my being able to say with certainty which part of it should be taken to be of a syncytial character.

In the cases of suballantoidal hemorrhage and in the surrounding parts, the nuclei of cells, lining the endothelium-walls of placental vessels and the foetal villi adhering to the blood-mass, are of a much darker hue than those of other parts of the placenta. As a

coating of these foetal villi I often observed a double layer of nuclei, viz. a layer of flattened nuclei, lying close together, coating the villus and, round this, a layer of round, large nuclei. Against the latter layer the extravasated blood is found. Between the nuclei of the inner series, cell-limits are sometimes noticeable (chorionectoderm).

Finally I want to offer a few remarks about the villi in the border-seam.

The mass of coagulated blood found near the edge of the placenta, and the bulk of which has the shape of amorphous, orange coloured lumps, is mainly situated between two very long villi, running in a slanting direction from the foetal surface of the placenta to the wall of the uterus. From these villi and from the part of the chorion that is lying between the bases of the two, a number of shorter villi, with ramifications, find their way into the above mentioned mass.

These villi are generally somewhat swollen and rounded off at their extremities. They are all conspicuous by their profuse vascularity.

The chorion between the bases of the two villi just described is covered with a layer of very darkly-stained nuclei, lying very close together. It remains doubtful whether this was a syncytium.

The secondary villi, passing into the above mass also have such a coating. In some cases two rows of nuclei could be seen on the surface, while it seemed to me that the row of nuclei turned towards the villus was not of such a dark hue as the outer one turned towards the blood-mass. At the extremities of the villi the nuclei are found to lie very closely together and to be more numerous.

Both in the protoplasm of the latter layer, covering the chorion, and in that of the villi one observes accumulations of orange-coloured particles.

Reabsorption of these corpuscles from the mass found in the border-seam of the placenta is therefore effected by means of the coating of the said layer of the chorion as well as that of the villi.

In one or two instances I found a coloured particle in the stroma of a villus or in the capillaries running into it.